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Canine and Feline Theriogenology

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dedicated to

. . . my beloved husband, Gary, our children, G-Man and Ali,
to Dorothy and Mother for their love

Shirley D. Johnston

. . . my parents, Arnold and Jeannette Root, my loving husband, Jason,
and our children, for their support and love

Margaret V. Root Kustritz

. . . my husband, Jerry, our children, Amy and Ron,
and my parents, Edwin and Naomi Schultz;
through their love, each has demonstrated their compassion and commitment for a chosen profession, and I am eternally grateful to each of them

Patricia N. S. Olson

. . . and to the many wonderful dogs, cats, clients, and students
from whom we have learned so much

the authors

Dr. Shirley Johnston and Grace

Dr. Margaret Root Kustritz and
(left to right)
Beatrice, Honda, and Esther

Dr. Patricia Olson and Emma

Preface

This book reflects our commitment to reproductive health of the dog and cat. The theriogenologist fulfills the charge of the Veterinarian's Oath to protect animal health and relieve animal suffering by learning normal reproductive physiology, understanding pathogenesis and treatment of reproductive disease, promoting sound reproduction in some patients and preventing reproduction safely in others. We are as committed to addressing the pet overpopulation problem in the United States with safe, effective contraception, as we are to furthering the understanding of how to improve and enhance reproductive performance. While promoting an expanded knowledge of normal reproductive physiology (learning from our patients), we also applaud advances in assisted reproduction (learning from our colleagues) with procedures such as artificial insemination with chilled and frozen semen, trans-cervical insemination, gamete cryopreservation, and in-vitro fertilization. We believe that every puppy and kitten born should have the best health, the best care, and the best opportunity for a good life, and are proud to be part of a profession that works to those ends.

The Authors

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NOTICE

Veterinary Medicine is an ever-changing field. Standard safety precautions must be followed, but as new research and clinical experience broaden our knowledge, changes in treatment and drug therapy become necessary or appropriate. Readers are advised to check the product information currently provided by the manufacturer of each drug to be administered to verify the recommended dose, the method and duration of administration, and contraindications. It is the responsibility of the treating veterinarian, relying on experience and the knowledge of the animal, to determine dosages and the best treatment for the animal. Neither the publisher nor the editor assumes any responsibility for any injury and/or damage to animals or property.

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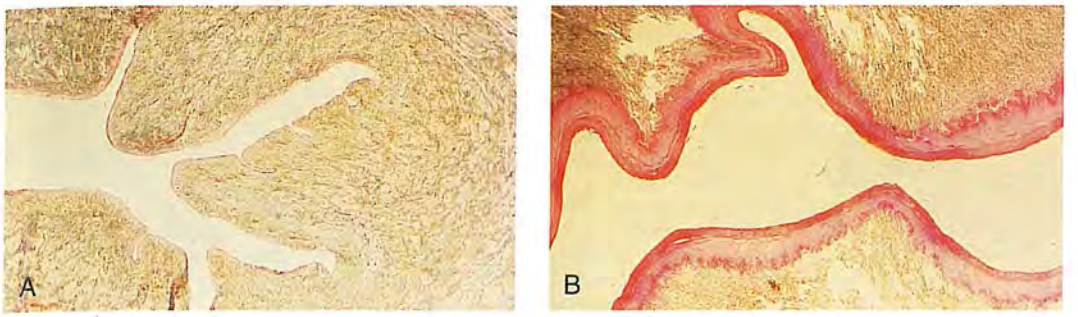


Figure 3–1. A: Histology of canine vaginal epithelium during anestrus. **B:** Histology of canine vaginal epithelium during estrus. Note the increased thickness of cells near the vaginal lumen. (Courtesy of Dr. Alvin Weber, University of Minnesota.)

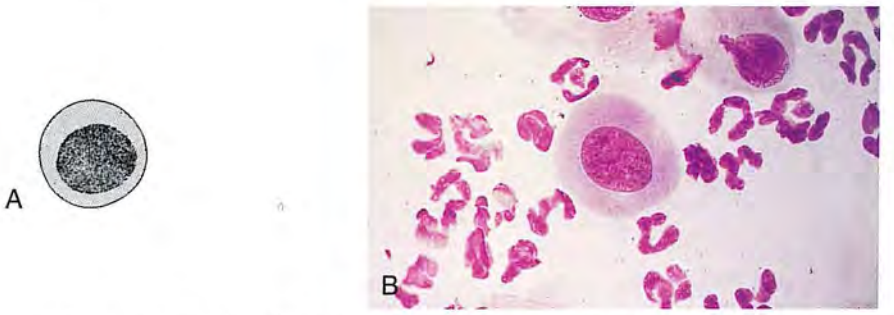
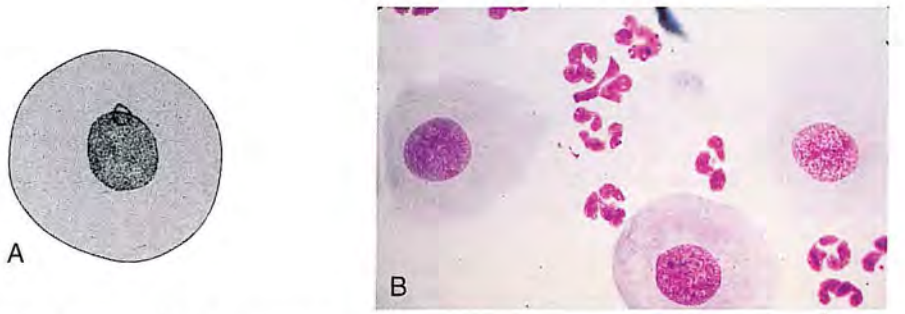


Figure 3–2. A: Schematic diagram of a parabasal epithelial cell from the canine vagina. (From Olson PN, Husted PW, Nett JM: The management of a successful mating between the bitch and the stud dog. Kal Kan Forum 2:15, 1983, with permission.) **B:** Parabasal epithelial cell (center) and neutrophils. Magnification; 1000×.



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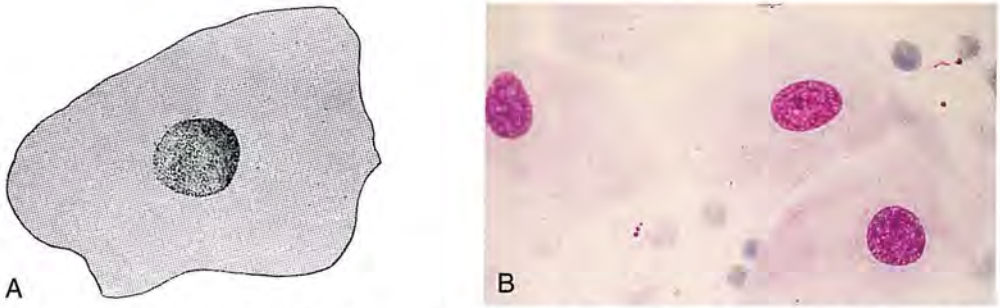

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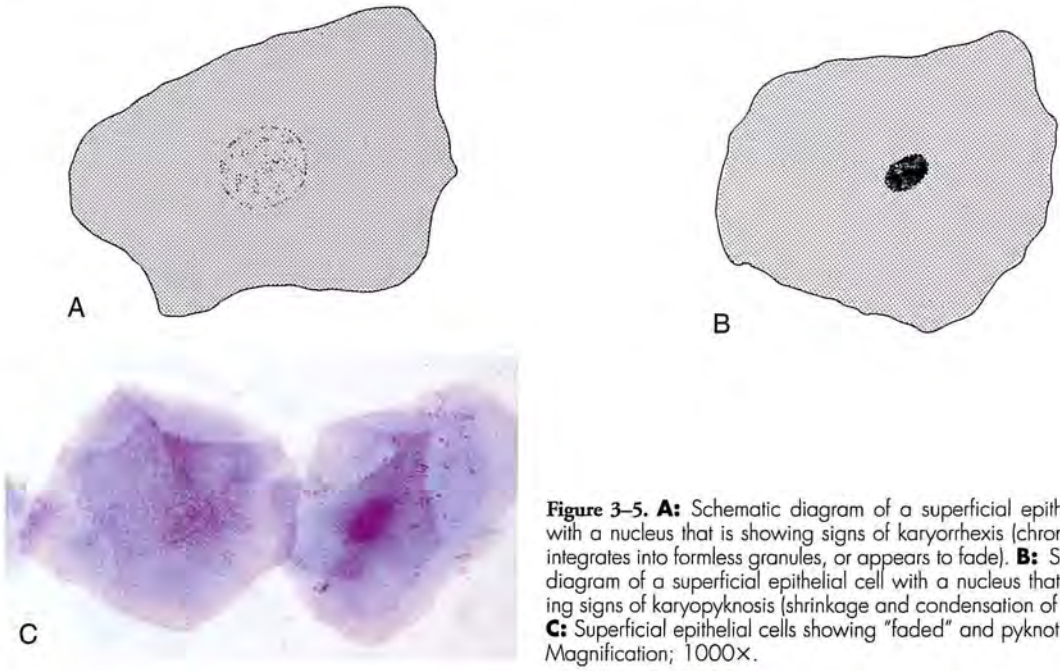

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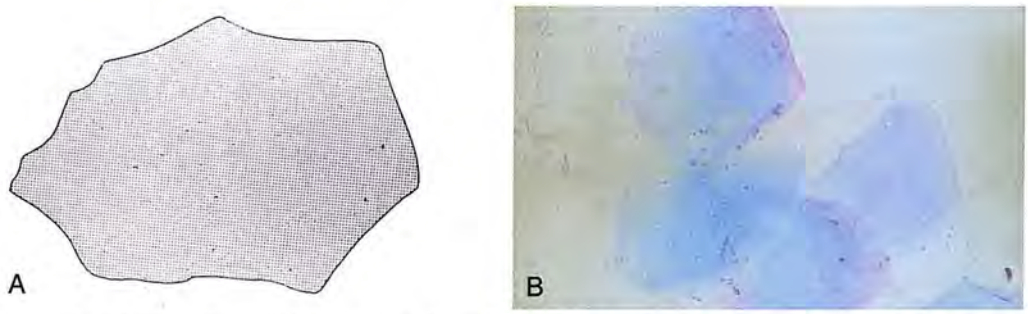

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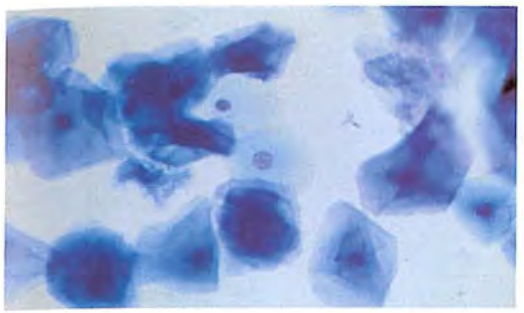

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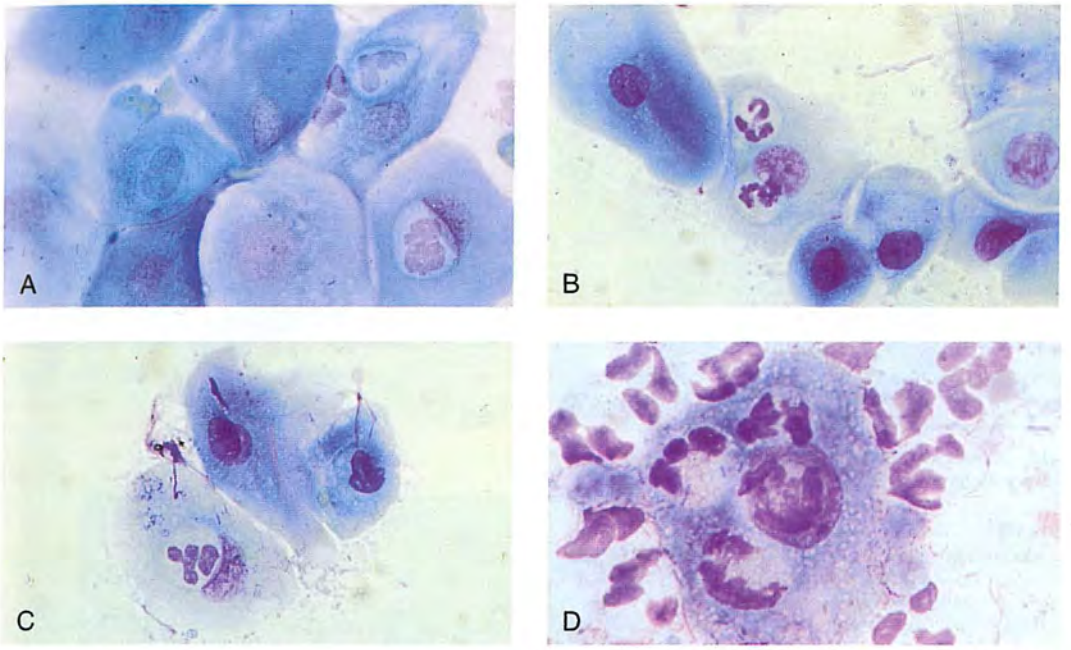

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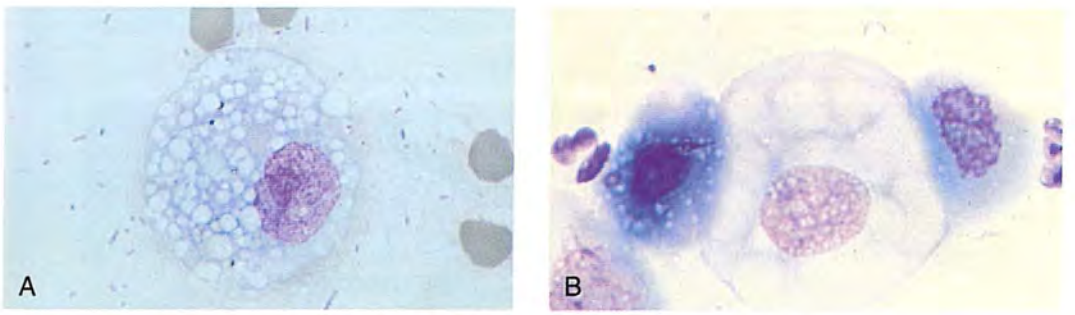
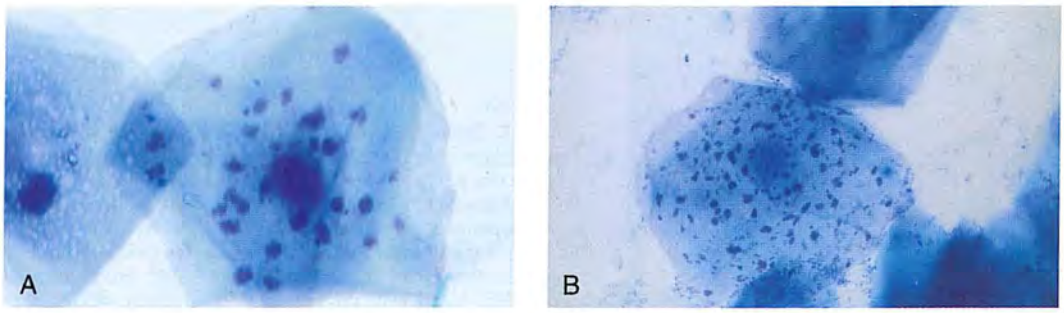


Figure 3–9. A and **B:** Foam cells. Magnification; 1000×.



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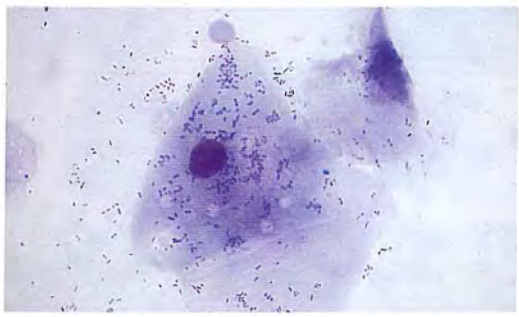

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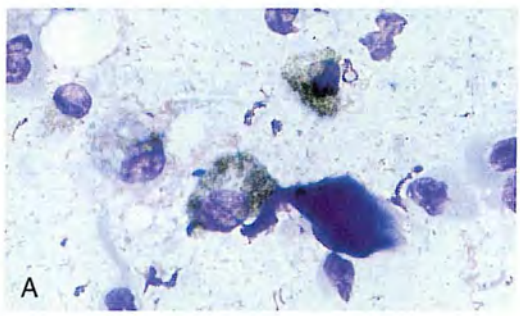

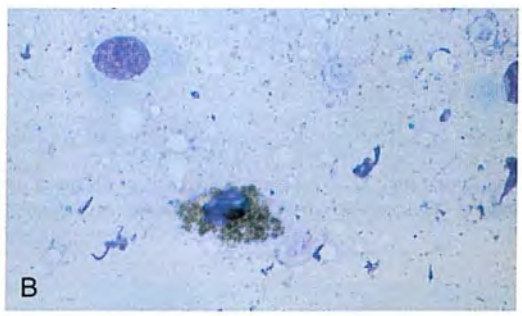

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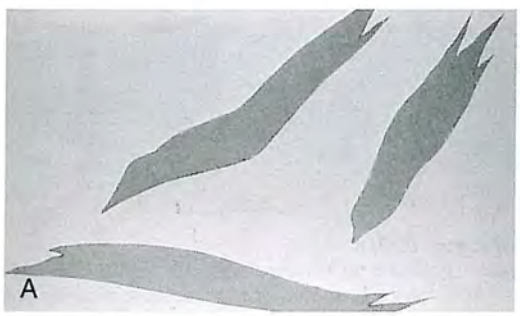

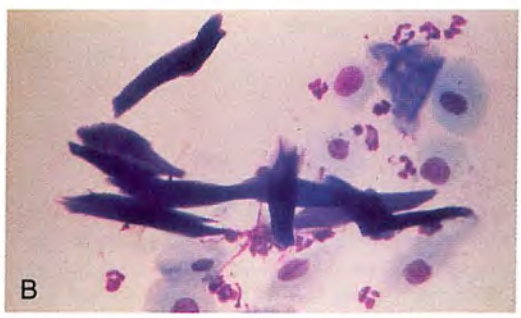

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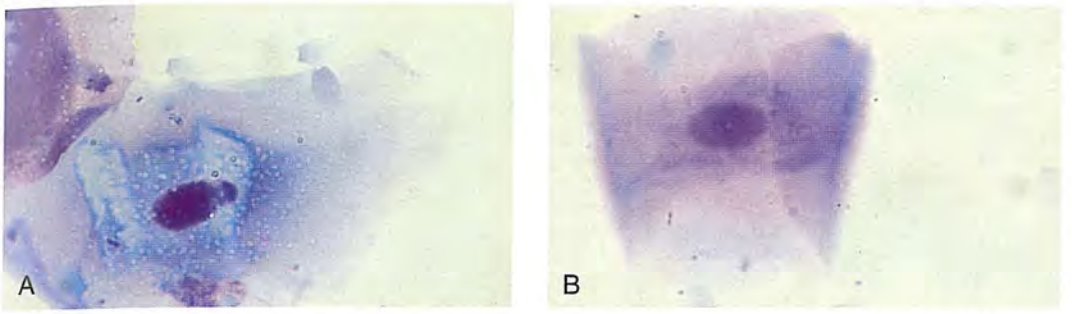

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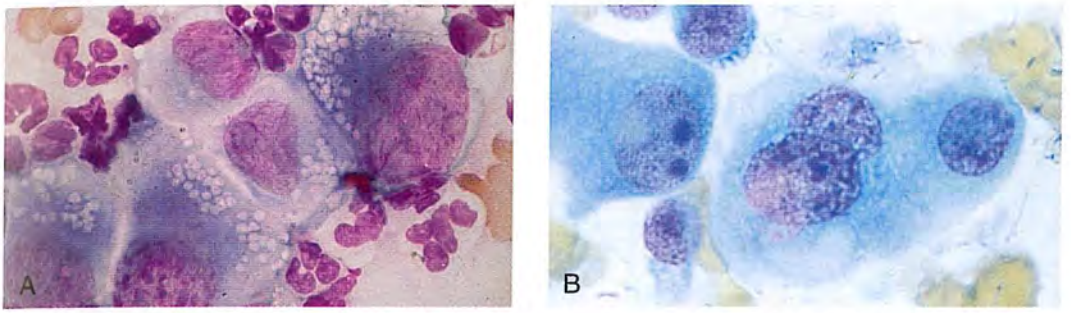

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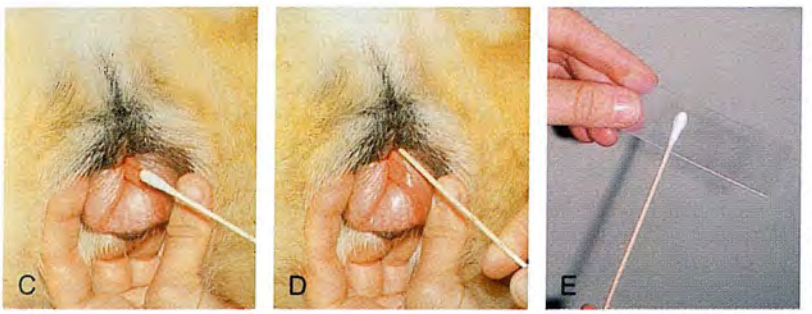


Figure 3–16. C and **D:** Caudal view, demonstrating the appropriate angle to pass a swab into the canine vestibule and vagina, avoiding the clitoral fossa. (From Olson PN, Thrall MA, Wykes PM, et al: Vaginal cytology. Part I. A useful tool for staging the canine estrous cycle. Compend Contin Educ Pract Vet 6:288–298, 1984, with permission.) **E:** Immediately after collection, the vaginal swab should be gently rolled over a clean glass. The smear should be allowed to air-dry prior to staining.

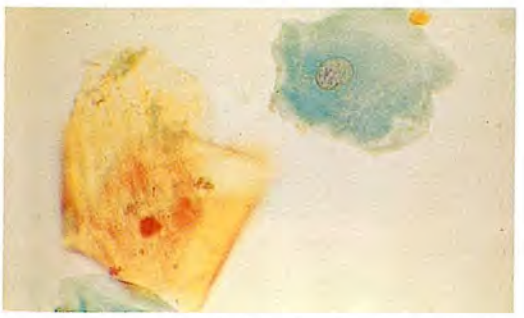


Figure 3–17 Vaginal epithelial cells that stain eosinophilic or cyanophilic with trichrome stain. Magnification; 100×.

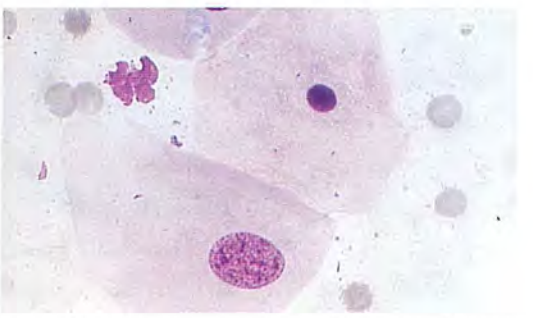
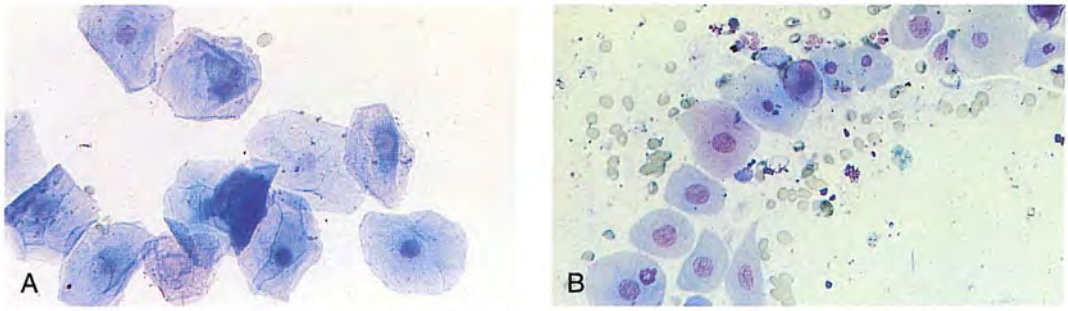


Figure 3–18 A large intermediate cell with normal-appearing nucleus and a superficial cell with a pyknotic nucleus. Magnification; 100×.



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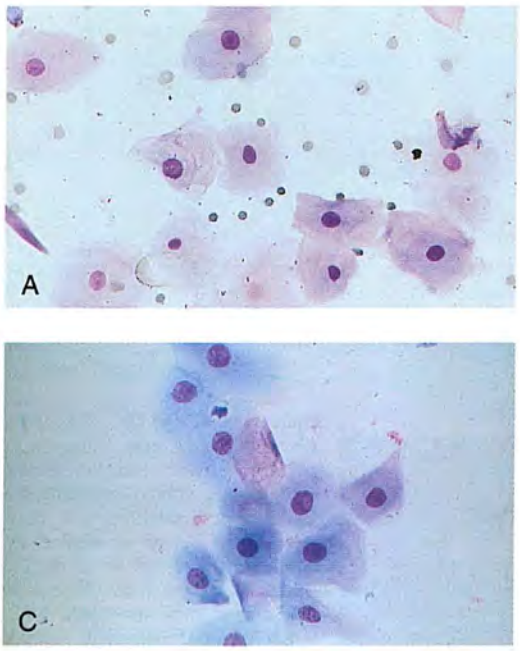


Figure 3–20. A: Vaginal smear from a bitch in early proestrus. The smear contains various types of epithelial cells, erythrocytes, and neutrophils. Magnification; 400×. **B:** Vaginal smear from a bitch in proestrus that contains numerous bacteria. Magnification; 400×. **C:** Vaginal smear from a bitch in proestrus that contains few to no erythrocytes. Magnification; 400×. (**B** and **C** from Olson PN, Thrall MA, Wykes PM, et al: Vaginal cytology. Part I. A useful tool for staging the canine estrous cycle. Compend Contin Educ Pract Vet 6:288–298, 1984, with permission.)

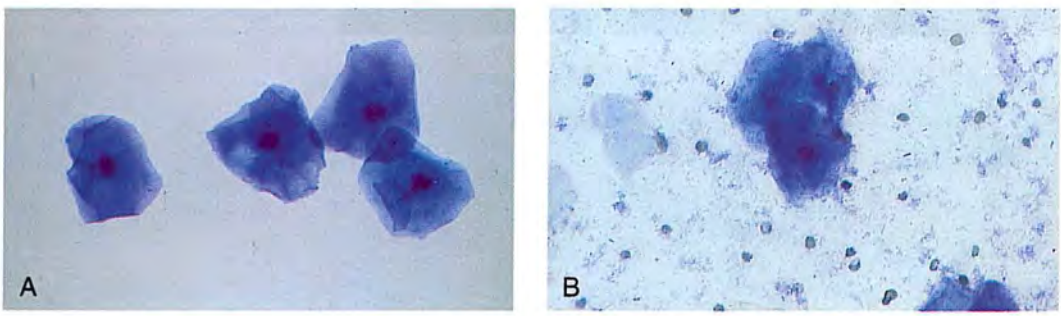


Figure 3–21. *legend on opposite page*

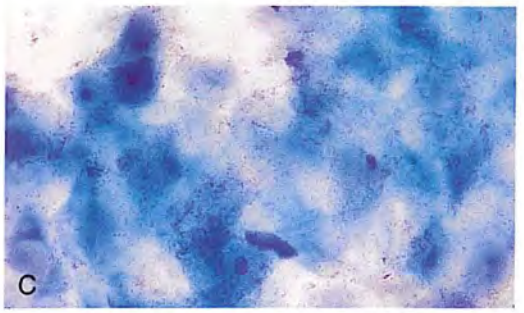
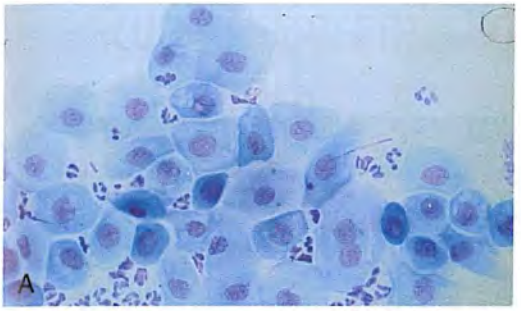


Figure 3–21. A: Vaginal smear from a bitch in estrus. Note the clear background, devoid of cellular debris. The epithelial cells have distinct borders. Magnification; 400×. **B:** Vaginal smear from a bitch in estrus that contains erythrocytes, numerous bacteria, and epithelial cells. The epithelial cells have indistinct borders. Magnification; 400×. **C:** Vaginal smear from a bitch on the last day of cytologic estrus. Note the indistinct cytoplasmic borders of the epithelial cells and the "sheets" or clumps of cells that have exfoliated. Magnification; 400×.



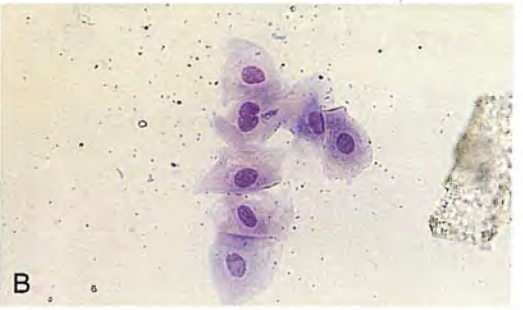


Figure 3–22. A: Vaginal smear from a bitch in early diestrus that contains neutrophils. Note that some parabasal cells stain a deep blue-purple. Magnification; 400×. **B:** Vaginal smear from a bitch in early diestrus that contains few to no neutrophils. Magnification; 400×. (**A** and **C** from Olson PN, Thrall MA, Wykes PM, et al: Vaginal cytology. Part I. A useful tool for staging the canine estrous cycle. Compend Contin Educ Pract Vet 6:288–298, 1984, with permission.)

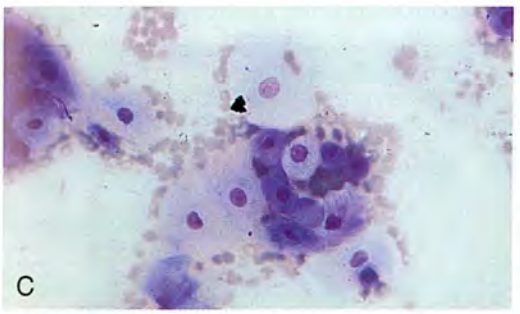


Figure 3–22. C: Vaginal smear from a bitch in early diestrus that contains erythrocytes. Magnification; 400×.

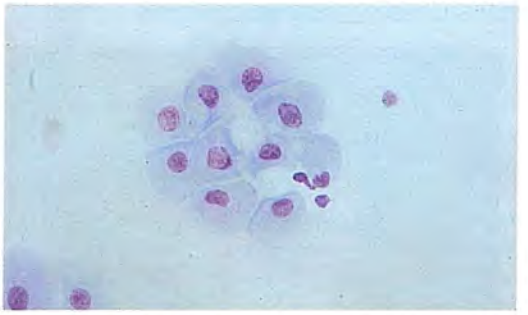
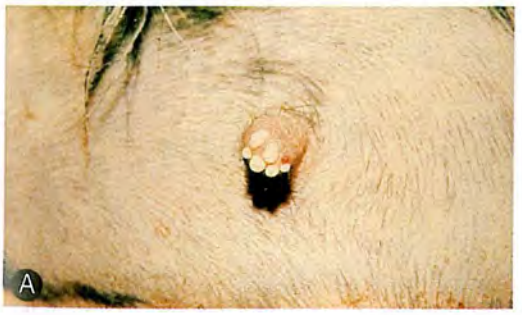


Figure 3–23. Vaginal smear from a bitch in anestrus. Magnification; 400×.



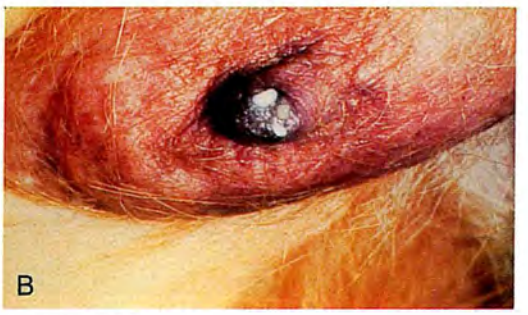


Figure 7–3. *See legend page 132.*

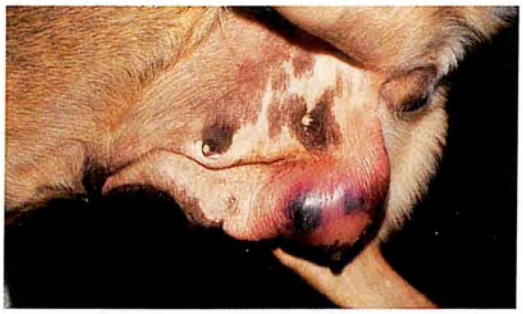


Figure 7–7. *See legend page 136.*

Figure 8–2. *See legend page 147.*

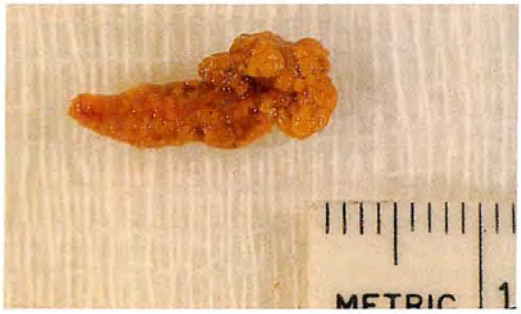


Figure 8–5. *See legend page 153.*

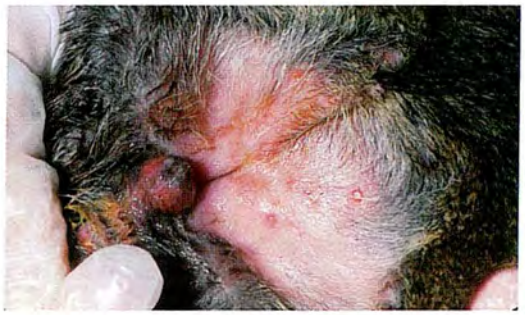


Figure 8–15. *See legend page 165.*

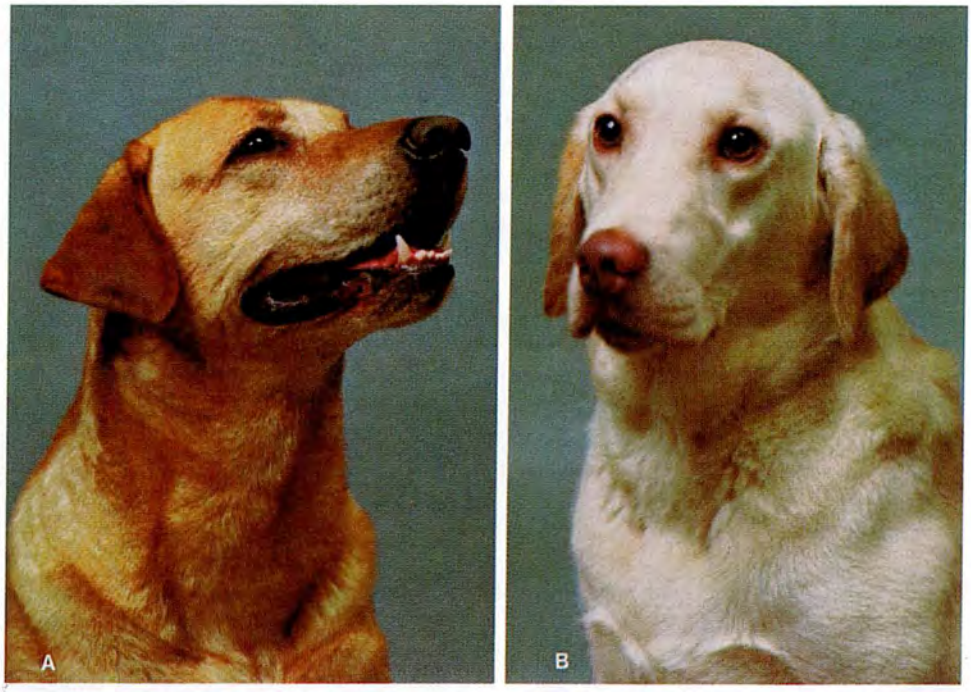


Figure 8–8. *See legend page 157.*

Section I

THE BITCH

Chapter 1

Sexual Differentiation and Normal Anatomy of the Bitch

Understanding normal sexual differentiation and anatomy of the bitch prepares the veterinary clinician for recognizing even the most subtle abnormalities of the reproductive system that result in dysfunction or disease. The following description of normal anatomy will emphasize those aspects that have special clinical relevance for the reproductive system.

Sexual Differentiation

Normal sexual development in the dog is dependent upon the normal development of chromosomal sex, gonadal sex, and phenotypic sex.[1] The diploid number of chromosomes in the somatic cells of the dog is 78, or 39 pairs.[2–4] The sex-determining gene, termed *SRY*, is on the Y chromosome. Male gonadal sex or testis formation occurs in the presence of a normal *SRY* and secondary pathways. In the absence of *SRY*, gonadal sex will be female. The accepted notation for the normal chromosomal sex in the bitch is 78,XX, referring to the total number of chromosomes (78) and the constitution of the single pair of sex-determining chromosomes (XX). The 38 pairs that are not the primary sex-determining chromosomes are somatic chromosomes, called autosomes. Clinical cytogenetics, or karyotyping, involves preparation and examination of neutrophils and mucosal cells for Barr bodies or evaluating mounted pictures of chromosomes in metaphase. Lymphocytes are used most often for karyotyping dogs[5] (see Chapter 8 for information on karyotyping).

Gonadal sex is undifferentiated in the early canine embryo. Although the sex of the embryo is determined at fertilization, it is not distinguishable morphologically until after day 30 of gestation.[6] If the sex chromosome constitution is XX, ovaries will develop; if the sex chromosome constitution is XY, testes will develop. The gonads develop from the ventromedial portion of the intermediate cell mass, or genital ridge, and migrate caudally during fetal development. The gonads eventually migrate to a scrotal position in the normal male dog. In the female, the ovaries remain in the abdominal cavity.

Phenotypic sex develops after gonadal differentiation. Paired paramesonephric (müllerian) ducts develop in both the male and female dog. In the bitch, the paramesonephric ducts open cranially into the peritoneal cavity as the abdominal openings of the uterine tubes (oviducts). Caudally, the paramesonephric ducts partially unite to form the uterine tubes and the bicornuate uterus and completely unite to form the vagina.[6] In the male, the paramesonephric ducts degenerate, except for those parts that remain as the vestigial appen-

Table 1–1. Homologies of Genital Organs in Male and Female Mammals

Male	Female
Testis	Ovary
Mesorchium	Mesovarium
Appendix testis	Abdominal ostium of uterine tube
Proper ligament of the testis, ligament of the tail of the epididymis	Proper ligament of the ovary, round ligament of the uterus
Prostatic urethra (cranial part)	Urethra
Prostatic urethra (caudal part)	Vestibule
Penis	Clitoris
Os penis	Os clitoridis (inconstant)
Glans penis	Glans clitoris
Corpus spongiosum penis	Vestibular bulb
Corpus cavernosum	Corpus cavernosum clitoridis
Scrotum	Labia
Scrotal raphe	Dorsal commissure of labia
Prepuce	Fold of fossa clitoridis

From Evans HE, Christensen GC: The urogenital system. *In* Christensen GC, Evans HE (eds): Miller's Anatomy of the Dog. Philadelphia, WB Saunders, 1993, p 547, with permission.

dix testis or uterus masculinus.[6] Regression of the paramesonephric ducts in the male results from the production of müllerian inhibiting substance by Sertoli cells of the fetal testes. Testicular differentiation in the dog has been observed at 36 days of gestation and temporally associated with regression of paramesonephric ducts.[7] In the absence of a testis, the paramesonephric duct system persists, resulting in the formation of the uterine tubes, uterus, and cranial vagina. Additionally, in the absence of a testis, testosterone is not produced in sufficient quantities to stabilize the mesonephric (wolffian) duct system (vasa deferentia and epididymides) and is therefore not converted to dihydrotestosterone (DHT) within the cells of the urogenital sinus, genital tubercle, and genital swellings. In the absence of DHT, the urogenital sinus, genital tubercle, and genital swellings become the caudal vagina and vestibule, the clitoris, and the vulva, respectively.[8]

Because of embryologic similarities during development, homologies of genital organs in male and female mammals exist (Table 1–1). Homologous structures may share certain functions (i.e., gonads producing gametes), produce similar substances (i.e., inhibin produced by the testis and folliculostatin produced by the ovary), and be stimulated by similar hormones (i.e., prepuce and vagina stimulated by estrogens; clitoris and penis stimulated by androgens).

Anatomy of the Reproductive Organs

Ovaries

The female gonads are paired oval organs, located in the abdominal cavity caudal to the kidneys. In a sexually mature, 11.4-kg dog, the left ovary is located approximately 12 cm caudal to the middle of the 13th rib and 1 to 3 cm caudal to the corresponding kidney. The right ovary is located approximately 10 cm caudal to the last rib of the right side.[6] The ovary lies within the ovarian bursa, which is an outpocketing of the peritoneal cavity. Unless the opening of the ovarian bursa is extended through an incision, the ovary may not be visible for inspection. Therefore, direct inspection of the ovaries with laparotomy or laparoscopy is often impossible. Because the uterine tubes lie in close proximity to the bursal slit, extending the bursal slit with an incision must be done with extreme care to avoid transecting the uterine tube (oviduct). The amount of adipose tissue in the bursal sheath is abundant in some older and obese dogs, obscuring the visualization of the uterine tubes (Fig. 1–1).

The blood supply of the canine ovary is provided by the ovarian and uterine arteries (Fig. 1–2). The ovarian artery is the larger of the two arteries and is the origin of a very rich vascular net in the ovarian stroma.[9] Caudally, the ovarian artery anastomoses with the uterine artery.[6] Through this anastomotic connection, the uterine artery may be considered as

Figure 1–1. Canine ovary exteriorized at surgery. Note that the ovary is surrounded by adipose tissue in the bursal sheath.

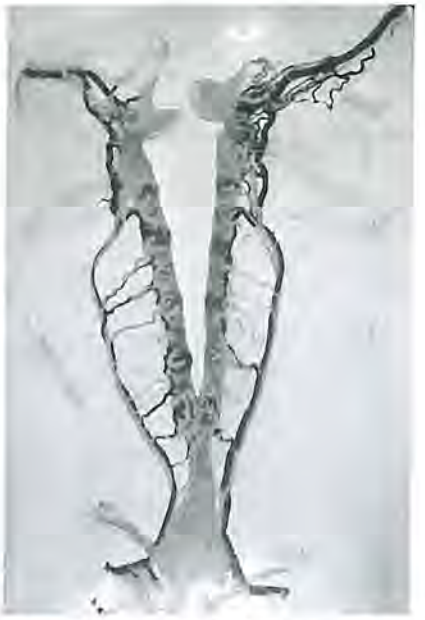


Figure 1–2. Arterial and venous blood supply to the canine uterus and ovaries. (Courtesy of Dr. Maurico Pineda, Iowa State University.)

a supplementary source of arterial blood to the ovary. Concentrations of progesterone in serum decrease immediately following hysterectomy in the diestrous bitch,[10] possibly resulting from a slight reduction of arterial blood from the uterine artery to corpora lutea at the time of surgery. The decreased progesterone concentration following hysterectomy is transient (i.e., 1 to 2 days). Ovarian function and estrous cycles continue unabated in the bitch following hysterectomy and removal of the uterine arterial blood supply.[10,11]

Uterine Tubes

The uterine tubes are structures that transport oocytes to the uterus, and are frequently referred to as oviducts. *Oviduct* is the correct term for birds and lower mammals, but *uterine tube* is the correct term for domestic animals. The ovarian extremity of the uterine tube, the infundibulum, is located near the edge of the opening into the ovarian bursa. During estrus a small (2 × 5-mm) reddish mass protrudes from the dorsal aspect of the bursal sheath into the peritoneal cavity. This is part of the fimbriated, everted mucosa of the infundibulum of the uterine tubes seen through the bursal slit.[12] The infundibulum has a small opening, the abdominal ostium, which is where ova enter the uterine tube following ovulation. The opening of the uterine tube into the horn of the uterus is called the uterine ostium.[6] Oocytes move down the uterine tube toward the uterus by peristaltic movements. A functional closure at the uterotubular junction prevents retrograde flow of fluid from the uterus back into the uterine tubes. When dye was injected into the uteri of over 100 hundred bitches hysterectomized at various stages of the estrous cycle, retrograde flow of the dye into the uterine tubes occurred in only 2 animals. The functional closure of the uterine tubes was maintained, even when the pressure at the time of dye injection was sufficient to result in an occasional uterine rupture (T. N. Thomas and P. N. Olson, unpublished data, Colorado State University, 1986).

Uterus

The uterus consists of a neck or cervix, a body, and two horns.[6] Uterine size varies considerably depending on the breed, age, and size of the animal; parity; and estrous cycle stage. Uterine size and weight increase as bitches mature and enter proestrus and estrus. Maximal uterine weight occurs during early diestrus in nonpregnant bitches and then decreases as the bitch enters anestrus.[13] Grossly, the uterus appears edematous during proestrus and estrus and has a characteristic "corkscrew" appearance during diestrus due to the increased endometrial stimulation from hormones. Extravasation of erythrocytes in the uterus, during both proestrus and estrus, may explain the apparent ease of hemorrhage observed when ovariohysterectomizing proestrous or estrous bitches.

Hysterographic studies on normal dogs have been reported.[14–16] During anestrus the uterine horns are relatively short, with minimal coiling. During proestrus and estrus, the radiographic outline of the uterus is increased in length and width as the uterus takes on a "large wave" of coiling. During diestrus, the radiographic outline is one of "short-wave" coiling, or the typical corkscrew appearance.

The thick, firm tissue between the uterus and the vagina is often referred to as the cervix. The cervix (cervix uteri) is composed of smooth muscle that contains the cervical canal and averages 1.5 to 2 cm in length.[6] The cervix may protrude 0.5 to 1 cm into the vagina but maintains an abdominal position. The canine cervix can frequently be abdominally palpated in proestrous and estrous bitches. The estrogen-stimulated cervix feels firm and lies

dorsal to the urinary bladder. The cranial vagina is limited by the fornix, which extends ventral and cranial to the cervix. The fornix is the deepest part of the vagina and is the place where fresh semen is frequently deposited during artificial insemination.

A dorsal median fold of tissue has been described that extends caudally from the vaginal portion of the cervix.[17] When viewed through a speculum, the caudal portion of the fold and constriction of the lateral and ventral vaginal walls give the appearance of a cervix (i.e., pseudocervix), with a ventral "fissure" (Fig. 1–3). The true vaginal portion of the cervix is cranial to, and often obscured by, the pseudocervix. The constriction of the vaginal walls and pseudocervix render intrauterine cannulation per vagina difficult. Additionally, the cervical canal is nearly perpendicular to the long axis of the vagina and uterine body, further impeding easy cannulation.[18] Muscular hypertrophy of the dorsal fold of tissue and cervix begins in proestrus, reaches maximal size in estrus, and begins to regress in early diestrus.[18]

Radiographic contrast medium placed in the vagina can pass through the cervix and on into the uterus during proestrus and estrus and following parturition[15] without the utilization of special cervical catheters (Fig. 1–4). Conversely, radiographic contrast medium fails to pass through the diestrous or anestrous cervix unless special equipment is utilized. With the aid of special equipment (i.e., Norwegian fox catheters, endoscopes, guiding devices, hysteroscopes), the canine cervix can be cannulated.[19–23] The ease of cannulation depends on the size of the dog (cannulation may be more difficult in larger bitches), the experience and training of the veterinarian, and the type of equipment utilized.

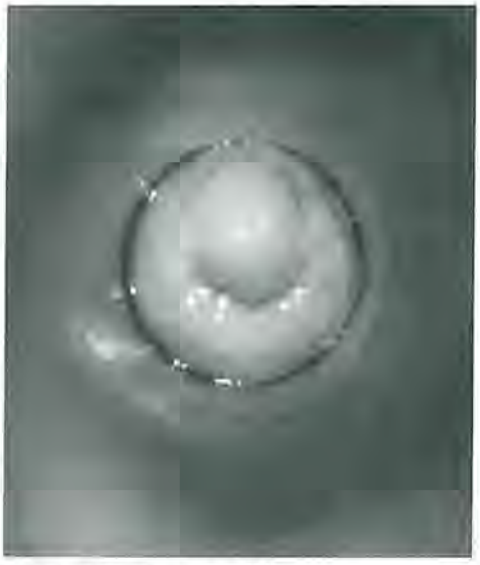


Figure 1–3. The appearance of the canine pseudocervix as viewed through a vaginoscope. (Courtesy of Dr. Maurico Pineda, Iowa State University.)

Vagina

The vagina is the musculomembranous canal that extends from the uterus to the vestibule (Fig. 1–5). The vagina can be considered as bottle shaped, with the vaginal portion of the cervix projecting caudally into the thin neck of the "bottle" or vagina.[24] Because the cranial aspect of the canine vagina, or "neck of the bottle," is relatively narrow, it may be difficult to pass some types of vaginoscopic equipment to the cervix. Cranially, the vagina is limited by the fornix and cervix. The fornix is the space cranioventral to the vaginal portion of the cervix, and is the space where fresh semen is often deposited during artificial insemination. The cranial portion of the vagina is covered dorsally by peritoneum that reflects onto the colon, and ventrally by peritoneum that reflects onto the bladder. Thus part of the vagina assumes an abdominal position. Fluid in the cranial vagina (i.e., vaginal abscess) can be mistaken for uterine fluid on radiographic and ultrasonographic examinations. In an 11.4-kg dog, the vagina averages 10 to 14 cm in length, a length similar to that for the uterine horns in a similarly sized nulliparous bitch.[6] Both the length and diameter of the vagina increase considerably during pregnancy and parturition. The caudal opening of the vagina into the vestibule is called the ostium vaginae.

The vaginal mucosa undergoes dramatic changes in appearance during the canine estrous cycle[24] (Fig. 1–6). Such changes are attributed to hormonally controlled alterations of the vaginal epithelium and of the fluid-retention properties of the mucosa, and are so characteristic that they can be used to define the following critical time points in the estrous cycle.[25] During proestrus, or at the time when concentrations of serum estrogens are elevated, the vaginal mucosa appears as large edematous pink or pink-white mucosal folds (period 1). As concentrations of serum estrogens fall in late proestrus, progressive shrinkage of the edematous folds occurs with the withdrawal of estrogen's tissue fluid-retention effect, and is accompanied by increasing mu-

cosal density and pallor. This initial mucosal shrinkage occurs without angulation of the mucosal borders (period 2). This period coincides with the initial decline in plasma estrogen : progesterone ratio and is significant because it spans the part of the estrous cycle from the preovulatory luteinizing hormone (LH) peak until up to 3 days after the LH peak, during which time ovulation is likely to occur. During period 3, mucosal shrinkage is accom-

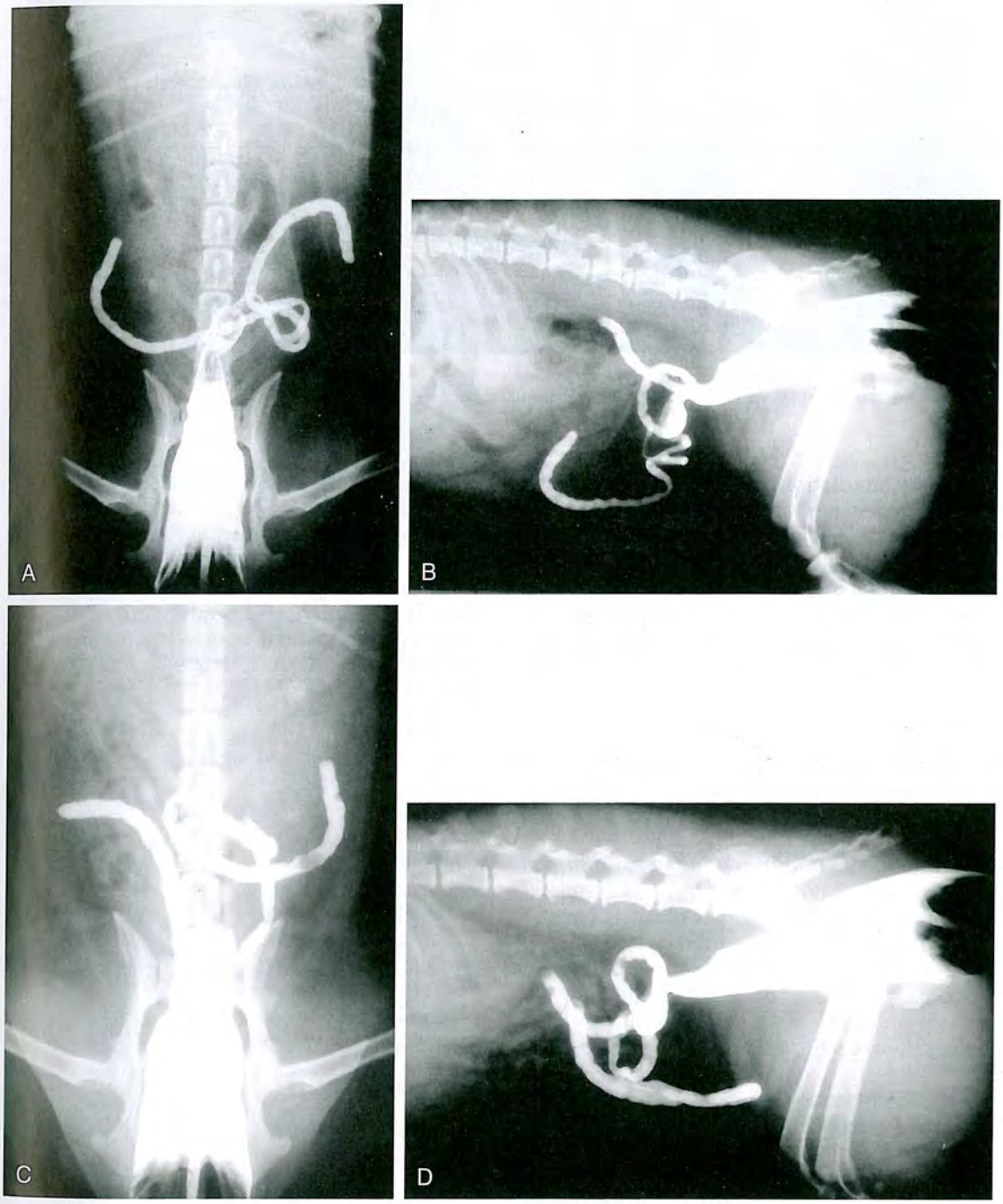


Figure 1–4. Estrous cycle as revealed by radiographic contrast medium. **A** and **B:** Ventrodorsal and lateral views of a vaginogram and hysterogram in a proestrous bitch. **C** and **D:** Ventrodorsal and lateral views of a vaginogram and hysterogram in an estrous bitch.

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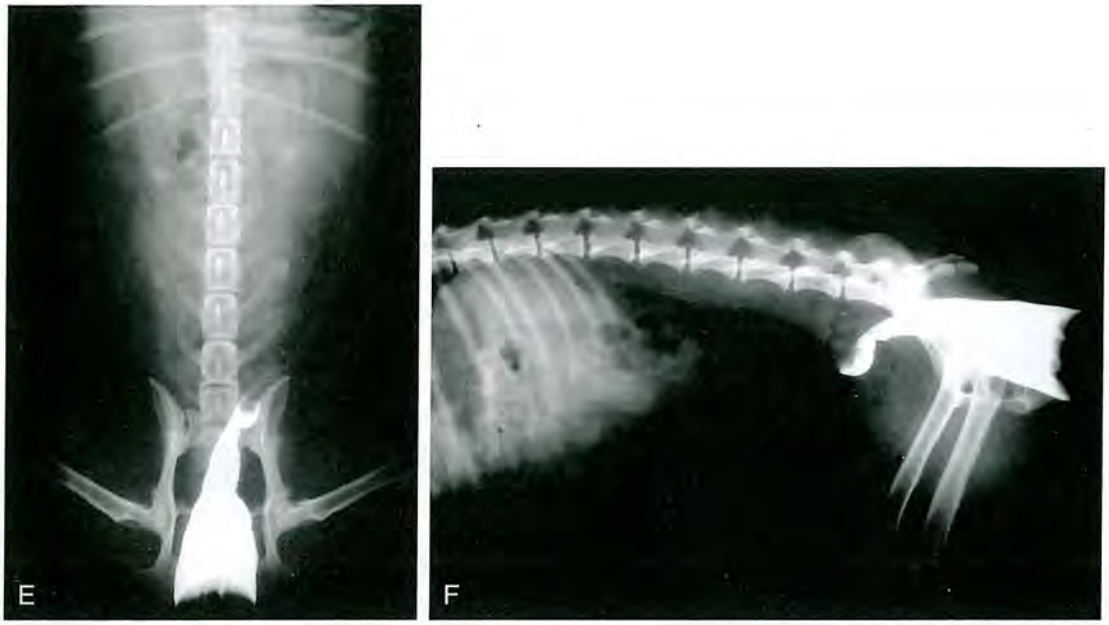


Figure 1–4 *Continued.* **E** and **F:** Ventrodorsal and lateral views of a vaginogram in a diestrous bitch (dye would not pass from vagina on into the uterus). (Courtesy of G.R. Johnston, University of Minnesota.)

panied by angulation of the mucosal folds. The mucosa is dense cream to paper white in color and the folds or borders become increasingly sharp. This period spans the fertilization period and coincides with dramatic increases in circulating progesterone concentrations, as well as behavioral estrus. During the last period (period 4), there is a cessation of mucosal shrinkage. This period marks the end of the fertilization period. Many angulated sharp borders may be seen during the early stage of period 4, but there is some thinning of the mucosa. During the later stages of period 4 there is more obvious rounding out of the mucosal borders, which occurs temporally with the transition of an "estrous" vaginal smear

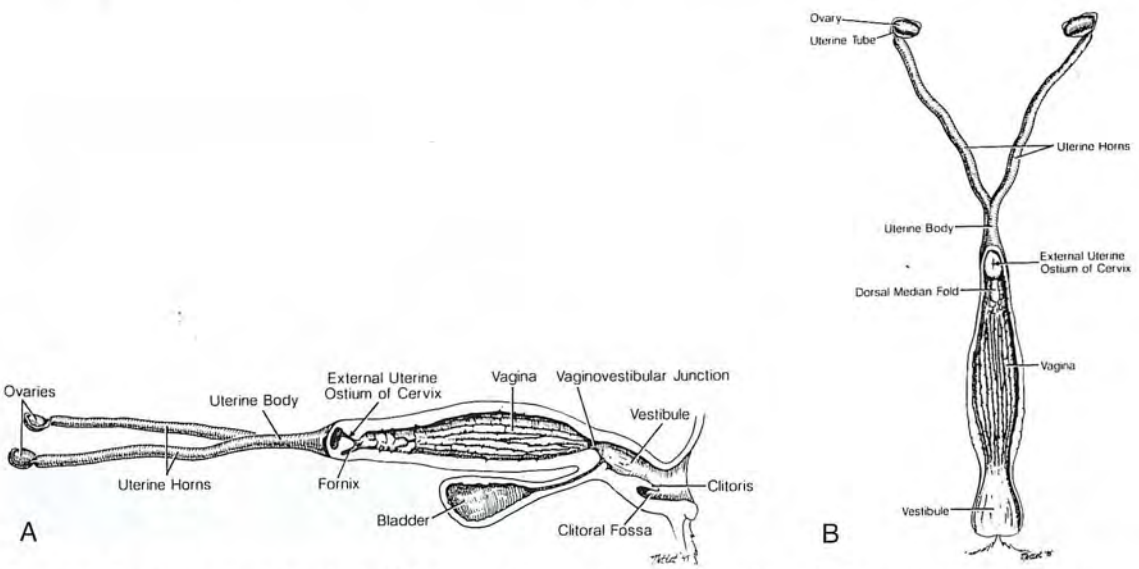
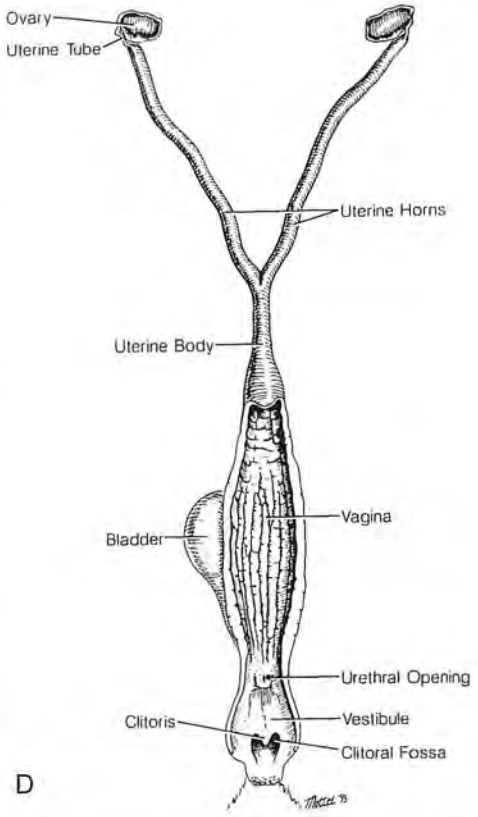


Figure 1–5. Reproductive tract of the bitch. **A:** Schematic diagram showing the lateral view. **B:** Schematic diagram showing the ventral view. *Illustration continued on opposite page*



D

Figure 1–5 *Continued.* **C:** Photograph showing the ventral view. **D:** Schematic diagram showing the dorsal view.

to a "diestrous" vaginal smear (see Chapter 3). The onset of diestrus is characterized by a patchy hyperemic mucosa that is irritable, and contact with the endoscope or vaginoscope provokes the formation of a "rosette" of pink-white folds.

Changes in the vaginal mucosa of the bitch are also the basis for utilizing vaginal smears to stage the canine estrous cycle. The vaginal epithelium increases in thickness from a few cells in early proestrus to 20 to 30 cells at the end of proestrus.[26] This change during estrus results in the sloughing of superficial cells, the increasingly impervious nature to leukocyte migration through the vaginal wall, and the increasing protection from injury at the time of mating. The cytologic changes of vaginal smears throughout the canine estrous cycle are discussed in detail in Chapter 3.

Vestibule

The vestibule is the part of the caudal reproductive tract that lies between the vulva and vagina in the bitch. In domestic animals, including the bitch, the vulva does not include the vestibule. The urethral tubercle is on the ventral floor of the vestibule, near the vaginovestibular junction, and contains the external urethral orifice. The cingulum is an annular, narrow, band-like area constricting the vaginovestibular junction (Fig. 1–5A).[24] The cingulum or vaginovestibular junction in some bitches (i.e., prepubertal, small, anestrous, or abnormal bitches) presents resistance to manipulative procedures such as vaginoscopy or digital palpation. Although the vaginovestibular junction relaxes during estrus in most bitches, failure to relax or dilate may cause some bitches to be nonreceptive to mating during estrus.

Nodules of lymphocytes are present in the subepithelial tissue of the vestibule in all species of domestic mammals.[27] Irritation of the vestibular mucosa by chemical or microbial agents induces hyperplasia and hyperemia of these lymphocytic nodules, which may be observed with vaginoscopy or felt during a digi-

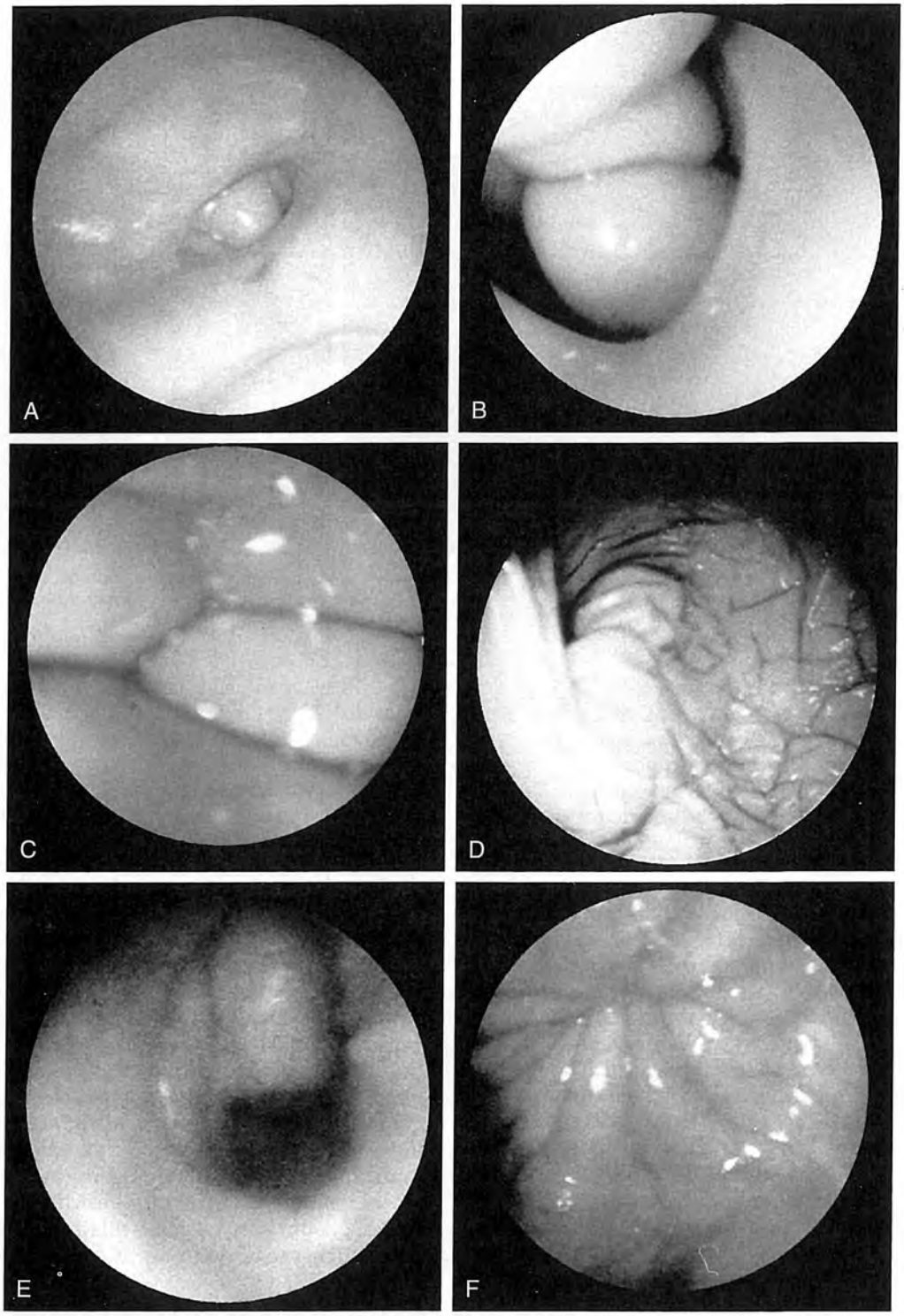


Figure 1–6. Photographs of the canine vagina as viewed during endoscopy. **A:** Cranial vagina during anestrus. In view is the round caudal tubercle of the dorsal median fold in center of field. **B:** Cranial vagina during early proestrus. Slightly oblique view of the dorsal median fold. Mucous membranes develop new folds and become edematous during proestrus. **C:** Cranial vagina during early proestrus. Note edematous folds of mucous membranes. **D:** Cranial vagina during late estrus (oblique view). Mucous membranes appear angulated during estrus. By the end of estrus, all folds are in their most shrunken and angular state. **E:** Cranial vagina as viewed on the first day of diestrus (first day of postestrous refusal). **F:** Cranial vagina showing the fully formed "rosette" that can be viewed with endoscopy during diestrus. (From Lindsay FEF: The normal endoscopic appearance of the caudal reproductive tract of the cyclic and non-cyclic bitch: Post-uterine endoscopy. J Small Anim Pract 24:1–15, 1983, with permission.)

tal examination of the vestibule. The presence of vestibular lymphocytic follicles cannot be associated with a specific disease, but is merely the sign of irritation or inflammation of the vestibular mucosa.

Clitoris

The clitoris, a homolog of the male penis, is composed of paired roots, a body, and a glans. The entire clitoris in normal bitches is relatively small, with the glans clitoridis projecting into the fossa clitoridis. The clitoris can enlarge in size, with an os clitoridis developing, in response to increased circulating levels of androgens that occur in some intersex dogs or from hormone therapy. The clitoris also enlarges in size from chronic inflammation in the vestibule or clitoral fossa. The clitoral fossa is encountered when the vulvar labia are separated. The clitoral fossa should not be confused with the vestibule, which lies dorsal and cranial to the clitoral fossa.

Vulva

The vulva consists of the labia, the commissures of the labia, and the rima vulvae between the labia. The cranial boundary of the vulva is also the cranial portion of the clitoral fossa. The vulva lies caudal to the vestibule. Unlike in humans, the vulva does not include the vestibule in domestic animals.

Mammary Glands

The mammary glands are typically arranged in two bilaterally symmetrical rows extending from the ventral thoracic region to the inguinal region. The number of glands varies from 8 to 12, with 4 to 6 glands on each side of the midline.[6] Typically, 10 glands are present, with the cranial 4 referred to as the thoracic mammae, the following 4 referred to as the abdominal mammae, and the caudal 2 referred to as the inguinal mammae. The number of ducts opening on a teat varies from 7 to 16.[28] Lymph drainage and topography of lymph vessels of the canine mammary glands has been reported.[29] In a study that evaluated 141 dogs, the most cranial thoracic mammae always drained to the axillary lymph nodes. The caudal thoracic mammae and the abdominal mammae drained to both the axillary and superficial inguinal lymph nodes (caudal thoracic: 100 per cent drained to axillary lymph nodes, 9.1 per cent drained to superficial inguinal lymph nodes; cranial abdominal: 93.4 per cent drained to axillary lymph nodes, 66.9 per cent drained to superficial inguinal lymph nodes; caudal abdominal: 8.3 per cent drained to axillary lymph nodes, 100 per cent drained to superficial inguinal lymph nodes). The inguinal mammae always drained to the superficial inguinal lymph nodes. The thoracic and cranial abdominal mammae also drain to the cranial sternal lymph nodes. No evidence existed in any of the 141 cases of lymph vessels connecting the two rows of mammary glands (Fig. 1–7). The lymphatic drainage of the canine mammary gland is of significance when determining the possible metastatic spread of mammary tumors.

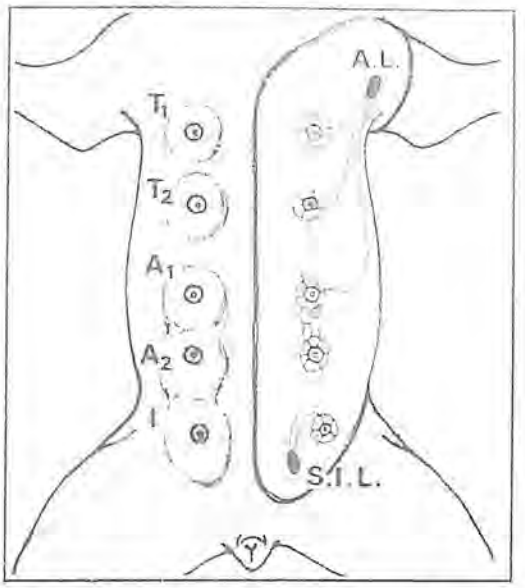


Figure 1–7. Schematic illustration of lymphatic drainage of the canine mammary glands. AL, axillary lymph node; SIL, superficial inguinal lymph node; T1 and T2, thoracic mammae; A1 and A2, abdominal mammae; I, inguinal mammae. (From Sautet JY, Ruberte J, Lopez C, et al: Lymphatic system of the mammary glands in the dog: An approach to the surgical treatment of malignant mammary tumors. Canine Pract 17[2]:30–33, 1992, with permission.)

Histology of the Ovary, Uterine Tubes, and Uterus during the Canine Estrous Cycle

Ovaries

Ovarian follicles are the largest during proestrus (Fig. 1–8). In addition to large ovulatory follicles, large numbers of small and medium-sized follicles are also present in the cortical

zone.[30] Both follicles and corpora lutea may be observed in the ovaries of estrous bitches, depending on whether or not ovulation has occurred. Preovulatory follicles contain luteinized tissue, accounting for the increased concentrations of serum progesterone observed in the bitch prior to ovulation (see Chapter 2).

Three stages of preovulatory follicular development have been described by Phemister et al.[31] The initial stage contained follicles lined by a thin but slightly folded layer of partly luteinized granulosa cells. Oocytes were attached to the follicular wall by a compact cumulus oophorus. Ovulation was estimated to be 2 to 3 days away. The intermediate stage included follicles that had a folded, luteinized granulosa layer. These follicles were surrounded by loosely arranged cumulus cells and contained oocytes that were nearly, but not completely, free floating. Ovulation was

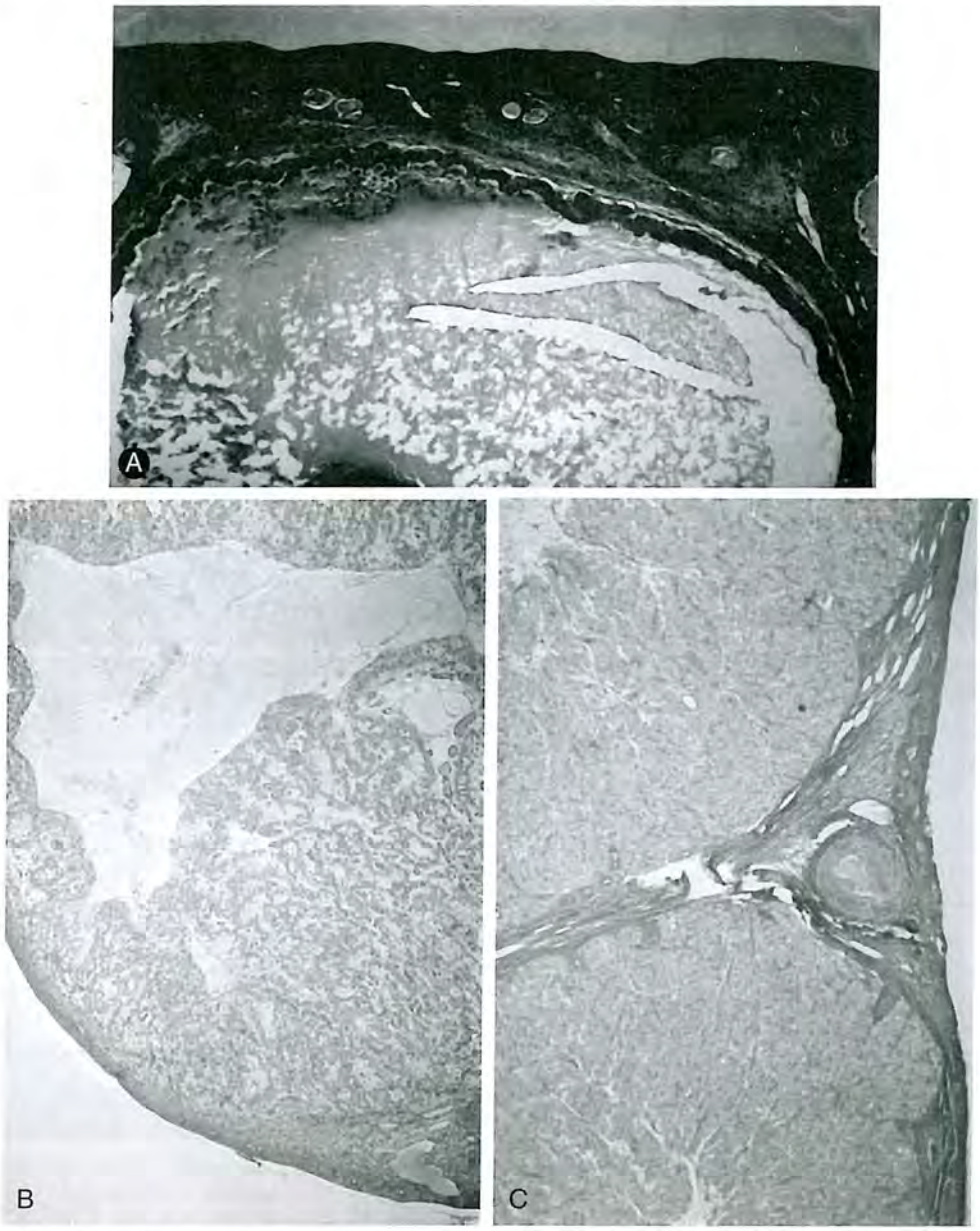


Figure 1–8. Histology of the canine ovary during the estrous cycle. **A:** Large ovulatory follicles are lined by complex, infolded mural granulosa cell layer in a bitch spayed on day 3 of proestrus. **B:** Section containing a corpus luteum with a large antrum in a bitch spayed on day 3 of estrus. **C:** Corpora lutea in a bitch spayed on day 12 after onset of estrus.

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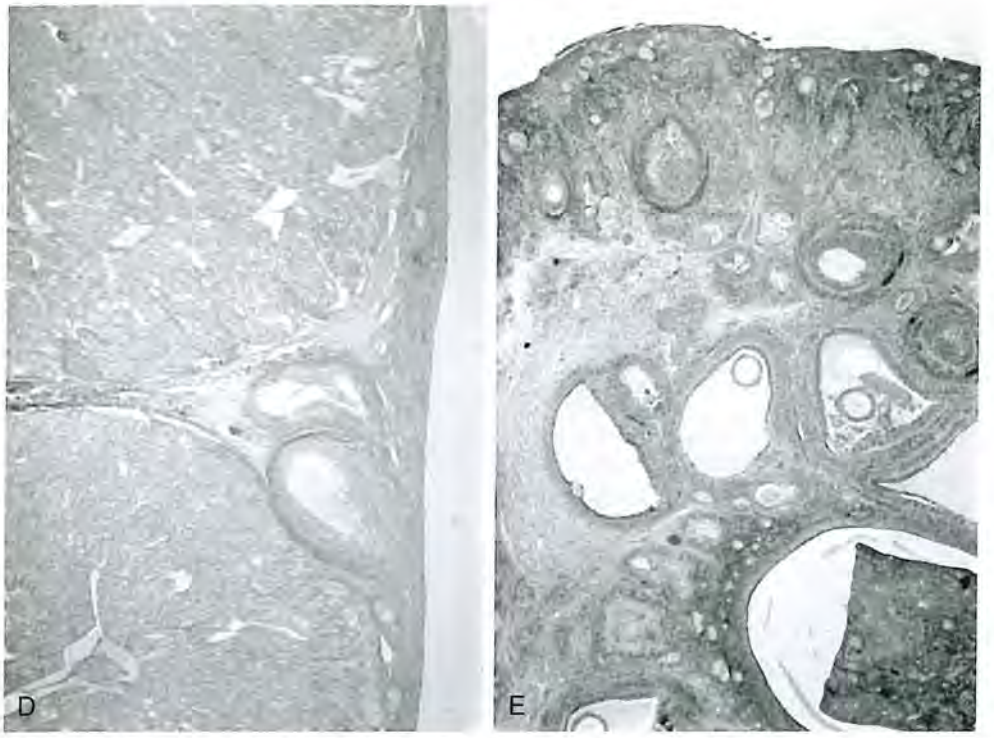

Figure 1–8 *Continued.* **D:** Corpora lutea in a bitch spayed on day 28 after onset of estrus. **E:** Section showing proliferation and small follicles along the germinal epithelium of the cortex in a bitch spayed during anestrus. (From Johnston SD: Steroid regulation of the canine uterus. PhD thesis, University of Minnesota, 1981.)

estimated to be 1 to 2 days away. The most mature stage included follicles having a focal area of marked thinning along a portion of the ovarian surface. The granulosa cells of these follicles were well luteinized and "thrown up in complex, loose folds." The oocytes were free floating in these follicles and the cells of the cumulus oophorus were widely dispersed around each oocyte. Ovulation was considered to be imminent.

Corpora lutea are present in the ovaries of both nonpregnant and pregnant bitches during diestrus, attaining the greatest size at approximately 20 days after the onset of proestrus.[13] Corpora lutea often contain antra during the first half of diestrus.[32] Follicular activity is also evident in ovaries from anestrous bitches and immature bitches.

Uterine Tubes

The epithelium of the uterine tube is hypertrophied during proestrus when compared to the low cuboidal epithelium present during anestrus (Fig. 1–9). Approximately 60 per cent of the cells contain cilia during proestrus.[30,33] During estrus, the uterine tube contains dilated secretory epithelial cells, has an increased number of mucosal folds, and reaches a maximum state of hypertrophy and differentiation.[30] The secretory cells and cilia atrophy during diestrus. In a study of prepubertal hormone-treated beagles, regression of the uterine tube epithelium occurred if estrogen treatment was terminated or progesterone was given along or in combination with estrogens.[34]

Uterus

Uterine size increases during proestrus as a result of interstitial edema. Myometrial thickness and endometrial thickness increase during proestrus, relative to anestrus. In late diestrus, endometrial thickness decreases but not to the degree of myometrial thickness, reflecting the effect of progesterone secretion on the endometrium.

The endometrial gland epithelium increases in thickness during proestrus, estrus, and early diestrus. Endometrial glands show great proliferation during diestrus, with coiling and branching of the basal glands. In late diestrus and anestrus, the glandular epithelial thickness again decreases in size[13,30] (Fig. 1–10).

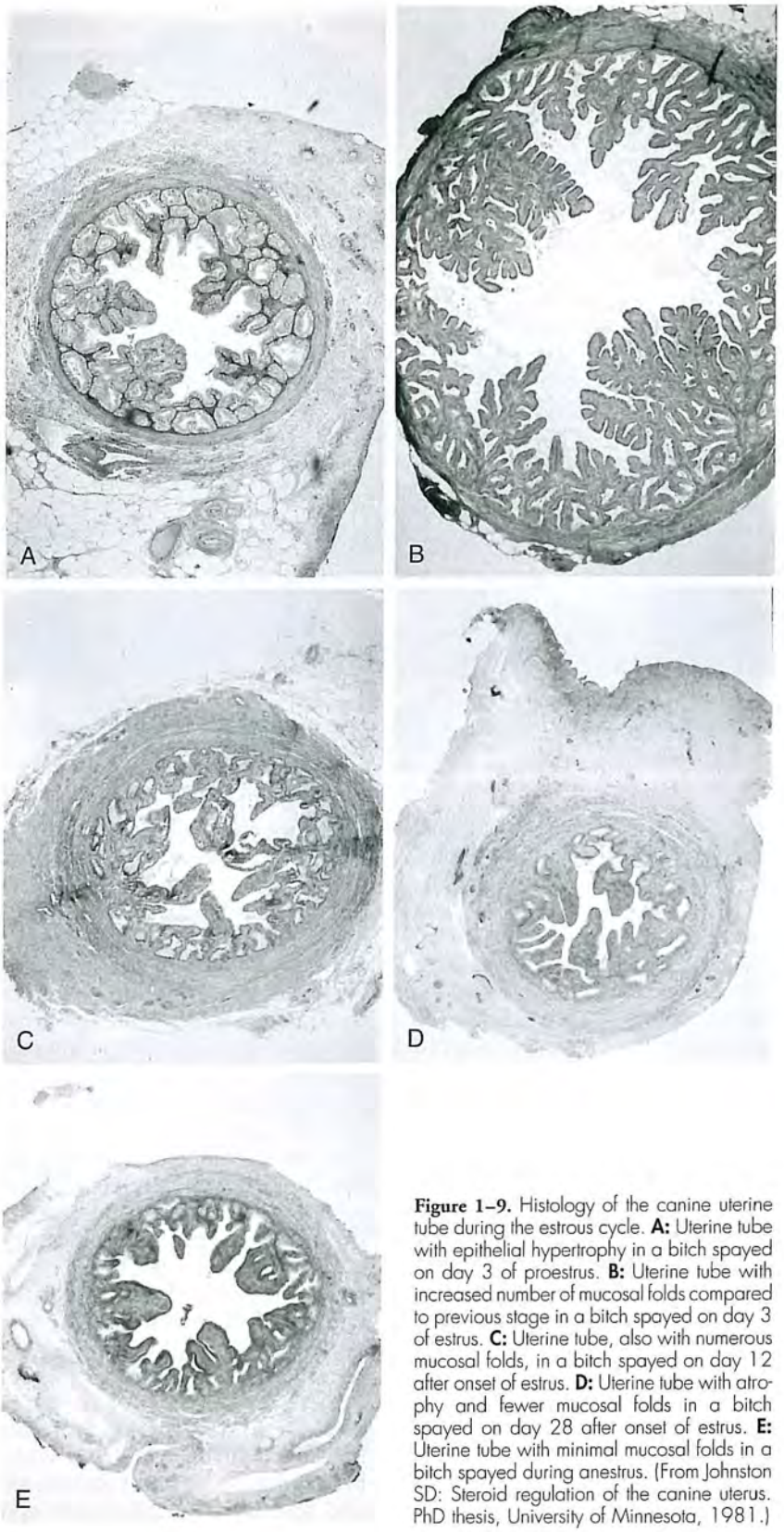

Figure 1–9. Histology of the canine uterine tube during the estrous cycle. **A:** Uterine tube with epithelial hypertrophy in a bitch spayed on day 3 of proestrus. **B:** Uterine tube with increased number of mucosal folds compared to previous stage in a bitch spayed on day 3 of estrus. **C:** Uterine tube, also with numerous mucosal folds, in a bitch spayed on day 12 after onset of estrus. **D:** Uterine tube with atrophy and fewer mucosal folds in a bitch spayed on day 28 after onset of estrus. **E:** Uterine tube with minimal mucosal folds in a bitch spayed during anestrus. (From Johnston SD: Steroid regulation of the canine uterus. PhD thesis, University of Minnesota, 1981.)

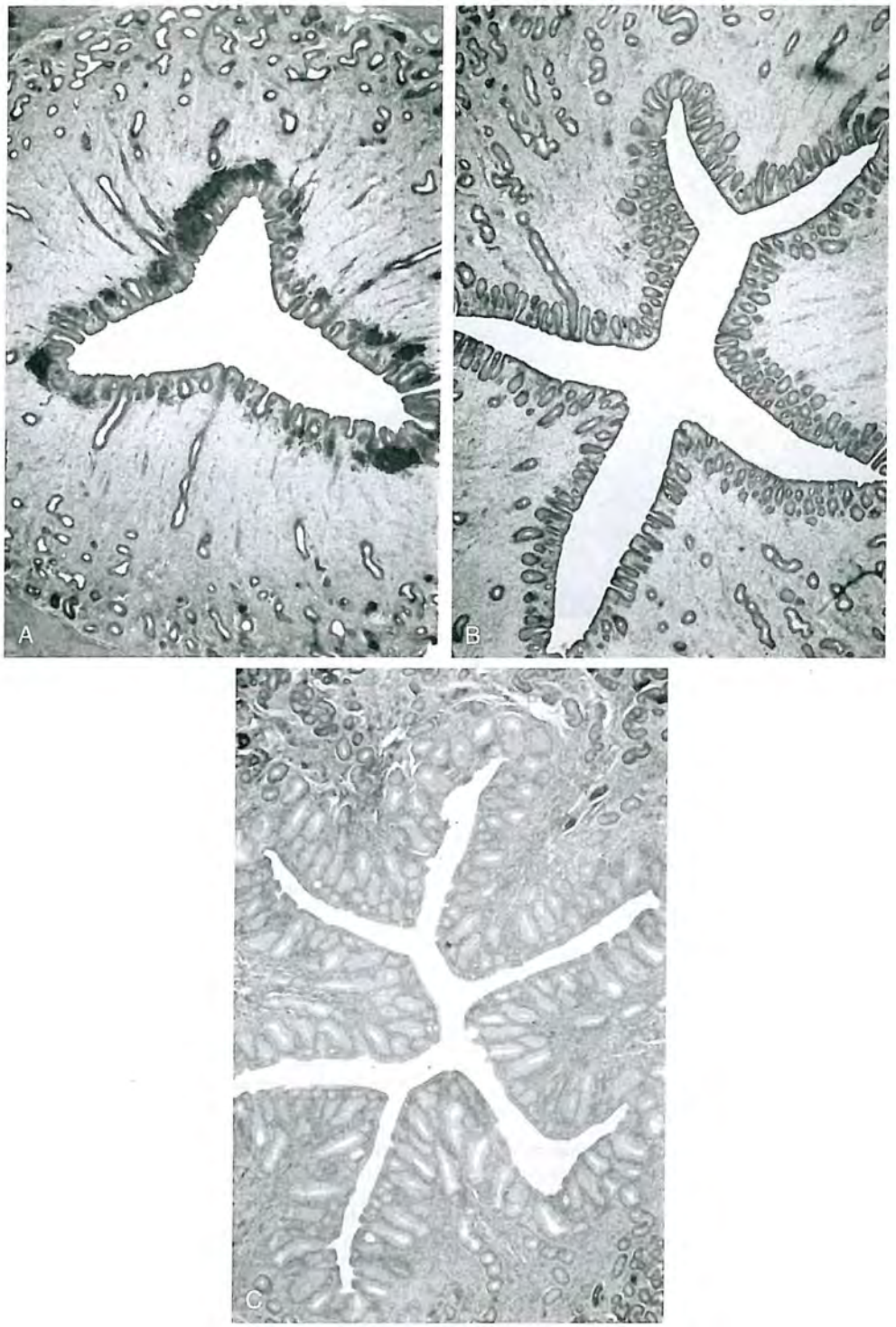

Figure 1–10. Histology of the canine endometrium during the estrous cycle. **A:** Endometrium in a bitch spayed on day 3 of proestrus. **B:** Endometrium in a bitch spayed on day 3 after onset of estrus. **C:** Endometrium with greatest proliferation of endometrial glands in a bitch spayed on day 12 after onset of estrus.

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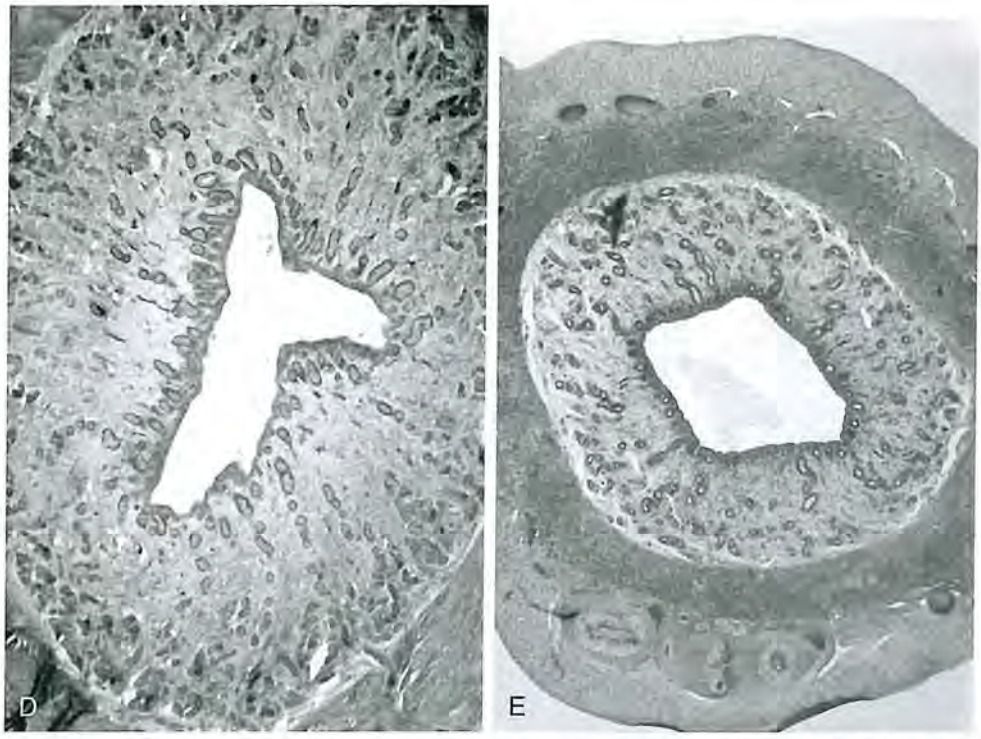

Figure 1–10 *Continued.* **D:** Endometrium with glandular epithelial cells that are atrophied when compared to those in **C** in a bitch spayed on day 28 after onset of estrus. **E:** Endometrium with atrophied epithelial cells in a bitch spayed during anestrus. (From Johnston SD: Steroid regulation of the canine uterus. PhD thesis, University of Minnesota, 1981.)

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Chapter 2

The Canine Estrous Cycle

Heape described the classical stages of the canine estrous cycle in 1900.[1] Because the classification was made prior to our understanding of hormones, the primary indicators that Heape used for defining the various stages of the estrous cycle were sexual behavior and physiologic changes observed to occur in the reproductive tract. Today, the canine estrous cycle can be defined in numerous ways, including definitions based on behavioral, clinical, physiologic, cytologic, and endocrinologic changes in the bitch (Table 2–1).

The Estrous Cycle

The estrous or reproductive cycle of the domestic dog (*Canis familiaris*) includes four stages: proestrus, estrus, diestrus,* and anestrus. The onset of proestrus usually is referred to as the beginning of the estrous cycle. There are typically two estrous cycles per year, with ranges of one to four cycles for some breeds or individual bitches. The canine estrous cycle is generally described as monestrus, referring to the completion of only one estrus per estrous cycle, and as nonseasonal, because dogs may exhibit estrus at any time of the year and litters are born during each month of the year.[2] Although most breeds of dogs can exhibit estrus at any time of the year, some authors have argued for trends of increased seasonal activity. Seasonal activity might be anticipated in the dog because seasonality is an attribute of other canine species. The wolf, northern coyote, wild dog (Australian dingo), and African basenji dog may display estrus only once per year,[3–5] with the season of estrus dependent on the hemisphere where the animal lives. Christie and Bell reported the results of a survey on the reproductive activity of 20 breeds of dogs in England and Wales.[6] These data, which represented 1561 cycles, indicated that proestrus starts in February to May more than at other times of the year. In another study,[7] the occurrence of estrus was evenly distributed over the year for Labrador retrievers and German shepherd dogs that were kept as family pets. However, for beagles kept in a colony and housed outdoors without heating or supplemental light, there were significant differences between the months when estrus occurred, with a peak occurrence of estrus in May. Therefore, environment may influence the seasonality of the estrous cycle in domestic dogs. A circannual rhythm in prolactin has been reported to occur in dogs housed outdoors,[8] which could influence the seasonality of the estrous cycle (see below). Environment can alter the seasonality of the wolf. Equatorial translocation results in a reversal of the wolf's breeding season by 6 months, and estrous periods of wolves occur later at higher altitudes.[9,10]

* Both the terms *diestrus* and *metestrus* are used to describe the stage of the canine estrous cycle that immediately follows estrus. Heape[1] described metestrus in the following way: "If conception does not take place during oestrus, the activity of the generative organs gradually subsides during a definite period, which I called the metoestrum." Although Heape correctly recognized that "some pecular substance in the blood" may contribute to various changes observed during the estrous cycle, it was after hormones were discovered that Heape's postestrous "subsiding" stage was found to be a very active stage relative to increased concentrations of progesterone in the blood. Additionally, increased progesterone concentrations were measured in the blood of postestrous bitches regardless of whether conception had occurred. Therefore, because the stage following estrus in the bitch is normally a very active stage, especially on the basis of concentrations of progesterone in the blood, the authors of this textbook will use the term *diestrus* when referring to the stage of the canine estrous cycle that normally follows estrus.

Table 2–1. Various Methods for Classifying Stages of the Canine Estrous Cycle

Method of Defining (Avg. Length)	Proestrus (9 d) (Range 0–27 d)	Estrus (9 d) (Range 4–24 d)	Diestrus (2 mo)	Anestrus (4 mo)
Behavioral	Bitch attracts male; not receptive to mating	Bitch is receptive to mating	Bitch is not receptive to mating	Bitch is not receptive to mating
Clinical	Vulva is swollen and turgid; serosanguineous discharge from the vulva	Vulva softens but is still enlarged; discharge from the vulva lightens in color	Vulva decreases in size; discharge from the vulva is variable during the first few days of diestrus (clear, purulent, or hemorrhagic); mammary development may be noted; lactation may occur at end of diestrus	Vulva further decreases in size
Hormonal (serum)	E2 peaks; P4 low until late proestrus or early estrus; LH pulses; FSH low	E2 drops to basal concentrations; P4 increases rapidly; LH surge occurs; FSH surges with LH	E2 low; P4 increases to peak concentrations (15–90 ng/ml) 3–4 weeks after diestrus onset, then declines to basal concentrations (<1 ng/ml) by end of diestrus; LH pulses increase in late diestrus; FSH low	E2 low until late anestrus when it starts to rise; P4 low; LH pulses increase in late anestrus; FSH pulses increase in late anestrus
Physiologic	Graafian follicles develop	Primary oocytes are ovulated; meiosis to secondary oocytes occurs; corpora lutea develop	Corpora lutea present in pregnant and nonpregnant bitch; morula enters uterus in pregnant bitch	Antral follicles in various stages of development and atresia; corpora lutea in various stages of regression; corpora albicans in late anestrus
Cytologic (vaginal smear)	Mixed types of epithelial cells; bacteria may be present; RBCs; WBCs may be present in early to midproestrus	>90% cornified cells; bacteria may be present and abundant; fewer RBCs than proestrus; few to no WBCs	>50% parabasal + intermediate cells on first day of diestrus; WBCs may be present and abundant in early diestrus (variable response); fewer RBCs than proestrus	>90% parabasal + intermediate cells; few WBCs; few bacteria

Abbreviations: E2, estradiol-17β; P4, progesterone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; RBC, red blood cell; WBC, white blood cell.

Although the canine estrous cycle may be "marginally" seasonal,[11] examination of breeding records from most breeds of dogs reveals that whelping occurs in all months. A study compiled at the American Kennel Club from 1971 to 1973 of 87,880 litter registrations shows that puppies of most breeds are born during each month of the year, with peak estrous activity estimated to occur in late winter and spring.[12] Litter registrations, however, may be influenced by owners who have a preference for the time of whelping.

The length of the interestrous interval appears to be variable within bitches, within breeds, and among breeds.[2,11] Although smaller breeds tend to have more estrous cycles per year than larger breeds, this is not always true. For example, the German shep-

herd bitch is likely to have more estrous cycles per year than the Boston terrier bitch.[2,13] Similarly, the German shepherd bitch is more likely to have more estrous cycles per year than the dachshund.[6] The heritability of the interestrous interval has been estimated at 35 per cent[11] and accounts for different lengths of interestrous intervals among breeds. However, the interestrous interval within a single bitch can also be extremely variable. Therefore, it is not always possible to predict the next estrus in a single bitch.[11]

Whelping has been reported by some investigators to increase the length of the interestrous interval (the time between the onsets of proestrus) over the interval for nonpregnant cycles.[6,7,14] However, Sokolowski reported that neither parity nor pregnancy significantly lengthened the interestrous interval in the bitch.[2] If interestrous intervals are lengthened following a pregnancy, as some authors suggest, it remains unknown if the increase in interestrous length results from the pregnancy or from the lactation that usually follows a pregnancy.

Age also has been suggested as a factor that influences the length of the interestrous interval. Various authors report no change in the length of interestrous intervals up to age 5 to 7 years.[15-19] In one study,[20] mean interestrous intervals increased from approximately 240 days to 332 days for 12 beagle bitches after they reached 8 years of age (128 estrous cycles were evaluated). The 12 bitches were maintained throughout their lives, with the oldest living to be over 16 years of age. Whether any health problems, such as hypothyroidism, contributed to the increased length of interestrous intervals in the older beagle bitches was not addressed. In addition to the extended anestrus, bitches over 8 years of age also had higher infertility rates and higher mortality rates for pups prior to weaning age.

Puberty

Puberty is defined as the period when the capability of reproduction is attained. For the bitch, puberty is recognized with the onset of the first proestrus. The onset of puberty has been associated with the time a bitch reaches a certain growth plateau.[2] Thus puberty begins between 6 and 10 months of age for bitches of many smaller breeds but may not begin for bitches of some larger breeds until they are nearly 2 years of age. In one study, however, there was no apparent relationship between weight and age at onset of first proestrus among Labrador retrievers and beagles,[21] and the onset of puberty appears quite variable within several small and large breeds of dogs[22,23] (Table 2–2). Fecundity, or maximal reproductive capability, may not be reached until the second, third, or fourth estrus, or approximately the third year of life.[17,24-28]

The duration and character of proestrus and estrus may differ among pubertal and mature bitches. Pubertal bitches may be less likely than mature bitches to demonstrate estrous behavior even when ovulation occurs.[21] Additionally, the duration of proestrus-estrus may be shorter in pubertal bitches, and reduced or inconsistent patterns of circulating concentrations of estradiol-17β (estradiol), luteinizing hormone (LH), and progesterone have been reported to occur in some pubertal cycles.[21,29]

The estrous cycle of the pubertal bitch also differs from those of mature bitches in that pubertal bitches are more likely to manifest a *split* or *false heat*.† During a split heat the bitch shows some of the signs of a true proestrus-estrus, such as a serosanguineous discharge passing from the vagina, vulvar swelling, and the attraction of male dogs. In some cases, bitches exhibiting split heats will even be receptive to mating. However, after a few days, the proestrous-estrous signs recede until the true estrus begins in several more days or weeks. During a split heat, proestrous-estrous behavior occurs in the absence of ovulation during the first part of the "split." However, conception can occur if the bitch is bred when ovulation occurs during the second part of the split heat, or true estrus. Pubertal bitches also are more likely than normal adults to manifest a *silent heat*‡ where ovulation occurs in the absence of proestrous-estrous behavior or notable clinical signs (see Chapter 14).

Proestrus

Proestrus has been clinically defined as that stage of the estrous cycle where recognizable external changes signify that estrus is immi-

† The exact cause for split/false heats is unknown. One possible explanation is that the increased levels of estradiol–17β from follicular development during anestrus, and prior to the onset of a "true" proestrus, may initiate the clinical signs.

‡ Concentrations of progesterone would be expected to increase following a silent heat and ovulation, remaining elevated above basal levels for approximately 2 months.

Table 2–2. Onset of Puberty for Selected Breeds of Dogs

Selected Breeds	Mature Weight (lb)	Onset of Puberty (mo)
Affenpinscher	7	8–14
Afghan hound	50	7–30
Airedale terrier	48	15
Akita	95	5+
American Staffordshire terrier	50	10
Australian shepherd	35	6–18
Basenji	21	10
Bearded collie	45	8–12
Belgian tervuren	70	10–12
Bernese mountain dog	65	9–12
Bichon frise	18	8–9
Bloodhound	90	12
Border collie	42	6–8
Borzoi	75	15–18
Boxer	62	8–24
Brittany spaniel	35	9-12
Bullmastiff	100	6–16
Bull terrier	45	7–11
Cavalier King Charles spaniel	18	6–9
Clumber spaniel	53	Up to 24
Deerhound	73	Up to 16
English setter	59	7–20
English toy spaniel	11	12–14
King Charles spaniel	11	12–14
Golden retriever	65	9–11
Great dane	100	Up to 18
Great Pyrenees	90	12
Greyhound	65	11–30
Griffon Bruxellois	8	Up to 18
Ibizan hound	48	8–24
Irish wolfhound	105	14–18
Italian greyhound	7	18–24
Keeshond	40	8–18
Komondor	110	12
Lakeland terrier	15	Up to 24
Mastiff	126	11–12
Norwegian elkhound	43	6–10
Pembroke Welsh corgi	25	9
Poodle (standard)	65	12–15
Pug	16	9–12
Rottweiler	85	8
Saint Bernard	150	9–15
Saluki	43	8–24
Samoyed	40	Up to 12
Schipperke	18	12–24
Scottish deerhound	75	12–14
Shetland sheepdog	20	7–17
Welsh springer spaniel	38	12
Whippet	22	12–24
Yorkshire terrier	7	8–16

Data from Evans and White[22] and Clark and Stainer.[23]

nent. The average duration of proestrus in mature bitches is 9 days, ranging from 0 to 27 days.[30] A serosanguineous discharge usually passes from a swollen and turgid vulva in the proestrous bitch (Fig. 2–1).

The proestrous bitch usually attracts male dogs but is not receptive to mating; the bitch will frequently turn on or growl at male dogs investigating her hind quarters, or sit down to prevent copulation (Fig. 2–2). The attraction of males by proestrous bitches likely results from the sex pheromones in vaginal secretions, anal sac secretions, or urine.[31–34] Three sexual reflexes can be observed to begin during proestrus in the bitch: (1) the upward tipping or "winking" of the vulva in response to touching the skin immediately dorsal to the vulva, (2) the ipsilateral curvature of the rear legs in response to tapping the skin to the right or left of the vulva, and (3) contralateral or vertical deviation or "flagging" of the tail in response to tapping the skin on either side of the vulva[35] (Fig. 2–3). These sexual reflexes are absent during anestrus, increase during proestrus, and reach their peak during early to midestrus.[35]

Vaginal smears obtained in early to midproestrus usually are characterized by the presence of erythrocytes and a mixture of epithelial cell types (Table 2–1). By mid- to late proestrus, the percentage of parabasal and small intermediate cells decreases and the per-

Figure 2–1. A serosanguineous discharge passing from the swollen vulva of a proestrous bitch.

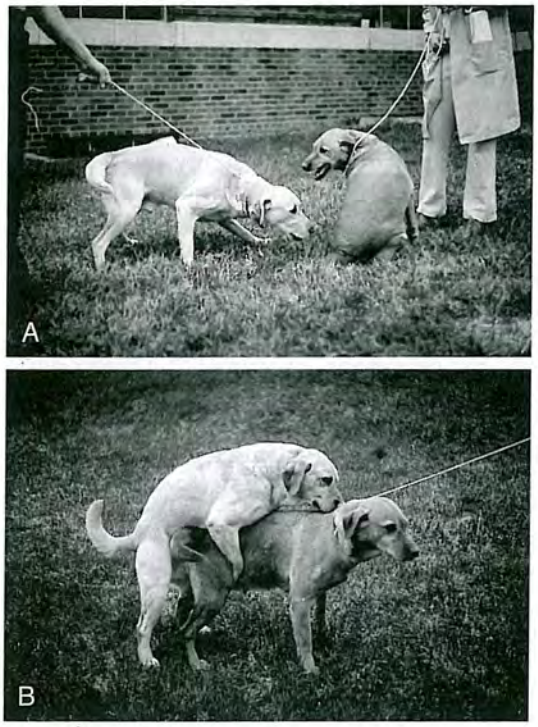


Figure 2–2. Changes in receptive behavior to mating. **A:** Although the male dog is attracted to the proestrous bitch, she growls and refuses to be mated. **B:** Same bitch during estrus allows male to mate.

Figure 2–3. Characteristic postural response of an estrous bitch to male dog. Note the deviation of the tail to one side (flagging).

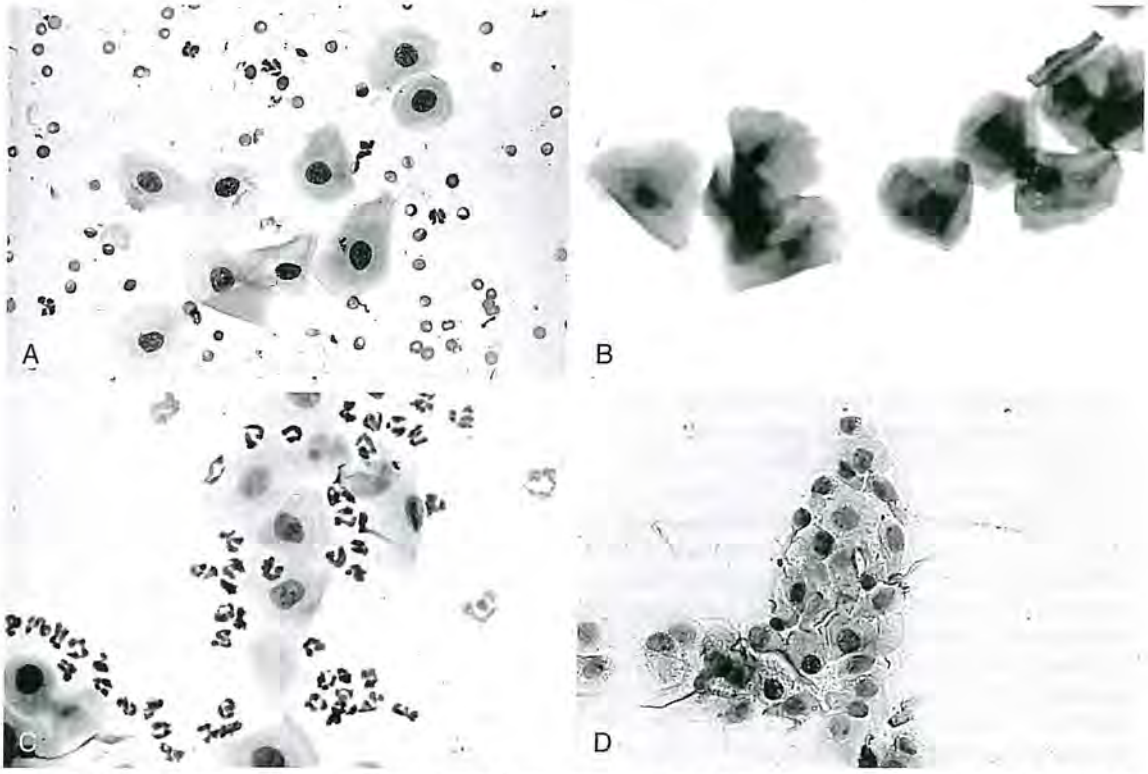


Figure 2–4. Vaginal smears (400×). **A:** Vaginal smear from a proestrous bitch. Note the nucleated vaginal epithelial cells and numerous red blood cells. **B:** Vaginal smear from an estrous bitch. Note the large superficial (cornified) epithelial cells that typify cytologic estrus. The nuclei of superficial cells may appear to be absent, pyknotic, or undergoing karyorrhexis. **C:** Vaginal smear from a diestrous bitch. Note the nucleated vaginal epithelial cells and numerous white blood cells. Although white blood cells may be abundant in vaginal smears during early diestrus, their presence is not a reliable indicator of diestrus. (From Nett TM, Olson PN: Reproductive physiology of dogs and cats. *In* Ettinger SJ [ed]: Textbook of Veterinary Internal Medicine, 2nd ed. Philadelphia, WB Saunders, 1983, p 1704, with permission.) **D:** Vaginal smear from an anestrous bitch. The epithelial cells are nucleated and smaller in size than those from bitches in late proestrus or estrus.

centage of superficial and large intermediate cells increases (Fig. 2–4). Although erythrocytes usually are abundant in vaginal smears from proestrous bitches, they may also be absent or few in number (see Chapter 3).

Endocrinologically, proestrus is that stage of the estrous cycle when serum concentrations of estradiol increase to levels that result in the clinical signs of proestrus. Following the increase, concentrations of serum estradiol decrease before the bitch becomes receptive to mating (i.e., estrus).[14,36–40] Serum concentrations of progesterone are at basal levels (<1 to 2 ng/ml) until late proestrus, when concentrations begin to rise. This increase in serum progesterone is related to preovulatory luteinization of follicles.[41,42] Bischoff[43] was the first to describe the folding of the follicle wall before rupture and believed that luteal tissue in the bitch began to form before ovulation occurred.

Concentrations of testosterone increase in the serum of bitches during late proestrus, reaching concentrations of 0.3 to 1.0 ng/ml at the time of the LH surge, levels similar to those found in the serum of intact male dogs.[44,45] Whether increased concentrations of testosterone contribute to behavioral estrus or are merely a result of increased steroidogenesis is unknown. Serum concentrations of LH remain near basal levels during most of proestrus, although increased concentrations of LH above basal levels have been reported to occur during late anestrus or early proestrus.[36,46] Serum concentrations of follicle-stimulating hormone (FSH) decrease during proestrus,[36] presumably the result of negative feedback on FSH by folliculostatin, a hormone produced by developing ovarian follicles that suppresses FSH.[47] Prolactin may be influential in the termination of anestrus and the initiation of proestrus, although concentrations of circulating prolactin have been reported as variable throughout proestrus.[36,48,49] Suppression of prolactin with the dopamine agonist bromocrip-

tine in early anestrus can result in precocious onset of proestrus.[50]

Estrus

Estrus is the stage of the estrous cycle characterized by the bitch's acceptance of the male for mating. The vulva remains enlarged during estrus but is usually softer than it was during proestrus. The discharge that passes from the vulva during estrus often contains less blood than the proestrous discharge, becoming more "straw colored" as the blood diminishes. Some bitches, however, may have a serosanguineous discharge throughout both proestrus and estrus. The average duration of estrus, when based on behavioral signs, is 9 days, ranging from 4 to 24 days.[30] The bitch appears to become receptive to the male after serum concentrations of estradiol begin to fall and concentrations of progesterone begin to rise.[35,51,52] Although estrogens alone can induce female sexual behavior, progesterone seems to further enhance[53] and synchronize this behavior. The decline in serum concentrations of estradiol precedes and may influence the LH surge that occurs near the onset of estrus and leads to ovulation.[54]

Although various hormonal and behavioral changes are temporally related in the bitch, the onset of estrus can be quite variable. For example, while some investigators have reported that the preovulatory surge of LH occurs on the first day of behavioral estrus,[37,55] others have found no correlation between the preovulatory surge of LH and the onset of behavioral estrus.[46,56] In some bitches the onset of estrus may occur as early as 2 to 3 days before the LH surge, while in many others it may not occur until 4 to 5 days after the LH surge. In the extreme case, a bitch in late proestrus may be mated by an aggressive male as early as 4 to 5 days before the LH surge, while some bitches may refuse a male until more than 6 days after the surge.[57]

Estrus also can be defined on the basis of cytology to begin when more than 90 per cent of the vaginal epithelial cells in a vaginal smear are superficial cells[58] (Table 2–1). However, the onset of cytologic estrus does not always correlate with the onset of sexual receptivity or the LH surge. For example, 22 of 24 mixed-breed research bitches were determined to be in cytologic estrus by vaginal smears but were not yet receptive to mating (P. N. Olson and R. A. Bowen, unpublished data, Colorado State University, 1989). In these same dogs, 18 of the 24 bitches were determined to be in estrus by vaginal smears prior to the onset of the preovulatory surge of LH. In another study of 12 beagle bitches, the first day of cytologic estrus ranged from 6 days before to 4 days after the LH surge (P. N. Olson and P. W. Concannon, unpublished data, Colorado State University and Cornell University, 1989).

Duration of the preovulatory surge of LH in the bitch appears to vary, with ranges of 24 to 96 hours reported.[36,37,42,46,55,59] Following the LH surge, concentrations of LH are lower during the remainder of estrus than those found during anestrus, early proestrus, or diestrus, due to depletion of pituitary LH.[60] There is also a surge in the serum concentration of FSH that occurs near the time of the LH surge, or shortly thereafter.[36,55]

Ovulation occurs approximately 2 to 3 days following the preovulatory LH surge. The average interval from LH surge to ovulation was 2 days for 10 beagle bitches.[41] Canine oocytes are ovulated as primary oocytes and fertilization cannot be completed until about 48 to 72 hours after ovulation, when they undergo the first meiotic division to become secondary oocytes. By this time the oocytes have descended through two thirds of the uterine tubes. The canine embryo appears to remain in the uterine tube for approximately 9 to 10 days after ovulation, entering the uterus either in the late morula or early blastocyst stage.[41]

Ovulation does not always bear a close temporal relationship to the onset of sexual behavior,[41] and may vary depending on breed.[61] Additionally, ovulation can occur in physiologically normal bitches that remain unreceptive to mating for a variety of reasons (see Chapters 4 and 14). As previously mentioned, ovulations have also been reported to occur in pubertal bitches failing to exhibit behavioral estrus.[21]

The gradual increase in serum concentrations of progesterone resulting from preovulatory luteinization of follicles continues until after the LH surge, when serum concentrations of progesterone undergo a more rapid increase. The bitch appears unique in that behavioral estrus is exhibited in the face of high concentrations of progesterone. The changing concentrations of progesterone during estrus (Table 2–3) can be utilized along with other parameters such as vaginal smears, LH assays, vaginoscopy, estrous behavior, electrical conductance of vaginal fluids, and arborization of

Table 2–3. Serum Levels of Progesterone Relative to the LH Surge

Day Relative to LH Surge	No. of Bitches	Concentration of Progesterone (ng/ml) (Mean ± SEM)
−4	6	0.3 ± 0.05
−3	6	0.5 ± 0.1
−2	6	0.7 ± 0.2
−1	6	0.7 ± 0.1
0	6	1.37 ± 0.1 (LH surge)
1	6	2.2 ± 0.2
2	6	2.8 ± 0.3
3	6	5.4 ± 0.6 (ovulation)
4	6	7.0 ± 0.5
5	6	11.8 ± 0.8
6	6	15.5 ± 2.1 (fertilization)
7	5	16.2 ± 1.9
8	4	19.4 ± 2.6
9	1	34.9 (diestrus)

From Olson PN, Bowen RA, Behrendt MD, et al: Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus, and estrus. Biol Reprod 27:1196–1206, 1982.

vaginal fluids to estimate the time of ovulation (see Chapter 4).

Various schematic representations of the canine estrous cycle have been proposed, depicting the changes in serum hormone concentrations, behavior, vaginal cytology, and physiologic events (Figs. 2–5 through 2–7). "Day 0" of various schematic representations has tended to vary, depending on whether the author has defined "day 0" as the day of the LH surge, ovulation, or onset of cytologic diestrus. The exact time of ovulation is difficult to determine, even with ultrasonographic technologies, but can be estimated by measuring progesterone or LH. The exact time of the LH surge is difficult to determine in a clinical setting because it can occur on a single day and may therefore be missed unless daily samples are obtained over several days to weeks. Because progesterone concentrations increase steadily for several weeks before and following the LH surge, identifying the increase is

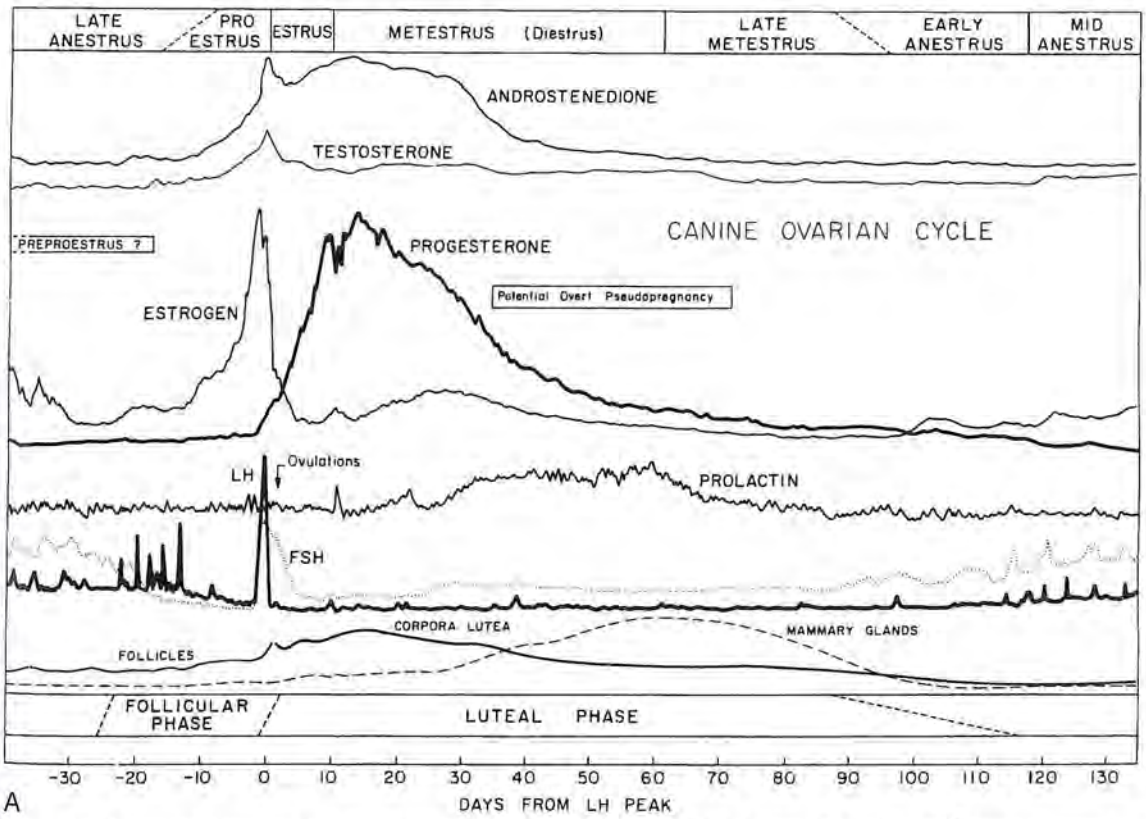
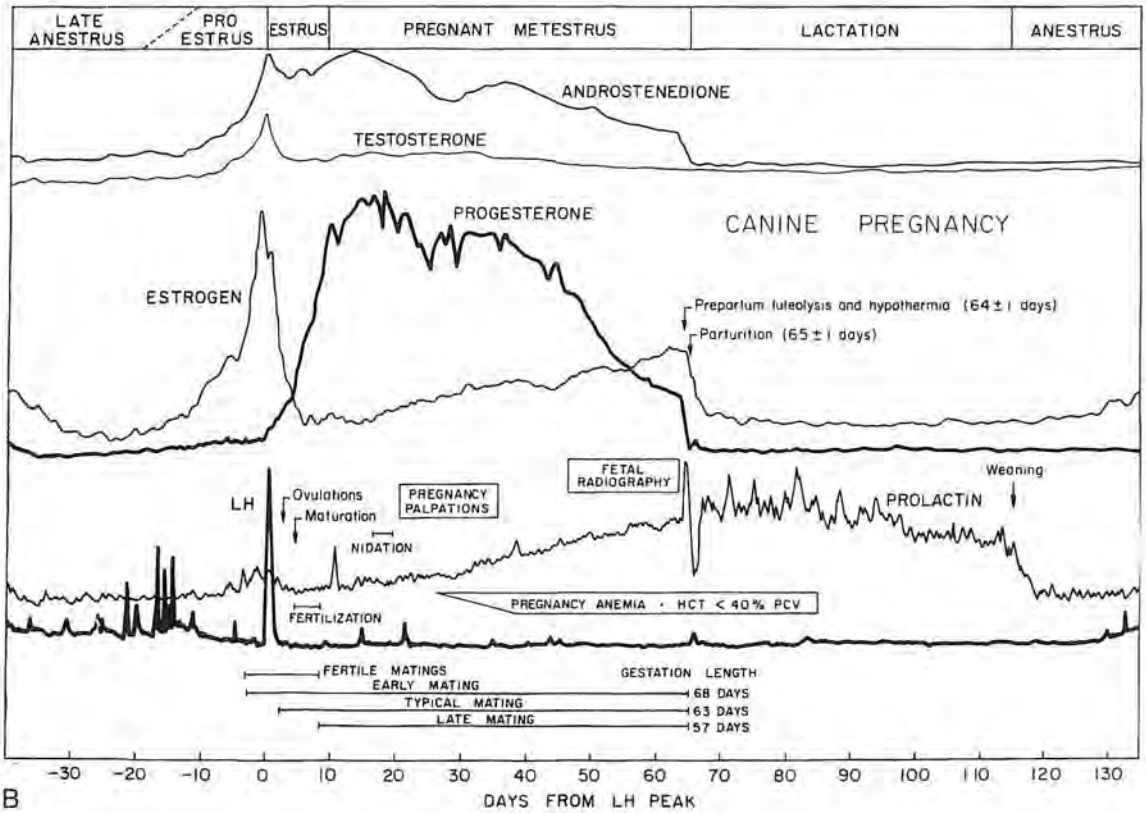
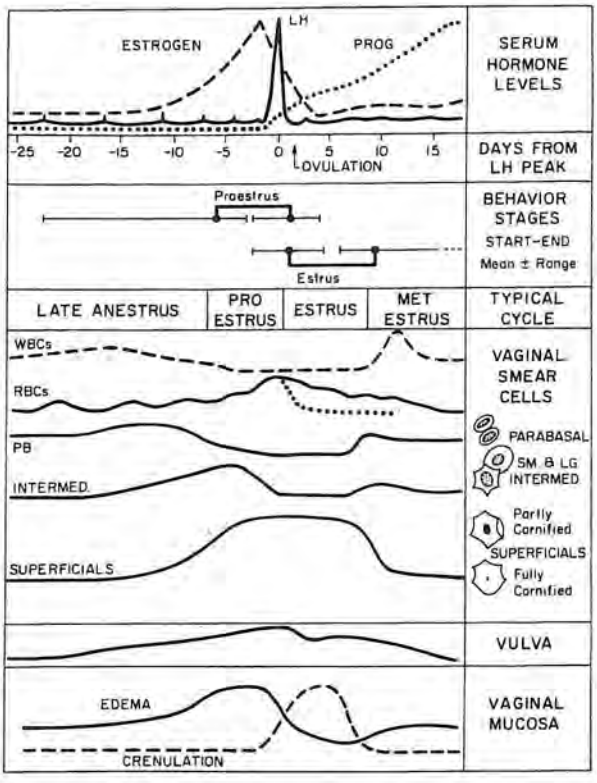


Figure 2–5. Schematic diagrams of the canine estrous cycle with day 0 = day of the LH surge. **A:** A schematic diagram representing hormonal and physiologic changes during the canine ovarian cycle.

Illustration continued on following page



B



C

Figure 2–5 *Continued.* **B:** A schematic diagram representing hormonal and physiologic changes during canine pregnancy and their relevance for breeding programs and the clinical management of pregnancy. **C:** A schematic diagram summarizing the temporal relationships between hormonal events, behavior, vulvar swelling, changes in vaginal mucosa, and changes in vaginal cytology. (From Concannon PW, Lein DH: Hormonal and clinical correlates of ovarian cycles, ovulation, pseudopregnancy, and pregnancy in dogs. Kirk RW, Bonagura JD (eds): In Current Veterinary Therapy X. Philadelphia, W.B. Saunders, 1989, pp 1269–1282, with permission.)

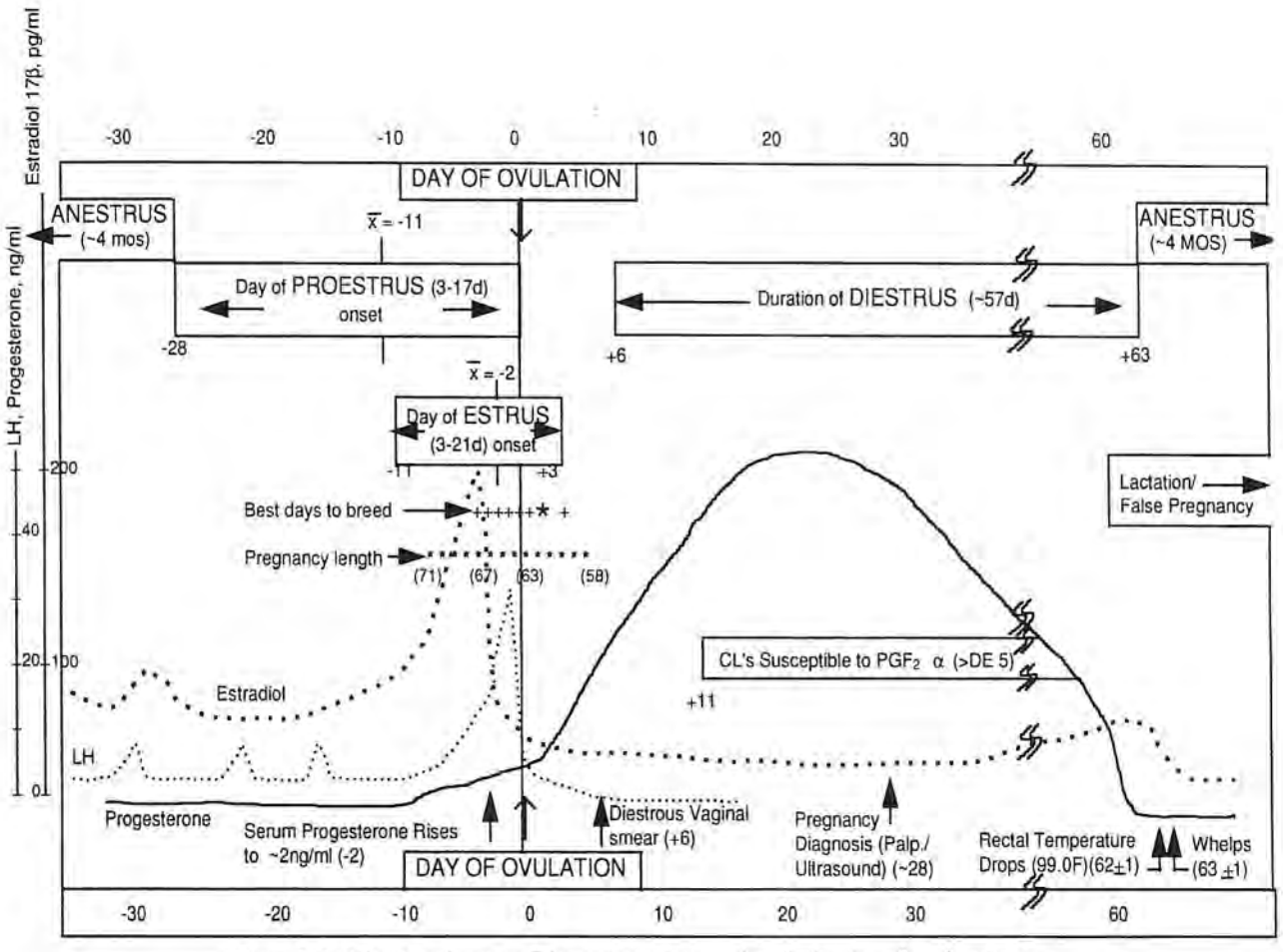


Figure 2–6. Schematic diagram of the canine estrous cycle with day 0 = day of ovulation.

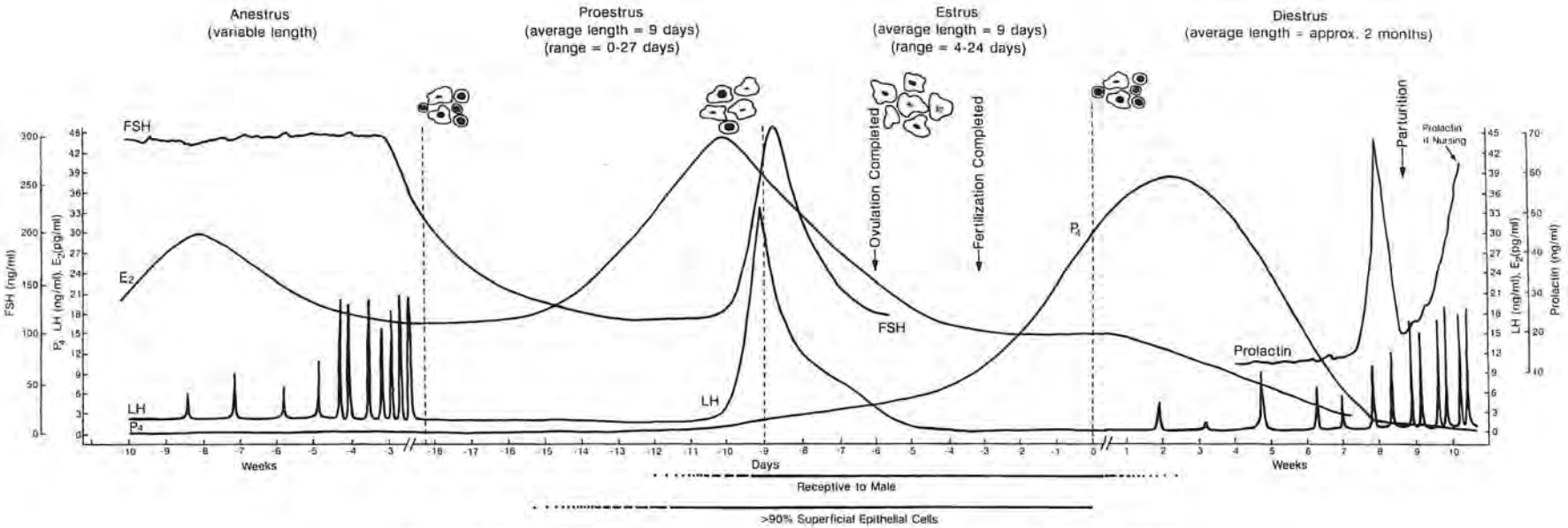


Figure 2–7. Schematic diagram of the canine estrous cycle with day 0 = onset of cytologic diestrus.

less likely to be missed. Measuring progesterone provides an indirect method that is useful for predicting the time of ovulation and the time of oocyte maturation (see Chapter 4).

Diestrus

It has been suggested that the onset of diestrus be defined by vaginal cytology rather than by mating behavior because the LH surge, ovulation, oocyte maturation, and whelping can be more accurately timed using vaginal cytology.[62] The onset of diestrus, as defined by vaginal cytology, occurs when there is a sharp decrease in the percentage of superficial cells and an increase in the percentage of small intermediate and parabasal cells in vaginal smears (Table 2–1, Fig. 2–4). The change in vaginal smears that signifies the onset of cytologic diestrus usually occurs about 3 days before the end of behavioral estrus, 2 to 5 days after oocyte maturation, 5 to 7 days after ovulation, and 8 to 9 days after the LH surge (Table 2–4). Although the onset of cytologic diestrus is easy for veterinary practitioners to identify by evaluating daily vaginal smears, the onset of cytologic diestrus occurs at a time when the bitch is no longer in her most fertile period. Therefore, the onset of cytologic diestrus can be used retrospectively to determine if matings occurred at appropriate times, but is not helpful for prospectively determining the best time of mating for optimal fertility.

Concentrations of serum progesterone rapidly increase to greater than 1 to 2 ng/ml before or during the preovulatory LH surge and continue to increase throughout estrus to reach initial peaks of 15 to 90 ng/ml by 15 to 30 days after the LH surge[57] (Figs. 2–5 through 2–7). After this time, serum concentrations of progesterone begin a gradual decline that continues for 5 to 6 weeks. The progesterone profile is similar for pregnant, nonmated, and hysterectomized bitches during diestrus.[63,64] Therefore, it is unlikely that the canine uterus or uterine prostaglandins play a vital role in the physiologic maintenance or regression of the corpora lutea in the bitch.[65,66] Maintenance of pregnancy is dependent upon ovarian production of progesterone in the bitch.[67] The corpora lutea appear to be the only source of circulating progesterone during pregnancy, and induction of luteolysis at any time in gestation causes termination of pregnancy or premature parturition.[68] An abrupt decline in serum progesterone to less than 1 to 2 ng/ml occurs prior to whelping and is necessary for a normal parturition to occur.[69]

Mammary development may be noted in both nonpregnant and pregnant bitches during diestrus, presumably due to the increased levels of circulating progesterone.[70] Pregnant bitches usually lactate near the time of parturition, and lactation also can occur in nonpregnant bitches near the end of diestrus. The onset of lactation correlates with the decrease in serum progesterone and the increase in serum prolactin that occurs near whelping or the end of diestrus.

It is still unclear as to whether canine corpora lutea require luteotropin support throughout all of diestrus. Luteotropins are hormones, such as LH and prolactin, that are necessary for the maintenance of the corpora lutea in certain species. A decline of circulating

Table 2–4. Relationship of Selected Reproductive Physiologic Events in the Bitch

	LH Surge	Ovulation	Oocyte Maturation	Onset of Cytologic Diestrus
LH Surge		Ovulation occurs 2–3 d after the LH surge	Oocyte maturation occurs 4–6 d after LH surge	Onset of diestrus occurs 8–9 d after LH surge
Ovulation	LH surge occurs 2–3 d before ovulation		Oocyte maturation occurs 2–4 d after ovulation	Onset of diestrus occurs 5–7 d after ovulation
Oocyte Maturation	LH surge occurs 4–6 d before oocyte maturation	Ovulation occurs 2–4 d before oocyte maturation		Onset of diestrus occurs 2–5 d after oocyte maturation
Onset of Cytologic Diestrus	LH surge occurs 8–9 d before onset of diestrus	Ovulation occurs 5–7 d before onset of diestrus	Oocyte maturation occurs 2–5 d before onset of diestrus	

progesterone in response to immunoneutralization of LH or to prolactin-lowering doses of a dopamine agonist (bromocriptine) led some investigators to conclude that luteal function in dogs during the last half of diestrus requires both LH and prolactin.[71] Others have suggested that prolactin, and not LH, is the primary luteotropin during the second half of the luteal period.[72] Luteal function in the bitch may be less dependent upon pituitary support during the first half of diestrus.[73–75] The reason for the apparent difference of pituitary luteotrophic support for young corpora lutea is currently unknown. However, differences do not appear to depend on fluctuations of LH or prolactin receptors in the luteal tissue.[60]

Declining concentrations of serum progesterone that occur in late diestrus do not appear to result from the withdrawal of circulating levels of LH or prolactin in the bitch. Concentrations of LH increase in the serum of pregnant and nonpregnant bitches during late diestrus.[63,76–78] Therefore, it seems unlikely that insufficient secretion of LH is responsible for the declining concentrations of serum progesterone that define the end of the luteal phase in the bitch. Similarly, serum prolactin concentrations increase two- to threefold in bitches during late diestrus over concentrations observed in early diestrus.[60] Regardless of whether prolactin is a primary luteotropin throughout diestrus or not, it appears to have a significant physiologic role in regulating the length of the canine estrous cycle. The interestrous interval can be shortened in bitches receiving a prolactin inhibitor,[50] but the shortening is caused primarily by a reduction in the length of anestrus rather than diestrus.

Unlike concentrations of serum progesterone, which are similar among pregnant, nonmated, and hysterectomized bitches, concentrations of serum immunoreactive relaxin differ between pregnant and nonpregnant dogs.[79] Serum immunoreactive relaxin concentrations are less than 0.25 ng/ml in diestrous bitches that are not pregnant. Mean serum immunoreactive relaxin concentrations increase to maximum concentrations (> 3.0 ng/ml) at about week 6 to 7 of gestation in pregnant bitches. While progesterone production is entirely of ovarian origin, relaxin production is primarily of placental origin.[79,80]

Concentrations of serum testosterone decline during diestrus in the bitch from levels present in proestrus, and remain low (< 0.1 ng/ml) throughout the luteal phase.[45] Serum androstenedione concentrations increase during proestrus, reaching a mean of 0.73 ± 0.13 ng/ml (mean ± standard error) near the time of the LH surge.[45] Concentrations of serum androstenedione decrease in most bitches during estrus, increasing again to reach peak values of 0.4 to 1.27 ng/ml between 6 and 18 days after the LH surge in both pregnant and nonpregnant animals. Both androstenedione and progesterone decrease abruptly at parturition.[45,57]

Anestrus

Anestrus is the quiescent phase of the canine reproductive cycle when defined by behavioral or clinical signs. The normal anestrous bitch does not attract male dogs and is not receptive to mating. The vulva is small during anestrus, with no or minimal passage of discharge. Parabasal and small intermediate cells are the predominant cell types present in vaginal smears from anestrous bitches (Fig. 2–4).

Anestrus is far from a quiescent phase when evaluated with endocrinologic parameters. Concentrations of LH in serum increase in pulsatile bursts in late anestrus, perhaps leading to the next proestrus.[36,45,81,82] Concentrations of serum FSH increase during anestrus, reaching levels in late anestrus that are as high as those present during the preovulatory FSH surge during estrus.[36]

Anestrus is often defined endocrinologically as the time following diestrus when concentrations of serum progesterone reach a basal level of less than 1 to 2 ng/ml. However, the definition of a "basal" progesterone level is arbitrary; it may be possible that further decreases in concentrations of progesterone to some yet-undefined level must occur before a new proestrus can be initiated.

Concentrations of serum estrogens during anestrus are controversial. In one study,[36] mean concentrations of estradiol in the serum of anestrous bitches were occasionally elevated between 7 and 9 weeks before the onset of proestrus. Although the anestrous bitches had increased serum concentrations of estradiol, they did not demonstrate notable clinical signs associated with the increased estrogen levels, such as vulvar swelling or a serosanguineous discharge. Papanicolaou (considered the father of clinical cytology) and Blau,[83] however, reported on rhythmic changes in vaginal smears of anestrous bitches in 1927. They suggested that some type of ovarian hormone caused the slight cornification of cells in vagi-

nal smears from anestrous bitches, which was less pronounced and shorter in duration than those observed during estrus.

Although concentrations of serum estradiol fluctuate during anestrus,[36] they increase again during proestrus with the development of graafian follicles. Increases in estrogen during anestrus in the bitch also have been reported by other investigators.[56,84] It seems likely that estrogen levels could increase with follicular development that occurs during anestrus (see discussion of split heat, above). However, estradiol also has been reported as low in mid-anestrus, increasing 1 month before the preovulatory surge of LH.[85,86] While the exact nature of ovarian function in the anestrous bitch remains unknown, the ovaries are definitely active and their products suppress pituitary gonadotropins. Concentrations of LH and FSH are increased dramatically in ovariohysterectomized bitches when compared to intact anestrous bitches.[87]

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Chapter 3

Vaginal Cytology

The following photograph is of Dr. P. A. Holst (left) and Dr. R. D. Phemister (right), two veterinary researchers who advanced the knowledge in canine reproduction through their outstanding work in correlating vaginal cytology with behavioral and physiological events of the canine estrous cycle.

The vaginal epithelium is one of several target tissues for ovarian hormones. Characteristic changes in exfoliated vaginal epithelial cells occur as a result of changing secretory patterns of estrogens. Increasing circulating levels of estradiol-17β stimulate the growth of the vaginal epithelium from a few cell layers in thickness during anestrus to 20 to 30 cell layers in thickness at the end of proestrus (Fig. 3–1, *see Color Plate*).[1] As the vaginal epithelium thickens, increased numbers of superficial epithelial cells slough into the vaginal fluids. The change in the percentage of superficial cells in a vaginal smear can be used to monitor the progression of proestrus and estrus in the bitch and predict the appropriate times for mating.

Typical Epithelial Cells Found in Normal Canine Vaginal Smears

Several investigators have described cell types found in canine vaginal smears.[1-17] A classification system based on cell morphology has been developed that describes the various cell types present in the epithelium at the time of maximal estrogenic stimulation, beginning with the deepest vaginal layer and progressing to the layer nearest the lumen.

Basal cells are small, round cells that ultimately give rise to all of the epithelial cell types observed in a vaginal smear. Because basal cells are on the basement membrane and are not usually exfoliated, they are rarely observed in a vaginal smear.

Parabasal cells are the smallest of the epithelial cells that are normally seen in vaginal smears (Fig. 3–2, *see Color Plate*). The cell diameter of a parabasal cell ranges from approximately 10 to 20 μm.[18] The parabasal cell is round to oval, has a normal-appearing nucleus, and possesses the largest nucleus:cytoplasm ratio of the vaginal epithelial cells that routinely exfoliate.

Intermediate cells vary greatly in size, leading to numerous terms for these cells. Smaller intermediate cells have been referred to as small, early, rounded, low, and middle intermediate cells (Fig. 3–3, *see Color Plate*). Larger intermediate cells have been referred to as large, late, polygonal, superficial, squamous, transitional, and upper intermediate cells (Fig. 3–4, *see Color Plate*). The cell diameter of a smaller intermediate cell exceeds 20 μm and the cell diameter of a larger intermediate cell exceeds 30 μm.[19] Although the smaller intermediate cell tends to be round to ellipsoid, angularity of the cytoplasmic border also has been reported.[6] Larger intermediate cells usually have an irregular or angulated cytoplasmic border. Both types of intermediate cells have prominent nuclei that appear normal. The large intermediate cell is sometimes confused with the

superficial cell because the size of the two cells is similar.

Superficial cells are large epithelial cells with diameters that range from 30[19] to 75 μm.[18] They are named for their superficial position in the vaginal epithelium at time of maximum estrogen stimulation. The cell borders of a superficial cell are irregular or angulated, similar to those of large intermediate cells (Fig. 3–5, *see Color Plate*). Unlike the large intermediate cell, which has a normal-appearing nucleus, the nucleus of the superficial cell is dark and pyknotic, faint, or not distinguishable from adjacent cytoplasma following staining (Fig. 3–6, *see Color Plate*).

Superficial cells also are referred to as cornified cells, whereas parabasal and intermediate cells are referred to as noncornified cells. Cornification refers to the degenerative process by which cells of a stratified squamous epithelium are converted into dead cells.[20] The pyknotic, faint, or nondistinguishable nucleus in a superficial cell reflects this degenerative process. Superficial cells that appear to lack a nucleus may be referred to as anuclear squames. Technically, all vaginal epithelial cells are derived from a stratified squamous epithelium, and are hence "squames." Although the intensity of staining for superficial cells varies from pale to dark blue-purple with modified Wright-Giemsa stains (see below), superficial cells frequently stain a dark blue-purple color during estrus (Fig. 3–7, *see Color Plate*). This dark staining may result in superficial cells appearing to lack a nucleus, even though a nucleus is present.

Other Cells Found in Normal Canine Vaginal Smears

Several other types of cells have been identified in vaginal smears from normal bitches.

Metestrum cells have been defined as noncornified cells that appear to contain a neutrophil within the cytoplasm (Fig. 3–8A through C, *see Color Plate*). These cells likely reflect the phagocytic properties of the vaginal epithelium. Although metestrum cells have been reported to occur only during the luteal phase of the canine reproductive cycle, they might be observed whenever neutrophils are present.[7,8] Metestrum cells may also be present in vaginal smears obtained from bitches with vaginitis, especially when neutrophils are abundant on the smears. Epithelial cells that appear to contain a neutrophil within the cytoplasm have also been observed in preputial swabs (Fig. 3–8D, *see Color Plate*).

Foam cells are parabasal cells or intermediate cells that contain cytoplasmic vacuoles (Fig. 3–9, *see Color Plate*). Their significance is unknown.[7,8]

Superficial cells with cytoplasmic bodies are cells that contain numerous dark-staining bodies in the cytoplasm.[13] These cells are commonly observed in smears obtained from estrous bitches, but the source and significance of the cytoplasmic bodies are unknown (Fig. 3–10, *see Color Plate*). Cytoplasmic bodies can readily be distinguished from bacteria in vaginal smears. Although bacteria may adhere to superficial cells, bacteria are smaller than cytoplasmic bodies and often are observed in an extracellular position (Fig. 3–11, *see Color Plate*).

Melanin-containing epithelial cells can be observed in vaginal smears from bitches with a pigmented epithelium (Fig. 3–12, *see Color Plate*).

Clitoral fossa epithelial cells are keratinized epithelial cells that can be observed in normal vaginal smears and can be abundant when swabs are collected from the clitoral fossa rather than from the vestibule or vagina (Fig. 3–13, *see Color Plate*). Clitoral fossa epithelial cells, which can be prevalent during both estrus and anestrus, are sometimes mistaken for superficial cells. Such confusion may occur because both clitoral fossa cells and superficial cells stain a dark blue-purple with modified Wright-Giemsa stain. Therefore, it is important that the clitoral fossa is not inadvertently swabbed when obtaining a vaginal smear to predict estrus (see below).

Spermatozoa are sometimes observed in vaginal smears from mated bitches (Fig. 3–14, *see Color Plate*). Although the presence of spermatozoa confirms a breeding, the absence of spermatozoa does not eliminate the possibility of a breeding. In one study,[19] sperm heads were identified in approximately 65 per cent of the vaginal smears obtained from bitches 1 day after a natural mating. Intact spermatozoa are observed for only a few hours following a mating.

Red blood cells may be present in vaginal smears from proestrous, estrous, or early diestrous bitches. During proestrus, serum concentrations of estradiol-17β increase as ovarian follicles mature. This increase in estradiol is believed to be responsible for the diapedesis of red blood cells through uterine capillaries. Therefore, the erythrocytes observed in vagi-

nal smears are thought to be of uterine origin. However, erythrocytes originate from the vagina also, since erythrocytes were observed in vaginal smears obtained from proestrous bitches that had been previously hysterectomized (i.e., ovaries remaining) (P. N. Olsen and R. A. Bowen, unpublished observations, 1981). The number of erythrocytes in vaginal smears from normal estrous bitches is usually less than in smears obtained from proestrous bitches. Erythrocytes also may be observed in vaginal smears from normal bitches in early diestrus, making it difficult to differentiate proestrus from diestrus when looking at only a single smear.

White blood cells may be present in vaginal smears obtained from normal bitches. The neutrophil is the predominant type of white blood cell observed in normal vaginal smears (lymphocytes and eosinophils are rarely observed in normal vaginal smears). Neutrophils may be abundant in vaginal smears obtained from normal bitches in early diestrus. Neutrophils are not observed in vaginal smears from normal bitches during estrus, presumably because diapedesis is impossible due to the thickened vaginal epithelium. If neutrophils are observed in vaginal smears from estrous bitches, inflammation of the uterus or vagina/vestibule should be considered.

Bacteria frequently are observed in vaginal smears obtained from normal bitches. The number of bacteria can increase logarithmically in vaginal samples obtained during estrus over samples obtained during anestrus, pregnancy, or postpartum[21] (see Chapter 12).

Neoplastic cells and other abnormal cells may exfoliate and be observed in vaginal smears[22] (see Chapter 12). Transitional cell carcinomas that have invaded the vagina, transmissible veneral tumors, squamous cell carcinomas, metastatic mammary adenocarcinomas, and lymphosarcomas are the more common types of tumors observed in vaginal smears (Fig. 3–15, *see Color Plate*).

Method to Obtain, Stain, and Store Vaginal Smears

Exfoliated cells are obtained by passing a cotton-tipped swab, spatula, glass rod, or bulb pipette into the vestibule or caudal vagina (Table 3–1). Although referred to as "vaginal" smears, most specimens contain cells from both the vestibular and vaginal epithelium. If care is taken to avoid the clitoral fossa, the number of keratinized cells from the clitoral fossa should be minimal and not confused with cornified or superficial cells of the vestibular or vaginal epithelium. To avoid swabbing the clitoral fossa, the cotton-tipped swab should be passed cranially and dorsally when entering the vaginal vault (Fig. 3–16A,B). A speculum can be passed before the swab is introduced, but generally is not necessary. The labia should be separated with a gloved hand to identify the clitoral fossa, and the swab directed into the vagina with the other hand so as to avoid swabbing the clitoral fossa (Fig. 3–16C,D, *see Color Plate*). By swabbing the dorsal vaginal wall, injury to the urethra can be prevented. Cells from the swab should be transferred to a clean glass slide by gently rolling the swab onto the slide, not by smearing, which may damage the cells (Fig. 3–16E, *see Color Plate*). Alternatively, smears may be prepared by using cells collected by pipette or spatula. Cells should not be collected by blot-

Table 3–1. How to Collect, Stain, and Store Vaginal Smears

1. Introduce a sterile cotton-tipped applicator swab into the dorsal vestibule or caudal vagina. The swab need not be moistened if the bitch has adequate vaginal fluids (i.e., proestrus, estrus); otherwise, the swab may be moistened with saline to allow easier passage.
2. The swab should be introduced into the vestibule or caudal vagina so as to avoid the clitoral fossa. The swab should be directed dorsocranially and rolled over the dorsal vestibular/vaginal mucosa so as to avoid injury to the ventral external urethral orifice.
3. The swab should be gently rolled over a clean microscope slide three to four times. The slide should be allowed to air-dry prior to staining.
4. The air-dried smear should be stained with a modified Wright-Giemsa stain such as Diff-Quik.* After staining, the smear can be gently blotted one or two times with a paper towel to dry. (*Note:* The smear may need to be dipped 10 to 15 times in the two staining solutions of the Diff-Quik stain.)
5. Scan the smear on 40× to 200× to determine if cells are adequately stained and under estrogenic stimulation. If cells are stained too lightly, the slide may again be immersed in the staining solutions. Use oil immersion (1,000×) if scanning for bacteria or spermatozoa.
6. Unstained or stained smears can be stored in a slide box and used at a later date. Immersion oil on stained smears should be removed prior to storage. Coverslips can be permanently added to stained smears for optimal storage.

* Manufactured by Baxter Healthcare Corporation, Dade Division, Miami, FL 33152; distributed by Baxter Healthcare Corporation, Scientific Products Division, McGaw Park, IL 60085.

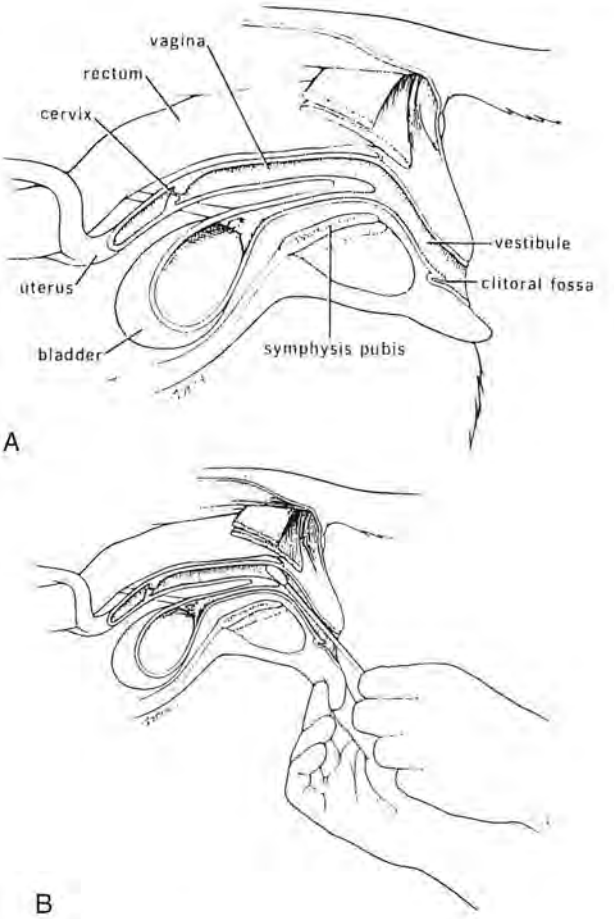

Figure 3–16. A and **B:** Schematic diagrams demonstrating the anatomy of the canine vagina and vestibule and the appropriate angle for introducing a swab into the vestibule and caudal vagina. The swab should avoid entering the clitoral fossa and should be directed to the dorsal vaginal wall so as to avoid possible trauma to the ventral urethral orifice (From Johnston SD: Examination of the genital system. Vet Clin North Am 11:543–559, 1981, with permission.)

ting the vulvar mucosa with a glass slide, as this technique may result in inaccurate diagnosis of cytologic estrus and diestrus.

Several commercially available stain preparations are suitable for staining vaginal smears. The veterinarian should select the stain that is easy to use and produces consistent results. Diff-Quik* is a rapid, modified Wright-Giemsa stain that reliably stains vaginal smears and is easy to use in a clinical setting. Air-dried smears are sequentially immersed in methanol and the two solutions that constitute the Diff-Quik stain. Vaginal smears should be dipped several times in the two staining solutions, since the time required to stain some vaginal cells is longer than that required to stain blood films. Smears stained with Diff-Quik can be stored for months to years if the immersion oil is removed after viewing.†

† Epithelial cells will become distorted over time if immersion oil is added directly to the smear and not removed after viewing. If immersion oil is repeatedly used, a coverslip should first be permanently fixed to the specimen.

New methylene blue stain is easy to use and smears can be read immediately after applying a drop of the stain and a coverslip to the slide. New methylene blue does not stain erythrocytes and provides only temporary staining so cytologic preparations cannot be stored for examination at a later date.

Trichrome and Papanicolaou stains can be utilized on vaginal smears to differentiate cyanophilic from eosinophilic cells (Fig. 3–17, *see Color Plate*). Although these stains can produce good results if cells are properly fixed and stains are prepared and maintained correctly, the protocol is more tedious than busy veterinarians may be willing to accept. Additionally, the percentage of eosinophilic epithelial cells, which is used to predict estrus, varies between smears obtained from the canine vestibule and vagina, whereas the percentage of superficial cells, as determined by a modified Wright-Giemsa stain, does not.[19]

The veterinarian can instruct the owner or breeder on the technique for obtaining vaginal smears. Vaginal smears that are air-dried and

stored in a slide box can be stained and evaluated at a later date if the bitch is unavailable for daily or alternate-day smears. Smears should not touch one another so as to prevent sticking of slides or contamination of cells from one smear to another. One swab of the vestibule or vagina usually contains sufficient cells to make at least two smears (e.g., one smear can be stained and viewed immediately and one smear can be stained and evaluated at a later time). If an owner repeatedly swabs the same location over a relatively short period of time within the same day, the relative number of cell types in the vaginal smear can change. The relative number of each cell type in the vaginal smear will vary with the extent of mucosal proliferation, the predominant surface cell type, and the number of cell layers removed in collecting the smear.[18]

Interpretation of Canine Vaginal Smears

Progression from Proestrus to Estrus

Serial evaluation of vaginal smears obtained on a daily or alternate-day basis can assist the veterinarian in estimating the appropriate time for breeding a bitch. Increasing numbers of superficial cells in a vaginal smear signify the progression of proestrus and estrus. Unfortunately, the time and intensity of maximum cornification (i.e., maximum percentage of superficial cells) varies among bitches, and precludes its use to prospectively predict the exact time of receptivity to mating, the luteinizing hormone (LH) surge, or ovulation. In addition to verifying the progression of proestrus and estrus, smears can be used to determine the onset of cytologic diestrus (see below), which allows retrospective determination if breedings occurred at appropriate times for maximal conception rates.

In evaluating vaginal smears obtained from 12 beagle bitches on a daily basis during proestrus, estrus, and early diestrus, the day of maximum cornification (i.e., maximum percentage of superficial cells) was variable, ranging from 91 to 100 per cent, and occurring between –1 to +7 days[‡] after the day of the LH surge. The LH surge occurs on an average of 2 to 3 days before ovulation and 4 to 6 days before optimal breeding times. The first day when at least 90 per cent of the epithelial cells were of a superficial type[§] ranged from –6 to +4 days after the LH surge.[19] For some bitches, maximum cornification may be less than 90 per cent superficial cells in a vaginal smear. Linde and Karlsson studied the correlation between vaginal smears and the calculated time of ovulation in 22 bitches, for a total of 24 estrous periods.[12] Maximum stimulation of the vaginal epithelium by estrogens was defined by the percentage of cells that were anuclear cells or superficial cells with a small pyknotic nucleus. In two thirds of the estrous periods the maximum cornification was over 90 per cent; in the remaining one third of the estrous periods the maximum cornification was between 80 and 90 per cent. Maximum cornification lagged behind the estradiol peak in all 24 estrous periods by at least 3 to 6 days. In 17 of the 24 (70.8 per cent) estrous cycles examined, ovulation was estimated to occur at approximately the same time maximum cornification occurred. In some bitches, "maximum" cornification appears to occur in two peaks.[12,13] Concannon has reported that maximum cornification varies among bitches and may be represented by nearly 100 per cent anuclear superficial cells; a high percentage of cells containing distinct, condensed nuclei; or by retention of some large, well-nucleated intermediate cells along with superficial cells.[14]

Depending on the type and intensity of staining, cells may appear anuclear when they actually contain a pyknotic nucleus (Fig. 3–7). Because cornified cells usually are defined to include all types of superficial cells, misinterpreting anucleated from nucleated superficial cells is generally not problematic when evaluating the progression of proestrus and estrus. One should not, however, place great significance on predicting the onset of estrus based solely on the relative number of cells that appear anuclear; the type and duration of staining may contribute to the appearance of anucleated epithelial cells. As proestrus progresses into estrus, the percentage of cornified cells in a vaginal smear often increases to at least 90 per cent at maximum cornification. Large intermediate cells may predominate in

‡ Original reference contains an error. Page 260 states that maximal cornification occurred from –1 to –7 days following the LH surge. This should have read "–1 to +7."

§ Superficial cells = cell diameter is 31 μm or more; nuclear area is 90 μm squared or less. Large (superficial) intermediate cells = cell diameter is 31 μm or more; nuclear area is more than 90 μm. Small intermediate cells = cell diameter is more than 20 μm and less than 31 μm. Parabasal cells = cell diameter is 20 μm or less.

vaginal smears obtained during proestrus, but their numbers usually decrease during the fertile period as numbers of superficial cells increase. Differentiating large intermediate cells from superficial cells is sometimes problematic for practicing veterinarians if only a single smear is evaluated (Fig. 3–18, *see Color Plate*). Evaluating serial smears, rather than only a single smear, allows the veterinarian to verify that the cycle is progressing normally as cell types change and maximal cornification occurs.

Progression from Estrus to Diestrus

Determining the onset of cytologic diestrus allows one to retrospectively predict if breedings occurred at optimal times for maximal conception rates, and to prospectively predict the time of whelping. Holst and Phemister[5] compared the percentage of superficial cells to the percentage of small intermediate and parabasal cells in daily vaginal smears collected from approximately 400 beagle bitches. The onset of cytologic diestrus was defined as the first day when the number of superficial cells decreased by at least 20 per cent. However, often the decrease was greater than 50 per cent (Fig. 3–19A,B, *see Color Plate*). The time of first refusal (onset of behavioral diestrus) occurred during the first 5 days of cytologic diestrus in 77 per cent of the bitches.

Conception rates following a single mating were greater than 95 per cent when the breeding occurred between 3 and 10 days before the onset of cytologic diestrus (Fig. 3–19C, *see Color Plate*). When gestation length was calculated from the onset of diestrus, the mean time of whelping for 93 bitches was 56.9 days after the onset of cytologic diestrus. Therefore, vaginal smears can prospectively predict the day of whelping and retrospectively determine if a bitch was mated at appropriate times to ensure maximum conception rates (see Chapter 4).

Proestrus, Estrus, Diestrus, and Anestrus

Although vaginal smears vary among and within bitches, the following descriptions describe the cell types that can be expected in vaginal smears from normal bitches throughout the estrous cycle.

PROESTRUS

Vaginal smears obtained in early to mid-proestrus usually are characterized by the presence of erythrocytes and a mixture of epithelial cell types (Fig. 3–20A, *see Color Plate*). Neutrophils may be present and bacteria may be abundant (Fig. 3–20B, *see Color Plate*). By mid to late proestrus, the percentage of parabasal and small intermediate cells decreases and the percentage of superficial and large intermediate cells increases. Although erythrocytes usually are abundant in vaginal smears from proestrous bitches, they may, alternatively, be absent or few in number (Fig. 3–20C, *see Color Plate*).

ESTRUS

Neutrophils usually are absent in vaginal smears obtained from estrous bitches. Erythrocytes often decrease in number, relative to proestrous smears, but can be observed throughout estrus and early diestrus in many bitches. Superficial cells are the predominant epithelial cell type during estrus. In most bitches, greater than 90 per cent of the epithelial cells are of a superficial type at the time of maximum cornification. Large numbers of bacteria frequently are observed in vaginal smears during estrus. In the absence of neutrophils, the presence of numerous bacteria during estrus should be considered normal. The background of estrous smears can be very clear and devoid of bacteria and cellular debris. Superficial cells may have well-defined cellular borders throughout estrus (Fig. 3–21A, *see Color Plate*). However, when numerous bacteria are present (Fig. 3–21B, *see Color Plate*), or if the vaginal smear is collected on the last day of estrus, superficial cells from bitches may have poorly defined cytoplasmic borders. Cells tend to clump (referred to as "sheeting") prior to the onset of cytologic diestrus[5] (Fig. 3–21C, *see Color Plate*).

DIESTRUS

An abrupt change in relative numbers of epithelial cell types in the vaginal smear marks the onset of cytologic diestrus. As previously noted, the relative number of superficial cells decreases and the relative number of parabasal and/or small intermediate cells increases. Parabasal cells may stain a dark blue-purple in vaginal smears obtained from bitches in early diestrus. Neutrophils may reappear in early diestrus (Fig. 3–22A, *see Color Plate*) but can precede or lag behind changes in epithelial cell types.[5] Although not phase specific, epithelial cells may occasionally appear to contain a neutrophil in the cytoplasm (see metestrum cell,

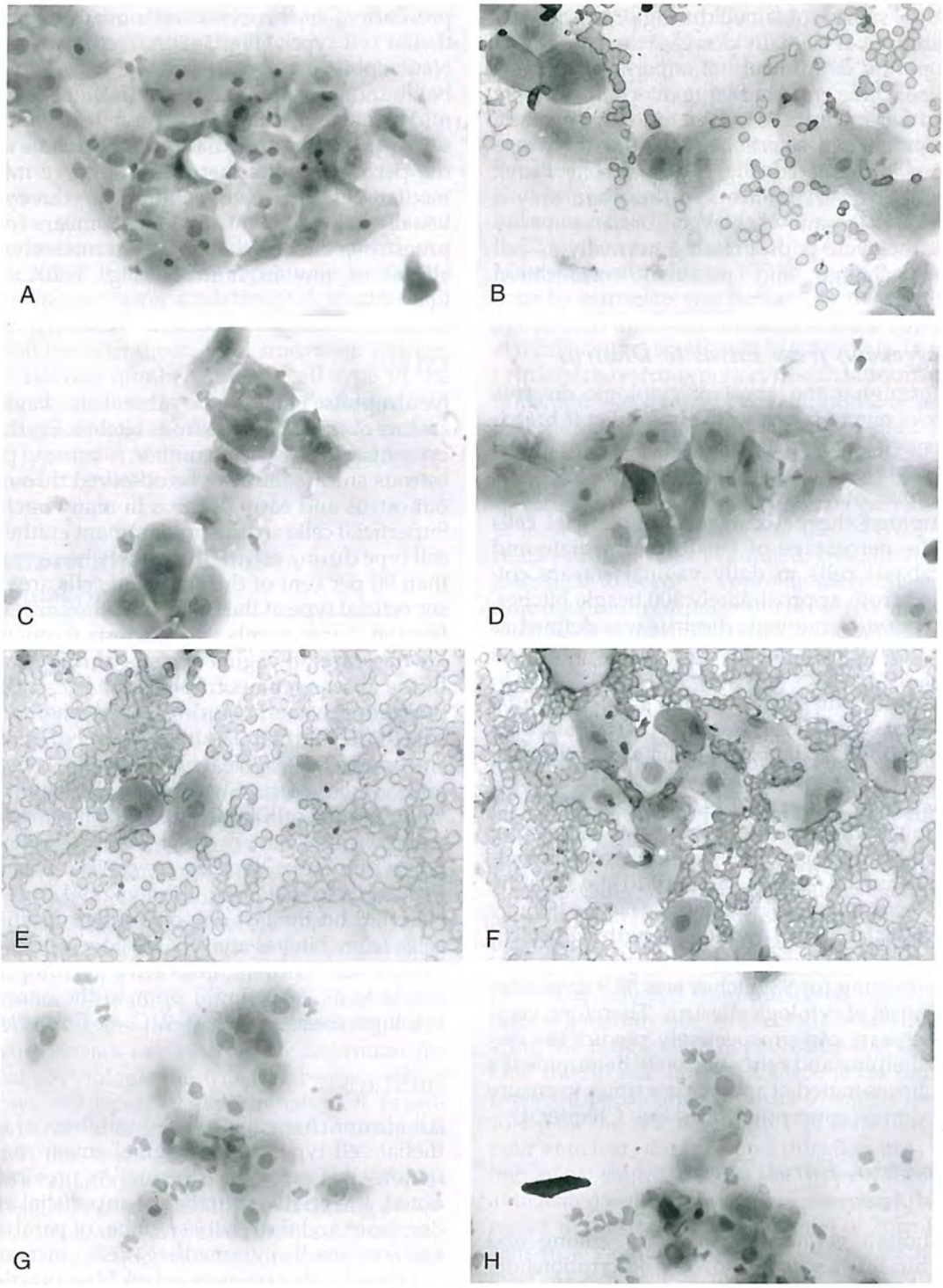

Figure 3–24. Eighteen vaginal smears obtained daily from a single bitch, beginning with the onset of proestrus. Vaginal smears (**A** through **H**) were obtained during proestrus. Note the great variability with regard to erythrocytes and white blood cells. Vaginal smears (**I** through **Q**) are consistent with cytologic estrus. Vaginal smear (**R**) would be considered the onset of cytologic diestrus. Although epithelial changes in this bitch were abrupt, in some cases the changes that signify the onset of cytologic diestrus are much more subtle. Magnification; 400×.

Illustration continued on opposite page

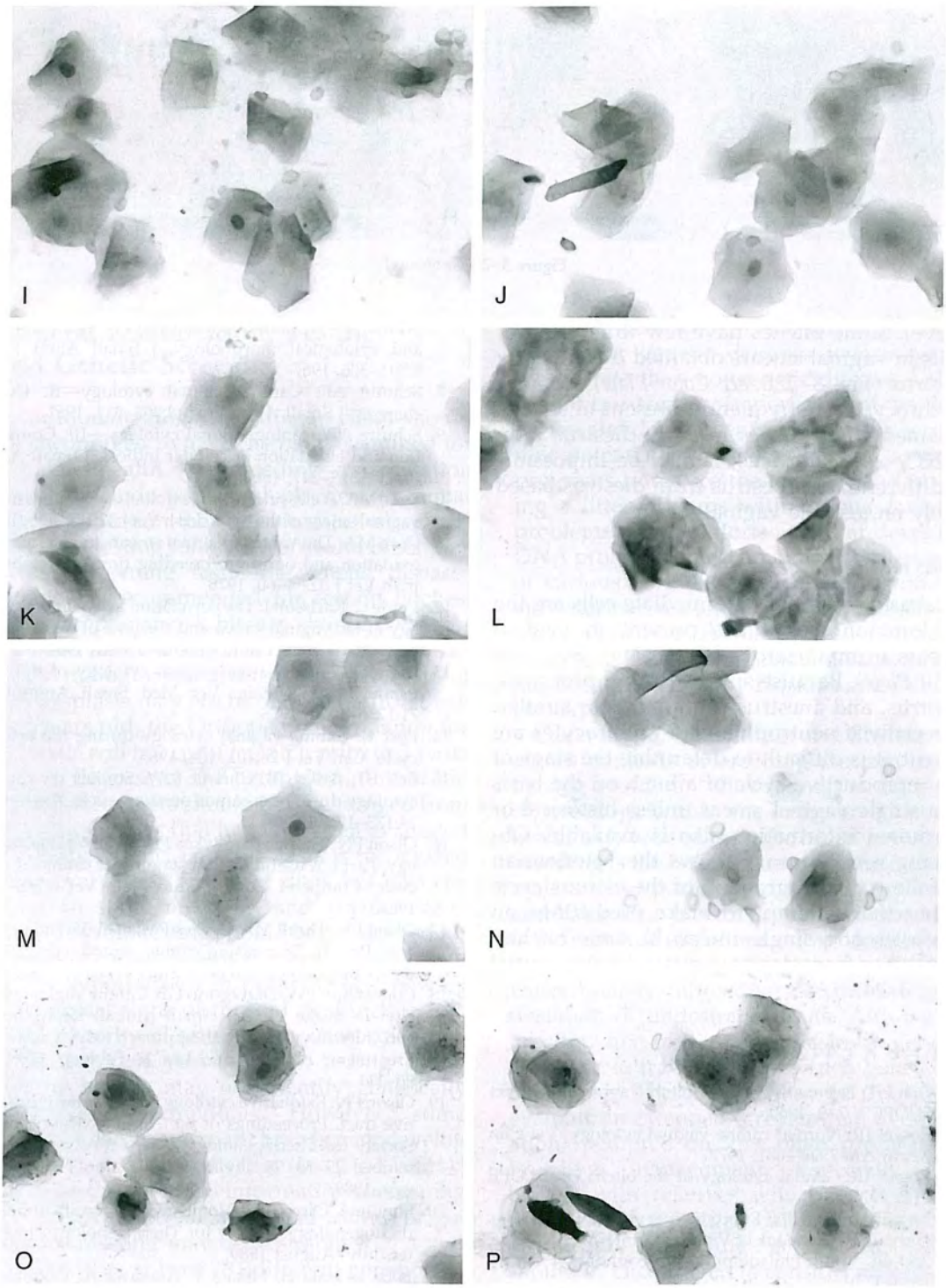

Figure 3–24 *Continued*

Figure 3–24 *Continued*

above). Some bitches have few to no neutrophils in vaginal smears obtained during early diestrus (Fig. 3–22B, *see Color Plate*). Because erythrocytes are frequently present in smears obtained from bitches in early diestrus (Fig. 3–22C, *see Color Plate*), it may be impossible to differentiate proestrus from diestrus based solely on a single vaginal smear.

ANESTRUS

Parabasal and small intermediate cells are the predominant cell types present in vaginal smears from anestrous bitches (Fig. 3–23, *see Color Plate*). Because smears during proestrus, diestrus, and anestrus can all appear similar, especially if neutrophils and erythrocytes are absent, it is difficult to determine the stage of the reproductive cycle of a bitch on the basis of a single vaginal smear unless historical or hormonal information also is available. Obtaining serial smears allows the veterinarian to follow the progression of the estrous cycle rather than attempt to make predictions on the basis of a single smear. In some bitches, variability from day to day can be substantial (Fig. 3–24).

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Chapter 4

Breeding Management and Artificial Insemination of the Bitch

General Examination, Testing, and Genetic Screening

Prior to managing a breeding, a client should be encouraged to have the bitch examined for general health. Prebreeding examination should include a general physical examination, verification that immunizations are current, and testing for heartworm and other parasites. Screening tests for genetic diseases should be recommended for certain bitches. For example, at-risk bitches should be radiographed and evaluated for hip dysplasia. Because radiographic changes characteristic of hip dysplasia may not be detected until a bitch is 2 years old, the Orthopedic Foundation for Animals will not certify a bitch prior to 2 years of age. Because of the heritable nature of hip dysplasia, owners should be discouraged from breeding bitches that have not been evaluated or are dysplastic. Hip dysplasia is recognized in several larger breeds of dogs but also can occur in some medium- and smaller sized dogs. Thyroid testing should be considered for bitches of certain breeds that are overrepresented for hypothyroidism, or for bitches showing any clinical signs of thyroid dysfunction. Because hypothyroidism usually affects older animals (i.e., > 4 years of age), testing young healthy bitches may not identify genetically predisposed individuals. However, some high-risk, large, and giant breeds may develop clinical signs at an earlier age (i.e., 2 to 3 years).[1] Based on the historical information concerning the bitch's relatives, additional testing or genetic counseling may be desirable.

As of 1988, at least 281 different genetic diseases were documented for the dog.[2] By 1994, Smith reported that over 400 genetic diseases had been described for the dog, with the frequency of genetic diseases increasing at a higher rate than nongenetic diseases.[3] Therefore, it is extremely important that the veterinarian and dog owner work together to recognize potential genetic diseases and formulate programs to reduce the likelihood of producing a litter of pups with serious congenital problems. Future efforts aimed at developing DNA probes to detect animals who are carriers of various heritable traits could significantly aid veterinarians and dog owners in eliminating or reducing particular diseases.[4] A comprehensive guide to congenital defects of dogs has been published by Hoskins and Taboada[5] (see Appendix).

Outcrossing, inbreeding, and line breeding are all methods of breeding that are utilized by dog breeders.[4,6] Outcrossing is the mating of two dogs within the same breed that are less closely related than the breed average. Inbreeding is the breeding of closely related animals, such as parents and offspring or brother and sister. Line breeding, a form of inbreeding, is the repeated use of one or two dogs (usually males) for breeding to increase a certain trait. Unfortunately, inbreeding also increases manifestation of undesirable traits. Although inbreeding does not create undesirable genes for undesirable traits, it allows such genes to be expressed.[6] Therefore, it may be desirable to evaluate an extended (greater than five-generation) pedigree on any bitch presented for breeding management, especially those bitches with relatives who have been diagnosed with a potentially inherited disorder. If significant inbreeding is present, owners should be encouraged to consider outcrossing. If the bitch is a likely carrier of a serious genetic disorder, breeding should be discouraged.

Examination and Testing That Pertains to the Reproductive System

Examination

All bitches presented for breeding should be carefully evaluated for abnormal vulvar conformation and vulvar discharge (see Chapter 12). Severe conformational problems can contribute to inability to breed, persistent inflammation of the caudal reproductive tract, and inability to whelp. Although vulvar conformation may improve during proestrus/estrus, those cases not influenced by hormones or severe cases may preclude a normal breeding (or whelping).

A digital vaginal examination should be performed on all bitches larger than 7 kg body weight in order to assess the adequacy of vaginal size for normal copulation (Fig. 4–1). The veterinarian should be mindful that normal anestrous bitches have a circular stricture at the vaginovestibular area, called the cingulum, that relaxes during estrus (see Chapter 1). Occasionally, however, the cingulum fails to fully relax, resulting in an "estrous" bitch who refuses to be mated. Digital vaginal examinations also can detect vaginal bands of tissue, tumors, foreign bodies, and other abnormalities that may prevent a normal mating (Fig. 4–2).

Figure 4–1. Technique of performing a digital vaginal examination. Estrous bitches rarely resent such an examination and may "flag" or deviate their tails during the procedure.

Vaginoscopy should be performed if a bitch historically has been nonreceptive to mating, has an abnormal discharge passing from the vulva, or has signs suggesting inflammation of the caudal reproductive tract (i.e., excessive licking of the vulva, scooting of the vulva area across floors, abnormal odor, and attacking male dogs even when not in estrus) (Table 4–1). A pediatric proctoscope (Fig. 4–3) can be used to perform vaginoscopy on medium- and large-size dogs and provides sufficient length to allow the examiner to visualize most of the vagina. The pediatric proctoscope cannot be used for vaginoscopy in small dogs and is even too large to pass through the narrow portion of the cranial vagina in large bitches. Therefore, because of the narrowing of the cranial vagina and positioning of the cervix, the external orifice of the cervix (ostium uteri externum) is not usually visualized when using the pediatric proctoscope. The proctoscope has an insufflator, allowing for air to be introduced into the vestibule and vagina to prevent mucosal tissues from obscuring the examiner's view. The pediatric proctoscope has a light source that allows for excellent visualization of the vaginal and vestibular mucosa.

Rigid endoscopes have been described for vaginoscopy.[7] The endoscope must be long (≥ 29 to 30 cm) and of small diameter (3.5 to 5.0 mm) to allow visualization of the entire vagina, including the narrow cranial fornix and paracervical area. Vaginoscopy with the rigid endoscope can be performed at any stage of the cycle; in one survey, 215 of 259 (83 per cent) attempts to visualize the entire vagina and cervix were successful.[8] In that study, dogs ranged in weight from 3.5 kg (Chihuahua) to 54.6 kg (mastiff). Complications are reportedly infrequent and include vaginitis, vaginal tears, and vaginal adhesions. Complications of vaginoscopy with a rigid endoscope occur most commonly when bitches undergo the procedure while in diestrus or anestrus, when the vaginal wall is thin.[8]

Human anoscopes of varying sizes can be utilized to visualize the vestibule and clitoral fossa of most bitches, and small anoscopes can be used to visualize the vagina in smaller bitches. Otoscopic equipment, nasal specula, arthroscopic specula, and endoscopes can all be utilized for examining various parts of the caudal reproductive tract. Modular endo-

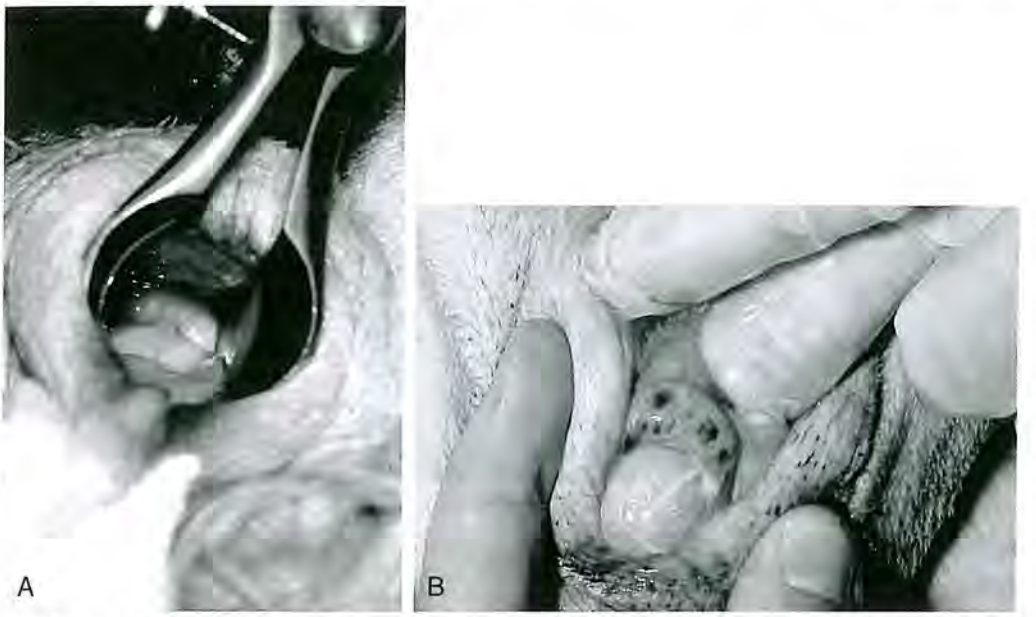


Figure 4–2. A: Vaginal examination with a speculum that reveals a narrow band of tissue (see Chapter 12). **B:** Digital examination that reveals a wider band of tissue in a location similar to that in **A.**

scopes that are portable and adaptable to otoscopic or ophthalmoscopic attachments are also available. (Medical Diagnostics Services, Inc., Brandon, FL.)

A careful evaluation of the mammae should be performed. All glands should be carefully palpated for tumors and other abnormalities.

Table 4–1. How To Perform Vaginoscopy in the Bitch

1. Depending on the stage of the estrous cycle and disposition of the bitch, sedation may be required. Generally, bitches in proestrus-estrus will tolerate the procedure without sedation. Analgesics or anesthetics are required if vaginal biopsies are obtained.
2. Use sterilized equipment.
3. Lubricate the vaginoscope, anoscope, endoscope, or other instruments if vaginal secretions are minimal or the bitch is in anestrus.
4. Separate the vulvar lips and introduce the instrument.
5. Gently pass the instrument in a dorsocranial position to avoid entering the clitoral fossa. Then pass the instrument cranially on into the vestibule and vagina. Remove the obturator (or open the vaginal speculum) after full insertion to view the mucosal folds.
6. Depending on the size of the bitch, instruments > 8 mm in diameter may not pass through the narrowing of the cranial vagina to allow for visualization of the external cervical os.
7. In large bitches, instruments as long as 25 cm may be needed to visualize the cranial vagina.

Abnormal secretions should be cytologically evaluated (see Chapter 13).

Serologic Testing

Serologic testing for canine brucellosis should be performed as part of the prebreeding examination (Table 4–2) (also see Chapter 5). Because canine brucellosis is transmitted by ingestion of bacteria as well as through venereal contact, all bitches (including virgin animals and those previously tested negative) should be tested for the presence of serum *Brucella canis* antibodies using the rapid card agglutination test (RCAT) (Canine Brucellosis Antibody Test Kit, Synbiotics Corporation, San Diego, CA). Because the RCAT is sensitive, animals that test negative may be presumed free of canine brucellosis unless there is a history of exposure within the past month. However, the RCAT is not specific. Therefore, if a patient's serum tests positive for *B. canis* antibodies, the test should be repeated using 2-mercaptoethanol (2-ME, available in the kit), which removes nonspecific agglutinins. Animals with positive test results on the RCAT that become negative after using 2-ME may be true negatives, or may be in the early stage of *B. canis* infection and should be retested in 30 days. Animals that remain positive following the addition of 2-ME to serum are presumptive positives, but still may be false positives, and should be fur-

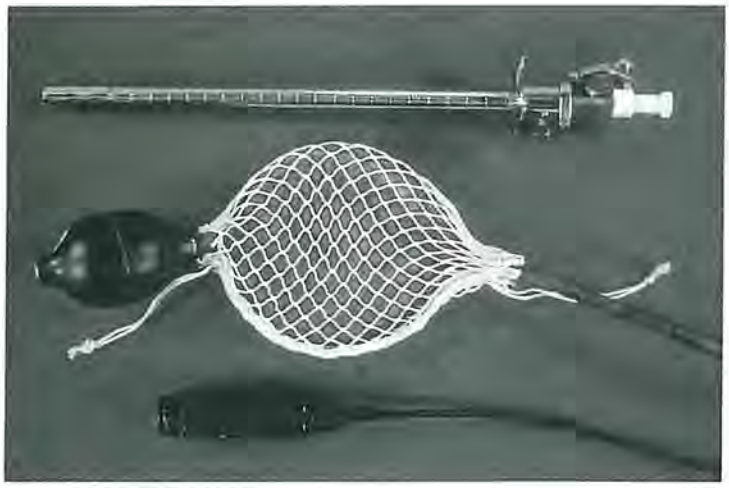


Figure 4–3. A pediatric protoscope (Welch Allyn, Skaneateles Falls, NY) can be used to perform vaginoscopic examinations on medium- and large-size dogs.

ther tested using the agar gel immunodiffusion test.*

Serologic testing for canine herpesvirus should be performed as part of the prebreeding examination on virgin bitches or bitches with a previous negative titer. Negative canine herpesvirus titers in the bitch prior to breeding mandate strict isolation from other dogs during the last 3 weeks of gestation and the first 3 weeks following whelping[9] (see Chapter 5).

Prebreeding Vaginal Cultures

Occasionally, owners will request that a vaginal culture be obtained from a bitch prior to mating. This request may originate with the owner of the bitch or be required by the owner of the male dog. Unfortunately, because a variety of microorganisms are present in the vaginas of bitches with and without reproductive diseases, it is often difficult to associate disease with a specific microbial isolate.[10] Approximately 60 per cent of normal bitches harbor aerobic bacteria in the cranial vagina, and approximately 90 per cent of normal bitches harbor similar organisms in the caudal vagina[11] (Table 4–3 through 4–8). Although most bitches have an established bacterial flora, the types and prevalence of organisms isolated may vary with the age of the bitch. Aerobic bacteria that were frequently (>15 per cent of cases) isolated from the caudal vaginas of 81 adult bitches included *Escherichia coli*, coagulase-positive staphylococci, coagulase-negative staphylococci, α-hemolytic streptococci, β-hemolytic streptococci, *Pasteurella*, and *Bacillus*.[11] The types of bacteria do not appear to vary with the stages of the estrous cycle in adult bitches. However, the numbers of bacteria isolated from bitches during proestrus and estrus are higher than at other times during the estrous cycle.[12] Similarly, many bacteria can be observed in the vaginal smears obtained from normal proestrous and estrous bitches. Although many bacteria are frequently present in vaginal smears obtained from normal estrous bitches, neutrophils should be absent or few in number until the bitch enters cytologic diestrus.

In one study[13,14] the aerobic bacterial flora of the genital tract were characterized in 59 bitches over 18 months. The bitches repre-

Table 4–2. How To Serologically Test for Canine Brucellosis Using Rapid Card Agglutination Test (RCAT)*

Negative result	Consider negative or early infection if history of recent exposure (retest in 30 d)
Positive result	Add 2-mercaptoethanol (2-ME) to serum and repeat the test
Negative after 2-ME	Negative or early infection if history of recent exposure
Positive after 2-ME	Submit serum for agar gel immunodiffusion test† for cell wall and cytosolic antigens

* Canine Brucellosis Antibody Test Kit, Synbiotics Corporation, San Diego, CA.
† Available at Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.

* Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY. In 1993, the American Association of Veterinary Laboratory Diagnosticians Interpretative Serology Committee agreed upon a resolution that the Diagnostic Laboratory at Cornell would serve as a *B. canis* reference laboratory.

Table 4–3. Classification of Vaginal Isolates from 20 Pups, 1 to 11 Weeks Old

Type of Isolate	No. of Isolates	Per Cent of Total Isolates	Pups with Isolate (%)
Escherichia coli	9	18.0	45.0
Coagulase$^+$ staphylococci	13	26.0	65.0
Coagulase$^-$ staphylococci	6	12.0	30.0
α-Hemolytic streptococci	3	6.0	15.0
β-Hemolytic streptococci	6	12.0	30.0
Nonhemolytic streptococci	4	8.0	20.0
Proteus	3	6.0	15.0
Bacillus	3	6.0	15.0
Corynebacterium	2	4.0	10.0
Pseudomonas	1	2.0	5.0
Total	50 (2.5 isolates/pup)		

From Olson PNS, Mather EC: Canine vaginal and uterine bacterial flora. J Am Vet Med Assoc 172:708–711, 1978, with permission.

sented four breeds from three kennels. All bitches whelped at least once during the study, with pregnancy rates, litter size, and pup mortality considered within normal limits. Swab specimens for culture were collected from the cranial vaginas of bitches once every month except during estrus, when specimens were collected weekly. Bacteria were isolated from most swabs (94.8 per cent) with *Pasteurella multocida*, β-hemolytic streptococci, and *E. coli* being the most commonly isolated microorganisms. Although the prevalence of *Pasteurella* in bitches housed closely together in a kennel may be higher than that found in bitches from single- or few-dog households,[15] repeated vaginal cultures from any bitch can vary from sample to sample, regardless of where the animal is housed. Therefore, the culturing of vaginal swabs for aerobic bacteria is of little value when bitches have no signs of genital disease.

Anaerobic bacteria also can be isolated from the vaginas of healthy bitches. This may explain why vaginal cultures occasionally yield no growth, yet bacteria are observed in vaginal smears. In one study,[12] Bacteroidaceae were the microorganisms isolated most frequently (55 percent) from vaginal swabbings of dogs. Other anaerobic bacteria isolated from the canine vagina include Peptococcaceae, *Lactobacillus, Bifidobacterium, Clostridium, Corynebacterium, Haemophilus*, unidentified gram-positive rods, and unidentified gram-negative rods.[12,16]

Mycoplasma and ureaplasma also can inhabit the canine vagina. In one study,[17] the genital mycoplasma and ureaplasma flora were compared among dogs with varied reproductive histories. Mycoplasma (*M. canis, M. maculosum, M. spumans, M. edwardii, M. cynos, M. molare*, and others) were isolated from 67 of 75 (89 per cent) bitches with varying reproductive histories. There was no significant difference in isolation rates between fertile bitches, bitches with histories of infertility, research animals, or animals with clinical vaginitis. Ureaplasma was isolated from 38 of the 75 bitches (51 per cent). Slightly higher isolation rates occurred from samples obtained from bitches with histories of infertility, espe-

Table 4–4. Classification of Vaginal Isolates from 21 Pups, 12 Weeks to 6 Months Old

Type of Isolate	No. of Isolates	Per Cent of Total Isolates	Pups with Isolate (%)
E. coli	8	17.0	38.1
Coagulase$^+$ staphylococci	14	29.8	66.7
Coagulase$^-$ staphylococci	5	10.6	23.8
α-Hemolytic streptococci	4	8.5	19.0
β-Hemolytic streplococci	3	6.4	14.3
Nonhemolytic streptococci	2	4.3	9.5
Proteus	1	2.1	4.8
Bacillus	3	6.4	14.3
Corynebacterium	2	4.3	9.5
Micrococcus	3	6.4	14.3
Neisseria	1	2.1	4.8
Klebsiella	1	2.1	4.8
Total	47 (2.2 isolates/pup)		

From Olson PNS, Mather EC: Canine vaginal and uterine bacterial flora. J Am Vet Med Assoc 172:708–711, 1978, with permission.

Table 4-5. Classification of Vaginal Isolates from 81 Postpuberal Bitches

Type of Isolate	Cranial Vaginal Swabbings			Caudal Vaginal Swabbings		
	Number of Isolates	Per Cent of Total Isolates	Bitches with Isolate (%)	Number of Isolates	Per Cent of Total Isolates	Bitches with Isolate (%)
E. coli	15	19.0	18.5	25	13.2	30.9
Coagulase$^+$ staphylococci	5	6.3	6.2	15	7.9	18.5
Coagulase$^-$ staphylococci	5	6.3	6.2	16	8.4	19.8
α-Hemolytic streplococci	8	10.1	9.9	18	9.5	22.2
β-Hemolytic streptococci	12	15.2	14.8	15	7.9	18.5
Nonhemolytic streplococci	3	3.8	3.7	10	5.3	12.3
Pasteurella	8	10.1	9.9	26	13.7	32.1
Proteus	4	5.1	4.9	5	2.6	6.2
Bacillus	3	3.8	3.7	13	6.8	16.0
Haemophilus	1	1.3	1.2	0	0	0
Corynebacterium	2	2.5	2.5	12	6.3	14.3
Pseudomonas	0	0	0	2	1.1	2.5
Moraxella	1	1.3	1.2	7	3.7	8.6
Acinetabacter	0	0	0	3	1.6	3.7
Flavobacterium	1	1.3	1.2	4	2.1	4.9
Lactabacillus	0	0	0	1	0.5	1.2
Micrococcus	1	1.3	1.2	3	1.6	3.7
Neisseria	2	2.5	2.5	7	3.7	8.6
Enterobacter	1	1.3	1.2	1	0.5	1.2
Klebsiella	0	0	0	0	0	0
Nonclassified spp.	7	8.9	8.6	7	3.7	8.6
Total	79 (0.975 isolate/bitch)			190 (2.35 isolates/bitch)		
No growth	30 (37% of total dogs)			7 (9% of total dogs)		

From Olson PNS, Mather EC: Canine vaginal and uterine bacterial flora. J Am Vet Med Assoc 172:708–711, 1978, with permission.

cially those that had a purulent vaginal discharge; the differences, however, were not significant ($p<0.05$).[17] Zoldag et al.[18] also reported on the isolation rate of mycoplasma from the genital tract of healthy bitches and those with histories of genital disorders. Mycoplasma were isolated from 106 of 145 bitches (73 per cent). On the basis of a clinical examination and history, 80.5 per cent of the bitches with a history of reproductive disease were infected with mycoplasma. However, 51.3 per cent of the clinically healthy bitches also were infected.

Therefore, because the vaginal flora of normal bitches and those with reproductive disease are often similar, prebreeding vaginal cultures are of little value. Additionally, most culture results provide little information that can be utilized to protect a male dog from acquiring transmissible reproductive disease. *Brucella canis* is the only bacterium known to be a specific cause of infertility that can be transmitted via venereal exposures.[19] Routine vaginal swabbings are rarely collected and cultured in a manner that promotes the isolation of *B. canis*. Serologic testing, not vaginal cultures, should be the diagnostic test of choice in determining if a bitch is potentially infected with *B. canis* (see above). Although canine herpesvirus infection can spread both via aerosolization and by venereal transmission, routine prebreeding vaginal cultures do not include viral isolation.

When owners of stud dogs are persistent in requesting vaginal cultures, fearful that a bitch could transmit an infectious disease to their stud dog at the time of mating, they should be reminded that the types of bacteria isolated from many bitches are similar to those isolated from the prepuces of normal male dogs (see Chapter 23).

Features of Reproductive Physiology That Aid Conception in the Normal Bitch

Several unique features of canine reproductive physiology ensure that conception occurs after

Table 4–6. Classification of Vaginal Isolates from 34 Anestrous Bitches

Type of Isolate	Cranial Vaginal Cultures			Caudal Vaginal Cultures		
	Number of Isolates	Per Cent of Total Isolates	Dogs with Isolate (%)	Number of Isolates	Per Cent of Total Isolates	Dogs with Isolate (%)
E. coli	6	26.1	17.6	8	10.5	23.5
Coagulase$^+$ staphylococci	2	8.7	5.9	8	10.5	23.5
Coagulase$^-$ staphylococci	1	4.3	2.9	9	11.8	26.5
α-Hemolytic streptococci	3	13.0	8.8	8	10.5	23.5
β-Hemolytic streptococci	5	21.7	14.7	9	11.8	26.5
Nonhemolytic streptococci	0	0	0	3	3.9	8.8
Pasteurella	1	4.3	2.9	8	10.5	23.5
Proteus	1	4.3	2.9	1	1.3	2.9
Bacillus	1	4.3	2.9	6	7.9	17.6
Haemophilus	1	4.3	2.9	0	0	0
Corynebacterium	1	4.3	2.9	6	7.9	17.6
Pseudomonas	0	0	0	1	1.3	2.9
Moraxella	0	0	0	4	5.3	11.8
Acinetobacter	0	0	0	1	1.3	2.9
Flavobacterium	0	0	0	1	1.3	2.9
Lactobacillus	0	0	0	1	1.3	2.9
Nonclassified spp.	1	4.3	2.9	2	2.6	5.9
Total	23 (0.7 isolate/dog)			76 (2.2 isolates/dog)		
No growth	18 (52.9% of total dogs)			3 (8.8% of total dogs)		

From Olson PNS: Canine vaginal flora. M.S. thesis, University of Minnesota, 1976.

Table 4–7. Classification of Vaginal Isolates from 25 Proestrous-Estrous Bitches

Type of Isolate	Cranial Vaginal Cultures*			Caudal Vaginal Cultures		
	Number of Isolates	Per Cent of Total Isolates	Dogs with Isolate (%)	Number of Isolates	Per Cent of Total Isolates	Dogs with Isolate (%)
E. coli	4	18.2	18.2	10	17.2	40.0
Coagulase$^+$ staphylococci	1	4.5	4.5	6	10.3	24.0
Coagulase$^-$ staphylococci	1	4.5	4.5	3	5.2	12.0
α-Hemolytic streptococci	3	13.6	13.6	6	10.3	24.0
β-Hemolytic streptococci	2	9.1	9.1	3	5.2	12.0
Nonhemolytic streptococci	0	0	0	3	5.2	12.0
Pasteurella	4	18.2	18.2	7	12.1	28.0
Bacillus	0	0	0	4	6.9	16.0
Corynebacterium	0	0	0	2	3.4	8.0
Moraxella	0	0	0	1	1.7	4.0
Acinetobacter	0	0	0	1	1.7	4.0
Flavobacterium	1	4.5	4.5	3	5.2	12.0
Micrococcus	0	0	0	2	3.4	8.0
Neisseria	2	9.1	9.1	4	6.9	16.0
Enterobacter	1	4.5	4.5	1	1.7	4.0
Nonclassified spp.	3	13.6	13.6	2	3.4	8.0
Total	22 (1.0 isolate/dog)			58 (2.3 isolates/dog)		
No growth	6 (27.3% of total dogs)			1 (4.0% of total dogs)		

* Cranial vaginal cultures were obtained on 22 of 25 dogs in this group.
From Olson PNS: Canine vaginal flora. M.S. thesis, University of Minnesota, 1976.

Table 4–8. Classification of Vaginal Isolates from 19 Diestrous or Pregnant Bitches

Type of Isolate	Cranial Vaginal Cultures			Caudal Vaginal Cultures		
	Number of Isolates	Per Cent of Total Isolates	Dogs with Isolate (%)	Number of Isolates	Per Cent of Total Isolates	Dogs with Isolate (%)
E. coli	3	13.6	15.8	4	8.7	21.1
Coagulase$^+$ staphylococci	2	9.1	10.5	2	4.3	10.5
Coagulase$^-$ staphylococci	3	13.6	15.8	4	8.7	21.1
α-Hemolytic streptococci	1	4.5	5.3	3	6.5	15.8
β-Hemolytic streptococci	2	9.1	10.5	2	4.3	10.5
Nonhemolytic streptococci	2	9.1	10.5	3	6.5	15.8
Pasteurella	2	9.1	10.5	7	15.2	36.8
Proteus	2	9.1	10.5	2	4.3	10.5
Bacillus	1	4.5	5.3	4	8.7	21.1
Corynebacterium	1	4.5	5.3	4	8.7	21.1
Pseudomonas	0	0	0	1	2.2	5.3
Moraxella	1	4.5	5.3	2	4.4	10.5
Acinetobacter	0	0	0	1	2.2	5.3
Micrococcus	0	0	0	1	2.2	5.3
Neisseria	0	0	0	3	6.5	15.8
Nonclassified spp.	2	9.1	10.5	3	6.5	15.8
Total	22 (1.2 isolates/dog)			46 (2.4 isolates/dog)		
No growth	6 (31.6% of total dogs)			5 (26.3% of total dogs)		

From Olson PNS: Canine vaginal flora. M.S. thesis, University of Minnesota, 1976.

a normal bitch and male dog mate. For example, the canine oocyte is viable for several days after ovulation because the bitch ovulates a primary oocyte rather than a secondary oocyte like many other species.[20] Although fertilization can only be completed after a primary oocyte matures into a secondary oocyte, some authors have suggested that the fertilization process might be initiated when spermatozoa penetrate primary oocytes. Because the life span of a secondary oocyte is relatively short (24 to 36 hours) compared to that of a primary oocyte (2 to 3 days), the process of oocyte maturation in the bitch could allow for an extended "fertile" period.

In vitro research supports such a suggestion because capacitated canine spermatozoa can penetrate the zona pellucida, enter the vitellus, and undergo nuclear decondensation in primary oocytes, with pronuclei forming after oocyte maturation has been completed and a secondary oocyte is formed.[21] In one study,[22] the kinetics of sperm penetration into the ooplasma did not differ between oocytes collected from preovulatory and anestrous follicles. However, the in vitro development of male and female pronuclei was observed more frequently in oocytes collected from preovulatory follicles. Farstad et al.[23,24] have reported that spermatozoa in vivo may penetrate and initiate decondensation prior to complete oocyte maturation in the vixen. Conversely, Tsutsui[25] concluded that the canine ovum will accept spermatozoa only after it has matured into a secondary oocyte. However, when 21 bitches were bred twice from 36 hours before ovulation to 84 hours after ovulation, each time to a different male, only 2 of the 21 pregnant bitches conceived pups from only the second male. Therefore, some advantage seemed to result from breedings that occurred prior to maturation of the primary oocyte to a secondary oocyte. The longevity of canine spermatozoa in the bitch's reproductive tract following a mating also contributes to the extended fertile period.[26]

Conception rates following a single mating in beagle bitches were greater than 95 per cent when the breeding occurred between 3 and 10 days before the onset of cytologic diestrus[27] (Fig. 4–4). Similarly, a 91 per cent conception rate occurred in mongrel and beagle bitches mated only once from 48 hours before ovulation to 108 hours after ovulation.[25] Thus, the period for maximal conception rates from a natural mating appears to last up to 7 days.

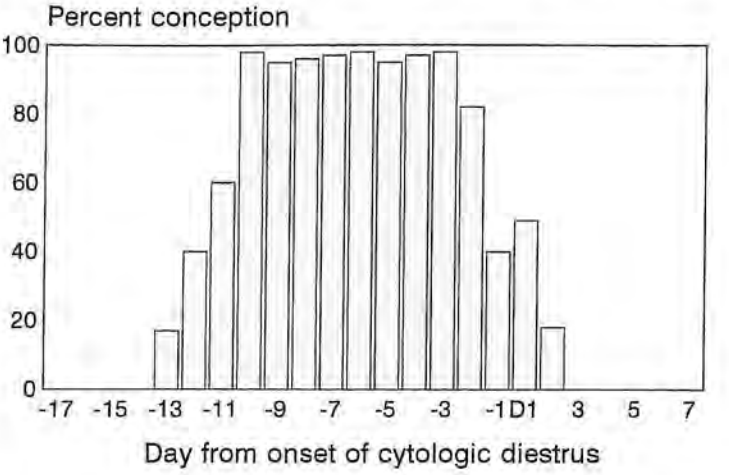


Figure 4–4. Conception rates relative to day of a single breeding (n = 267 beagle bitches). (From Holst PA, Phemister, RD: Onset of diestrus in the beagle bitch: Definition and significance. Am J Vet Res 35:401–406, 1974, with permission.)

Because most bitches are receptive to mating over several days during estrus, conception is further ensured to occur following a natural mating. The fertile period is decreased when chilled extended or frozen semen is used rather than a natural mating,[28] arguing for the importance of sperm viability.

Timing Ovulation and Mating in the Bitch

The period in which a natural mating yields maximal conception rates spans up to 7 days for the dog. Therefore, in many dogs, one or more breedings during behavioral estrus will result in pregnancy. However, when considering various methods of breeding or insemination for the best reproductive performance overall (conception rate and litter size), optimal fertility can be anticipated if a bitch is bred 2 to 4 days after ovulation (Fig. 4–5). This would include bitches that can be mated only once because of sire availability or those bitches that must be bred near the time of oocyte maturation because chilled or frozen semen is being used. The day of ovulation in any given bitch may be quite different from that of the average bitch.[29] Although ovulation occurs approximately 12 days after the onset of proestrus or 2 to 3 days after the onset of estrus in the average bitch, ovulation was predicted to occur from 7 to 22 days after the onset of proestrus in 50 bitches who were presented for breeding management assistance, and who subsequently became pregnant on the basis of the management advice (Fig. 4–6).[30] Therefore, predicting the time of ovulation in a bitch can greatly aid in managing a breeding (Table 4–9).

Indications for determining the day of ovulation for managed breedings in the bitch include the following[28–31]:

1. The use of artificial insemination (AI) with fresh, extended, or frozen semen, when cost of each insemination is high and/or timing is critical
2. The managed breeding of bitches with historical failure to show standing behavior, historical conception failure, historical small litter size, or need to inseminate with poor quality semen
3. The use of natural breedings that are difficult or expensive, so that a high chance of conception occurs even with only a single breeding or few breedings
4. Need for predicting whelping dates
5. Shipping bitches after the luteinizing hormone (LH) surge to avoid a decrease in LH that might result from the stress of shipment

Various methods to predict ovulation in the bitch have been described.

Progesterone Assays

Measuring the concentrations of serum progesterone in samples obtained during late proestrus and estrus allow one to predict the optimal breeding times for a bitch. The bitch is unique among domestic species because concentrations of serum progesterone begin to increase 2 to 3 days before ovulation. Serum progesterone is less than 1.0 ng/ml during anestrus and most of proestrus (Fig. 4–5). Values rapidly increase to greater than 1 ng/ml just

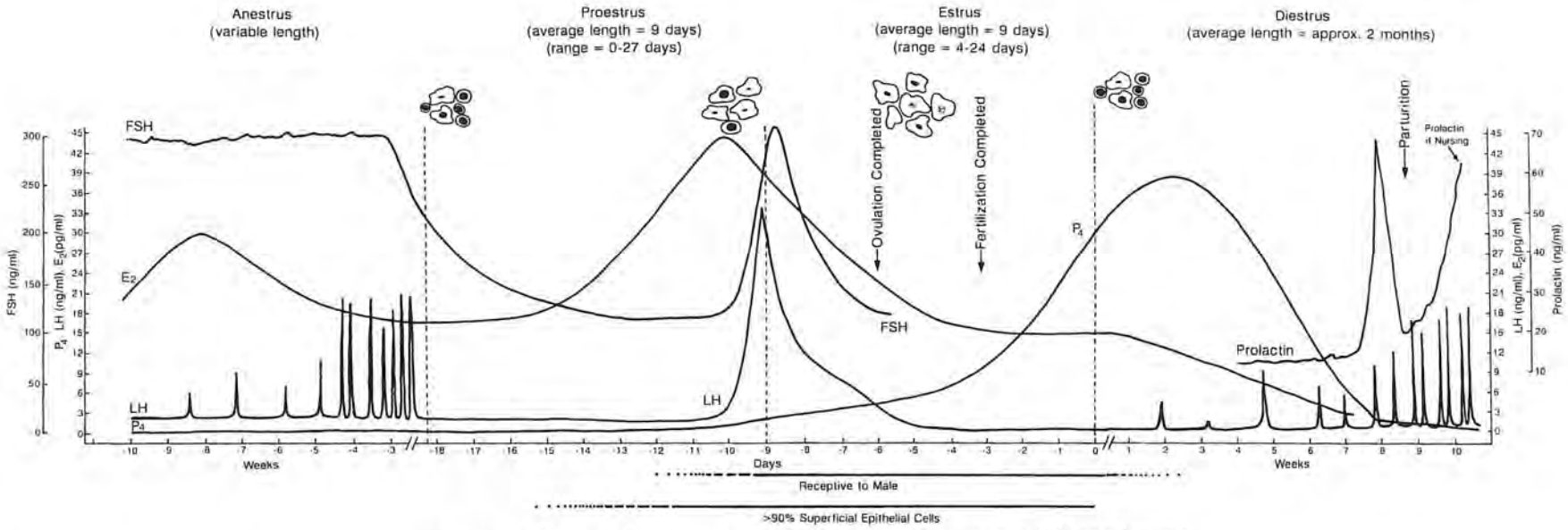


Figure 4–5. Timing of physiologic events with progesterone measurements and vaginal smears.

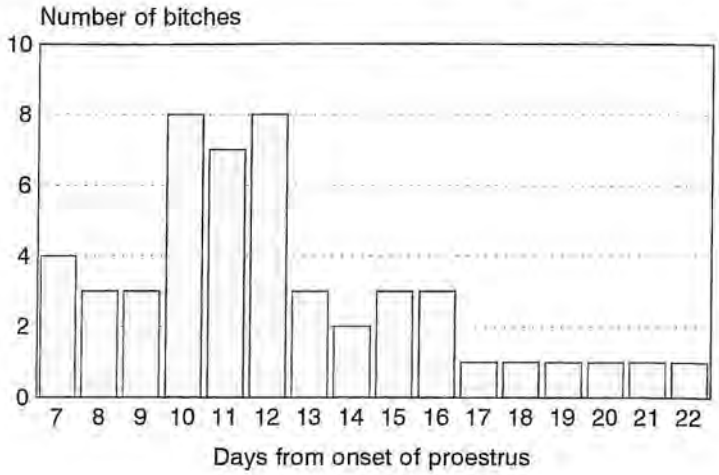


Figure 4–6. Predicted time of ovulation for 50 bitches, based on serum progesterone concentrations. More than 20 breeds were represented. [S. Johnson and M.V. Root-Kustritz, University of Minnesota, 1995. (Personal communication.)]

before and during the preovulatory LH surge and continue to increase throughout estrus to reach initial peaks of 15 to 90 ng/ml by 15 to 30 days after the LH peak.[32,33] At the time of ovulation, serum levels of progesterone range from 4 to 10 ng/ml.[34] By measuring the concentration of progesterone during proestrus or estrus, one can predict the best time to mate a bitch (Table 4–9). Conception rates may exceed 95 per cent following a single natural mating from approximately 3 days before to 4 days after ovulation.[27] However, to further assure maximal chances for conception if only one breeding or insemination can occur, the best time for breeding is 2 days following ovulation or 2 days after progesterone reaches a concentration of 4 to 10 ng/ml.[30]

Although the range of progesterone concentrations at the time of ovulation (2 to 3 days after the LH surge) is usually from 4 to 10 ng/ml (Fig. 4–7), concentrations may vary among bitches. Diurnal variation of serum progesterone has been reported to occur in pregnant bitches, with concentrations being approximately twice as high in the morning as in the afternoon.[35] If similar diurnal variations occur in nonpregnant animals during estrus, variability in progesterone levels may also relate to sampling times.

Serum progesterone concentrations can be measured by commercial radioimmunoassay (RIA), chemiluminescence, or by the use of in-house enzyme-linked immunosorbent assay (ELISA) kits.† Radioimmunoassay is generally the most accurate and reproducible of the two techniques. However, RIA is generally the more expensive of the two methods and has historically been associated with a longer turnaround time. Fortunately, increasing numbers of RIA laboratories are now providing same-day progesterone results. Radioimmunoassay provides the veterinarian with quantitative results, whereas the ELISA is qualitative or semi-quantitative, with accuracy dependent upon the level of progesterone in the sample.[34,36] The decision on which progesterone-measuring technique to use should be influenced by the case being managed. For example, a qualitative estimate of changing concentrations of

† ELISA progesterone kits are available from Synbiotics Corporation, San Diego, CA.

Table 4–9. Guidelines for Interpretation of Serum Progesterone Concentration in the Estrus Bitch Measured by RIA

Serum Progesterone Concentration (ng/ml)	Estimated Day of Ovulation	Best Breeding Days*	Estimated Parturition from Ovulation Day†
1.0–1.9	+ 2 days	+ 4 days (3–6)	+63 (62–64) days
2.0–3.9	+ 1 day	+ 3 days (2–5)	+63 (62–64) days
4.0–10.0	0 day	+ 2 days (1–4)	+63 (62–64) days

* Single breeding recommended; use a day in parentheses if best day is inconvenient.
† Estimate 80% patients to whelp in this interval.
From Johnston SD, Root MV: Serum progesterone timing of ovulation in the bitch. *In* Proceedings of the Annual Meeting of the Society for Theriogenology, San Antonio, TX, September 13–15. 1995. Nashville, Society for Theriogenology, 1995, pp 195–203, with permission.

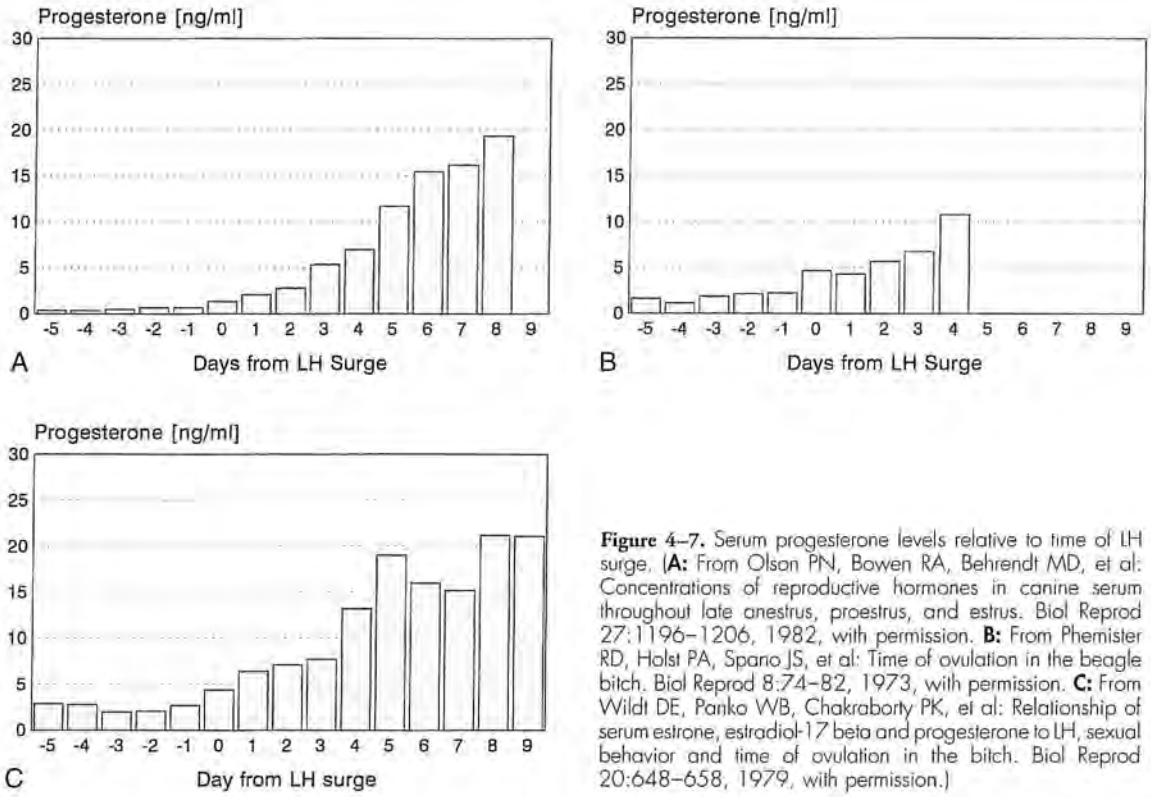


Figure 4–7. Serum progesterone levels relative to time of LH surge. (**A:** From Olson PN, Bowen RA, Behrendt MD, et al: Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus, and estrus. Biol Reprod 27:1196–1206, 1982, with permission. **B:** From Phemister RD, Holst PA, Spano JS, et al: Time of ovulation in the beagle bitch. Biol Reprod 8:74–82, 1973, with permission. **C:** From Wildt DE, Panko WB, Chakraborty PK, et al: Relationship of serum estrone, estradiol-17 beta and progesterone to LH, sexual behavior and time of ovulation in the bitch. Biol Reprod 20:648–658, 1979, with permission.)

progesterone may be adequate for predicting when a bitch should first be introduced to an available male. However, if frozen semen is to be surgically deposited in the uterus of a bitch (see Chapter 16), the exact concentration of progesterone should be determined to more accurately predict the best time for insemination.

Luteinizing Hormone Assays

Determining the time of the LH surge is helpful in predicting the time of ovulation, oocyte maturation, and fertilization. Ovulation usually occurs 2 to 3 days after the LH surge and oocyte maturation and fertilization occur 4 to 6 days after the LH surge (see Table 2–4). Unlike serum progesterone, which increases steadily in the bitch during estrus, the LH surge may be increased for only 24 hours. A single serum sample that is obtained on any day during estrus and assayed for LH may not provide information on whether or not the LH surge has occurred. However, the LH surge is likely to already have occurred if concentrations of progesterone are measured in the sample and are found to be greater than 2.0 ng/ml. Historically, LH assays took several days to perform, and were of little value in prospectively determining the optimal time for mating. However, rapid radioimmunoassays[37] and in-clinic ELISA tests‡ have now been developed for measuring LH in serum to help determine optimal mating time (Table 4–10). Samples may need to be collected daily, beginning in proestrus when concentrations of progesterone are low, so that the LH surge is not missed.

Estrogen Assays

Frequently veterinarians will inquire about the usefulness of assaying serum samples for estrogens to determine the appropriate time of breeding a bitch, or to determine if a bitch's nonreceptive behavior resulted from abnormally low estrogen levels in the blood. Unfortunately, concentrations of various estrogens (estradiol-17β, estrone, and estriol) are 1000-fold less in blood than progesterone and more difficult and expensive to assay. Additionally, concentrations of estrogens, just as LH, may be increased in the blood of some bitches for a relatively short period of time. Therefore, a single sample assayed for concentrations of estrogens provides little information on timing

‡ Status-LH, an in-clinic test for detection of canine LH, is available from Synbiotics Corporation, San Diego, CA.

Table 4–10. Recommendations for Breeding and Anticipated Time of Whelping

	Time for ≥ 1 Natural Breedings between Dogs with Maximal Fertility	Optimal Time for a Single Breeding or Insemination, or When Breeding Dogs with Reduced Fertility	Anticipated Time of Whelping
LH Surge	1 d before the LH surge to 6 d after the LH surge	4–6 d after the LH surge	64–66 d after the LH surge
Ovulation	3 d before ovulation to 4 d after ovulation	2–4 d after ovulation	62–64 d after ovulation
Oocyte Maturation	6 d before oocyte maturation to 1 d after oocyte maturation	At the time a secondary oocyte is formed to 2 d after	60–62 d after oocyte maturation
Onset of Cytologic Diestrus	3–10 d before the onset of cytologic diestrus	2–5 d before the onset of cytologic diestrus	56–58 d after the onset of cytologic diestrus

of ovulation or hormonal causes for nonreceptive behavior. Because estradiol-17β is normally elevated in late proestrus and not estrus, it is normal to find basal (i.e., low) concentrations of estradiol-17β during the period when normal bitches are receptive to mating. Changes in vaginal cytology during estrus provide a reliable and inexpensive indicator to predict if estrogen levels were elevated (see Chapter 3).

Vaginal Cytology

Increasing circulating levels of estradiol-17β during proestrus stimulate the growth of the vaginal epithelium from a few cell layers in thickness during anestrus to 20 to 30 cell layers in thickness at the end of proestrus. As the vaginal epithelium thickens, increased numbers of superficial cells slough into the vaginal fluids. The change in the percentage of superficial cells in a vaginal smear can be used to monitor the progression of proestrus and estrus and is a valuable tool to predict the fertile period of the bitch (see Chapter 3). Unfortunately, the time and intensity of maximum cornification (i.e., maximum percentage of superficial cells) vary among bitches and preclude the use of maximum cornification to precisely predict the exact time of receptivity, the LH surge, or ovulation. Nevertheless, when used along with hormone assays, vaginal smears provide crucial information on whether estrus is progressing normally and if the bitch is still in her fertile period (Fig. 4–5).

Because concentrations of progesterone vary among bitches, using only progesterone data may occasionally lead to erroneous conclusions when predicting optimum breeding times. For example, a serum concentration of 10 ng/ml could mean a bitch is near the time of ovulation, oocyte maturation, and optimal fertilization, or is in early diestrus. However, if, in addition to the progesterone results, the preponderance of epithelial cells in a vaginal smear are noncornified cells (i.e., nonsuperficial cells), the bitch is beyond the time of ovulation, oocyte maturation, and optimal fertilization, and has already entered cytologic diestrus.

Bitches bred for the first time in diestrus have a significantly reduced conception rate[27] (Fig. 4–4). However, identifying the onset of diestrus with vaginal smears can still be a very useful tool for veterinarians and owners. The onset of cytologic diestrus is temporally associated with the LH surge, ovulation, oocyte maturation, and whelping. Retrospectively, identifying the onset of cytologic diestrus allows a veterinarian to determine if breedings did, in fact, occur at the appropriate times. This can be done by evaluating daily vaginal smears, inexpensively collected by owners. Owners can be instructed on the proper technique and advised to obtain smears throughout the bitch's receptive period and for approximately a week after the last breeding. In addition to predicting if conception likely occurred from the breedings, one can predict if litter size will be maximal. Live litter size and pups per corpus luteum may decrease if bitches are bred too early or too late relative to the onset of cytologic diestrus (Figs. 4–8 and 4–9). Prospectively, identifying the onset of cytologic diestrus allows the veterinarian to predict the time of whelping with greater accuracy than by using breeding dates alone (Fig. 4–10).

Ferning Patterns of Vaginal Fluid

The phenomenon described as "ferning" of cervical mucus is utilized by physicians to determine the fertile period in women. Fluid col-

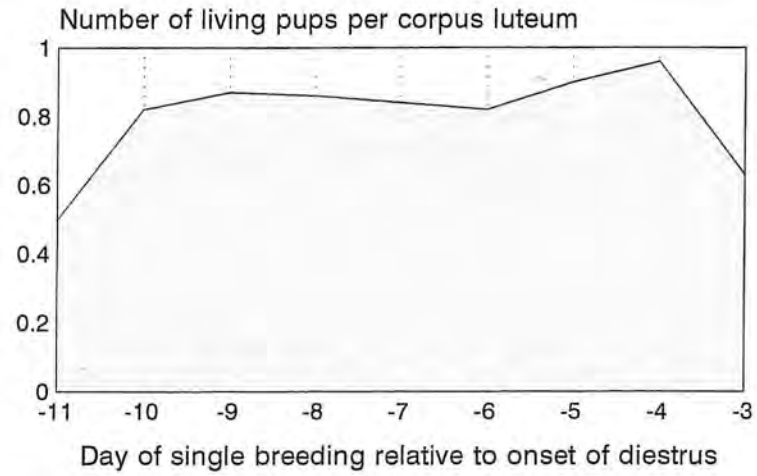
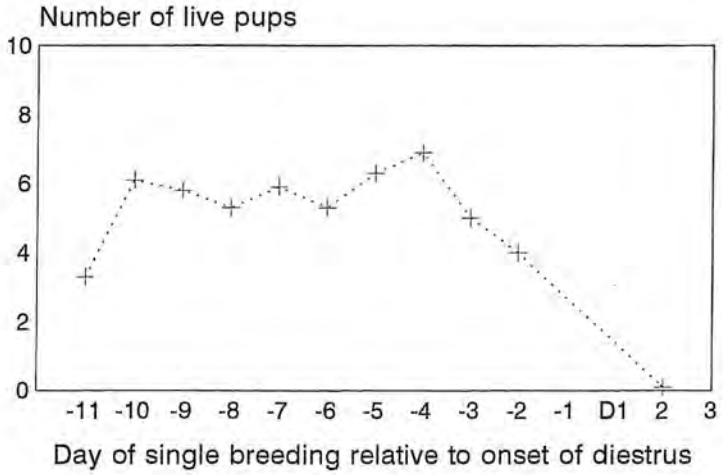


Figure 4–8. Pups per corpus luteum (n = 72 bitches bred only once). (From Holst PA, Phemister RD: Onset of diestrus in the beagle bitch: Definition and significance. Am J Vet Res 35:401–406, 1974, with permission.)

Figure 4–9. Live litter size relative to day of breeding (n = 74 bitches bred only once). (From Holst PA, Phemister RD: Onset of diestrus in the beagle bitch: Definition and significance. Am J Vet Res 35:401–406, 1974, with permission.)



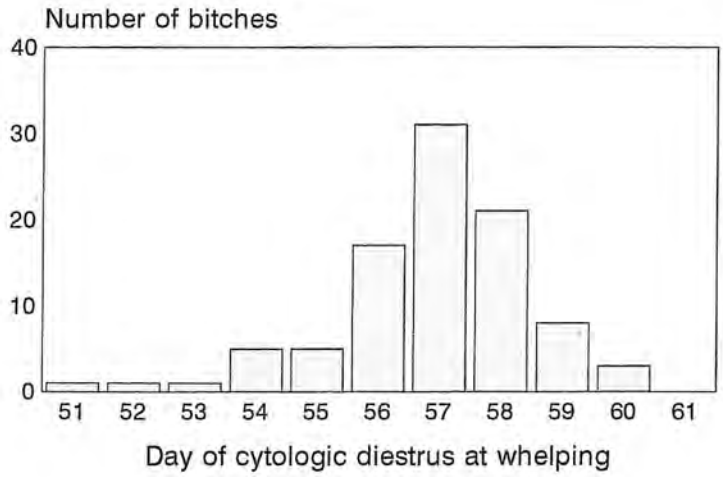


Figure 4–10. Day of whelping in relation to onset of cytologic diestrus (n = 93 bitches). (From Holst PA, Phemister RD: Onset of diestrus in the beagle bitch: Definition and significance. Am J Vet Res 35:401–406, 1974, with permission.)

lected from the cranial vagina of an estrous bitch also will appear to have the pattern of a fern if placed on a microscope slide and allowed to air-dry.[38] Vaginal fluid is collected by passing a sterile insemination pipette into the cranial vagina. A 5-ml syringe attached to the pipette provides the suction necessary to collected enough fluid to place on a tilted microscope slide. The fluid is allowed to spread on the slide and air-dry at room temperature. Enough fluid generally is left from the first slide to prepare a second smear for vaginal cytology. The first slide can be examined at 400× magnification when completely dry for ferning patterns. In one study,[39] sporadic ferning began in proestrus for 9 of 50 bitches tested. In 42 of the 50 bitches, the ferning index reached a peak that lasted for several days before declining. The mean interval between the calculated LH surge and the peak ferning (or midpoint of the ferning plateau) was 2.3 ± 2.1 days, with a range of −2 to +8 days.

Changes in Gross Appearance of the Vaginal Mucosa

As concentrations of estradiol and progesterone change in blood during proestrus and estrus in the bitch, various target tissues for these hormones are altered. Endoscopic observations of the changes in vaginal mucosa have been reported in detail by Lindsay[7] (see Chapter 1). These changes can be observed using a vaginoscope, fiberoptic endoscope, or pediatric sigmoidoscope.[40] During proestrus, two stages of changes may be observed. During the early stage, folds of vaginal mucosa become edematous, followed by a later "shrinkage" stage when folds become less edematous.

During estrus, the "shrinking" of the vaginal mucosa, which began in proestrus, intensifies. By the end of estrus, folds are maximally shrunken and angulated (crenulated). The onset of diestrus is signaled when a "rounding out" or smoothing out of the vaginal mucosa commences and the mucosa develops a variegate coloration of red and white areas, at about the same time that the percentage of nonsuperficial epithelial cells abruptly increases in the vaginal smear. The vaginal mucosa in a spayed or anestrous bitch is thin and susceptible to trauma. Occasionally the mere passage of vaginoscopic equipment during anestrus will result in slight submucosal hemorrhage.

Changes in Vaginal Resistance/Conductance

Changes in the electrical resistance of the vaginal fluids have been used successfully to determine the optimal time for inseminating the blue and silver fox. More than 80 per cent of all foxes inseminated in Norway are tested with a heat detector that measures changes in electrical resistance in vaginal fluids.[41] Changes in vaginal resistance also have been evaluated in dogs (P. W. Concannon, unpublished data, New York State College of Veterinary Medicine) and proved accurate when the probe was consistently placed in the same position in the vagina. The wide range of vaginal lengths in various breeds of dogs may render the procedure less useful in the bitch than the vixen.

Ultrasonographic Evaluation

Follicular development, ovulation, and development of corpora lutea can be evaluated by using ultrasonography.[42–44] In one study,[44] changes in vaginal cytology and plasma concentrations of estradiol, LH, and progesterone were compared to the ultrasonographic findings from scanning the ovaries of 13 bitches during proestrus and estrus. A rapid disappearance of the follicular antrum, corresponding to ovulation, was detected in only 2 of the 13 bitches. Although follicular rupture was not detected in the remaining 11 bitches, there was a gradual thickening of the antral wall that began at the time of the LH surge. The progressive thickening noted throughout estrus likely resulted from the pre- and postovulatory development of luteal tissue. The phenomenon of preovulatory luteinization in the bitch was first described by Bischoff[45] in 1845 and later confirmed by other investigators.[46,47] The gradual development of luteal tissue prior to ovulation gives the antral wall rigidity, possibly contributing to the lack of abrupt ultrasonographic changes observed at the time of follicular rupture and ovulation. The antrum may not be completed obliterated by luteal tissue until the 15th through the 20th days of diestrus in the bitch.[47]

How To Breed the Bitch

Using Natural Mating

Goals of managed breeding of the bitch are to achieve the best conception rate and litter size with the least labor and cost. Three general

strategies may be used to accomplish these goals: (1) achievement of alternate-day breedings over the period of receptivity (estrus) of the bitch, (2) determination of approximate day of ovulation and achievement of two breedings 2 to 4 days after possible ovulation, and (3) accurate determination of day of ovulation and achievement of one breeding 2 days after ovulation. Strategy 3 should be used with AI.

Strategy 1 is indicated when the male and female are owned by the same person, or are housed at the same site, and when animals are young, sound, and have no history of infertility. In general, for strategy 1, the male and female should be housed in different rooms when the bitch is in heat, and brought together on leads for short periods daily to observe for the presence of estrous behavior and to accomplish mating. If the approximate ovulation day of the bitch is unknown, she should be brought to the male (for "teasing") starting about 5 days after onset of proestrual serosanguineous vulvar discharge, and every day or every other day thereafter until estrous behavior is observed. The average bitch first exhibits estrous behavior 10 days after proestrus onset, but normal bitches may first exhibit this behavior as early as proestrus onset or as late as 27 days after proestrus onset. If the approximate ovulation day of the bitch is known from previous reproductive history, teasing may begin at or just before the expected day of ovulation; however, because some but not all bitches ovulate at the same time of every estrous cycle, careful observation is necessary at every managed breeding. Estrous behavior includes standing still, with the tail deflected up or to the side of the perineum ("flagging") when the male sniffs the vulva and/or attempts to mount (see Chapter 2). If the bitch exhibits estrous behavior in the presence of the male, both animals should be permitted to play off-lead and to mate naturally. Such behavior is permitted every other day until the bitch refuses copulation.

With natural copulation, the bulbus glandis of the male engorges inside the vagina of the bitch, resulting in an "inside" tie, which refers to the position of the bulbus glandis inside the female. Following ejaculation of the presperm and sperm-rich fractions of semen, the male dog will dismount from the bitch with the erect penis still inside the vagina, and turn and stand end to end with her in the copulatory lock (Fig. 4–11).[48] If the bulbus glandis engorges outside of the vulva and ejaculation occurs, the bitch is said to be bred with an "outside" tie. With the outside tie, semen is deposited in the middle of the vagina; conception rate is improved with outside ties if the bitch's hindquarters are elevated for 5 minutes following insemination, as is done with vaginal AI as described below.

If the bitch exhibits estrous behavior but refuses mating, or if the male appears unable to achieve mating with a receptive bitch, the animals should be presented to the veterinarian for examination. Primary causes of mating failure in the estrous bitch include estrous behavior and mating attempts prior to ovulation, congenital vaginal anomaly that prevents normal intromission, female dominance over the male, arthritis of the hips or lumbar spine of the male causing pain on mounting, and prostatic pain in the male that causes him to dismount and pull away from the bitch at onset of erection. At examination of the bitch, vaginal cytology should be evaluated for magnitude of vaginal cornification, serum progesterone should be measured to determine day of ovulation, and a digital vaginal examination should be performed to determine whether vaginal size permits normal mating. The penis of the male should be protruded for examination, and rectal palpation of the prostate and physical examination of the hip joints and lumbar spine performed. If the animals appear normal and day of ovulation has not occurred, natural breeding may be attempted later, depending on serum progesterone concentration (Table 4–9). Alternatively, AI with fresh semen may be indicated depending on timing of ovulation and abnormalities found.

Strategy 2, determination of approximate day of ovulation, is used when semiquantitative methods of determination of ovulation are used. These may include use of commercial ELISA kits for determination of serum progesterone in the veterinary practice, or endoscopic observation of gross changes in the vaginal mucosa. Commercial ELISA kits[§] for serum progesterone measurement generally show a color change in test wells around the time of change in progesterone concentration. Color endpoints may indicate serum progesterone concentration in low (< 1.0 to 2.0 ng/ml), medium (around 2.0 ng/ml), or high (> 5 or 7 ng/ml) concentration, or may indicate progesterone concentration less than or greater than a standard such as 2 ng/ml. Accuracy of the

§ ELISA kits for serum progesterone measurement include the Target Canine Ovulation Timing Test kit and the Icagen Status-PRO Canine Ovulation Timing Test kit from Synbiotics Corporation, San Diego, CA.

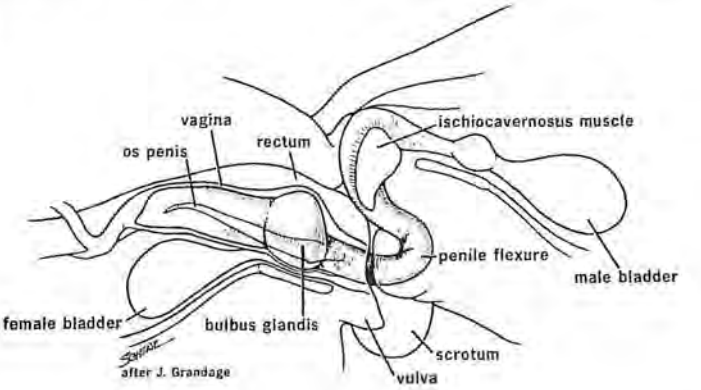


Figure 4–11. Anatomy of the male and female reproductive tracts during copulation in the dog. (Modified from Grandage.[48] Reprinted from Johnston SD: Examination of the genital system. Vet Clin North Am 11:543–559, 1981, with permission.)

color endpoint as an indicator of serum progesterone concentration varies with the kit and with the true serum progesterone concentration, and may range from 62 to 96 per cent.[34] The semiquantitative nature of the ELISA results, coupled with inaccuracy of the results, makes this mode of ovulation timing an approximate one. Different kit manufacturers recommend different protocols for sampling, but, in general, suggest that blood be drawn starting around day 5 after proestrus onset or at onset of complete vaginal cornification. Samples are then evaluated every other day until the color change is observed, and two breedings, 4 and 6 days after the suspected LH surge, are recommended. Although cost to the veterinarian of the ELISA reagents is generally about half of the cost of RIA, client cost of the ELISA progesterone analyses is similar because of in-house labor costs.

Strategy 3, accurate determination of day of ovulation, means measuring serum progesterone by RIA, with sampling interval adequate to detect a concentration between 1.0 and 10.0 ng/ml in the presence of a cornified vaginal smear. Guidelines in Table 4–9 then may be used to choose a single day for breeding, which should be within 1 to 3 days following ovulation. Strategy 3 is indicated when cost or labor of multiple matings exceeds cost of two to three serum progesterone measurements, when there is a history of infertility, when the male or female are aged and a future attempt at mating may not be possible, or when the stud dog is in high demand and minimum numbers of inseminations are desired. In the bitch with unknown ovulation time, vaginal smears should be examined twice weekly starting 5 days after onset of proestrual bleeding. Following predominant vaginal cornification, blood is drawn twice weekly and submitted to a commercial laboratory for progesterone measurement by RIA until a serum concentration exceeding 1.0 ng/ml is detected. Table 4–9 then may be used to choose a single date for mating or insemination. Determination of multiple serum progesterone concentrations after first rise to greater than 1 ng/ml may be indicated in those bitches with abnormal ovarian function; the majority of bitches bred naturally or by AI, however, have normal ovarian function but unknown time of ovulation.

Future availability of commercial canine serum LH assays or ovarian ultrasonography may offer other strategies for accurately planning a single managed breeding. One study evaluating use of an in-house ELISA for LH (Status-LH) demonstrated good correlation between measurement of serum LH with the ELISA kit and measurement by RIA.[49] Because the elevation in LH occurs as a single peak about 24 hours in length in normal dogs prior to ovulation, collection of daily blood samples, at about the same time of day, is required to ensure recognition of the LH peak and to predict ovulation day. Anecdotal reports of lack of definition of the LH peak despite daily blood sampling, with subsequent proof of ovulation by elevation in serum progesterone concentrations, suggest that some bitches may have an LH peak of less than 24 hours' duration.

Conception rate in normal bitches bred a single time between 4 days before and 3 days after ovulation (determined as 3 to 10 days prior to onset of the diestrus vaginal smear) exceeds 95 per cent (Fig. 4–4).[27] Conception rates from 20 to 80 percent occur in normal bitches bred a single time between 7 days before and 7 days after ovulation (Fig. 4–4).[27]

Using Artificial Insemination with Fresh Semen

The Italian physiologist and priest Abbe Lazzaro Spallanzani (1729–1799) is reported to be

the first scientist to report AI in the dog.[30] He siphoned semen from the vagina of a naturally bred bitch following mating and infused it into the vagina of another estrus bitch. The second bitch conceived and delivered normally.

Artificial insemination with fresh semen is indicated in animals that will not or cannot copulate normally. Some brachiocephalic breeds with deep chests and narrow pelves, such as the English bulldog, are routinely bred by AI; AI also is performed in some canine research colonies, because it can be done more quickly than natural mating. If copulation failure is due to congenital vaginal anomaly (see Chapter 12), the owner of the bitch should be advised of the probable need for cesarean section should pregnancy be accomplished with AI. In general, bitches with vaginal anomalies that become pregnant following AI require cesarean section. However, the authors have observed occasional bitches with vaginal strictures precluding normal copulation that whelped normally following AI, and required AI for insemination at seasons following the normal whelping. Secretion of the hormone relaxin at the time of parturition may permit relaxation of fibrous connective tissue vaginal strictures as it does fibrous connective tissue of the pubic symphysis.

Success in achieving pregnancy with fresh semen AI in the dog relies on three factors: normal semen quality, accurate timing of insemination, and appropriate site of deposition of the semen. If possible, prebreeding examination of the male should be accomplished prior to proestrus onset of the female, so as to confirm the availability of normal semen at the time of insemination. The procedure for collecting and evaluating canine semen prior to or at the time of AI is described in Chapter 16. Because of the difficulty in synchronizing estrus in the bitch, most fresh semen AI is done with the entire ejaculate, which may contain 250 to 2500 $\times$ 10^6 sperm. The suggested minimum insemination dose for achieving conception in the dog with normal litter size is more than 150 $\times$ 10^6 motile sperm, although pregnancy has been achieved with fewer numbers. At the time of semen collection for insemination, adequate prostatic fluid should be collected to bring the total volume to more than 2 to 4 ml, which is a manageable volume for AI. If semen quality has not been evaluated prior to day of insemination, it should be evaluated quickly prior to insemination on that day. *Veterinarians are cautioned not to inseminate semen until it has been examined microscopically for presence of motile spermatozoa.* Insemination of poor-quality semen prevents the option of using a different male at that season. Insemination of an azoospermic sample wastes the client's money. Insemination of a sample with dead spermatozoa secondary to bacterial infection may endanger the future reproductive health of the bitch.

Accurate timing of insemination should be performed using serum progesterone measurement as described under strategy 3 above.

The appropriate site of deposition of fresh semen in canine AI, as depicted in Figure 4–12, is at the external cervical os. Sterile insemination pipets‖ or canine urethral catheters may be attached to syringes and used to deposit semen at the external cervical os. Necessary length of the insemination pipet may be determined by abdominal palpation of the estrous cervix, which usually can be identified as a walnut-sized muscular mass in the caudal abdomen, and estimating its distance from the vulva. Alternatively, the inseminator may estimate the approximate distance from the costal arch to the vulva of the bitch and divide that distance by 2 to determine approximate pipet length. A 6- or 12-ml syringe (depending on semen volume collected) should be attached to the insemination pipet, and 1 to 2 ml air aspirated before aspirating the entire semen sample.

The insemination pipet is inserted at the dorsal commissure of the vulva and directed craniodorsally until it is over the ischial arch, after which time it may be directed in a more cranial direction. Sometimes putting a gloved finger into the vulva facilitates inserting the pipet (along the side of the finger) to the external cervical os; lubricant should not be used on the insemination pipet or the gloved finger, however, because sterile aqueous lubricant is spermicidal. Semen is deposited, the 1 to 2 ml air in the syringe is flushed through the pipet, the pipet is withdrawn, and the finger in the vulva is used to tickle the ceiling of the vagina to promote contractions of the female tract. Thereafter, the hindquarters of the bitch are elevated for 5 minutes to facilitate pooling of the semen at the external cervical os.

Conception rate in bitches bred by AI with fresh semen varies with accuracy of timing the ovulation day, quality of semen inseminated, site of insemination, and normality of the fe-

‖ Large animal infusion pipette, 0.205-inch outer diameter $\times$ 22 inches long, flexible, rounded insertion end; product no. 3577, Harford Veterinary Supply Co., Darlington, MD.

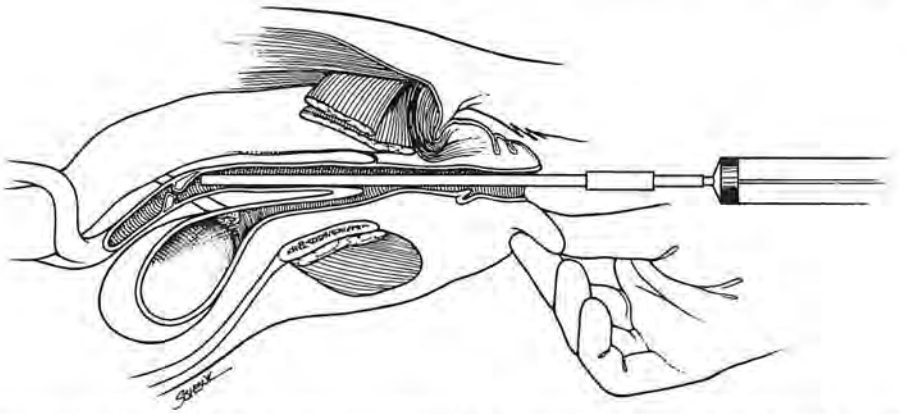


Figure 4–12. Position for placement of the insemination catheter for canine artificial insemination with fresh or chilled extended semen. Semen should be deposited at the external cervical os, and the bitch's hindquarters thereafter elevated for 5 minutes to cause pooling of semen around the external cervical os.

male reproductive tract. Conception rates in small numbers of research bitches inseminated using various experimental protocols were reported to range from 70 to 84 per cent, with normal litter size.[51-56] Conception rate in 405 bitches inseminated with fresh semen by one investigator in Sweden between 1978 and 1987 was 65.7 per cent overall, and 83.8 per cent when corrected for stage of estrus at the time of AI (determined using measurement of serum progesterone) and for semen quality.[57] Pregnancies were obtained with fresh semen of inferior quality, but litter size was reported smaller.[57] Conception rate in 527 bitches inseminated one to four times with fresh semen by 40 Swedish veterinarians in 1990 and 1991 was 54.7 per cent overall, and 62.3 per cent when corrected for stage of estrus at time of AI and semen quality.[58] Corrected pregnancy rate in 256 bitches bred a single time by AI was 55.5 per cent, and pregnancy rate increased significantly with increased number of AIs.[58]

Using Artificial Insemination with Chilled Extended Semen

Chilled extended semen is semen to which an extender is added in order to prolong life span and to facilitate transport to a recipient bitch at a distant site. The long life span of dog sperm when compared to other species, the frequent client need to inseminate bitches that are distant from the dog, and the availability of rapid global courier delivery services make the technique of chilled extended semen AI particularly attractive in this species.

In 1954, Harrop reported extending canine semen with heat-treated pasteurized milk, holding the sample at 4°C for 5 days, and then inseminating it into a greyhound bitch, who whelped two puppies 64 days later.[59] Harrop subsequently reported air transport of chilled extended canine semen from England to New York and from England to New Zealand; at both destinations it was inseminated into estrous bitches and resulted in successful pregnancies.[60,61] In 1958, Bendorf and Chung reported one canine pregnancy (in eight attempts) following shipment of extended semen from California to Hawaii.[62]

Success in achieving pregnancy following AI with chilled extended semen into normal bitches relies on the three factors important with fresh semen AI (normal semen quality, accurate timing of insemination, and appropriate site of deposition of the semen), as well as on method(s) of semen handling and time from collection until insemination. Chilled extended semen is inseminated without warming into the cranial vagina of the bitch using the same AI technique and ovulation timing regimen as for fresh semen.

A description of types of extenders for chilled semen is included in Chapter 16; one of these is 4 parts cream (12 per cent fat) to 1 part egg yolk.[63] One milligram of dihydrostreptomycin and 1000 IU of benzyl penicillin usually are added per milliliter of extender. Suggested dilution of 4°C semen to 4°C extender is 1:3 to 1:5.[63] Alternatively, commercial extenders for canine semen are available for purchase. As with AI with fresh semen, the entire ejaculate usually is used; if not, insemination of more than 150×10^6 sperm is recommended. Extended semen is shipped in thermos bottles or styrofoam boxes containing cold

packs or ice by express mail or courier service. Motility of extended normal dog sperm was 80 per cent after 96 hours at 4°C when extended, and 35 per cent after 120 hours at 4°C when extended, whereas motility of undiluted normal dog semen was 18.3 per cent after 72 hours at 4°C (Table 4–11).[64] These data suggest that extended semen should be inseminated as soon as possible after collection, and ideally in less than 4 days.

Conception rate in bitches bred by AI with chilled extended semen, as with fresh semen, varies with accuracy of timing the ovulation day, quality of semen inseminated, and normality of the female reproductive tract; in addition, poor semen handling technique, such as failure to maintain cleanliness, improper extender preparation, temperature fluctuation, or prolonged shipping time of the semen, probably will cause decline in conception rate. Conception rates were 33 per cent ($n = 12$) in bitches bred with chilled extended semen on the fourth and sixth days after the LH surge, and 89 per cent ($n = 9$) in bitches bred with chilled extended semen on the fifth and seventh days after the LH surge in one study.[49] Conception rate in 15 bitches inseminated on days 2 and 4 of estrus with semen diluted 1:4 with a skim milk diluent stored 3 days at 4° to 8°C was 53 per cent (8 of 15).[65] Pregnancy rate in 109 bitches bred by AI by Swedish veterinarians with chilled extended semen was 47.8 per cent, and 55.3 per cent when corrected for poor ovulation timing or semen quality.[58] Information was not provided on possible reproductive pathology of inseminated bitches.

Table 4–11. Motility of Spermatozoa in Dog Semen Kept at 4°C after Being Frozen and Thawed, Extended, or Left Undiluted*

Time (h)	Motility (%)†		
	Frozen-thawed	Extended	Undiluted
0	65 (7.6)	100 (0)	100 (0)
24	45 (8.7)	98 (1.7)	91.7 (1.7)
48	16.7 (7.3)	90 (2.9)	36.7 (12.0)
72	8.33 (6.0)	87 (1.7)	18.3 (15.8)
96	0	80	0
120	0	35	0

* Extender for both frozen-thawed and extended semen was a Tris-citrate buffer with glycerol and egg yolk, diluted to give 80×10^6 spermatozoa/ml.
† Values are means with standard error in parentheses; five ejaculates from three beagle dogs.
From Morton DB, Bruce SG: Semen evaluation, cryopreservation and factors relevant to the use of frozen semen in dogs. J Reprod Fertil Suppl 39:311–316, 1989, with permission.

Extenders and degree of cooling varied; however, most contained egg yolk in combination with powdered milk, cream, or a Tris buffer.[58]

Using Artificial Insemination with Frozen Semen

First canine pregnancies following AI with frozen thawed dog sperm were reported in 1957, 1970, 1972, and 1973.[66–69] The first frozen semen puppy registered by the American Kennel Club in 1981 was an Irish Setter puppy born as a single-pup litter to a bitch inseminated with frozen thawed sperm at Colorado State University.[70] Less than 500 canine pregnancies worldwide from frozen semen insemination were reported in 1989.[70] The major obstacles to success using frozen semen AI in the dog in early studies were the difficulty in timing day of ovulation accurately, the short post-thaw life span of frozen dog sperm, and the anatomy and location of the cervix of the bitch, which prevent easy cervical cannulation for intrauterine insemination of sperm. In addition, canine semen freezing service is not widely available and, when available, is expensive. Advantages of using frozen semen in the bitch and other species include the ability to store genetic material indefinitely, which permits transport of semen to bitches around the world and storage of semen until optimal breeding time, and storage of sperm well beyond the life span of the donor dog.

Success in achieving canine pregnancy following AI with frozen semen depends on the factors essential for success with fresh or chilled extended semen, which are normal semen quality, accurate timing of insemination, and appropriate site of deposition of the semen; in addition, semen handling procedures at time of collection, addition of the buffer/extender, freezing, storage, transport, thawing, and at insemination, are critical. Semen of marginal quality that may result in conception when inseminated fresh may not survive the freeze-thaw process; therefore, normal semen quality should be demonstrated prior to freezing, and a test thaw after freezing should be performed to demonstrate ability of the semen to withstand the process. Timing of insemination is particularly critical when frozen semen is used in the dog because of the very short sperm life span post-thaw (Table 4–11) when compared to fresh sperm life span in the tract of the estrous bitch; in general, insemination of frozen semen in the bitch is recommended at 3 to 4 days after day of ovulation so as to assure presence of secondary oocytes in the

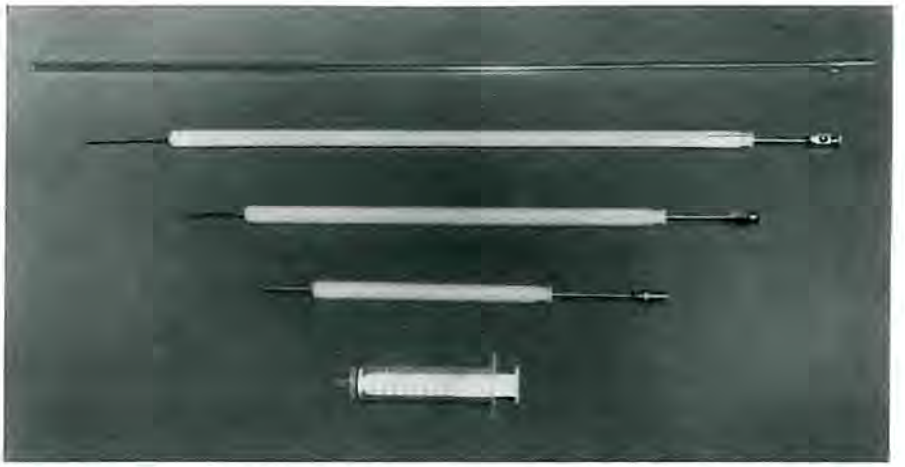


Figure 4–13. Insemination catheters for vaginal and intrauterine insemination in the bitch. *Top:* The plastic catheter used for deep vaginal inseminations in the bitch. *Bottom:* Three sizes of the Norwegian catheter for intrauterine inseminations. A 5- to 10-ml syringe should be used for the semen. (From Linde-Forsberg C: Achieving canine pregnancy by using frozen or chilled extended semen. Vet Clin North Am 21:467–485, 1991, with permission.)

uterine tube at time of insemination. Accurate timing of ovulation day using serum progesterone measurement by RIA (strategy 3 above) is recommended for all use of frozen semen in this species.

Appropriate site of deposition of frozen thawed sperm in the dog is the lumen of the uterus.[57,58,63,70,71] Many investigators have reported that frozen thawed dog sperm, like frozen thawed sperm of many mammalian species, cannot easily traverse the cervix of the estrous female. Although a few canine pregnancies of small litter size have been achieved with vaginal insemination of frozen thawed semen, investigators around the world agree that intrauterine insemination of frozen thawed sperm results in higher conception rate and increased litter size in this species.

In the bitch, intrauterine insemination of frozen semen has been reported by transcervical intrauterine insemination and by surgical injection of semen through the uterine wall at time of laparotomy (surgical insemination). Two methods of transcervical insemination are described. The first method of transcervical insemination utilizes 20- to 50-cm-long steel catheters with 0.5- to 1.0-mm-diameter tips, which have been adapted from catheters used for fox insemination in Norway (Fig. 4–13)[63]. The method of fixing the cervix through the abdominal wall and applying downward traction so as to change the angle of the cervical canal to a horizontal one is depicted in Figure 4–14.[63] After fixation of the cervix, the tip of the catheter is gently moved with small movements in search of the opening of the cervical canal. The sensation when the opening of the external cervical os is found has been described as the sensation of touching cartilage, that is, "crispy."[63] Thawed semen is then injected through the catheter into the cervical canal or uterine lumen (see semen handling procedures below). Practice with saline infusion in estrous bitches has been recommended

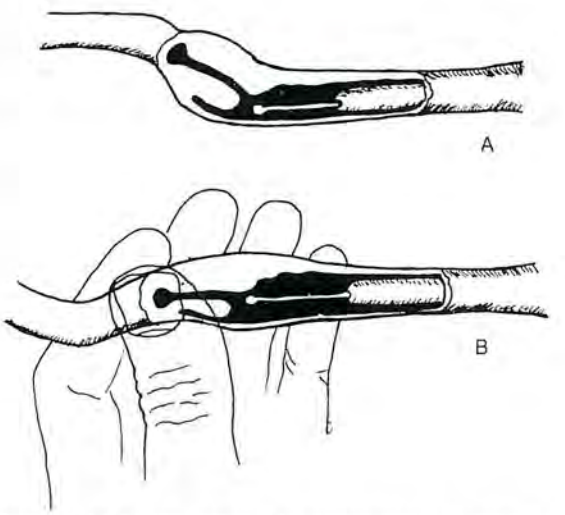


Figure 4–14. Sagittal section of the canine cranial vagina and cervix with the Norwegian intrauterine insemination catheter in position. **A:** The cervical canal is at an angle of approximately 45 to 60 degrees to the cranial vagina. **B:** To facilitate catheterization, the cervix is fixed between the thumb and index finger by abdominal palpation and tilted in a more horizontal position. (From Linde-Forsberg C: Achieving canine pregnancy by using frozen or chilled extended semen. Vet Clin North Am 21:467–485, 1991, with permission.)

using this technique, as has elevation of the hindquarters of the bitch for at least 10 minutes after insemination with frozen semen so as to facilitate movement of the semen to the uterine tube.[63] Advantages of this insemination method are that it may be performed in the nonanesthetized bitch, client cost is minimal, multiple inseminations may be performed inexpensively, and good conception rates in hundreds of pregancies have been reported by skilled inseminators.[57,58,63] Disadvantages of this method are the lack of availability of these insemination catheters in the United States, the need for extensive practice before veterinarians can fix and cannulate the cervix (the authors of this book have never been able to do so), and the potential danger that inexperienced clinicians may traumatize the cervix or rupture the vaginal fornix with the insemination catheter (Fig. 4–15).

A second type of transcervical insemination involves visualization of the cervix with a rigid endoscope, passage of a 6- to 8-Fr polypropylene urinary catheter through the cervix, and subsequent insemination.[72,73] The bitch is restrained in a standing position; sedation is very rarely necessary. The endoscope is passed carefully along the length of the vagina. Air insufflation is rarely required. The cervical os appears as a dimple in a rosette of transcervical folds. The urinary catheter is passed through the cervix with the aid of the endoscope, which is manipulated to straighten the cervical canal. The catheter is passed as far as it will go without force and the semen deposited. Advantages of this technique are that it is not blind, minimizing trauma to the reproductive tract, and that multiple inseminations can be performed in a given heat cycle. In one study, 32 of 40 (80 per cent) bitches inseminated with frozen thawed semen using this technique became pregnant.[72]

Surgical insemination requires general anesthesia for about 20 minutes. Both injectable barbiturate and inhalation anesthesia regimens, each following atropine premedication, have been used successfully for frozen semen inseminations in the bitch. A 5-cm midline abdominal incision is made, starting midway between the umbilicus and the pelvic brim.[71] When the surgeon begins the incision, an assistant should thaw the semen (see semen handling techniques below). The uterine body is identified and exteriorized using a Snook ovariohysterectomy hook. The surgeon then uses a sterile 3- to 6-ml syringe attached to a 22-gauge needle to aspirate thawed semen from the thawing vessel provided by the assistant. With an index finger under the exteriorized uterus, the surgeon then slides the needle through the uterine wall at an oblique angle and injects a small volume of semen (about 0.25 ml) in order to observe filling of the uterine lumen.[71] After confirmation of uterine filling, the rest of the semen is injected. Because the uterine lumen is a potential space, not normally occupied by fluid or air at the time of insemination, even small volumes of semen (0.5 ml) will distend the uterus of very large dogs. Following insemination, the uterus is returned to the abdominal cavity, and the abdomen closed routinely. Advantages of this insemination method are that it may be performed easily by any veterinarian able to perform abdominal surgery, appropriate site of insemination is confirmed visually, and good conception rates have been reported by skilled inseminators.[71] Disadvantages of this

Figure 4–15. Lateral radiograph of the abdomen of an estrous beagle bitch used for research following attempt at transcervical injection of contrast radiographic medium into the uterine lumen. Contrast medium both in the uterine horns and free in the peritoneal cavity indicates rupture of the vaginal fornix by catheter trauma. This bitch recovered uneventfully from the vaginal rupture without therapy.

method are the need to time ovulation accurately so as to prevent need for more than one insemination, the relatively high client cost, and the anesthetic and surgical risk to the bitch; in some countries anesthesia and surgery in dogs for the purpose of insemination are considered unethical.[63]

Semen handling at the time of insemination requires the ability to keep the semen frozen until time of insemination, knowledge of the number of sperm in shipped samples (straws, ampoules, vials), and knowledge of optimal thawing protocol. Frozen semen is transported in tanks containing liquid nitrogen, some of which are called "dry shippers" because liquid nitrogen cannot be spilled if they are tipped over. Some transport tanks will maintain semen samples in the frozen state for up to 4 to 6 weeks. If samples will not be transferred to a larger holding tank, the inseminating veterinarian should speak to the semen shipper to find out how long the samples will remain frozen within the tank used. Number of sperm per straw, ampoule, or vial also should be ascertained from the shipper, as well as post-thaw motility from test thaw information. These numbers can be used to assure that an adequate volume of semen will be thawed to yield more than 150×10^6 motile sperm post-thaw. Pregnancy has been reported in bitches inseminated surgically into the uterine horn with as few as 20×10^6 fresh, or with 100×10^6 frozen thawed sperm.[70] Optimal thawing procedure for frozen canine semen should be provided by the person or commercial facility that performed the freezing. Several thawing procedures have been reported as satisfactory[70]; these include thawing pellets or straws in a 37°C water bath for 30 to 90 seconds or until the semen is liquid, or in a 75°C water bath for 6 seconds.

Conception rate in bitches bred by AI with frozen thawed semen varies considerably with accuracy of timing the day of ovulation, quality of semen inseminated, buffer/extender used, freezing protocol used, site of insemination, number of sperm inseminated, number of inseminations, and normality of the female reproductive tract. Canine conception rates reported in the literature using intravaginal or intrauterine AI with frozen semen vary from 0 to 100 per cent with sperm numbers of 39 to 800×10^6.[70,71] Early studies of intravaginal deposition of 700 to 800×10^6 sperm frozen in lactose–egg yolk buffer in pellets reported 40 to 97 per cent conception rates.[74,75] Surgical frozen semen inseminations of high-quality semen into the vagina or uterus of research beagles on the first and third days of estrus yielded pregnancy rates of 11 and 46 per cent, respectively.[71] A pregnancy rate of 51.1 per cent was reported in 45 bitches inseminated transcervically with frozen semen by eight Swedish veterinarians.[58] A conception rate of 73.6 per cent was reported in 19 bitches inseminated one to three times with frozen thawed semen using transcervical insemination based on serum progesterone concentration.[76]

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Chapter 5

Canine Pregnancy

Physiology and Endocrinology

Maintenance of Pregnancy

Maintenance of pregnancy in the bitch depends on the secretion of progesterone throughout gestation. The ovaries are the major, if not the only, source of progesterone for maintaining canine pregnancy. Ovariectomy during pregnancy results in resorption or abortion.[1] Although the canine fetoplacental unit can metabolize progesterone that is injected into experimental fetal pups,[2] the canine placenta does not appear to naturally synthesize large amounts of progesterone.[3]

Secretion of progesterone from corpora lutea is regulated by both luteotropic and luteolytic factors. The major source of luteotropic hormones is the pituitary gland. Although canine pregnancy at any stage is terminated by hypophysectomy,[4] pregnancy termination takes longer when hypophysectomy is performed early in diestrus. Although canine corpora lutea may be less dependent on pituitary support during the first half of diestrus, pituitary luteotropic support is necessary for maintaining the secretion of progesterone during the last half of diestrus. Luteal secretion of progesterone can be depressed by the administration of anti–luteinizing hormone (LH) serum or by prolactin-lowering drugs in the last half of diestrus, arguing for at least two pituitary luteotropins in the dog.[5] Progesterone secretion is not, however, affected when LH is suppressed through the administration of a gonadotropin-releasing hormone agonist, arguing that LH is not the primary luteotropic factor in the bitch.[6] However, it is possible that even when LH secretion is suppressed, low concentrations of LH remain that are adequate to maintain luteal function.

Concentrations of LH in serum increase during the last half of diestrus in the bitch as concentrations of progesterone decline.[7,8] Although the physiologic cessation of diestrus and pregnancy does not appear to result from an absolute lack of LH, because concentrations of LH are increasing when concentrations of progesterone are declining, luteolytic factors (i.e., prostaglandins) could override the luteotropic effect of pituitary luteotropins. Thus the absolute role of LH in maintaining secretion of progesterone throughout diestrus remains unclear.

Concentrations of serum prolactin also increase during the second half of diestrus when concentrations of progesterone are declining. Concentrations of prolactin in pregnant bitches in late diestrus are much higher than those in nonpregnant bitches. Drugs that lower prolactin levels cause progesterone levels to decrease, resulting in pregnancy termination in bitches that are treated during the last half of diestrus.[5,6] Although high doses of certain prolactin-decreasing drugs decrease secretion of both prolactin and LH in some species, concentrations of progesterone decreased in bromocriptine-treated bitches whose prolactin levels decreased when LH levels remained unchanged. Thus prolactin appears certain to be a luteotropic hormone in the bitch. The exact luteotropic role for LH is not as clear.[6]

The uterus appears to have little or no effect on luteal function in bitches; hysterectomized bitches cycle normally and have normal luteal phases.[9] Uterine luteotropic factors have not been identified in the bitch, and the absence of uterine luteolytic factors does not appear to lengthen diestrus. However, prostaglandin $F_{2\alpha}$ administered in pharmacologic doses is luteolytic in the bitch, and physiologic increases in

prostaglandins by the fetoplacental unit probably result in the prepartum luteolysis that precedes whelping.[10,11]

Clinically Relevant Physiologic and Endocrinologic Events of Pregnancy

The timing of the physiologically important and clinically relevant events of canine pregnancy is presented in Table 5–1.[13]

The gestation length in the dog varies considerably and depends on the physiologic or behavioral event that serves as "day 0." As depicted in Table 5–1, parturition occurs approximately 65 days after the LH surge, 63 days after ovulation, and 57 days after the onset of cytologic diestrus (as determined by vaginal cytology). Gestation length, when based solely on breeding dates, can be extremely variable (also see Chapters 4 and 6). For example, gestation length, when defined as the length of time from the day of first mating to the day of whelping, has been shown to range from 57 to 72 days.[12]

HORMONE PROFILES

The endocrine events of pregnancy in the bitch (Fig. 5–1)[13] have been studied most frequently in beagles. Although major endocrinologic differences have not been reported between those breeds studied, endocrine data are unavailable for many breeds of dogs.

Progesterone. Progesterone concentrations in serum or plasma are similar in pregnant and nonpregnant diestrous bitches[14,15] and therefore cannot be used as indicators of pregnancy status. Although some authors have reported pregnancy-specific increases in pro-

Table 5–1. Timing of Selected Events of the Fertile Ovarian Cycle and Pregnancy of the Domestic Dog in Relation to the Day of Preovulatory LH Surge, Ovulation, and Onset of Cytologic Diestrus

Selected Reproductive Events	Days Before (−) or After (+) the LH Surge	Days Before (−) or After (+) Ovulation	Days Before (−) or After (+) Onset of Cytological Diestrus
Onset of proestrus	Variable with regard to clinical signs (i.e., −25 to −3)	Variable with regard to clinical signs	Variable with regard to clinical signs
Maximum vaginal cornification	−1 to +7	−4 to +4	−10 to −2
Onset of estrous behavior	−4 to +5	−7 to +2	−13 to −4
Estradiol peaks	−3 to −1	−6 to −4	−10 to −12
Progesterone increases to 1–2 ng/ml	−1 to 0	−4 to −3	−9 to −10
LH surge	0	−2 to −3	−8 to −9
Matings that result in maximum conception rates	−1 to +6	−3 to +4	−3 to −10
Initial crenulation of vaginal mucosa	−1 to +1	−2 to −4	−8 to −10
Maximum crenulation of vaginal mucosa	+2 to +6	−1 to +3	−3 to −7
Ovulation of primary oocytes	+2 to +3	0	−5 to −7
Fertilization pronuclei formation	+4 to +6	+2 to +4	−2 to −5
Two-cell embryo in uterine tube	+6 to +10	+3 to +7	−3 to +1
Onset of cytological diestrus	+8 to +9	+5 to +7	0
Onset of behavioral diestrus	+10 to +14	+7 to +11	+1 to +5
Zygotes enter uterus	+11 to +12	+8 to +9	+2 to +3
Attachment sites established, zona pellucidae shed	+16 to +18	+13 to +15	+7 to +9
Ultrasound detection of amniotic cavities possible	+19 to +22	+16 to +19	+10 to +13
Pregnancy can be verified by abdominal palpation in some bitches	+20 to +25	+17 to +22	+11 to +16
Ultrasound detection of fetal heartbeats possible	+22 to +25	+19 to +22	+13 to +16
Onset of pregnancy anemia	+25 to +30	+22 to +27	+16 to +21
Radiographic detection of radiopaque fetal skull and head possible	+44 to +46	+41 to +43	+35 to +37
Prepartum luteolysis and hypothermia	+63 to +65	+60 to +62	+55 to +57
Parturition	+64 to +66	+62 to +64	+56 to +58

Adapted from work published by Concannon and Lein.[13] Relationship to ovulation and onset of cytological diestrus have been added. Values for maximal cornification, time of fertile matings, onset of cytologic and behavioral diestrus, and entry of zygotes into the uterus have been added or modified.

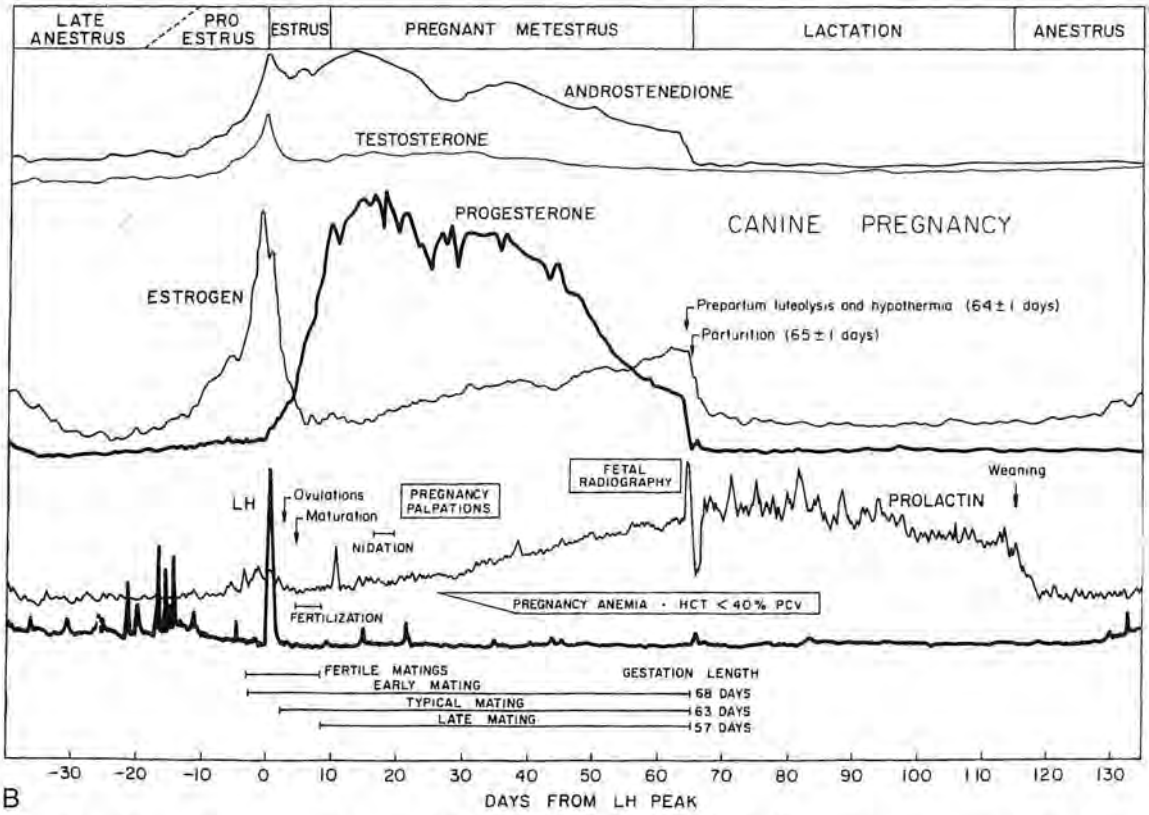

Figure 5–1. Schematic representation of typical changes in serum or plasma levels of estrogen, progesterone, LH, prolactin, testosterone, and androstenedione reported or presumed to occur during pregnancy and lactation in the bitch. (From Concannon PW, Lein DH: Hormonal and clinical correlates of ovarian cycles, ovulation, pseudopregnancy, and pregnancy in dogs. *In* Kirk RW [ed]: Curr Vet Ther Small Anim Pract 10. Philadelphia, WB Saunders, 1989, p 1271, with permission.)

gesterone after implantation and placental development,[16–18] the difference is not great enough to provide a diagnostic test for determining pregnancy. Concentrations of progesterone overlap between pregnant and nonpregnant bitches throughout diestrus.[10] Whether total progesterone secreted by corpora lutea varies among pseudopregnant and pregnant bitches remains to be determined.

Peak concentrations of progesterone occur during early to mid-diestrus, then gradually decline to basal values 51 to 82 days after the LH peak in nonpregnant bitches or 24 to 48 hours prior to parturition.[7,14,16,17,19–24] The luteal phase, or diestrus, ends when concentrations of progesterone decrease to, and remain at, a level inadequate to support pregnancy (i.e., < 1 to 2 ng/ml).

Peak concentrations of progesterone during diestrus are variable among bitches, ranging from 10 to 70 ng/ml.[19] Total progesterone secreted by multiple corpora lutea in pregnant bitches with large litters has not been compared to total progesterone secreted by pregnant bitches with small litters. The number of corpora lutea should be equal to the number of fetuses, assuming that conception was normal, fetal loss did not occur, and splitting of the embryo did not result (i.e., no twinning). The total number of corpora lutea is normally less in breeds of dogs that produce smaller litters than in those with larger litters. Theoretically, the total number of corpora lutea and litter size might be less in a bitch of any breed if ovulation were incomplete. Total progesterone secreted per corpus luteum has not been reported for different breeds, nor for animals with reduced ovulation rates.

The abrupt decline in serum progesterone prior to parturition results from prepartum luteolysis, which can be monitored by observing a decline in rectal temperature in the bitch. The transient hypothermia parallels the decline in blood progesterone concentration with a delay of about 12 hours, with rectal temperatures falling about 1°C (1.6°F). In one study,[18] a distinct transient prepartum temperature drop occurred in 78 of 80 pregnancies in which rectal

temperatures were recorded daily or more frequently. The mean low temperature recorded between 8 and 24 hours prior to parturition was 98.8°F (range = 98.1° to 100.0°F) for 40 pregnancies. The hypothermia was transient, with temperatures rising during or immediately after parturition (see Chapter 6).

During the last week of pregnancy, the pattern of uterine electrical activity also changes in the bitch as progesterone concentrations decrease.[25] The total duration of electromyographic activity per hour and the burst frequency per hour increase prior to whelping and coincide with a significant drop in body temperature, drop in plasma progesterone, and drop in unconjugated estradiol-17β.[24]

Prolactin. Prolactin concentrations were reported[8] as low (mean concentration <2 ng/ml) throughout most of diestrus in nonpregnant bitches, increasing two- to threefold during late diestrus. Although little information exists on the hormone levels in nonpregnant bitches demonstrating signs of pseudopregnancy (i.e., lactation, nesting behavior) in late diestrus, increased levels of prolactin have been suggested to be responsible for these physiologic changes. During the last week of pregnancy, serum prolactin concentrations in seven pregnant beagle bitches were variable within and among the bitches and averaged 40 ± 7 ng/ml.[20] In each bitch, prolactin values increased by 195 ± 29 per cent during the 16- to 56-hour prepartum period and reached peak levels (117 ± 24 ng/ml) at 8 to 52 hours prepartum in six of seven bitches and 24 hours postpartum in the remaining bitch. Concentrations of prolactin decreased by 36 hours after the peak, before again increasing in response to suckling by pups.[20]

Estrogens. Data on serum concentrations of estrogens in pregnant and pseudopregnant bitches are variable, possibly resulting from the few animals sampled in many studies and from varying methodologies used to measure estrogens (i.e., total estrogens vs. specific estrogens). Total unconjugated estrogens were reported to increase in nonmated bitches following estrus, peaking in mid-diestrus.[26] Higher concentrations of estradiol also were measured in the serum of nonpregnant bitches.[14]

Concentrations of total extracted immunoreactive estrogens in plasma were reported to be constant during diestrus in nonpregnant bitches and elevated during the last 3 weeks of diestrus in pregnant bitches.[17] However, when specific estrogens were assayed, different hormone profiles were identified for estrone and estradiol-17β among nonpregnant and pregnant bitches.[27] Concentrations of estradiol were low in pregnant bitches and were higher in nonpregnant bitches during early to mid-diestrus and did not reach a nadir until week 5 of diestrus. Conversely, mean serum concentrations of estrone remained low during diestrus in nonpregnant bitches but were increased in pregnant bitches, reaching a peak by week 5 of diestrus. To date, research data have not supported the canine placenta as a major contributor of estrogens. The contribution of the adrenal gland to hormone production throughout diestrus has not been well studied.

Androgens. Serum concentrations of testosterone increase during proestrus in the bitch, with the highest mean concentration occurring on the day of the LH surge.[28] Concentrations of testosterone then decline to basal levels throughout the remainder of the luteal phase.[29] Androstenedione concentrations increase during estrus and early diestrus and then decrease by day 30 to 40 in pregnant and nonpregnant bitches.[29]

Relaxin. Concentrations of immunoreactive relaxin in the plasma of pregnant and lactating dogs have been reported.[30–33] Immunoreactive relaxin was not detected in the plasma of male dogs, anestrous bitches, or nonpregnant diestrous bitches.[30] Immunoreactive relaxin was detected in pregnant bitches, reaching peak concentrations (4 to 6 ng/ml) 2 to 3 weeks before parturition, declining somewhat before parturition, and persisting (0.5 to 2.0 ng/ml) for 4 to 9 weeks after whelping. Plasma concentrations of relaxin were higher and persisted longer in Labrador retrievers than in beagles during lactation. The persistence of plasma relaxin postpartum initially led to the suggestion that the ovary, not the placenta, was a source of relaxin. However, Tsutsui and Stewart[31] reported that immunoreactive relaxin concentrations remained increased in ovariectomized bitches whose pregnancies were maintained with progesterone, arguing against the ovary as the sole source of relaxin. Tsutsui and Stewart also reported that immunoreactive relaxin concentrations became undetectable within 2 days after pregnant or postpartum dogs were hysterectomized, suggesting that the uterus contributed to relaxin production.

Although the highest tissue concentrations of immunoreactive relaxin were found in the canine placenta,[31] relaxin bioactivity has been detected in both canine placentas and ova-

ries.[32,33] The 6-kDa polypeptide relaxin also has been identified in the milk of lactating bitches who were ovariohysterectomized at the time of cesarean section, arguing that the source of milk relaxin is neither the ovary nor the placenta.[34] The mammary gland is a source of milk relaxin in some other species[35,36] and may be a source of biologically active relaxin in the milk of the bitch. Relaxin may be produced by several tissues in the dog.

Measuring immunoreactive relaxin has been suggested as a pregnancy test for the dog[33] because concentrations are elevated in pregnant, but not in nonpregnant, diestrous bitches. Because immunoreactive relaxin is not detected in plasma until the third or fourth week of pregnancy,[30] or after a time when pregnancy can be diagnosed by other means (i.e., abdominal palpation, ultrasonography), its usefulness as an early pregnancy test is limited. Relaxin measurements might provide useful information on whether a pregnancy had, in fact, been established or if fetal death and resorption occurred.

Luteinizing Hormone. Serum concentrations of LH increase in nonpregnant and pregnant bitches during late diestrus.[7,27,37] Endogenous opioids may partly control LH release in the bitch. LH release has been studied in bitches given the opioid antagonist naloxone (1.0 mg/kg intravenously) during various stages of the reproductive cycle. LH release following naloxone administration was minimal in the early luteal phase but increased during days 21 to 63 after the LH surge in pregnant and nonpregnant diestrous dogs.[38] These data suggest that opioids may be involved in controlling the release of LH during certain times of diestrus.

Follicle-Stimulating Hormone. Very little information has been published on the concentrations of FSH in blood during the canine luteal phase. Reimers et al.[15] reported that mean concentrations of FSH in serum of pregnant and nonpregnant diestrous bitches were similar up to day 16 following the LH surge. After that time, concentrations were higher on days 28 to 30 and days 55 to 58 for two pregnant bitches than for four nonpregnant diestrous bitches.

PHYSIOLOGIC CHANGES OF PREGNANCY THAT CAN BE CONFUSED WITH PATHOLOGIC CONDITIONS

Numerous physiologic changes occur during a normal canine pregnancy that can be confused with pathologic conditions if unrecognized. Some physiologic changes that have clinical relevance for veterinarians are listed in Tables 5–2 and 5–3.[39–45] Physiologic alterations in the pregnant bitch may dictate that modifications of an anesthetic protocol be considered for a cesarean section or that dosing regimens of other drugs (i.e., antibiotics) be modified (see Chapter 6).

Embryonic and Fetal Events: Transuterine Migration, Implantation and Placentation, Superfecundation and Superfetation

Transuterine Migration of Embryos

The number of corpora lutea in one ovary and the number of fetuses in a corresponding uterine horn may not be well correlated because of transuterine migration of embryos.[46] In one study of 192 bitches of unknown age and parity, transuterine migration was assumed to have occurred in 92 animals (47.9 per cent).[47] This assumption was based on a greater number of fetuses in a given horn than corpora lutea in the ipsilateral ovary.

Implantation and Placentation

Implantation in the bitch was observed to occur 17 to 22 days after breeding[48,49] or 16 to 18 days after the LH surge (Table 5–1). The canine placenta is composed of two parts: the fetal placenta or allantois chorion, and the maternal placenta or endometrium. Because the allantois chorion and endometrium are in intimate contact, diseases or abnormalities of either may affect the other. The dog has endotheliochorial placentation, meaning the endothelium of the uterine vessels lies adjacent to the fetal chorion.[50] The maternal and fetal circulations are separated by four layers: the maternal endothelium of uterine vessels, the chorion, the fetal mesenchyme, and the fetal endothelium.[50] The canine placenta forms a zonary band or girdle about 2.5 to 7.5 cm in width around the circumference of the uterine lumen in the middle of the oval chorionic sac (Fig. 5–2). Placental hematomas or extravasations of blood components are observed as green and brown borders or margins of the zonary placenta, and are considered to play a role in the nutrition of the fetus; the stagnant maternal

Table 5-2. Physiologic Changes during Pregnancy That Might Be Confused with Pathologic States in the Bitch

Event	Time of Occurrence	Magnitude of Change	Breeds Studied
Anemia (normocytic, normochromic—may reflect increase in plasma volume rather than decrease in whole-body red cell number)	7–9 wk postestrus in nonpregnant and pregnant diestrous bitches	Decrease in packed cell volume in pregnant bitches was 24% to 33%[39, 40] and in nonpregnant bitches was 17% to 21%[39] Decrease in hemoglobin in pregnant bitches was 23% to 36%,[39, 40] and in nonpregnant bitches was 18% to 22%[39]	Beagles[39]; Brittany spaniels, Labrador retrievers, and beagles[40]
Increased plasma cholesterol	3–8 wk postestrus in nonpregnant and pregnant diestrous bitches	Increase in plasma cholesterol of 75 to 94%[39]	Beagles[39]
Plasma protein changes	Increase at 3–8 wk postestrus in nonpregnant and pregnant diestrous bitches; decrease at day 56 from mating and at whelping	Increase in plasma proteins of 13% to 20% at 3–8 wk postestrus[39] Decrease in total protein and albumin at late gestation and whelping[41]	Beagles[39]
Increased factor VII	3 wk postconception to 1 wk postwhelping	Increase in factor VII in pregnant, but not in nonpregnant, diestrous bitches; maximum increase of 100% occurred between weeks 4 and 6 postconception[42]	Mixed breeds[42]
Increased factor IX	3 wk postbreeding to whelping	Increase in factor IX in pregnant, but not in nonpregnant, diestrous bitches; maximum increase of 60% occurred between weeks 4 and 6 postbreeding[42]	Mixed breeds[42]
Increased factor VIII	3 wk postbreeding to whelping	Increase in factor VIII in pregnant, but not in nonpregnant, diestrous bitches; no defined peak of activity; activity was increased 50% throughout the period of gestation[42]	Mixed breeds[42]
Increased factor XI	5 wk postbreeding	Increase in factor XI in pregnant, but not in nonpregnant, diestrous bitches; activity was increased 20% over controls[42]	Mixed breeds[42]
Increased gastrin	Weeks 1–8 of pregnancy; gastrin also increases in response to suckling	Increase in plasma gastrin in pregnant bitches; peak value occurred week 7 of pregnancy (range 60–327 pmol/L)[43]	Beagles[43]
Increased somatostatin	Midpregnancy	Increase in plasma somatostatin in pregnant bitches; peak value occurred during middle of pregnancy (range 12–260 pmol/L)[43]	Beagles[43]
Increased cholecystokinin	Weeks 2–8 of pregnancy; also increases in response to suckling	Increase in plasma cholecystokinin in pregnant bitches; peak value at week 3 of pregnancy (27 pmol/L)[43, 44]	Beagles[43, 44]
Increased resistance to insulin	Day 55 of pregnancy	Increased insulin resistance during pregnancy; an intravenous dose of insulin (0.1 U/kg) was less effective in reducing plasma glucose levels at day 55 of pregnancy than at day 55 of nonpregnant luteal phase or during lactation at day 25 postpartum[45]	Beagles[45]
Decreased serum calcium	Day 56 from mating and at whelping	Decreased serum calcium level may reflect decreased serum albumin level reported to occur during late gestation and at whelping[41]	

Table 5–3. Summary of Physiologic Alterations in the Pregnant Bitch

Parameter	Change
Heart rate	Increased
Cardiac output	Increased
Blood volume	Increased
Plasma volume	Increased
Packed cell volume	Decreased
Hemoglobin concentration	Decreased
Plasma protein concentration	Decreased
Arterial blood pressure	Unchanged
Central venous pressure	Unchanged (increased during labor)
Minute volume of ventilation	Increased
Oxygen consumption	Increased
Arterial blood gases and pH	pH and O_2 tension unchanged, CO_2 tension decreased
Total lung capacity and vital capacity	Unchanged
Functional residual capacity	Unchanged
Gastric emptying time	Increased
Intragastric pressure	Increased
Gastric motility	Decreased
pH of gastric secretions	Decreased
Gastric Cl^- and enzyme concentration	Increased
SGOT	Increased
LDH	Increased
BSP retention time	Increased
Plasma cholinesterase concentration	Decreased
Renal plasma flow	Increased
Glomerular filtration rate	Increased
BUN	Decreased
Creatinine	Decreased
Na^+ and water balance	Unchanged

Abbreviations: SGOT, serum glutamic-oxaloacetic transaminase; LDH, Lactate dehydrogenase; BSP, Bromsulphalein; BUN, blood urea nitrogen.
From Benson GJ, Thurmon JC: Anesthesia for cesarian section in the dog and cat. Mod Vet Pract, Jan. 30, 1984, with permission.

blood provides a source of iron for the developing fetus.[50] This pigment, called lochia or uteroverdin, may be observed passing from the vulva at the time of parturition and signifies placental separation for one or more pups.

In the bitch, the amnion containing the fetus floats free in the allantoic cavity and is attached only by the umbilical stalk. Therefore, pups are sometimes covered with the amnion at birth. Unless promptly removed, pups can suffocate if the amnion lies over the nostrils and mouth. In the cow, sow, and ewe, the amnion is attached to the allantois at several sites and is less likely to cover the fetus at birth.[50]

The type of placentation is a major factor influencing the passage of immunoglobulins

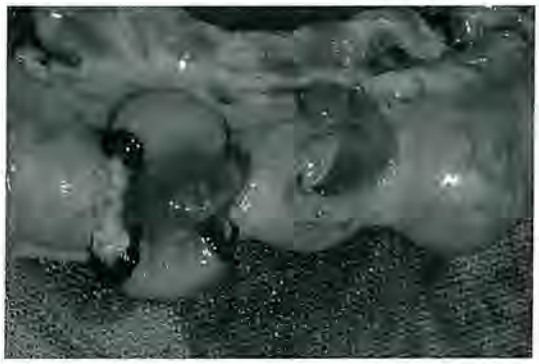

Figure 5–2. A canine uterus from a bitch that was approximately 30 days pregnant. Incisions have been made in the uterus to demonstrate a fetus and fetal membranes. Note the zonary placental band of tissue, with dark margins of blood components, that surrounds the developing fetus.

in utero. In the dog, only 5 to 10 per cent of the total immunoglobulins provided by the bitch is transferred to the pup through the endotheliochorial placenta.[51–53] Most of the passive immunity in neonatal pups is derived through colostrum.[54,55] The placenta serves to protect the developing fetuses, transmit nutrients from the dam to the fetuses, remove fetal waste products, and synthesize substances and enzymes necessary to support the pregnancy.

Superfecundation and Superfetation

Superfecundation occurs when two or more ova ovulated during a single estrus are fertilized by spermatozoa from different males. Because behavioral estrus and mating can occur over many days in the bitch, it is common for a litter to contain pups with different sires. In a study[56] of 21 bitches bred to two male dogs with different blood types, superfecundation was confirmed in 7 of 15 bitches mated for the second time by 60 hours after ovulation. None of six bitches mated for the second time at 72 or 84 hours after ovulation exhibited superfecundation.

Superfetation occurs when a pregnant female, carrying one or more live fetuses, is bred again and a second conception occurs. Superfetation has not been demonstrated in the bitch and is unlikely to occur following the establishment of zonary placentae in both uterine horns; spermatozoa could not reach the uterine tubes even if ovulation had occurred, because the placentae would prevent sperm transit. Although owners frequently question if pups of different sizes are the result of different gestational ages, it is unlikely that superfetation is the cause of such size disparities in this species.

Because most ova are ovulated over a period of only 1 to 2 days in the bitch, differences in the size of the pups are not likely the result of different gestational ages. Other factors, such as placental disease, placental attachment area, inherited disorders, and nutrients available to individual fetuses, may account for disparity in size among pups.

Diagnosis of Pregnancy

Abdominal Palpation

The embryos and chorioallantoic vesicles in the bitch form a series of ovoid swellings in the early gravid uterus, the most caudal of which can be identified by palpation through the abdominal wall as early as 17 to 22 days after ovulation.[57,58] In an average-sized dog (20 kg) the swellings are approximately 2 inches in length at 28 to 30 days after ovulation, which is the time when the swellings are most easily and accurately palpated (Fig. 5–3). By days 35 to 45 after ovulation, these swellings increase in size and elongate, and the fetal vesicles in the diffusely enlarging uterus become more difficult to identify. In general, the bitch should be palpated approximately 31 to 33 days after the LH surge and initial sharp rise in concentration of serum progesterone, or about 28 to 30 days after the suspected day of ovulation. Even when abdominal palpation is conducted at an optimal time, it may be difficult to determine pregnancy in some larger bitches, in bitches with extremely tense abdomens, or in bitches carrying only one pup or a few pups in the cranial abdomen.

Pregnancy palpation should always be as gentle as possible. The effect of repeated or excessively harsh palpation during early pregnancy in the bitch on fetal resorption is unknown. Increased resorption rates were observed in a kennel of Shetland sheepdog bitches when owners palpated their bitches daily during early pregnancy (P. N. Olson, unpublished observation, Colorado State University, 1988). Many factors may have led to the increased resorption rate in the kennel, and subsequent recovery when bitches were no longer palpated by the owners. Nevertheless, palpation of fetal membranes and amniotic vesicles during early pregnancy is associated with increased fetal attrition in other species.[59]

Figure 5–3. A uterus removed from a pregnant bitch that had mated approximately 30 days prior to ovariohysterectomy.

Radiographic Diagnosis of Pregnancy

Up to 21 days after ovulation, there may be no radiographic signs of uterine enlargement. Between 21 and 42 days after ovulation, enlarged, fluid-filled horns can be observed radiographically in many bitches. Fetal calcification of the spine and skull begins at 43 to 46 days after the LH surge and initial sharp rise in concentrations of serum progesterone (42 to 50 days after the first mating).[13] Therefore, this initial calcification of canine fetal skeletons occurs 20 to 22 days before the onset of parturition.[13,58] The calcification of the scapula, humerus, and femur is not observed until 46 to 51 days after the LH surge. The calcification of the radius, ulna, tibia, pelvis, and other structures is observed even later (Table 5–4). Although fetal teeth have been reported to become radiopaque 58 to 63 days after the LH surge or 3 to 8 days prior to whelping, and could serve as a useful indicator for staging advancing pregnancies, identifying fetal teeth may be difficult, depending on the radiographic techniques used. First fetal calcification observed radiographically may not occur until as late as 54 days after the first breeding.

Radiography of the bitch in late pregnancy may assist in determination of litter size and size of fetal skulls in relation to the bony maternal birth canal. Although significant increases in the size of fetal skulls (i.e., large single pups, fetal monsters) can be detected radiographically and serve to predict potential dystocia (see Chapter 6), less dramatic changes may be difficult to assess. Fetal skull size, determined by radiographic evaluation, may be influenced by the time of gestation, radiographic technique, and radiographic positioning. Radiographic evaluation is deemed desirable for bitches who are at high risk for, or who are experiencing, dystocia.

Table 5–4. Radiographic Detection of Uterine Enlargement and Fetal Mineralization in the Bitch*

Event	Days after Preovulatory LH Surge	Days Prepartum	Days after First Mating
Bitch first radiographed	29 (24–33)	36 (33–41)	30 (26–34)
Uterus first observed	30 (28–34)	35 (32–36)	32 (28–37)
Circular uterine swellings	35 (31–38)	30 (27–33)	35 (31–38)
Tubular/ovoid uterine swellings	41 (38–44)	24 (22–27)	41 (36–45)
Mineralized fetus first observed—spine, skull, ribs	45 (43–46)	21 (20–22)	46 (42–50)
Scapula, humerus, femur observed	48 (46–51)	17 (15–18)	50 (45–54)
Radius, ulna, tibia observed	52 (50–53)	11 (9–13)	54 (49–59)
Pelvis observed	54 (53–57)	11 (9–13)	56 (52–63)
13 pairs of ribs observed	54 (52–59)	11 (7–12)	56 (51–66)
Caudal vertebrae, fibula, calcaneus, paws observed	61 (55–64)	5 (2–9)	63 (58–70)
Teeth observed	61 (58–63)	4 (3–8)	63 (60–68)
Whelping	65 (64–66)	0	66 (63–71)

*Values are means (and ranges) of the day the indicated changes were first evident in the pregnancies studied. Note that the range was often reduced in magnitude when timing was related to the day of the preovulatory LH surge or to the day of parturition in comparison to that observed when timing was related to the time of mating.
From Rendano VI Jr, Lein DH, Concannon PW: Radiographic evaluation of prenatal development in the beagle—correlation with time of breeding, LH release, and parturition. Vet Radiol 25:132–141, 1984, with permission.

Radiography may be associated with some risks to the fetuses, even if performed late in gestation. In experimental studies, the risk for neoplasia or hematopoietic alteration was increased for beagle pups whose dams were irradiated during pregnancy.[60,61] Although the experimental bitches received doses much greater than what most bitches would be exposed to during routine veterinary diagnostic radiography, it is probably wise to avoid unnecessary radiographs during pregnancy.

Ultrasonographic Diagnosis of Pregnancy

Real-time ultrasonography has proven to be a valuable tool for diagnosing canine pregnancy and assessing fetal viability.[62–71] Ultrasonography does not always determine the number of fetuses accurately, because only one sector of the abdomen is imaged at a time. Therefore, pups may be inadvertently imaged a second time (giving too high a number) or missed when scanning (giving too low a number). In one study,[64] 51 pregnant bitches (Labrador retrievers, golden retrievers, and their crosses) were evaluated with B-mode ultrasonography. In only 31.8 per cent of the pregnancies was the number of pups that whelped accurately determined. Although the greatest tendency was to underestimate the number of conceptuses, inaccurate counts due to overestimation of fetal numbers occurred in 20 per cent of the pregnancies. The authors did not comment on whether fetal death may have accounted, in part, for any overestimation of fetal numbers.

Serial ultrasonographic examinations of beagle bitches from 20 to 60 days of pregnancy (day 0 = day of LH surge) provided information on detection of various fetal features.[62] Extrafetal structures also were identified (Table 5–5), of which chorionic cavity diameter was the most accurate for estimation of gestational age. Of fetal structures, head diameter was the most accurate for estimation of gestational age. In another study,[65] the variability for predicting gestational age during the last half of pregnancy for Labrador retrievers, golden retrievers, and their crosses was 2.84 days when a prediction table was constructed that compared fetal trunk diameter and fetal biparietal head diameter (Table 5–6). Variability of gestational age was less when comparing fetal trunk diameter and fetal biparietal head diameter than using either parameter alone.

Acute-Phase Proteins for Diagnosing Pregnancy

Acute-phase proteins in the dog include C-reactive protein (CRP), haptoglobin, acid glycoprotein, and fibrinogen, some of which may be useful indicators for detecting canine pregnancies. Serum fibrinogen concentrations increase during pregnancy in dogs;[72] the rise in fibrinogen or other acute-phase proteins is the basis for a canine pregnancy test available in the United Kingdom[73] and the United States. Fibrinogen is one of several acute-phase proteins that may increase due to inflammation at the time the embryo is invading the endometrium. Because serum fibrinogen also increases

Table 5–5. Gestational Age Range at First Ultrasonographic Detection of Selected Features in Pregnant Beagles

Pregnancy Feature	Days after LH Surge
Gestational sac	20
Uterine wall	
Echogenic gestational sac	20–23
Placental layers	22–24
Zonary placenta	27–30
Embryo position	
Apposed to uterine wall	23–25
Dependent in chorionic cavity	29–33
Fetal membranes	
Yolk sac membrane	25–28
Allantoic membrane	27–31
Yolk sac tubular shape	27–31
Yolk sac folded cross section	31–35
Embryo and fetus	
Heartbeat	23–25
Bipolar shape	25–28
Anechoic area in head	25–31
Choroid plexus	31–35
Limb buds	33–35
Fetal movement	34–36
Dorsal sagittal tube	30–39
Skeleton	33–39
Bladder	35–39
Stomach	36–39
Lung hyperechoic vs. liver	38–42
Liver hyperechoic vs. abdomen	39–47
Kidney	39–47
Eyes	39–47
Umbilical stalk	40–46
Intestine	57–63
Relative size relationships	
Body diameter 2 mm > head	38–42
Body diameter:chorionic cavity diameter >1:2	38–42
Crown-rump length > placenta	40–42
Body diameter:outer uterine diameter > 1:2	46–48
Parturition	63–65

From Yeager AE, Mohammed HO, Meyers-Wallen V, et al: Ultrasonographic appearance of the uterus, placenta, fetus, and fetal membranes throughout accurately timed pregnancy in beagles. Am J Vet Res 53:342–351, 1992, with permission.

with other causes of inflammation that are not specific to pregnancy, such as uterine infection, positive test results do not prove a pregnancy is present.

An increase in the concentration of CRP was detected in all of nine pregnant bitches studied,[73] increasing at approximately 20 to 25 days after ovulation. In two nonmated control animals, no CRP was detected in any of the serum samples taken during the 10 weeks following estrus. Similarly, in two of three bitches who were mated but failed to whelp, the concentration of CRP was negligible throughout the 10 weeks following estrus. In one bitch that was mated and diagnosed pregnant on day 28 following ovulation but failed to whelp, CRP increased but returned to negligible by day 25 following estrus.

Serum fibrinogen concentrations rise to greater than 250 mg/dl by day 21–30 of gestation.[74–76] Assay of serum fibrinogen as a pregnancy test has been reported to be 98% accurate with a value of greater than 280 mg/dl indicative of pregnancy,[74] and nearly 100% accurate with a value of greater than 300 mg/dl indicative of pregnancy.[76] The latter study described enhanced accuracy if samples drawn 28 days post-breeding were compared to those drawn pre-breeding.[76] In that study, samples were analyzed in-house on the QBC AutoRead (IDEXX Laboratories, Westbrook, ME). The only commercially available assay for fibrinogen in the U.S. was reported to be 93% accurate, but has since been withdrawn from the market.[77]

Hormone Assays for Canine Pregnancy Diagnosis

Pregnant bitches do not produce a pregnancy-specific gonadotropin, such as human chorionic gonadotropin (hCG) in women or equine chorionic gonadotropin (eCG) in mares.

PROGESTERONE

In all normal dogs, serum progesterone concentrations remain high in diestrus regardless of breeding status. Maintenance of corpora lutea occurs because of absence of release of an effective luteolysin from the uterus as is seen in other species after the time of pregnancy recognition.[78] This prolonged luteal phase in dogs precludes ability to use measurement of serum progesterone concentrations for pregnancy diagnosis. Although some studies have documented either higher serum progesterone concentrations in early diestrus in pregnant dogs compared to non-bred dogs,[79] or differences in pattern of progesterone secretion over diestrus when comparing fertile-mated to sterile-mated dogs,[80] the majority of studies have reported either no statistically significant difference between pregnant and non-pregnant dogs or such wide overlap in the absolute values between the groups so as to make interpretation of a single value impossible.[81–86]

Table 5–6. Combined Regression Table of Fetal Biparietal Diameter and Fetal Trunk Diameter for the Prediction of the Number of Days before Parturition in Labrador Retrievers, Golden Retrievers, and their Crosses

Fetal Trunk Diameter (cm)	Fetal Biparietal Head Diameter (cm)																					
	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3
0.6	26	25	24	24	23	23	22	21	21	20	20	19	18	18								
0.8	25	24	24	23	23	22	21	21	20	20	19	18	18	17	17							
1.0	24	24	23	23	22	21	21	20	20	19	19	18	17	17	16	16						
1.2	24	23	23	22	22	21	20	20	19	19	18	17	17	16	16	15	14					
1.4	23	23	22	22	21	20	20	19	19	18	17	17	16	16	15	14	14	13				
1.6	23	22	22	21	20	20	19	19	18	17	17	16	16	15	15	14	13	13	12			
1.8	22	22	21	20	20	19	19	18	17	17	16	16	15	15	14	13	13	12	12	11		
2.0	22	21	20	20	19	19	18	18	17	16	16	15	15	14	13	13	12	12	11	10	10	
2.2	21	21	20	19	19	18	18	17	16	16	15	15	14	13	13	12	12	11	10	10	9	9
2.4	21	20	19	19	18	18	17	16	16	15	15	14	13	13	12	12	11	11	10	9	9	8
2.6	20	19	19	18	18	17	16	16	15	15	14	14	13	12	12	11	11	10	9	9	8	8
2.8	19	19	18	18	17	16	16	15	15	14	14	13	12	12	11	11	10	9	9	8	8	7
3.0	19	18	18	17	17	16	15	15	14	14	13	12	12	11	11	10	9	9	8	8	7	7
3.2	18	18	17	17	16	15	15	14	14	13	12	12	11	11	10	9	9	8	8	7	7	6
3.4	18	17	17	16	15	15	14	14	13	12	12	11	11	10	10	9	8	8	7	7	6	5
3.6	17	17	16	15	15	14	14	13	13	12	11	11	10	10	9	8	8	7	7	6	5	5
3.8	17	16	16	15	14	14	13	13	12	11	11	10	10	9	8	8	7	7	6	5	5	4
4.0	16	16	15	14	14	13	13	12	11	11	10	10	9	8	8	7	7	6	6	5	4	4
4.2	16	15	14	14	13	13	12	11	11	10	10	9	8	8	7	7	6	6	5	4	4	3
4.4	15	14	14	13	13	12	11	11	10	10	9	9	8	7	7	6	6	5	4	4	3	3
4.6	14	14	13	13	12	12	11	10	10	9	9	8	7	7	6	6	5	4	4	3	3	2
4.8	14	13	13	12	12	11	10	10	9	9	8	7	7	6	6	5	4	4	3	3	2	2
5.0		13	12	12	11	10	10	9	9	8	7	7	6	6	5	5	4	3	3	2	2	1
5.2			12	11	10	10	9	9	8	8	7	6	6	5	5	4	3	3	2	2	1	0
5.4				10	10	9	9	8	8	7	6	6	5	5	4	3	3	2	2	1	0	0
5.6					9	9	8	8	7	6	6	5	5	4	3	3	2	2	1	0	0	
5.8						8	8	7	6	6	5	5	4	3	3	2	2	1	1	0		
6.0							7	6	6	5	5	4	4	3	2	2	1	1	0			
6.2								6	5	5	4	4	3	2	2	1	1	0				
6.4									5	4	4	3	2	2	1	1	0					

From England GCW, Allen WE, Porter DJ: Studies on Canine pregnancy using B-mode ultrasound: Development of the conceptus and determination of gestational age. J Small Anim Pract 31:324–329, 1990, with permission.

Table 5-7. Optimal Time for Diagnosing Canine Pregnancy with Various Testing Procedures

Test	Optimal Time for Diagnosing Pregnancy	Comments
Abdominal palpation	28–30 d after ovulation 31–33 d after LH surge	Variable
Radiograpic diagnosis of calcified feti	> 42 d after ovulation > 45 d after LH surge or first mating	Variable
Ultrasonographic diagnosis	> 22 d after ovulation > 25 d after LH surge	Can also determine fetal viability
Acute-phase proteins	> 25 d after ovulation > 28 d after LH surge > 28 d after mating	Not specific
Serum relaxin	> 25 d after ovulation > 28 d after LH surge > 21 d after mating	Variable

RELAXIN

Relaxin is a hormone produced primarily by the canine placenta and is, therefore, the nearest thing to a pregnancy-specific hormone known in the dog.[78,87] Serum relaxin concentrations in pregnant dogs rise significantly compared to non-pregnant dogs, beginning at 20 to 30 days of gestation,[74,77,78] and peak at mid-gestation.[77,78] An enzyme-linked immunosorbent assay (ELISA) for canine relaxin (Repro-CHEK, Synbiotics Corporation, San Diego, CA) is reported to detect pregnancy as early as 21 days after breeding. False negatives may occur when bitches are tested as early as 21 days of gestation; negative results in early gestation should be re-checked by repeating the test 7 to 10 days later.

PROLACTIN

Serum prolactin concentrations rise spontaneously in the latter half of diestrus, with a significantly greater elevation in pregnant dogs than in non-pregnant dogs by day 30 to 45 of gestation.[78,86,88,89] In early pregnancy, serum prolactin concentrations can be stimulated to rise with administration of the opioid antagonist naloxone.

FOLLICLE STIMULATING HORMONE

Serum follicle stimulating hormone (FSH) concentrations fall to less than 150 ng/ml post-implantation (16 to 18 days after conception) in non-pregnant dogs and remain greater than 150 ng/ml in pregnant dogs.[77]

ESTROGEN

Total estrogen concentrations in urine have been reported to be increased 21 days post-mating in pregnant dogs compared to non-pregnant dogs.[81] Assay of estrogen in urine may be marketable as an in-house assay for veterinarians or an in-home pregnancy diagnostic test for clients.

Care of the Pregnant Bitch

Proper client education is essential in managing a normal canine pregnancy and parturition. It is desirable to examine the bitch for pregnancy diagnosis at about 4 weeks of gestation (Table 5-7). The decision to examine the bitch at other times is based on the potential risk for dystocia for the individual or breed, historical information on past diseases or disorders that could complicate the pregnancy, and the risk of exposure to infectious agents and stress at a veterinary clinic. For example, while it might be desirable to examine a young bitch during the last week of pregnancy to determine whether pelvic size is sufficient for the delivery of pups, it also is desirable to prevent a previously unexposed bitch from contracting herpesvirus infection during the last 3 weeks of pregnancy (see Canine Herpesvirus Infection below). Any pregnant bitch showing significant signs of illness should be examined. Some diagnostic testing, such as fecal examinations and urinalyses, can be performed on samples without exposing the bitch to potential infectious agents found in veterinary clinics.

Pregnant bitches should receive moderate exercise and good nutrition (see below). Vac-

cines, especially modified-live vaccines, should be avoided during pregnancy unless there is a substantial risk of exposure to an infectious disease in a previously unvaccinated animal. Clients should be encouraged to have bitches properly immunized prior to breeding to avoid potential harm to fetuses by the administration of a modified-live vaccine during pregnancy.

If a previous dystocia has occurred, the veterinarian should instruct the client on obtaining the bitch's rectal temperature two to three times per day, beginning 54 days after breeding. The method of obtaining accurate temperatures should be taught, and the client should be instructed to plot the temperatures on graph paper. Along with other signs, a temperature chart can help predict the onset of parturition (see Chapter 6).

Nutritional Requirements during Pregnancy

Owners are most likely to err by overfeeding bitches during early pregnancy and underfeeding them during lactation. The best chances of a normal delivery occur when bitches receive good nutrition and moderate exercise. A normal maintenance diet, in normal amounts, should be fed to bitches during the first two thirds of pregnancy.[90] Less than 30 per cent of fetal growth occurs during the first 5 to 6 weeks of gestation. Therefore, there need be little or no change in the bitch's body weight or nutritional needs during early pregnancy.

Fetal size rapidly increases during the last 3 to 4 weeks of gestation. As a result, the body weight of the bitch should increase 25 to 30 per cent by the time of whelping. Therefore, during the last 3 to 4 weeks of pregnancy, the amount of food should gradually be increased so that the bitch is receiving 25 to 30 per cent more food by whelping. The exact increase in food will vary, depending on the number of fetuses. Bitches in late pregnancy also should be gradually switched to a diet that contains higher levels of protein, carbohydrates, and minerals than required for maintenance (i.e., a growth/lactation-type diet).[91] Nutrient-dense foods (3.6 kcal/kg food dry matter or higher) may be necessary in late pregnancy for bitches that have decreased stomach capacity resulting from carrying a large litter.[90] The consumption of smaller meals at increasing frequency is another method of compensating for the reduced stomach capacity. A bitch frequently will decrease food consumption when she enters the first stage of labor. Prior to whelping, however, it is important to monitor that food intake continues to be normal. Some bitches carrying large litters may develop severe and life-threatening ketoacidosis if food consumption becomes inadequate, especially during late gestation (see Pregnancy Toxemia below).

Appetite reduction and decreased food intake can occur in normal bitches during early to midpregnancy. In one study,[41] food consumption decreased during estrus and again by the 10th day of gestation (Table 5–8). At about 3 weeks of gestation, bitches underwent another short period of partial appetite loss for 3 to 10 days. By week 6 of gestation, food consumption had increased by 30 to 50 per cent over that during early proestrus. In many bitches, food intake decreased substantially within 24 to 48 hours of whelping, or during stage I of labor.

Although calcium requirements increase in late gestation and early lactation, they usually are met by feeding a properly balanced diet. Excessive calcium supplementation during late gestation has been incriminated in predisposing the bitch to eclampsia and dystocia and causing soft-tissue calcification, physical abnormalities, and gastric dilation/volvulus in neonatal pups.[90,91] Although the optimal

Table 5–8. Daily Food Consumption during the Reproductive Cycle and Pregnancy

	Relative Food Intake*					
Breed Size	Early Proestrus	Peak Estrus	Day 10 of Pregnancy	Day 25 of Pregnancy	Day 42 of Pregnancy	Day 62 of Pregnancy
Small	1†	−20%	+14%	−36%	+50%	−75%
Medium	1	−14%	+12%	−21%	+35%	−77%
Large	1	−17%	+12%	−33%	+30%	−83%

* Based on a dry dog food containing 1600 calories/lb of dog food.
† Food consumption during the reproductive cycle and pregnancy was compared to that during proestrus. Hence, proestrus was assigned a numerical value of "1" for the purpose of this table.
Adapted from Bebiak et al.[41]

amount of calcium for prepartum bitches of various breeds has not been well studied, such studies have been conducted in other species. Giving large amounts of dietary calcium to dairy cows during late pregnancy predisposes to hypocalcemia. However, even when dietary calcium is constant, diets that increase the pH of blood may predispose cows to develop postpartum hypocalcemia. Parathyroid hormone is apparently less able to stimulate bone calcium release and production of 1,25-dihydroxyvitamin D when the blood is alkaline.[92] Although acidifying the diet by increasing dietary anions reduces the incidence of milk fever in cows, similar studies in dogs are lacking. Until further studies have been reported for dogs, it is probably prudent to avoid excessive calcium and vitamin D supplementation during pregnancy. Whether acidifying the diet would reduce the risk of hypocalcemia in dogs, as it does for cows, remains unknown (see Chapter 7).

Whelping Area

The whelping area may vary, depending on whether the area is designed by a client with a single bitch or by a commercial kennel. All whelping environments should protect the bitch and pups from injury and disease. Excellent sanitation is essential for optimal reproductive efficiency.

New animals with unknown vaccination or disease histories should not be introduced to pregnant bitches or newborn pups. Contact with visitors or with fomites that transmit infectious agents should be prevented; traffic through the whelping area should be minimized for the first 3 weeks of the pup's life. The whelping area should be in a familiar environment that has privacy and is free from drafts, moisture, and excessive cold or heat.

A child's plastic swimming pool (Fig. 5–4A) serves as a good whelping box that can be readily disinfected. The sides of the whelping box should be high enough to prevent the neonates from escaping, and should be lined with bedding that the mother can tear up and rearrange as part of her nesting behavior prior to delivery. The bitch should be introduced to the whelping box about a week prior to whelping. After whelping, the shredded bedding can be replaced with clean towels that can be washed several times per day, if necessary.

Owners may add external sources of heat to keep newborn pups warm (Fig. 5–4B). Although this may be desirable, one must be careful to protect the bitch and pups from excessive heat, dehydration, or burns.

Drug Administration

Administration of drugs to pregnant bitches should be avoided if possible. There is very little information available on the safety of numerous drugs that are administered to pregnant dogs. Many physiologic changes occur during pregnancy that affect drug availability and toxicity.[93] For example, changes in serum albumin may affect the volume of distribution for a drug that is highly protein bound. Changes in cardiac output, renal blood flow, and glomerular filtration rates during pregnancy can alter the availability of drugs eliminated through the kidneys. When treating a pregnant bitch, each drug selected for therapy should be evaluated carefully in light of the physiologic changes of pregnancy (Table 5–3).

Figure 5–4. Examples of whelping "boxes" prepared for the pregnant bitch and neonatal pups **A:** A child's plastic swimming pool serves as a good whelping box and can be disinfected easily **B:** A whelping box prepared with an external heat lamp. While it may be desirable to provide an external source of heat to newborn pups, one must be careful that heating devices are securely attached and do not cause burns to the pups.

Doses may need to be altered and the patient may need to be monitored carefully to ensure that a therapeutic effect is achieved and that toxicity is minimized.

In addition to considering the effect of drugs on the bitch, drugs also must be selected with the developing pup in mind. The developing embryo or fetus becomes an inadvertent recipient of many drugs administered to a pregnant bitch. Adverse drug effects may be embryotoxic or teratogenic, causing abortion or congenital malformations. The critical time for embryotoxicity in the bitch is from 6 to 20 days following the LH surge. During this time, prior to implantation, the embryo is bathed in uterine fluid that attains drug concentrations reflecting those of the maternal extracellular fluids.[93]

Once the placenta has formed, nutrients and drugs given to the dam must cross the placenta to reach the fetus. Although there is no "true placental barrier," there are numerous factors that govern the transfer of drugs across the placental membranes (Table 5–9). Many drugs used to treat pregnant bitches should produce no teratogenic effects (Table 5–10), especially if they are administered for a short time at relatively moderate doses.[93] Therefore, even if a drug reaches the fetus, toxicity will not necessarily occur. Over the years, many pregnant bitches have been treated with numerous drugs, and relatively few reports of drug-induced teratogenicity have been published. Nevertheless, it is prudent to advise clients of the potential danger and select drugs carefully.

Papich reported on numerous drugs used in treating pregnant dogs and cats, and classified them on the basis of drug safety (Table 5–10).[93] Class A drugs are those that are probably safe for use during pregnancy, although specific studies may not have proved the safety in dogs and cats. Class B drugs are safe if used cautiously; studies in some laboratory animals may have revealed some risk. Class C drugs have potential risks; these drugs should be used cautiously and only as a last resort when the benefit of therapy clearly outweighs the risk. Class D drugs are contraindicated during pregnancy; these drugs have been shown to cause congenital malformations or embryotoxicity.

Table 5–9. Factors That Influence the Transfer of Drugs across the Placenta

1. Placental blood supply
2. Age of gestation
3. Placental drug-metabolizing capabilities
4. Drug size
5. Drug's lipid solubility
6. Drug dose
7. Duration of drug exposure
8. Maternal/fetal pH differential
9. Maternal/fetal drug protein-binding differences
10. Species of animal

From Papich MG: Effects of drugs on pregnancy. *In* Kirk RW (ed): Curr Vet Ther Small Anim Pract 10. Philadelphia, WB Saunders, 1989, p 1297, with permission.

Physical Examination and Diagnostic Testing

If the risk for dystocia or disease is great, it is desirable to examine the pregnant bitch one or more times during gestation. In addition to a complete and thorough physical examination, it is desirable to perform ultrasonographic and/or radiographic examinations; obtain a complete blood count, serum chemistry profile, urinalysis, and fecal examination; and submit blood for endocrine testing. For example, measurement of concentrations of serum progesterone may be indicated in bitches who have repeatedly delivered premature, but otherwise normal, pups that died because of their immaturity (see Hypoluteoidism below). Depending on the geographic location, fecal examinations of pregnant bitches may be indicated, especially in young bitches who are more likely to pass parasites to their offspring. Although the diagnostic approach is tailored for each animal, special consideration should be given to disorders that are associated with, or worsen during, pregnancy.

Diseases Associated with or Exacerbated by Pregnancy

Pregnancy Toxemia

At least two types of pregnancy toxemia have been described in different species. In humans, pregnancy toxemia (pre-eclampsia) is associated with hypertension and proteinuria. The condition, as observed in the human, can be induced experimentally in dogs[94] but has not been reported to occur spontaneously in the bitch. Pregnancy toxemia that occurs naturally in pregnant dogs is associated with a relative lack of carbohydrates or alteration in carbohydrate metabolism.

Ketosis usually develops during late gestation in bitches on inadequate nutrition or in those who cannot eat enough carbohydrates to meet energy demands. Bitches carrying large numbers of pups are predisposed to preg-

Text continued on page 86

Table 5–10. Safety of Drugs in Pregnancy in the Dog and Cat

Drug	Recommendation*	Comments
Antimicrobial Drugs		
Amikacin	C	Aminoglycoside antibiotics easily cross the placenta and may cause 8th nerve toxicity or nephrotoxicity.
Ampicillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Amoxicillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Carbenicillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Cephalosporins	A	Crosses the placenta but has not been shown to be harmful to fetus.
Chloramphenicol	C	May decrease protein synthesis in fetus, particularly in bone marrow.
Ciprofloxacin	D	Do not use during pregnancy; quinolones have been associated with articular cartilage defects.
Clavulanic acid–amoxicillin (Clavamox, Beecham)	A	Crosses the placenta but has not been shown to be harmful to fetus.
Clindamycin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Cloxacillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Dicloxacillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Doxycycline	D	Tetracyclines can cause bone and teeth malformations in fetus and may cause toxicity in mother.
Enrofloxacin	D	See ciprofloxacin.
Erythromycin	A	Appears to be safe except for erythromycin estolate, which has been shown to increase the risk of hepatoxicity in women.
Gentamicin	C	Aminoglycoside antibiotics easily cross the placenta and may cause 8th nerve toxicity or nephrotoxicity. However, specific toxicities from gentamicin have not been reported, and it may be used for a serious infection in place of a suitable alternative.
Hetacillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Kanamycin	C	Aminoglycoside antibiotics easily cross the placenta and may cause 8th nerve toxicity or nephrotoxicity.
Lincomycin	A	Crosses the placenta but has not been shown to cause problems in fetus.
Metronidazole	C	Teratogenic in laboratory animals, but there is no information for dogs and cats. It should be avoided during the first three weeks of pregnancy.
Neomycin	A	Not absorbed sufficiently to cause systemic effects after oral administration.
Oxacillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Oxytetracycline	D	Toxic to fetus and may increase risk of hepatitis in mother (see tetracycline).
Penicillin G (benzyl penicillin)	A	Crosses the placenta but has not been shown to be harmful to fetus.
Streptomycin	D	See gentamicin. Streptomycin is associated with higher incidence of 8th nerve toxicity than other aminoglycosides.
Sulfonamides	B	Sulfonamides cross the placenta and have produced congenital malformations in rats and mice, but problems have not been reported in dogs or cats; in people, they have caused neonatal icterus when administered near term. Avoid long-acting sulfonamides.
Tetracycline	D	Tetracyclines can cause bone and teeth malformations in fetus and may cause toxicity in mother.
Trimethoprim-sulfadiazine (Tribrissen, Coopers)	B	Manufacturer states that it is safe during pregnancy in dogs; see also trimethoprim and sulfonamides.

Table continued on following page

Table 5–10. Safety of Drugs in Pregnancy in the Dog and Cat *Continued*

Drug	Recommendation*	Comments
Trimethoprim	B	Teratogenic in rats but probably safe in other species. Folate antagonism and bone marrow depression are possible with prolonged use.
Ticarcillin	A	Crosses the placenta but has not been shown to be harmful to fetus.
Tobramcyin	C	Aminoglycoside antibiotics easily cross the placenta and may cause 8th nerve toxicity or nephrotoxicity.
Tylosin	B	No information is available.
Antifungal Drugs		
Amphotericin-B	C	There are no known teratogenic effects, but amphotericin is extremely toxic. Use only if the disease is life threatening, in absence of a suitable alternative.
Griseofulvin	D	Teratogenic in rats; causes multiple skeletal and brain malformations in cats.
Ketoconazole	B	Teratogenic and embryotoxic in rats; antiandrogenic; stillbirths have been reported in dogs.
Miconazole	A	Apparently safe if applied topically.
Antiparasitic Drugs		
Amitraz	C	Manufacturer states that reproduction studies have not been done; no information available.
Diethylcarbamazine	A	Manufacturer states that the drug may be given to dogs throughout gestation.
Dithiazanine iodide (Dizan, TechAmerica)	B	No information is available; iodide salts may cause congenital goiter if administered for prolonged periods during pregnancy.
Fenbendazole	A	Safe. Has been administered to pregnant bitches without producing adverse effects.
Dichlorvos (Task, Solvay)	B	Caution is advised when administering cholinesterase inhibitors to pregnant animals; it should not be administered to puppies or kittens, but studies in pregnant dogs and cats suggest that there are no adverse effects during pregnancy.
Ivermectin	A	Safe. Reproduction studies in dogs, cattle, horses, and pigs have not shown adverse effects.
Levamisole	C	No information available.
Mebendazole	A	Safe. In reproduction studies in dogs, it was not teratogenic or embryotoxic.
Piperazine	A	Safe. No known contraindications for the use of piperazine.
Praziquantel	A	Safe. No adverse effects were seen when tested in pregnant dogs and cats.
Thiacetarsamide (Caparsolate sodium, CEVA)	C	No specific information regarding toxicity to fetus is available. It can be hepatotoxic and nephrotoxic, and heartworm adulticide should be postponed until after parturition.
Bunamidine	A	Has been administered to pregnant bitches without problems and is safe in pregnant cats. Slight interference with spermatogenesis has been seen in male dogs.
Pyrantel	A	Safe. Toxicity studies have not shown any adverse effects.
Thenium	A	Safe. Manufacturer states that except in young puppies, there are no known contraindications.
Thiabendazole	B	Thiabendazole is not teratogenic in laboratory animals, but high doses have produced toxemia in ewes.
Trichlorfon	C	Caution is advised when administering organophosphates to pregnant animals. Congenital toxicoses have been reported following administration to pregnant sows. Manufacturer states that trichlorfon should not be administered to pregnant mares, but there are no recommendations for dogs and cats.
Anticancer Drugs		
Doxorubicin hydrochloride (Adriamycin, Adria)	C	May produce malformations in newborn or embryotoxicity.

Table continued on opposite page

Table 5-10. Safety of Drugs in Pregnancy in the Dog and Cat *Continued*

Drug	Recommendation*	Comments
Azathioprine	C	May produce congenital malformations but has been used in pregnant women safely. It may be a suitable alternative to other drugs when immunosuppresive therapy is required.
Chlorambucil	C	May produce malformations in newborn or embryotoxicity.
Cisplatin	C	May produce congenital malformations, embryotoxicity, or nephrotoxicity.
Cyclophosphamide	C	May produce malformations in newborn or embryotoxicity.
Methotrexate	C	May produce malformations in newborn or embryotoxicity.
Vincristine	C	May produce malformations in newborn or embryotoxicity.
Analgesic Drugs		
Acetaminophen	C	Safety not established in dogs; toxic in cats.
Aspirin	C	Embryotoxicity has been seen in laboratory animals but not in other species. Late in pregnancy, it may produce pulmonary hypertension and bleeding problems.
Flunixin meglumine	C	Safety in pregnancy has not been determined.
Gold (aurothioglucose)	D	Laboratory animal studies clearly show increased congenital malformations.
Ibuprofen	C	Safety in dogs and cats not established.
Indomethacin	C	Can be toxic in adult dogs; can cause premature closure of ductus arteriosus if administered near term.
Phenylbutazone	C	Safety has not been established. Long-term use can depress bone marrow.
Salicylates	C	Embryotoxicity has been seen in laboratory animals but not in other species. Late in pregnancy, it may produce pulmonary hypertension and bleeding disorders.
Anesthetic and Preanesthetic Drugs		
Acepromazine	B	Phenothiazines should be avoided near term; they may produce neonatal CNS depression.
Atropine	B	Crosses the placenta and has been used safely but may cause fetal tachycardia.
Butorphanol	B	Safe for short-term use. Neonatal depression can be treated with naloxone.
Codeine	B	Safe for short-term use. Neonatal depression can be treated with naloxone.
Diazepam	C	See anticonvulsants.
Fentanyl	B	Safe for short-term use. Neonatal depression can be treated with naloxone.
Glycopyrrolate	B	Safe. Does not cross placenta as readily as atropine. Studies in rats and rabbits have not revealed teratogenic effects.
Halothane	C	Decreased learning ability has been reported in rats after *in utero* exposure; depression may be seen in neonates after cesarean section; excessive uterine bleeding may be seen when administered during cesarean section.
Isoflurane	B	Probably safe. Depression may be seen in neonates after cesarean section.
Ketamine	B	Probably safe. Depression may be seen in puppies delivered by cesarean section; may increase intrauterine pressure and induce premature labor.
Lidocaine	A	All local anesthetics appear to be safe when used for a local nerve block or epidural anesthesia.
Meperidine	B	Opiates can produce neonatal sedation and respiratory depression, but the effects can be reversed with the administration of naloxone.
Methoxyflurane	C	Neonatal depression is seen when used for cesarean section.

Table continued on following page

Table 5–10. Safety of Drugs in Pregnancy in the Dog and Cat *Continued*

Drug	Recommendation*	Comments
Morphine	B	Opiates can produce neonatal sedation and respiratory depression, but the effects can be reversed with the administration of naloxone.
Naloxone	A	Has been shown to be safe when administered to newborns within a few minutes after birth.
Nitrous oxide	B	Probably safe. Used frequently for cesarean section without adverse effects.
Oxymorphone	B	Opiates can produce neonatal sedation and respiratory depression, but the effects can be reversed with the administration of naloxone.
Pentobarbital	D	Associated with high incidence of neonatal mortality.
Thiamylal	C	Easily crosses the placenta; all barbiturates produce respiratory depression in fetus; however, thiobarbiturates are not as toxic as pentobarbital.
Thiopental	C	Easily crosses the placenta. All barbiturates produce respiratory depression in fetus; however, thiobarbiturates are not as toxic as pentobarbital.
Gastrointestinal Drugs		
Antacids	A	Safe. Not absorbed systemically.
Antiemetics	B	Probably safe if administered short term.
Cimetidine	B	Safety has not been established, but no reports of toxicity in humans.
Dimenhydrinate	B	Safe if used short term.
Diphenhydramine	B	Safe if used short term.
Diphenoxylate	C	Studies have reported adverse effects in laboratory animals, but no adverse effects have been reported in pregnant dogs, cats, and humans.
Laxatives	B	All laxatives, except Castor Oil (Squibb), are considered safe if they are used short term. Castor Oil causes premature uterine contractions.
Loperamide	C	Same comment as diphenoxylate.
Metoclopramide	B	Safe in laboratory animals, but no studies available for cats or dogs.
Methscopolamine	C	Safety not established.
Misoprostol	D	Synthetic prostaglandin, causes a termination of pregnancy.
Prochlorperazine	B	No reports of toxicity when administered short term.
Ranitidine	B	Safety has not been established, but no reports of toxicity were reported in humans.
Sucralfate	A	Probably safe. Not absorbed systemically.
Sulfasalazine	B	Salicylate component is not absorbed enough to produce adverse effects; sulfonamide may produce neonatal icterus when used near term.
Cardiovascular Drugs		
Atropine	B	Probably safe but may produce fetal tachycardia.
Captopril	C	Has been shown to be embryotoxic in laboratory animals and goats.
Digitalis	A	Probably safe. No adverse effects seen in humans and laboratory animals.
Furosemide	B	No adverse effects have been reported.
Dopamine	B	Probably safe at therapeutic doses.
Heparin	B	Does not appear to cross placenta.
Hydralazine	B	Probably safe. There have been reports of minor toxicity in rats, but it has been administered safely to pregnant women.
Isoproterenol	C	May cause fetal tachycardia; beta-adrenergic drugs inhibit uterine contractions.
Lidocaine	B	Probably safe. May cause fetal bradycardia.
Nitroglycerin	C	No information available.
Nitroprusside	C	There is a risk of fetal cyanide toxicity with prolonged use.
Procainamide	B	Probably safe. May cause fetal bradycardia.
Propranolol	C	May cause fetal bradycardia, respiratory depression, and neonatal hypoglycemia; avoid use near term.
Quinidine	B	Probably safe. May cause fetal bradycardia.

Table continued on opposite page

Table 5–10. Safety of Drugs in Pregnancy in the Dog and Cat *Continued*

Drug	Recommendation*	Comments
Theophylline	B	No reports of adverse effects.
Thiazide diuretics	C	May cause increased incidence of perinatal mortality.
Warfarin	D	Causes embryotoxicity and congenital malformations, neural tube defects in laboratory animals and humans.
Anticonvulsant Drugs		
Diazepam	C	Has been associated with congenital defects in mice, rats, and people.
Phenobarbital	B	Has been associated with rare congenital defects and bleeding tendencies in newborn but may be safer than other anticonvulsants.
Phenytoin	C	Teratogenic in rats, mice, and people.
Primidone	C	Same risks as phenobarbital and has been associated with increased incidence of hepatitis in adult dogs.
Valproic acid	C	May cause congenital malformations.
Muscle Relaxants		
Dantrolene	C	Safety not established.
Dimethyltubocurarine	B	Quarternary base with negligible placental transfer; it does not affect the fetus unless administered in large doses.
Gallamine	B	Quarternary base with negligible placental transfer; it does not affect the fetus unless administered in large doses.
Methocarbamol	C	Safety not established; manufacturer states that it should not be administered during pregnancy.
Pancuronium	B	Quarternary base with negligible placental transfer; it does not affect the fetus unless administered in large doses.
Succinylcholine	B	Quarternary base with negligible placental transfer; it does not affect the fetus unless administered in large doses.
Endocrine Drugs		
Betamethasone	C	Corticosteroids have been associated with increased incidence of cleft palate and other congenital malformations, and they may induce premature labor and abortion in dogs.
Cortisone	C	Corticosteroids have been associated with increased incidence of cleft palate and other congenital malformations, and they may induce premature labor and abortion in dogs.
Dexamethasone	C	Corticosteroids have been associated with increased incidence of cleft palate and other congenital malformations, and they may induce premature labor. Dexamethasone has caused abortion and fetal death in dogs.
Diethylstilbestrol (DES)	D	Malformation of male and female genitourinary systems.
Estradiol cypionate (ECP)	D	Malformation of male and female genital tracts and bone marrow depression.
Flumethasone	C	Corticosteroids have been associated with increased incidence of cleft palate and other congenital malformations, and they may induce premature labor and abortion in dogs.
Mitotane (*o, p*-DDD)	D	Adrenocortical necrosis.
Prednisolone	C	Although prednisolone has been administered to pregnant women without adverse effects, caution is advised (see dexamethasone). Prednisolone may be used in serious diseases in absence of a suitable alternative.
Stanozolol	D	Manufacturer states that it should not be administered to pregnant dogs and cats.
Testosterone	D	Causes masculinization of female fetus.
Thyroxine	B	Does not cross placenta easily and has not been associated with any problems.

Table continued on following page

Table 5-10. Safety of Drugs in Pregnancy in the Dog and Cat *Continued*

Drug	Recommendation*	Comments
Miscellaneous Drugs		
Ammonium chloride	B	May cause fetal acidosis; discontinue use during pregnancy.
Aspartame (NutraSweet)	A	No risk.
Dimethylsulfoxide (DMSO)	C	Teratogenic in laboratory animals; manufacturers state that it should not be applied to breeding animals.

* Drugs are classified as follows:
A: Probably safe. Although specific studies may not have proved the safety of all drugs in dogs and cats, there are no reports of adverse effects in laboratory animals or in women.
B: Safe for use if used cautiously. Studies in laboratory animals may have uncovered some risk, but these drugs appear to be safe in dogs and cats or these drugs are safe if they are not administered when the animal is near term.
C: These drugs may have potential risks. Studies in people or laboratory animals have uncovered risks, and these drugs should be used cautiously, as a last resort when the benefit of therapy clearly outweighs the risks.
D: Contraindicated. These drugs have been shown to cause congenital malformations or embryotoxicity.
From Papich MG: Effects of drugs on pregnancy. *In* Kirk RW (ed): Curr Vet Ther 10. Philadelphia, WB Saunders, 1989, pp 1293–1296, with permission.

nancy toxemia during late pregnancy. In one study,[95] pregnant bitches that were fed a carbohydrate-restricted diet had decreased blood glucose levels and corresponding increases in blood β-hydroxybutyrate compared with bitches fed a diet containing 44 per cent metabolizable energy from carbohydrates. The total number of pups between the two groups was similar, but fewer pups from the bitches on the carbohydrate-restricted diet were born alive (63 vs. 96 per cent).

Owners should be concerned if a pregnant bitch develops anorexia during late gestation. Although temporary anorexia can occur during midgestation and during labor in normal bitches, sustained anorexia during the last 2 weeks of pregnancy should alert owners and veterinarians to the possibility of pregnancy toxemia. Diagnosis of pregnancy toxemia is based on the presence of urine ketones in the absence of urine glucose, unlike diabetes mellitus, which also can occur during pregnancy and is associated with an increase of both urine ketones and glucose. Additionally, some bitches with pregnancy toxemia have decreased concentrations of blood glucose,[96] unlike the diabetic bitch with increased concentrations of blood glucose.

Pregnancy toxemia can occur in any breed of dog, but has been observed by the authors in several Yorkshire terriers and Labrador retrievers. If the condition is recognized early in the course of the disease, supplemental nutrition may be all that is required for a full recovery. If bitches are severely affected (i.e., systemically ill, increased liver enzymes), it may be necessary to terminate the pregnancy so that energy demands are reduced and the dam's life is spared. If bitches with pregnancy toxemia require surgery (i.e., cesarean section), analgesics and anesthetics must be selected carefully because hepatic lipidosis or fatty liver syndrome may be present, resulting in diminished metabolism of some drugs. Owners should be advised that pregnancy toxemia is potentially life threatening for the pups and bitch.

Parturition in bitches with pregnancy toxemia may be induced medically through administering glucocorticoids (see Chapter 6), which may also enhance the development of pulmonary function in premature pups. In all cases, careful monitoring of the bitch's condition is required. Repeated testing for urine ketones is helpful in monitoring the effect of treatment. Increased consumption of carbohydrates and decreased urine ketones in a bitch whose attitude is good signifies progress in treating pregnancy toxemia.

Diabetes Mellitus

Pregnant bitches are at risk for alterations in glucose homeostasis and should be evaluated for diabetes mellitus. Because of the increased challenge to maternal carbohydrate homeostasis, it is not uncommon for a bitch to be first diagnosed with diabetes mellitus when pregnant.

In previously diagnosed diabetic animals, insulin requirements often increase during pregnancy, making regulation of the diabetic bitch difficult. In a colony of golden retrievers, insulin requirements increased during the second half of pregnancy but dramatically decreased when whelping began.[97] In some cases, exogenous glucose was necessary to maintain euglycemia during labor.

Because insulin requirements also increase for nonpregnant bitches during diestrus, pro-

gesterone has been implicated in causing the altered carbohydrate metabolism during pregnancy. Diabetes mellitus or altered glucose metabolism in dogs has been experimentally induced with the administration of progestogens.[98–100] While a single injection of progesterone caused a moderate glucose intolerance in dogs, administration of estrogen caused an increased hypoglycemic response to exogenous insulin.[101] Increased concentrations of serum progesterone during diestrus and pregnancy stimulate the secretion of growth hormone in the bitch,[102] which acts as an insulin antagonist.[103] Thus, when concentrations of progesterone decrease at term, insulin requirements may also decline as the mechanism for insulin antagonism is reversed. Macrosomia (large body size) of neonates has been noted in pups born to diabetic bitches, possibly as a result of increased insulin produced by fetal pancreatic tissue in response to excessive availability of glucose and other metabolic fuels from the diabetic dam.[97]

Pyelonephritis

Although not common, pyelonephritis has been observed in pregnant bitches. Pyelonephritis during pregnancy may result from alterations in the urinary system, such as dilation of the ureters and collecting tubules, resulting from hormonal stimulation or urine stasis from increased pressure in the distended abdomen of pregnancy (S. D. Johnston, unpublished class notes, University of Minnesota, 1989). Clinical signs of pyelonephritis include polyuria, polydipsia, vomiting, anorexia, and an unusual gait. Some of these signs are also observed during normal pregnancy, making early suspicion of this disease by owners unlikely. Thus a routine urinalysis should be performed as a screening test on any pregnant bitch.

Cardiac Disease and Hypertension

During pregnancy, there are many physiologic changes in the cardiovascular system, including a decrease in total peripheral resistance and increases in blood volume, cardiac output, stroke volume, and heart rate. Decreases in arterial pressure and pulmonary vascular resistance also occur.[104] The effect of these alterations upon a dog with pre-existing cardiac disease may vary. Although owners should be discouraged from breeding bitches with cardiac disease, such breedings do occur.

Mitral valve insufficiency is the most common cause of congestive heart failure in dogs. In women, pregnancy ameliorates rather than adversely affects the hemodynamics associated with mild to moderate mitral insufficiency.[105] Parturition was reported to be normal in a colony of boxers with idiopathic hypertrophic subaortic stenosis.[104] Similarly, in a colony of Siberian huskies with hypertension, there were no apparent adverse effects on fertility, pregnancy, or delivery.[104] Pregnancy was associated with the onset of acute cardiomyopathy in a 2-year-old Doberman pinscher,[106] although other factors, including the breed predisposition to the disorder, were not ruled out.

Pregnancy Loss

Definitions

Pregnancy loss may occur at any stage of gestation in the dog, and may be manifested by (1) embryonic death and resorption, (2) abortion of a live or dead fetus, (3) stillborn pups, or (4) fetal death, mummification, and retention in the dam's uterus or peritoneal cavity beyond the normal time of parturition. Whether the fetus is resorbed, aborted, born dead, or retained as mummified tissue depends on the cause of pregnancy loss, the stage of gestation at which death occurs, and maternal and fetal responses.[107]

The exact incidence of embryonic and fetal loss is difficult to determine. If fetal death occurs during the first half of pregnancy, *resorption* or unobserved abortion occurs. Owners may be uncertain if a pregnancy was established, since external signs of resorption may be minimal. Because there is no early pregnancy test available for the bitch (i.e., first 1 to 2 weeks of pregnancy), veterinarians also may be uncertain that resorption occurred. In examining the uteri of 22 pregnant beagle bitches from 22 to 54 days postcoitum, 13 of 117 embryos and fetuses (11 per cent) were found to be in different stages of resorption.[108] In another study,[109] 13 resorptions were present among 98 fetal implantation sites (13 per cent) in 12 beagle bitches examined by hysterotomy at 48 days of gestation.

If fetal death occurs during the second half of pregnancy, *abortion* or birth of stillborn pups occurs. Abortion is defined as the expulsion of a dead conceptus or a living one incapable of independent life. Although abortion is asso-

ciated with a hemorrhagic uterine discharge passing from the vulva, the diagnosis of an abortion may be difficult, especially if the bitch consumes or hides the aborted fetuses.[110] Signs of clinical illness may be absent in bitches who abort, and aborted fetuses may have relatively few gross pathologic changes, or may be macerated or mummified. If a fetus dies in late gestation and is not aborted, fetal emphysema and maceration often result. Bitches who retain macerated fetuses frequently have a foul and fetid uterine discharge and may become systemically ill, showing signs of toxemia or septicemia.

The incidence of *stillbirth* in the dog is reported as 2.2 to 4.6 per cent, occurring with similar incidence in male and female fetuses.[107] The incidence of stillborn pups increases with dystocia (see Chapter 6). In one clinical study,[111] the mortality rate of pups was 22.3 per cent for 159 bitches who were experiencing dystocia. Weak or diseased pups also may be born alive but die during the neonatal period (see Chapter 8).

Fetal death occurring in late gestation that is not associated with abortion or maceration may be followed by fetal *mummification*. Fetal mummification does not occur during the first half of pregnancy because embryonic or fetal death prior to the development of fetal bones usually is followed by resorption. Mummification results when autolytic changes in the fetus, absorption of placental and fetal fluids, and involution of the maternal placenta occur. Conditions necessary for fetal mummification include the maintenance of a dead fetus within a uterus that also contains a normal viable fetus or fetuses or the persistence of luteal tissue although canine mummified fetuses may persist after luteolysis.[50] Although fetal mummification and retention in utero have been described in the bitch, the actual incidence is unknown, but presumed to be low.

Causes of Embryonic/Fetal Death in the Dog

There are both noninfectious and infectious causes of embryonic and fetal loss in the dog. Infectious causes of embryonic and fetal loss have received more attention in the literature, either because of a higher incidence or because they are easier to diagnose.[110] The diagnosis of embryonic or fetal death can be difficult, especially if tissues are improperly collected or submitted to a diagnostic laboratory. An aborted fetus, and the placenta if possible, should be chilled (4°C), but not frozen, and submitted to a diagnostic laboratory within 24 hours of death. If several fetuses are aborted, more than one fetus should be submitted to the laboratory to increase the possibility of a diagnosis. Histologic, microbiologic, toxicologic, and chromosomal analyses can be performed on the fetal tissues. Serum from the dam should also be submitted for serologic testing, pending the results of the necropsy on the fetus (Table 5–11).

INFECTIOUS CAUSES OF EMBRYONIC OR FETAL DEATH

Bacteria

Brucella canis. In 1966, *Brucella canis* was identified as the causative agent of canine brucellosis,[112] a contagious disease characterized by embryonic death, abortions, testicular atrophy, epididymitis, scrotal dermatitis, infertility, and generalized lymphadenitis in both sexes.[113,114] Nonreproductive tissues also can become infected, such as the kidney, eye, meninges, intervertebral disk, and skin.[115] Diskospondylitis also has been associated with chronic *B. canis* infection.[116] Infected dogs can remain asymptomatic; fever is not a character-

Table 5–11. Samples to Submit for Diagnosis of Fetal Loss in the Dog (Abortion, Premature Pups, Stillborn Pups)

Sample	Comments
Whole fetus	Chilled to 4°C and submitted within 24 h of abortion or death
Fetal tissues Heart Lung Stomach and contents Gut Kidney Liver Brain	Chilled to 4°C and submitted within 24 h of abortion or death
Bitch's serum	5 ml for various serologic tests, pending necropsy results of fetus
Bitch's vaginal or uterine discharge or swab of discharge	Transport medium will vary depending on suspected pathogen (i.e., Amies transport medium for mycoplasma, viral transport medium for viral isolation, etc.). Contact laboratory for collection and shipping instructions.
Placenta	Chilled to 4°C and submitted within 24 h of abortion or death

istic finding. Canine brucellosis has been diagnosed in many breeds and in all parts of the United States, as well as in several countries.

Abortions at 7 to 9 weeks of gestation, and prolonged vaginal discharge following abortion, are characteristic of canine brucellosis. Canine brucellosis should be suspected whenever a healthy bitch aborts approximately 2 weeks before term, or if a bitch fails to whelp after apparent successful matings. Some infected bitches give birth to stillborn or weak pups at term.

The most common mode of transmission of canine brucellosis is by the ingestion of organisms in aborted placental tissues and vaginal discharges of infected bitches. Mammary secretions, urine, saliva, nasal secretions, and semen may contain organisms and serve as potential fluids for transmission.[117–119] *Brucella canis* infects susceptible animals via mucosal surfaces such as the oral cavity, vagina, and conjunctiva. The minimal oral infectious dose is 10 to 100 times greater than the minimal infectious dose for conjunctival transmission.[120] Venereal transmission appears to occur most frequently when infected males are bred to susceptible females and somewhat less when susceptible males are bred to infected females.[119] Although neutered animals with brucellosis are less likely to transmit the disease, transmission between sexually mature male dogs has been reported to occur after 4 to 6 months of cohabitation.[119]

Definitive diagnosis of canine brucellosis can be made by isolating the organism from infected tissues or blood. It is important to submit specimens that maximize the chances of recovering the organism on culture (Table 5–12). Within 2 to 4 weeks after infection, blood cultures are positive for *B. canis* and remain positive for at least 30 weeks and sometimes years.[121] The majority of dogs (80 per cent) have positive blood cultures for at least 1 year; less than 25 per cent of infected animals have positive blood cultures after 58 months.[115,120]

Serologic testing is available for presumptive, but not definitive, diagnosis of canine brucellosis (Table 5–13). A rapid card agglutination test (RCAT) modified with 2-mercaptoethanol is available for veterinarians to use in house. (Synbiotics Corporation, San Diego, CA). The RCAT is a sensitive but not specific serologic test to screen dogs for canine brucellosis. A definitive diagnosis of canine brucellosis should not be made solely on the basis of the RCAT because false positives are common. Cell wall antigens of *B. canis* are not unique, and are similar to antigens of *Brucella ovis*, *Brucella abortus*, mucoid *Pseudomonas*, mucoid *Staphylococcus* species, and *Bordetella bronchiseptica*.[121] Antibodies produced against any of these organisms can cross react in the *B. canis* test, yielding a false-positive test result. In fact, *B. ovis* is the antigen that was used in the RCAT for years. False-positive test results may decrease if M-strain *B. canis* becomes commercially available for the RCAT.

To reduce the number of false-positive results in the current RCAT, 2-mercaptoethanol (2-ME) can be added to the dog's serum before testing. The 2-ME eliminates the cross-reaction of some nonspecific agglutinins and immunoglobulin M (IgM). Thus a negative test result after 2-ME has been added indicates that the first result was a false positive or that the dog is in the early stage of infection with *B. canis* where only IgM is present.

Because false positives can occur with the RCAT, serum should be retested with the agar gel immunodiffusion (AGID) test. In 1993, the American Association for Veterinary Diagnostic Laboratories agreed that the Cornell Diagnostic Laboratory would serve as a *B. canis* reference laboratory. Therefore, serum samples from RCAT test–positive dogs should be sent to the Cornell Diagnostic Laboratory for further testing. The Cornell Diagnostic Laboratory will re-evaluate the serum with the AGID test using cytoplasmic protein antigens. Because the cytoplasmic protein antigens are more unique to *Brucella* species than the cell wall antigens, the AGID test using cytoplasmic antigens is more specific. However, the AGID test may be positive in dogs that have been exposed to *B. ovis*, *B. abortus* and *Brucella suis*[115] because cytoplasmic antigens may be shared among the *Brucella* species. The disadvantage of the AGID test is that it is less sensitive than the RCAT in detecting early infections and may not become positive until 4 weeks after the RCAT. A blood culture at the early stage of infection should, however, be positive, but a negative culture at this time does not rule out the disease.

Antibiotic therapy can be considered for infected bitches, but has not been proven to result in a cure. Therapy is often unsuccessful in eliminating the organism because of the intracellular location of *B. canis*. Eradication of infected animals has been suggested as necessary, especially in kennel situations.[115] Therapy can be attempted for a pet animal, providing the owner is aware that canine brucellosis can cause disease in humans. Immunocompetent humans tend to be more resistant to *B. canis*

Table 5-12. Confirmation of *Brucella canis* Infection in the Dog

Material to Culture	Time to Culture	Expected Results
Postabortion discharge	When present	Positive
Placenta	When present	Positive
Abortus	When present	May be negative
Semen	3–11 weeks after infection	Positive
	12–60 weeks after infection	Positive but few organisms are shed
	>60 weeks after infection	Negative
Blood	5–30*weeks after infection	"100%" positive
Blood	6–12 months after infection	>80% positive
	28–48 months after infection	50%–80% positive
	48–58 months after infection	25%–50% positive
	>58 months after infection	<25% positive
Epididymis	35–60 weeks after infection	50%–100% positive
	>100 weeks after infection	Negative
Urine	8–30 weeks after infection	Usually positive; more organisms shed by male than by female
Prostate	Until 64 weeks after infection	Usually positive
Lymph node, spleen, and bone marrow	When animal is bacteremic	Usually positive
	When animal is abacteremic	Positive or negative
Eye	When uveitis is present	Usually positive
Intervertebral disk	When diskospondylitis is present	Positive or negative

* Blood cultures are intermittently positive 30 weeks after infection.
Modified from Johnson CA, Jacobs J, Walker RD: Diagnosis and control of *Brucella canis*. *In* Proceedings of the Annual Meeting of the Society for Theriogenology, San Diego, August 16–17. Nashville: Society for Theriogenology, 1991, p 239, Reprinted from Johnson CA, Walker RD: Clinical signs and diagnosis of *Brucella Canic* infection. Compend Contin Educ Pract Vet 14:767, 1992, with permission.

than to other *Brucella* species and generally respond well to appropriate therapy if infected; immunocompromised individuals, pregnant women, and children should avoid exposure. The majority of infections in people result from laboratory accidents where someone has been directly exposed to the organism, or from the handling of infected animals. Symptoms in infected humans include fever, sweats, weakness, weight loss, headache, lymphadenopathy, and splenomegaly. Agglutination tests that utilize only *B. abortus* antigen are negative for persons with *B. canis* infection.[122] Tetracycline, demethylchlortetracy-

Table 5-13. Serologic Tests for *Brucella canis* in the Dog

Serologic Test*	Antigen	Time Frame for Positive Results	Comments
2-ME–RCAT	Cell wall	8–12 weeks after infection to 3 months after the animal is abacteremic	Very sensitive; false-positive results are common; few (1%) false-negative results are reported; easy and fast
2-ME–TAT	Cell wall	10–12 weeks after infection to 3 months after the animal is abacteremic	Semiquantitative; false-positive results are possible
AGID test	Cell wall	12 weeks after infection to 4 months after the animal is abacteremic	Test procedure is complex: more specific than 2-ME–RCAT
AGID test	Cytoplasmic	12 weeks after infection to 36 months after the animal is abacteremic	Most specific serologic test but not sensitive; detects chronic cases when other tests give negative results
ELISA	Cell wall	Unknown (expect time to be similar to that observed with the TAT)	Very specific; less sensitive than TAT; limited availability

* RCAT, card slide agglutination test; 2-ME, 2-mercaptoethanol; TAT, tube agglutination test; AGID, agar gel immunodiffusion; ELISA, enzyme-linked immunosorbent assay.
Data from Carmichael LE, Greene CE: Canine brucellosis. *In* Green, CE (ed): Infectious Diseases of the Dog and Cat. Philadelphia, WB Saunders, 1990, pp 573–584.

cline, doxycycline, lymecycline,[123] and minocycline hydrochloride have been used for treating brucellosis in humans.

A synergistic effect between tetracycline and streptomycin was demonstrated in brucella-infected mice.[124] Minocycline administered orally at a dose of 25 mg/kg once daily for 2 weeks and dihydrostreptomycin administered intramuscularly at a dose of 5 mg/kg every 12 hours for 1 week was associated with temporary remission of canine brucellosis. Using this regimen, 15 of 18 experimentally infected dogs had negative blood cultures at the time of euthanasia (6 to 28 weeks following therapy).[125] However, relapse can occur weeks to months following treatment. An apparent cure of 94 per cent of dogs with canine brucellosis also has been reported following therapy with tetracycline hydrochloride (30 mg/kg twice daily for 28 days) and intramuscular streptomycin (20 mg/kg once daily for 14 days).[126] Unfortunately, dogs that appear "cured" may relapse after cessation of therapy, during estrus, or during pregnancy. Animals that were treated with antibiotics and are still infected may have negative blood cultures and negative serologic tests for weeks to months when a true cure has not occurred.[127] Some infected bitches who were treated with antibiotics have gone on to whelp living pups.[128] It is unknown if pups born to infected bitches can seroconvert upon reaching puberty.

Other antibiotics, such as ampicillin, trimethoprim, sulfadiazine, rifampin, declomycin, sulfadimethoxine and fluoroquinolones alone or in combination also have been used without complete success to treat infected bitches.[116,125] Because the reproductive tract is one reservoir of organisms, pet owners wishing to treat their animals are encouraged to have the pet neutered and reminded that therapy will likely result in remission but not cure. Pets that are treated and neutered are less likely to discharge the large numbers of organisms necessary to result in human infection.

Other* Brucella *species Infections. *Brucella abortus, B. suis,* and *B. melitensis* all have been associated with abortion in the bitch,[110,129,130] but the role of these *Brucella* species in causing reproductive diseases in dogs is not great. Exposure to infected livestock usually is a key part of the history.

Abortion at 38 to 39 days of canine gestation caused by ingestion of *B. abortus* in infected milk, udders, or offal has been reported in bitches in the United States, Germany, and Great Britain.[131] Natural infection also has been reported to occur following ingestion of contaminated bovine placentas and aborted fetuses.[120] *Brucella abortus* biovar 4 was isolated from 14 of 14 dogs on 10 farms with *Brucella*-infected cattle.[132] Only 3 of the 10 farms had two or more dogs, including a sexually intact female, and none of these dogs had a history of whelping or reproductive disease. Infected dogs may have the potential to infect cattle, and possibly humans, and pose a threat for longer duration of disease transmission in cattle than previously assumed. The RCAT used to screen for canine brucellosis does not provide diagnostic information on infection for some of the other brucella species, so specific screening for each organism may need to be performed.

Campylobacter. *Campylobacter* species, bacteria that have been associated with abortions and stillbirths of sheep and cattle, also have been isolated from tissues of aborted pups as well as from apparently healthy dogs.[133] Ninety-five *Campylobacter* species isolates were isolated from rectal swabs collected from 362 healthy dogs, with prevalence greater for dogs less than 6 months of age than adults, for dogs living under high density and cohabitation housing conditions, and during the autumn season.

Campylobacter jejuni was isolated from a Wheaton terrier bitch who aborted, and *Campylobacter* of undetermined species was isolated from aborted poodle pups.[134] The Wheaton terrier entered premature labor on the 45th day of gestation and required treatment for dystocia. One live and two dead fetuses were removed through the vagina. The live fetus died within minutes of delivery. Other than the abortion, the bitch had no signs of illness. The owner of the Wheaton terrier indicated that two children in the family had been ill with an undiagnosed gastrointestinal disease approximately 2 weeks before the bitch aborted. Pure cultures of *C. jejuni* were isolated from the liver and stomach contents obtained from one of the pups. A swab of the dam's cervical area was cultured; the cervical swab yielded colonies of mixed flora, but colonies of *Campylobacter* predominated.

Tissues of aborted pups from another bitch were submitted for histologic evaluation from a kennel experiencing diarrhea and vomiting in adult dogs and the birth of weak and dead pups during an 8-month period.[134] Histologic

examination revealed pneumonic lesions, including generalized collapse, acute bronchiolar epithelial necrosis, focal hyperplasia, and numerous macrophages with alveoli. The request for additional samples was made. Three premature poodle pups from a subsequent litter were then submitted. Although specific microscopic lesions were not observed in the poodle pups, a *Campylobacter* species was isolated from fetal lung and liver tissues. The culture was lost before final identification of the species could be made.

Campylobacter species may be zoonotic and have public health significance. Animal-to-human transmission is generally implied, but infections also may go from humans to animals. However, in an epidemiologic study of human campylobacteriosis, 38 dogs, 9 pups, and 13 cats were examined.[135] None of the dogs or cats had diarrhea, and *Campylobacter* was not recovered from the animals. Because *Campylobacter* has public health significance, it is prudent to culture fetal tissues (liver, lung, stomach), placenta, and vaginal swabs obtained from an aborting bitch. Because *Campylobacter* requires special culture media, the diagnostic laboratory conducting the testing should be contacted for instructions for proper sample submission and alerted that *Campylobacter* is a consideration. It has been recommended that fetal or pup tissue, including stomach or stomach contents, as well as placenta and vaginal swabs of the bitch, be submitted for bacterial culture from all cases of aborted or premature nonviable pups.[134]

If campylobacterosis is identified in a kennel, adult dogs may be treated with oral erythromycin (10 to 15 mg/kg every 8 hours) or oral neomycin (2.5 to 5 mg/kg every 12 hours).[110] Other antibiotics that have been reported as effective include tetracycline and chloramphenicol. The animal should be treated for at least 5 days or 1 to 3 days beyond resolution of clinical signs (i.e., resolution of diarrhea). Antibiotic treatment may not eradicate the bacteria in a kennel, and reinfection can occur. Intermittent or chronic infections may need to be treated for weeks.

Salmonella *species.* Salmonellae are mobile, non-spore-forming, gram-negative bacilli of the family Enterobacteriaceae. Salmonellae are found worldwide and have important public health implications, causing mild to severe gastrointestinal disease in humans.[136] *Salmonella* infections may be severe and life threatening in immunocompromised people.

The most common source of infection, which occurs through the gastrointestinal route, is contact with contaminated food, water, or fomites. The frequency of fecal isolation from healthy or hospitalized dogs has been reported as 1 to 36 per cent.[137-140] The ability of salmonellae to infect a dog and cause clinical illness depends on many factors. Antibiotic therapy may reduce the resistance of a dog to salmonellosis and prolong the course of illness. Dogs developing salmonellosis in a hospital outbreak were at risk if they had received prior antimicrobials, especially ampicillin.[141] Age is another important variable. Pups less than 1 year of age are more susceptible to infection and clinical illness than are adult dogs.[136]

Salmonella panama has been isolated from the fetuses of two related boxer bitches.[142] In one case, a boxer pup was aborted at day 52 of pregnancy, with two dead and seven live pups later delivered by cesarean section. In the second case, one dead and five live pups were delivered by cesarean section on day 56 of gestation. On macroscopic examination, the placenta and organs of the aborted fetus from the first case were partially autolyzed. The fetal liver was enlarged and adhered to the body wall. Cultures were obtained from the placenta and fetal heart, liver, lung, kidney, and spleen. The fetus from the second case was well preserved without visible gross abnormalities. No placenta was submitted from the second case, but similar fetal tissues were submitted for culture as for the fetus in the first case. In addition, stomach contents and meconium were cultured from the second case. A pure culture of *S. panama* was isolated from organs sampled and the placenta. Subsequent examination of rectal swabs from the two bitches, surviving pups, and other dogs from the same establishment failed to produce isolates of *S. panama*. Possible sources of infection considered included the feeding of inadequate cooked offal and the introduction of an imported male into the kennel.

Salmonella species have been reported present in 12.1 per cent of 4752 canine fecal samples.[143] Of those positive, 9 per cent were *S. panama*. Their role in fetal death may be that of a primary pathogen or a secondary pathogen, ascending into the uterus at the time of fetal death. *Salmonella* species are not considered to be part of normal vaginal flora (see Chapter 12).

Treatment depends on the clinical signs. Animals that are bacteremic should receive appropriate antibiotics. Drugs that have been

suggested for treating salmonellosis include chloramphenicol, trimethoprim-sulfonamides, and amoxicillin.[110] There is strong evidence that antibiotic treatment can prolong the *Salmonella* carrier state in humans. The traditional anti-*Salmonella* drugs for treating humans have been ampicillin, chloramphenicol, and furazolidone.[141] However, these drugs may promote the carrier state. Conversely, treatment of *Salmonella* enteritis with trimethoprim-sulfamethoxazole or oral polymyxin B has been reported to shorten the period of *Salmonella* shedding and to cure permanent carriers.[145] Animals with only diarrhea should not be given antibiotics because the gastroenteritis is self-limiting and several antibiotics promote a carrier state. Additionally, antibiotic therapy may promote the development of bacterial resistance.

Prevention depends on isolating infected animals from other animals and providing excellent sanitation to reduce the spread of the organisms. Airborne transmission occasionally may occur. Dogs usually acquire the infection from fomites or by drinking contaminated water and foods. Salmonellae can survive for relatively long periods outside the host.[136] Prevention can be compromised by the indiscriminate use of antibiotic therapy. It takes 1 million times more *Salmonella* organisms to produce disease in an untreated animal than in an animal pretreated with an antibiotic.[144] Therefore, it seems prudent to avoid giving prophylactic antibiotics to pregnant bitches, especially those who are clinically normal, without signs of infectious disease.

Escherichia coli. *Escherichia coli* is the most common bacterium isolated from the canine vagina. It is also commonly cultured from the uteri of bitches with metritis and pyometra. *Escherichia coli* was isolated from the vaginal swabs and uterine exudate obtained from a 5-year-old Airedale bitch who aborted two fetuses at day 41 of pregnancy and subsequently delivered two live and three dead pups by cesarean section on day 61 of pregnancy.[146] At the time of abortion, the bitch had a hemorrhagic discharge passing from the vulva and was anemic. The discharge subsided with antibiotic therapy. Concentrations of progesterone at the time of abortion were considered normal for the stage of pregnancy (i.e., 20.5 ng/ml). The two fetuses alive at the time of cesarean section died within 2 days. The author speculated that endotoxins induced decidual hemorrhage and caused complete placental separation for the two fetuses that aborted. *Escherichia coli* produces an endotoxin that has been shown to be an abortifacient in other species and may result in pregnancy termination in the bitch.

Bitches with pyometra occasionally have concurrent purulent material and fetuses present in the uterus (P. N. Olson, unpublished observations, Colorado State University, 1981–1989). The fetuses may be viable, or in various stages of degeneration. In some cases, antibiotic therapy may prevent further fetal loss as monitored by ultrasonography and eventual birth outcome. In other cases, ultrasonography may reveal progressive death of fetuses in spite of antibiotic therapy, necessitating medical or surgical treatment for the pyometra (i.e., prostaglandin therapy or ovariohysterectomy).

Streptococci. β-Hemolytic streptococci are common isolates from the normal canine vagina that have been associated with infertility, uterine disease, abortion, and neonatal septicemia. Mantovani and co-workers reported the presence of a β-hemolytic streptococcus (type L) from the vaginal swabs ($n = 5$) and lymph node aspirates ($n = 2$) of five collie bitches from a kennel with a history of abortion, infertility, and neonatal death.[147] For 2 years, abortion in the kennel occurred mainly between days 30 and 40 of gestation. During the third year, however, abortions were mainly observed during the last 10 days of pregnancy. Abortion or neonatal death occurred when the streptococci isolated from the clinical cases were later inoculated into experimental bitches.

β-Hemolytic streptococci, like many other potential pathogens, are part of the normal vaginal flora in dogs. Therefore, merely isolating a β-hemolytic streptococcus from a vaginal swab does not necessarily establish the cause for reproductive failure or pregnancy termination. Twenty-one β-hemolytic streptococcal isolates, types C or G, were isolated from vaginal swabs obtained from 125 normal adult bitches in various stages of the estrous cycle.[148]

Other Bacteria. Various bacteria have been associated with abortion in dogs. Because bacterial organisms of various types are normally present in the caudal reproductive tract of healthy bitches, it is difficult to determine whether an organism was the primary factor in the fetal death and abortion or invaded after cervical patency and fetal compromise occurred.

Viruses

Canine Herpesvirus Infection. Canine herpesvirus (CHV) infection was first reported in 1965 as the cause of a fatal viral disease in newborn pups, characterized by generalized focal necrosis and hemorrhage.[149] The disease in the adult dog is mild or inapparent and generally restricted to the respiratory and genital tracts. The spread of CHV is primarily the result of licking (saliva and nasal mucus) or coughing (aerosol). Other modes of infection include in utero transmission and venereal spread.[150] When a pregnant bitch is infected, the virus can spread to the fetus and cause fetal death, mummification, abortion, premature birth, and stillbirths. In one study,[151] fetal infection was established in 28 of 33 pups obtained from seven pregnant bitches inoculated with CHV on days 47 to 53 of gestation. Of the 33 pups born, 2 were stillborn, 5 were born alive and remained healthy throughout the observation period, and 26 pups died in the neonatal period. Three pups (the two stillborn pups and one live-born pup) were runted. Fourteen of the fetal placentae had variable degrees of necrotizing lesions. The lesions included degenerative changes and focal necrosis of the placental labyrinth. Lesions were present in the walls of allantoic blood vessels, and intranuclear inclusions were evident in degenerating trophoblastic cells and in the cells of maternal and fetal blood vessels. Infective virus and viral antigens were detected in various organs of the pups (e.g., liver, spleen, kidney, lung).

Abortion and premature birth may occur in pregnant bitches infected with CHV during the second third of gestation.[152] Abortion occurred approximately 2 to 3 weeks after a pregnant bitch was inoculated with CHV on day 30 of gestation. Two other bitches inoculated with CHV on day 30 of gestation delivered dead, mummified, or live pups by cesarean section. Infection by CHV was confirmed histologically by the presence of focal areas of necrosis associated with intranuclear inclusion bodies in heart muscle sections of one dead fetus; CHV was not recovered from other organs. When two bitches were inoculated on day 40 of gestation, they gave birth to 10 premature pups, born 5 to 7 days prior to the anticipated whelping dates. CHV was isolated from the liver, spleen, kidneys, and lungs of the premature pups.

Abortion from CHV should be self-limiting after the bitch develops immunity. It is generally believed that bitches experiencing fetal loss or neonatal death from CHV will not have subsequent litters affected. Although many adult dogs have been exposed to CHV and have immunity, it is very important to prevent exposure of those pregnant bitches who may not have immunity (i.e., young bitches, highly confined or isolated bitches). Treatment is generally unrewarding (see Chapter 8).

Canine Parvovirus Type 2. Although the ability to cause transplacental infections is an important pathogenic trait of most autonomous parvoviruses, the role of canine parvovirus type 2 (CPV-2) in reproductive performance in the pregnant bitch appears less than in some other species. In one study,[153] an examination of breeding records for a 7-year period demonstrated a sudden decrease in reproductive efficiency after CPV-2 was introduced into a kennel. However, conception rate, rate of stillborn pups, average litter size, and average number of pups weaned did not change when CPV-2 became established in another kennel.[154] Although experimental infection with CPV-2 in utero has been associated with myocarditis,[155] natural transplacental infection and disease from parvovirus is much rarer in dogs than in other species.

Minute Virus of Canines. Minute virus of canines (MVC or canine parvovirus type 1) has been shown to cause transplacental infections and resorption, especially in bitches given parenteral inoculations of MVC during the first half of gestation.[156] Direct exposure of embryos or fetuses to MVC via the amniotic sac resulted in embryonic/fetal death approximately 2 weeks after exposure. Although the role of MVC as a natural cause of fetal resorption in the bitch requires additional studies, resorption did occur in three of eight experimental bitches infected via the oral-nasal route, suggesting a mode of natural transmission. When infection was induced during the last half of gestation, MVC was incriminated as a potential cause for abortion and pups who were born dead or dying. The disease in the dead or dying pups was manifested as anasarca, lung consolidation, nonsuppurative pneumonitis, pulmonary vasculitis, and myocarditis[157] (see Chapter 8).

Canine Distemper Virus. Canine distemper virus (CDV) can cross the placenta, resulting in

abortion and congenital infections in newborn pups.[158] Abortion can result from the severe systemic effects of CDV infection in the bitch or from a direct infection of the placenta or fetuses. Viral antigens were not identified in pups aborted solely because of maternal systemic effects, such as fever. Viral antigens were, however, identified in tissues and lesions observed at necropsy in aborted pups that were infected with CDV in utero.

Mycoplasma and Ureaplasma. Mycoplasma and ureaplasma are two genera of the family Mycoplasmataceae. Ureaplasma have the ability to hydrolyze urea and were formerly called T-strain mycoplasmas because of their tiny ("T" standing for tiny) size. Both mycoplasmas and ureaplasmas can be part of the normal mucosal flora of the nasopharyngeal and reproductive tracts of dogs. However, urogenital diseases caused by mycoplasmas and ureaplasmas have been recognized for years in humans and cattle,[159] and in dogs have been associated with reproductive disease, including poor conception, early embryonic death or fetal resorption, abortion, stillborn pups, weak pups, and neonatal death.[159,160] Bitches may not show clinical signs of genital infection from mycoplasma or ureaplasma, but embryos, fetuses, or newborn pups may be infected in utero following breeding, during pregnancy, or at the time of whelping if exposed to organisms in the vaginal cavity.[110]

Diagnosis is based on isolation of the organisms from fetal tissues or uterine discharge. Culture swabs should be placed in Amies (without charcoal) transport medium (Difco Laboratories, Detroit, MI), chilled but not frozen, and shipped by overnight express to a laboratory with expertise and experience in isolating mycoplasma and ureaplasma.

Although several species of mycoplasma and serologic groups of ureaplasma have been isolated from dogs, the exact role of each species and strain in reproductive dysfunction has not been established. It is thought that mycoplasma and ureaplasma are not a significant cause of reproductive dysfunction in single-housed dogs or those maintained under strict hygiene. Reproductive dysfunction may be more likely to occur in kennels under intense management, especially when animals are housed closely together. One suggested method of controlling reproductive dysfunction in affected dogs is to alter the husbandry and management, isolating bitches and dogs into smaller, less intense groups and treating with appropriate antibiotics.[161] Although tetracyclines, chloramphenicol, and quinolones are effective against mycoplasma and ureaplasma, they are not considered as safe in pregnant bitches as some other drugs.[161] Quinolones are recommended if treatment during pregnancy is necessary. The veterinarian should be aware that reinfection is common after treatment, because mycoplasma and ureaplasma are ubiquitous in nature.

Parasites

Toxoplasma gondii. *Toxoplasma gondii* infects many species of animals, including dogs. Transmission can occur through congenital infection, ingestion of infected tissues, and ingestion of oocyst-contaminated food or water. Little is known of transplacental toxoplasmosis in dogs or cats, although its prevalence is thought to be less common than that occurring in sheep and goats.[163]

Toxoplasma gondii has been reported to infect pups in utero following experimental inoculation (intravenous or intraperitoneal) of the pregnant bitch with a suspension of tachyzoites harvested from infected mice.[164] Infected bitches generally showed systemic illness 3 to 5 days after inoculation with the tachyzoites, including depression, anorexia, diarrhea, and ocular and nasal discharge. Infected pups were delivered 4 to 6 days after the bitches were inoculated and died soon after birth.

Neospora caninum. *Neospora caninum*, a recently recognized protozoal disease that may have been previously confused with canine toxoplasmosis, has been reported to be transplacentally transmitted in dogs.[165] An 8-year-old beagle bitch obtained from a parasite-free breeding colony was inoculated both subcutaneously and intramuscularly with tachyzoites of *N. caninum* on day 35 of pregnancy. Eight pups were born on the 28th day after *N. caninum* inoculation. The smallest of the eight pups was enclosed in fetal membranes at birth and thought to have died in utero. Myocarditis was detected in the pup on microscopic examination of cardiac tissues. Whether *N. caninum* is a significant cause of naturally occurring canine abortion is unknown.

NONINFECTIOUS CAUSES OF EMBRYONIC OR FETAL DEATH

Maternal Endocrine Abnormalities

Hypoluteoidism [Inadequate Luteal Phase]. Because the maintenance of canine pregnancy

is dependent upon serum progesterone concentrations of greater than 1 to 2 ng/ml, and because abortion often is accompanied by concentrations of progesterone lower than those necessary for maintaining a pregnancy, some authors have suggested that resorption, abortion, and premature deliveries in the bitch may be the result of inadequate production of progesterone from luteal tissue.[130,166] This suggestion may have merit when infectious and other causes for fetal death are absent, or when a bitch that previously experienced a pregnancy loss carries a normal pregnancy to term after receiving progesterone supplementation. However, there is no documented confirmation of abnormal luteal function leading to naturally occurring pregnancy termination in the dog.

Caution must be exercised when making the diagnosis of hypoluteoidism, recognizing that a decrease in progesterone is a normal physiologic response that results from fetal distress and that may accompany premature delivery or abortion from any cause. Administration of progesterone to maintain pregnancy in bitches carrying fetuses with primary abnormalities, bitches with placentitis, or those with intrauterine infections can result in maintenance of an abnormal pregnancy, dystocia, pyometra, and septicemia.

The type or dose of progesterone to administer to bitches with proposed hypoluteoidism remains uncertain. Progesterone in oil, 4 to 6 mg/lb intramuscularly every 1 to 2 days, has been suggested as treatment for hypoluteoidism, discontinuing injections 1 week prior to anticipated whelping to permit normal parturition.[151] Owners should be warned that progesterone therapy might masculinize female pups. Female pseudohermaphrodism of pups has been cited to occur when progesterone is administered to racing greyhounds during gestation.[167]

The use of ally-trenbolone as a progestational agent to maintain pregnancy in the bitch also has been reported.[168] The administration of ally-trenbolone at a dose of 0.088 mg/kg/day per os successfully maintained pregnancy in three of three bitches ovariectomized on days 34 to 42 of gestation and in one of three bitches ovariectomized on day 8 or 9 of gestation. Therapy was discontinued 2 days before the anticipated whelping date, as determined by use of vaginal cytology to predict the onset of cytologic diestrus. Bitches whelped between 0 and 2 days after ally-trenbolone therapy was discontinued. All pups born to one bitch the day treatment was discontinued died because of dystocia due to oversized pups. Four of six pups born to one bitch 1 day after treatment was discontinued survived, and all 12 pups born to two bitches 2 days after treatment was discontinued survived. Milk production was minimal at parturition but increased within 2 days of whelping.

Hypothyroidism. Although the role of iodine and the thyroid gland in successful reproduction has been established in many species, there is little information correlating hypothyroidism with infertility or fetal loss in the dog. Results of studies in women suggest a correlation between the failure of the normal rise in thyroid hormone concentrations during early pregnancy and abortion.[169] Although pregnant women with hypothyroidism are reported to have a stillbirth rate twice that of euthyroid patients, other hypothyroid patients have been reported to carry normal pregnancies to term. In one study of 66 hypothyroid dogs,[170] which included male dogs and neutered bitches, only 1 dog had a history of abortion.

Hypothyroidism is the most commonly diagnosed endocrinopathy in dogs. Lymphocytic thyroiditis and idiopathic thyroid atrophy are the most common causes of canine hypothyroidism.[171,172] Reproductive function in male dogs varies depending on the type of thyroid dysfunction. Lymphocytic thyroiditis has been associated with lymphocytic orchitis and sterility in male dogs.[173] Daily sperm output remained normal, however, when hypothyroidism was induced in male beagle dogs with surgery and propylthiouracil (S. F. Soderberg, unpublished results, Colorado State University, 1984) or with iodine-131[174] (see Chapter 23).

In a colony of hypothyroid borzois, infertility was reported as the major problem.[104] Bitches cycled every 10 to 12 months, but did not show normal behavioral estrus and were unreceptive to breeding. Conception rates were poor, ranging from 33 to 50 per cent. Abortion at midgestation and stillbirths at term were common. Mummified fetuses were also observed. Lymphocytic hypothyroidism was identified in the borzoi colony and thought to result from a single-gene recessive trait.

Other Endocrine Disorders. Diabetes mellitus, adrenal insufficiency, and hyperadrenocorticism are other maternal endocrinopathies that may affect embryonic and fetal development in women, and possibly dogs.

Exogenous Drugs. Drugs that may have an adverse effect on canine pregnancy were discussed above. When using drugs to treat a pregnant bitch, one should consider potential effects of the drug on the developing embryo or fetus.

Immunologic Factors. Unexplained abortions account for 30 to 50 per cent of all miscarriages in women. Immune factors are now considered one explanation for some of the previously unexplained human abortions.[175] Systemic lupus erythematosus is known to be associated with an increased incidence of spontaneous abortion, fetal death, and preterm delivery with neonatal death in humans. The role of immunologic factors in embryonic and fetal death in dogs is unknown.

Genetic Factors. In one study,[176] chromosome abnormalities were observed in 31.9 per cent (29 of 91) of the poor-quality human embryos evaluated. The abnormalities included mosaicism (diploid/haploid, diploid/triploid, diploid/aneuploid), pulverized chromosomes, aneuploidy, prematurely condensed chromosomes, and structural rearrangements of chromosomes. The types of fetal chromosome abnormalities associated with resorption, abortion, stillbirth, or mummification in dogs and cats have been compared to those observed in humans (Table 5–14).[106]

In the dog, death and mummification were detected in a 117,XXX malamute pup delivered by cesarean section 5 days after its due date.[177] This was a single pup conceived from insemination with thawed frozen semen 65 days earlier. At necropsy, multiple somatic abnormalities were identified, including ectopic abdominal viscera; atresia ani; diaphragmatic hernia; arthrogryposis of the left foreleg; absence of the right humerus, radius, ulna, carpus, metacarpus, and phalanges; lordosis of thoracic vertebrae; open cranial fontanelles; cleft palate; and persistent right aortic arch.

Two mosaic littermate Munsterlander pointer pups were reported to be delivered spontaneously with nine normal pups after a 61-day gestation.[178] One mosaic pup (117,XXY/156,XXYY) was stillborn, small in size, and hydrocephalic; had hypospadias; and was bilaterally cryptorchid. The other mosaic pup (78,XY/156,XXYY) was born alive but died a few hours after birth; this pup also had hydrocephalus.

Chromosome abnormalities of the embryo due to gamete aging predispose to miscarriage in humans and may be a cause of small litter size in the dog. Holst and Phemister reported a ratio of more than 0.8 for the number of pups to the number of corpora lutea in bitches bred a single time at 4 to 10 days before the onset of cytologic diestrus.[179] However, the ratio of pups to corpora lutea was 0.5 in three bitches bred a single time 11 days before the onset of cytologic diestrus and 0.63 in four bitches bred a single time 3 days before the onset of diestrus (Fig. 5–5). Aging of both sperm and ova before fertilization is accompanied by a higher probability of abortion in humans, and postovulatory aging of ova in laboratory animals has been found to be associated with decreased litter size, increased polyspermia, and chromosomal abnormalities.[180] It is possible that the reduced ratio of pups to corpora lutea for bitches bred too early or too late in estrus also may result from sperm aging or ova aging, respectively.

There is evidence from women having in vitro fertilization that oocyte quality also declines with the age of the patient.[181] A marked reduction in implantation rates has been reported,[182] with delivery rates in women over the age of 40 being one third to one half those in younger women. The ability of female beagles to reproduce decreases with age, beginning at 4 to 8 years of age.[183] During this time, the number of bred bitches failing to whelp increases, the number of pups whelped per litter decreases, and the pups lost prior to weaning increases. The waning ability to reproduce further decreased after 8 years of age.

Because over 400 genetic diseases have been described in dogs that survived gestation,[184] it is likely that resorption and abortion may occur when these defects are more severe and result in embryonic or fetal death. Neonatal pup mortality is reported to be higher in purebred dogs than in hybrid dogs, supporting the hypothesis that inbreeding may contribute to poor reproductive performance.[185] Mean conception rates were studied in a foxhound colony when 14 outbred and 4 inbred male dogs were bred naturally to 544 outbred and 52 inbred bitches.[186] Mean conception rates, total number of pups whelped, and number of pups born alive were greater ($p < 0.01$) in outbred compared to inbred lines. If repeated resorption or abortion occurs in a breeding pair of

Table 5–14. Types of Fetal Chromosome Abnormalities Associated with Fetal Death (Resorption, Abortion, Stillbirth, or Mummification)

Fetal Karyotype	Species		
	Human (2n = 46,XX 46,XY)	Canine (2n = 78,XX 78,XY)	Feline (2n = 38,XX 38,XY)
Autosomal trisomy (2n + 1)	47,XX+ – 47,XY, + – (51.9%)*	NR	39,XY, + 11
Monosomy X (2n – X)	45,XO (18.9%)*	[77,XO]†	[37,XO]†
Triploidy) (3n)	69,XXY 69,XXX 69,XYY (15.5%)*	117,XXX	NR
Tetraploidy) (4n)	92,XXYY 92,XXXX (5.6%)*	NR	NR
Translocation	45,XX,t(--) 45,XY,t(--) (3.8%)*	[77,XX,rob(1q;9q]† [77,XX,t(13q;17q)]†	NR
Mosaicism 2n/2n xn/xn	46,XX/46,XY (1.5%)*	177,XXY/156,XXYY 78,XY/156,XXYY [78,XX/78,XY]†	38,XX/37,XX, – E 38,XX/39,XX, + D 38,XX/37,XX, – F [38,XX/38,XY]†

* Percentage denotes portion of 3040 human abortions with cytogenetic abnormalities.
† Brackets denote that chromosome abnormality has been reported in species indicated, but not in association with fetal death. All abnormalities listed as present in human abortuses have also been described in humans who survived past birth.
NR, not reported.
From Johnston SD, Raksil S: Fetal loss in the dog and cat. Vet Clin North Am Small Anim Pract 17:538, 1987, with permission.

dogs, a different bitch or male dog should be used in future breedings. Owners should receive genetic counseling and be encouraged to mate dogs that are not highly inbred or line bred, and therefore are less likely to produce pups with genetic abnormalities.

Environmental Factors. There is increasing awareness that environmental factors may affect birth outcomes in humans and animals. The cause(s) of approximately 60 per cent of all human birth defects are unknown,[187] and epidemiologic studies are just beginning to correlate environmental and occupational exposures to reproductive dysfunction and adverse birth outcomes. Studies of adverse developmental outcomes such as spontaneous abortion, low birth weight, and birth defects have frequently assessed maternal drug, smoking, alcohol, infectious, and occupational

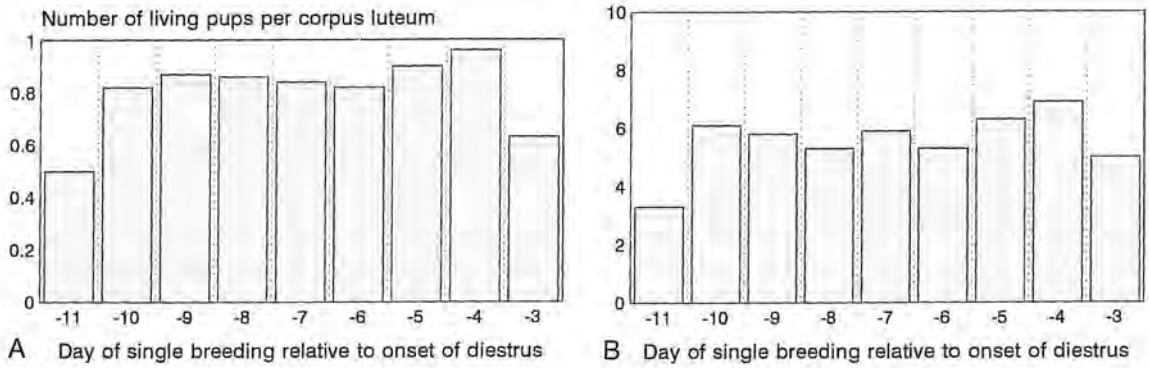

Figure 5–5. A: Number of living pups produced per corpus luteum **B:** Number of pups per litter. (From Holst PA, Phemister RD: Onset of diestrus in the beagle bitch: Definition and significance. Am J Vet Res 35:401–406, 1974, with permission.)

exposures. Additionally, paternal exposures are now also considered potential causes of human abortion and adverse birth outcomes.[188–190] The first indications that paternal occupational exposures may be linked with an increase in spontaneous abortion in people were derived from studies of paternal exposure to vinyl chloride, anesthetic gases, and dibromochloropropane. Positive associations have been reported for fetal loss and fathers who are smelter workers, plate and steel industry workers, or who are exposed to mercury, lead, zinc, and copper. Additionally, community-based and industry-specific studies have found an excess of spontaneous abortions among wives of men with occupational exposures to rubber, plastics, and solvents.[188] These examples serve to illustrate the importance of considering a wide variety of potential causes for reproductive loss in dogs, including environmental histories on bitches and stud dogs.

Nutritional Factors. In recent years, some owners of breeding animals have expressed concern over the use of ethoxyquin, a synthetic antioxidant, in pet food as a cause of reproductive dysfunction. Butylated hydroxytoluene and butylated hydroxyanisole are also used as antioxidants in food, and some studies have raised concerns about their safety.[191] Although scientific data are lacking, there are no reported clinical or epidemiologic studies that demonstrate increased infertility or pregnancy loss with various antioxidants used in pet foods.

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Chapter 6

Canine Parturition—Eutocia and Dystocia

Understanding the physiology and endocrinology of normal parturition (eutocia) in the bitch is necessary for preventing, diagnosing, and treating abnormal parturition (dystocia). Although the exact mechanisms that lead to the initiation of parturition in the bitch are not completely known, studies in the bitch and other species have provided data that allow veterinarians to understand normal birth and to identify dystocia.

Endocrinology of Canine Parturition

Corticosteroids

Both fetal and maternal factors contribute to the initiation of normal parturition. Increased release of fetal corticotropin-releasing hormone near parturition stimulates release of fetal adrenocorticotropic hormone (ACTH) and subsequent release of fetal cortisol, both of which are important in initiating parturition.[1,2] Fetal hypophysectomy or bilateral fetal adrenalectomy leads to prolonged gestation in the sheep. Stimulating the fetal adrenal by infusing ACTH, or directly administering a glucocorticoid hormone into the fetal lamb in utero, leads to premature delivery.[2] Fetal pituitary and adrenal responsiveness increase during late pregnancy and are necessary for normal parturition. In addition, fetal adrenal cortisol output may result, in part, from the effect of tropic substances other than ACTH, or from withdrawal of inhibitory substances.[1]

Pregnancy-specific increases in plasma cortisol concentrations occur during late pregnancy in the bitch.[3] Increased concentrations of cortisol are, however, erratic, and fail to parallel the decline in progesterone and subsequent onset of parturition. This may suggest that higher concentrations of cortisol at the fetoplacental level are more intimately involved in the mechanism of prepartum luteolysis and initiation of parturition than are plasma concentrations.

A prepartum rise in plasma 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM, the major metabolite of prostaglandin $F_{2\alpha}$ [PGF]) occurs in the bitch.[4] The PGFM increase may reflect increased synthesis and release of prostaglandins by the pregnant uterus, perhaps in response to increased fetal cortisol secretion. Prostaglandins can stimulate uterine contractions directly, or can enter the maternal circulation to stimulate release of oxytocin from the pituitary gland.

Progesterone

A prepartum decrease in serum progesterone to less than 1 to 2 ng/ml is required before parturition can occur in the bitch.[5] Parturition is blocked if the prepartum decrease is prevented through administration of exogenous progesterone; such administration results in prolonged gestation, fetal death, and maternal compromise.[5] Measurement of serum progesterone concentration has been suggested as a test for determining the onset of parturition or for selecting the time to perform an elective cesarean section, although data are not available to confirm usefulness of this technique.[6]

Prolactin

Canine serum prolactin concentrations increase from 40 ± 7 ng/ml during the week before parturition to 117 ± 24 ng/ml within 32 hours prior to parturition.[3] The prepartum rise in serum prolactin is reported to occur when serum progesterone concentrations decline.[3]

Predicting the Onset of Parturition

Predicting day of whelping can be difficult, because canine gestation length from a single breeding can range from 57 to 72 days.[7] In addition, many bitches are bred multiple times during estrus so that the range of possible whelping dates is great. When canine gestation length is timed from ovulation, however, duration is much more precise, ranging from 62 to 64 days.[7] Predictors of delivery date, which are listed in Table 6–1, include date(s) of breeding; estimated date of ovulation at time of breeding using serum luteinizing hormone or progesterone concentrations, or the diestrous vaginal smear; radiographic or ultrasonographic fetal development during pregnancy; rectal temperature decline at term; decline in serum progesterone concentration at term; and signs of impending parturition, to include lactation, nesting behavior, cervical relaxation, and lochial vulvar discharge.[3,5,8–13] Methods of determining day of ovulation and of assessing fetal development using radiographic or ultrasonographic imaging are described in Chapters 4 and 5, respectively.

The prepartum rectal temperature drop is a particularly useful clinical tool in predicting onset of whelping, because it is a clinical indicator of the rapid decline in serum progesterone, the thermogenic hormone that maintains pregnancy, at the end of gestation. The average rectal temperature declines gradually in pregnant (Figs. 6–1 and 6–2) beagle bitches during late diestrus.[14] A transient and abrupt prepartum temperature drop occurred in 78 of 80 pregnancies in which rectal temperatures were recorded daily or more frequently.[5] Mean low temperature recorded for 40 pregnancies between 8 and 24 hours prior to parturition was 98.8°F (range 98.1° to 100.0°F). Rectal tempera-

Table 6–1. Predictors of Onset of Parturition in the Bitch

Predictor	Interpretation
Breeding Dates	Parturition may occur 57–72 d from a single breeding
Ovulation Date, as Determined by	
Serum luteinizing hormone (LH)	Parturition occurs 64–66 d after the serum LH surge at time of estrus
Serum progesterone	Parturition occurs 64–66 d after serum progesterone concentration of 1–1.9 ng/ml Parturition occurs 63–65 d after serum progesterone concentration of 2–3.9 ng/ml Parturition occurs 62–64 d after serum progesterone concentration of 4–10 ng/ml Parturition occurs 12–24 h after a drop in circulating progesterone concentration to < 1–2 ng/ml in normal bitches[3,5]
Diestrous vaginal smear	Parturition occurs approximately 57 d after onset of cytologic diestrus[6]
Rectal temperature	A drop in rectal temperature to a mean of 98.8°F (range 98.1°–100.0°F) occurs between 8 and 24 h prior to parturition[5]
Radiographic Appearance of Fetuses	Mineralized fetal spine, skull, and ribs first seen 20–22 d prepartum; caudal vertebrae, fibula, calcaneus, and paws observed 2–9 d prepartum; teeth observed 3–8 d prepartum[9] (see Chapter 5, p 74)
Ultrasonographic Appearance of Fetuses	Combined regression of fetal biparietal diameter and fetal trunk diameter can be used to predict the number of days before parturition in some breeds[10] (see Chapter 5, p 76)
Onset of Lactation	Onset of secretion of milk by the bitch varies from 2 wk before until several days after parturition.[11] Colostrum may appear yellow-tinged and more opaque than later milk.[12]
Nesting Behavior	Onset ranges from 5 to 7 d prior to onset of parturition to the time of first stage of labor
Cervical, Vaginovestibular, and Vulvar Relaxation	Increased serum relaxin concentrations near parturition cause increased cervical distensibility and relaxation.[13] The vaginovestibular junction and vulva also relax.
Lochia	Greenish black discharge passes from the vulva following placental separation; whelping should occur within 1–2 h of its presence

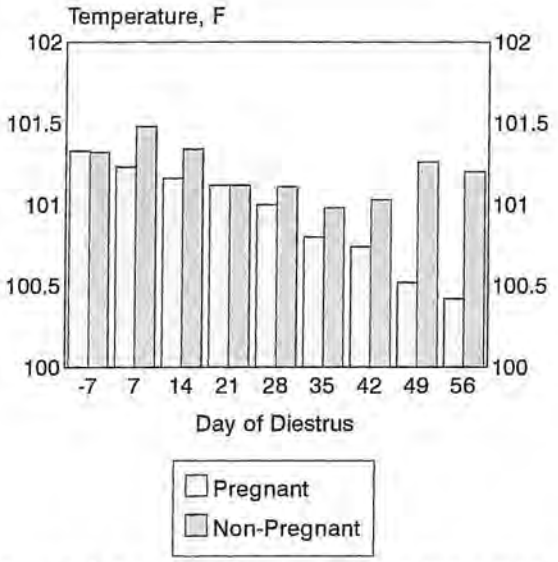


Figure 6–1. Average body temperature during diestrus. (From Holst PA: Canine Reproduction—A Breeder's Guide, Loveland, CO, Alpine Publications, 1985, p 195, with permission.)

tures subsequently increased at parturition and were above those recorded prior to the transient prepartum hypothermia for at least 4 days postpartum. Rectal temperature declines abruptly, at least 1 full degree and often to less than 99°F, about 14 hours after prepartum luteolysis with drop in serum progesterone to less than 1 ng/ml. Temperature thereafter begins to rise as the bitch enters stage I labor, and whelping starts 8 to 24 hours after the temperature drop.[5]

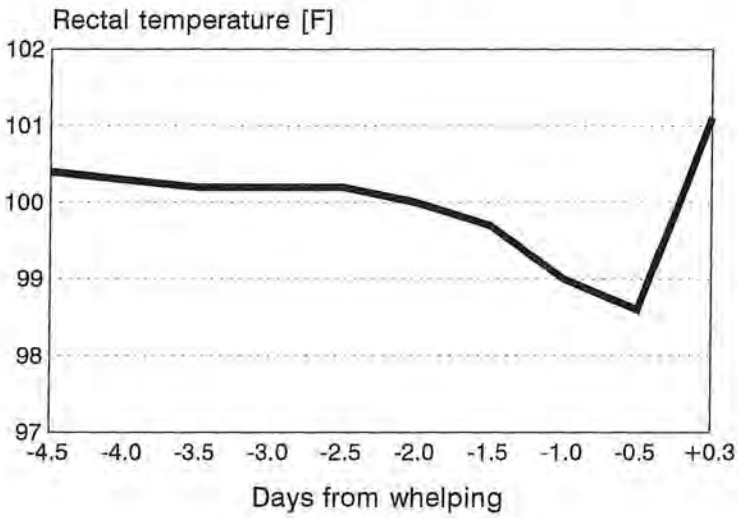
Owners can be instructed to monitor rectal temperature, starting 54 to 55 days following breeding, if prediction of onset of whelping is desired. Temperature should be taken three times daily in a consistent manner. Small temperature drops may be observed during the last week of pregnancy as serum progesterone declines, and considerable variation in rectal temperature values may occur between bitches. Occasional bitches do not demonstrate a detectable prepartum rectal temperature drop even when monitored three times daily. Clients should be advised that temperature will increase following the drop to about 99°F, and that parturition should follow 8 to 24 hours later.

Concentrations of serum progesterone decline to less than 1 ng/ml 24 to 48 hours before parturition.

Commercial external monitoring devices that record uterine activity and fetal heart rates are available (WhelpWise; Veterinary Perinatal Specialties, Wheat Ridge, CO). The bitch wears the monitor for 1-hour periods and an operator identifies fetal heartbeats with a hand-held Doppler unit twice daily beginning the eighth week of gestation. The trace from the monitor is transmitted via modem to a technician skilled in interpretation of the intrauterine pressures and fetal heart rates recorded, and the attending veterinarian is contacted with information regarding stage and progression of labor and viability of the pup. The cost to the client for rental of this external monitor is usually less than the price of one puppy.[15]

Uterine contractions begin before actual labor is initiated. Recognizable prelabor patterns of uterine contraction can be interpreted by trained personnel, allowing appropriate man-

Figure 6–2. Mean rectal temperature in the peripartum period. (From Concannon PW, Powers ME, Holder W, et al: Pregnancy and parturition in the bitch. Biol Reprod 16:517–526, 1977, with permission.)



agement of whelping. Fetal distress is recognized by decline of fetal heart rates. Diagnosis of fetal distress may differentiate eutocia, or normal delivery, from dystocia before the bitch shows clinical evidence of dystocia. Normal fetal heart rate is 150 to 160 beats per minute or greater.

Stages of Canine Parturition

Stage I

The first stage of parturition is the time of synchronous uterine contractions leading to complete cervical dilation. Active contractions of the longitudinal and circular muscle fibers of the uterine wall occur, but these contractions are not visible externally. Stage I labor lasts about 6 to 12 hours in the bitch, and is associated with clinical signs of anorexia, restlessness, apprehension, panting, shivering, and occasional vomiting. Obsessive nesting behavior may be observed shortly before birth of individual pups.[16] The bitch may seek seclusion. The previous experience and temperament of the bitch play a significant role in the length of the first stage of labor. Nervous primiparous bitches often experience a prolonged stage I that can extend up to 36 hours.

Stage II

The second stage of labor is the time when the cervix is completely dilated, and the puppies are moving through the birth canal (cervix, vagina, vestibule, and vulva) to be born. As the fetus enters the birth canal and stretches the cervix, a neuroendocrine reflex (Ferguson's reflex), which is mechanical distention of the cervix, uterus, and vagina leading to oxytocin release, occurs.[17] Cervical distention causes the "urge to push," leading the bitch to contract her voluntary abdominal musculature and show abdominal straining that is visible externally.

The contractions of the uterus occur just cranial to the most caudal fetus, forcing it through the cervix into the birth canal, while the rest of the uterus remains quiescent.[11] The process then is repeated for the most caudal fetus in the other horn, or the fetus immediately cranial to the one just expelled. The longitudinal fibers of the uterine horn contract, but the circular fibers remain relaxed so that the next fetus may pass through. This shortens the uterus as parturition progresses, so that each fetus in turn is brought nearer the cervix. A bitch may lie down as each pup is born, getting up and moving around the whelping area between the birth of pups.

In utero, the puppy is covered with two fetal membranes: the inner allantoamnion, and the outer allantochorion. The outer membrane breaks as the puppy enters the birth canal, and a clear fluid may pass from the vulva. The inner membrane usually invests the puppy at birth and must be removed by the mother or by persons in attendance. The bitch normally licks the newborn puppy vigorously, removing the amnion and severing the umbilical cord (Fig. 6–3). Her licking stimulates cardiovascular and respiratory function in the pups. The normal bitch often ingests the placentas of her puppies, which may lead to vomiting. Aspiration pneumonia following such vomiting has been reported in the bitch, leading some authors to advocate removal of placentas from the whelping area.[18] If the bitch does not sever the umbilical cord, it can be ligated with thread or suture material about 1 inch from the puppy and cut. The umbilicus should be disinfected with 2% iodine or other antiseptic.

Greenish-black fluid called lochia or uteroverdin is discharged from the vulva following separation of fetal membranes; this fluid is a breakdown product of biliverdin from the placental margins (Fig. 6–4). Vulvar discharge of lochia usually precedes the birth of the first pup, because placental separation precedes the birth (Fig. 6–5). Lochia also may be observed passing from the vulva of normal bitches for up to 3 weeks postpartum, and can be distinguished from abnormal uterine and vaginal discharges cytologically.

Presentation, posture, and position describe the relationship between the puppy in stage II labor and the maternal birth canal. *Presentation* refers the relationship of the spinal axis of the fetus to that of the dam.[12] Normal presentation is longitudinal (Fig. 6–6A,B); abnormal presentation is transverse (Fig. 6–6G). In one study, transverse presentation was the most common abnormality of presentation, posture, or position in 28 bitches with fetal dystocia (Table 6–2).[19] Presentation also refers to the portion of the pup that is approaching or entering the birth canal. Approximately 60 per cent of all pups are normally born in a cranial presentation, with the head entering the birth canal before the body (Fig. 6–6A); the remaining 40 per cent of pups are born in a caudal presentation (rear limbs first) (Fig. 6–6B), which does not predispose to dystocia in this species.

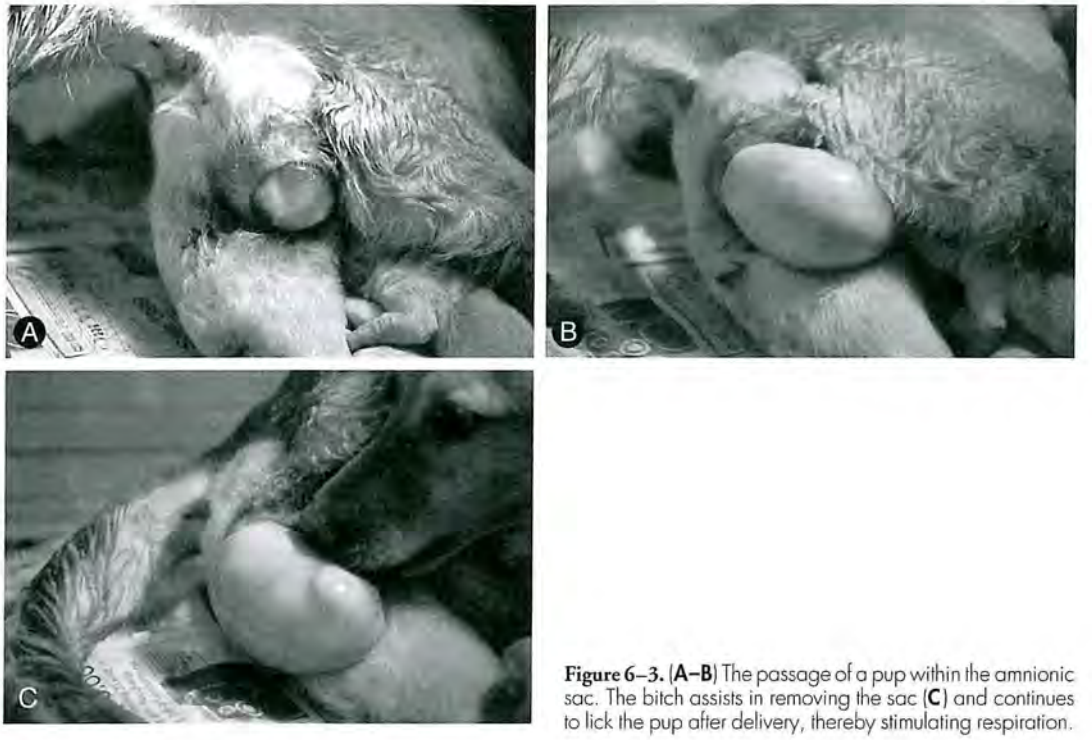


Figure 6–3. (A–B) The passage of a pup within the amnionic sac. The bitch assists in removing the sac **(C)** and continues to lick the pup after delivery, thereby stimulating respiration.

Posture refers to the relationship of the fetal extremities or the head and neck to the body of the fetus.[20] Normal posture (Fig. 6–6A,B) occurs when front and hind fetal limbs are extended. Abnormal postures include flexion of front or hind limbs as the puppy enters the birth canal (Table 6–2, Fig. 6–6C,D). The breech posture (Fig. 6–6D), which has been associated with dystocia in the bitch, occurs when a puppy is born in caudal presentation with the hind limbs flexed and under the body. Abnormal flexion or extension of the head and neck (Fig. 6–6E,F) are fetal postures that can result in dystocia in this species.[20]

Position refers to the relationship of the dorsum of the fetus in a longitudinal presentation, or the head of the fetus in a transverse presentation, to the quadrants of the maternal pelvis. The normal position for pups during parturition is dorsosacral, with the dorsum of the pup next to the sacrum of the bitch. Dorsoilial and dorsopubic positions have been reported to result in canine dystocia.[20]

A fetus that dies before reaching the pelvic inlet may not be able to assume the normal presentation, posture, or position for delivery.[21,22] Although pups usually are delivered every 30 minutes to 1 hour until whelping is

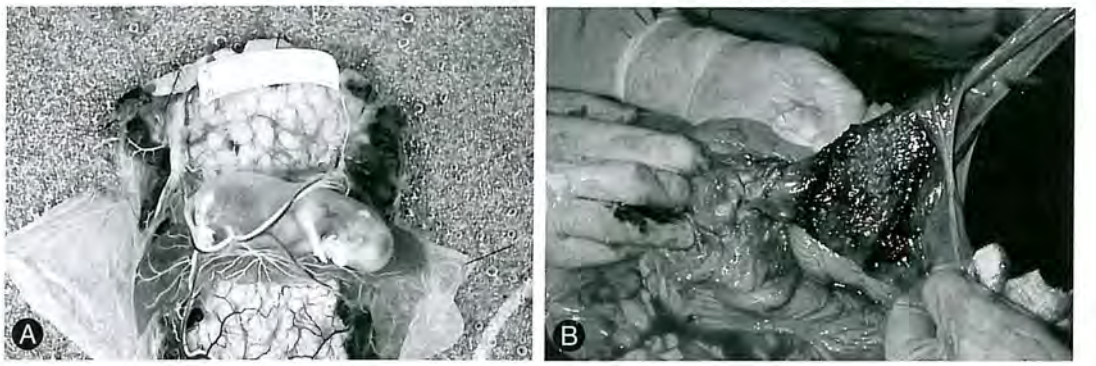


Figure 6–4. A: A fetal pup and membrane. Note the dark margin of the placenta, which is the area that releases uteroverdin or lochia at the time of placental separation. (Courtesy of Dr. Howard E. Evans, Cornell University, Ithaca, NY.) **B:** A canine placenta being removed at the time of cesarean section. Note the dark margins, reflecting blood breakdown pigments (uteroverdin or lochia).

Figure 6–5. Uteroverdin or lochia passing from a term bitch.

completed, the interval can be variable, with up to 4 hours between the birth of pups in some eutocic bitches. Because pups may be delivered alternately from each uterine horn, two pups may be delivered consecutively before the passage of a placenta. In one study where pups were marked in utero, it was reported that, after the expulsion of one pup, the next pup was expelled from the contralateral horn 78.2 per cent of the time.[23] In six of eight bitches in which each horn contained unequal numbers of pups, the first pup was born from the horn containing the most pups. Although stage II of labor usually is completed within 6 hours, delivery of an entire litter, especially when large, can extend to 24 hours without obvious complications.

Stage III

The third stage of labor is the time when fetal membranes are expelled, which usually occurs during stage II labor or slightly thereafter. Most canine placentas pass with the puppy or 5 to 15 minutes after birth. Retained placentas may occur in the bitch, but are uncommon.

Some authors include postpartum uterine involution in stage III labor. Uterine involution occurs for several weeks after whelping, with complete endometrial involution occurring by 3 months postpartum.[24] Even at 3 months, slight pigmented bands representing former placentation sites may be observed grossly in the endometrium (Fig. 6–7).

Dystocia

Abnormal parturition, or dystocia, occurs frequently in the dog. Owners may present a bitch for possible dystocia because of perceived failure to start parturition on time, or because of perceived failure to progress normally with delivery of puppies once labor has begun. Complete history and physical examination are important in establishing whether parturition is progressing normally, or in verifying presence, extent, and duration of dystocia. The following criteria are helpful guidelines in diagnosing canine dystocia.

Predisposing Factors That Lead to Dystocia

There are numerous fetal and maternal factors that contribute to canine dystocia, and many of these factors may occur together.[25,90] For example, fetal oversize, a fetal factor, may predispose the bitch to secondary uterine inertia, a maternal factor (Fig. 6–8). When diagnosing or predicting dystocia, it often is helpful to characterize the maternal and fetal factors associated with the dystocia in the case in question.

MATERNAL FACTORS

Primary Uterine Inertia. Primary uterine inertia is characterized by a failure to expel normal-sized fetuses through a birth canal that is normal except for an incompletely dilated cervix. Although the etiology of uterine inertia has not been defined precisely, it may be multifactorial, with mechanical, hormonal, physical, and genetic components as potential factors.[26] Primary uterine inertia is complete if no signs of second-stage labor occur. Lochia may be observed in the vagina or vestibule in affected bitches. Partial primary uterine inertia occurs when parturition begins normally, but uterine contractions stop before expulsion of the puppy. Primary uterine inertia has been associated with an inherited breed predisposition in terrier breeds; with overstretching of the uterus containing a large litter; with inadequate uterine stimulation in one- or two-pup litters; with systemic disease such as hypocalcemia, obesity, uterine infection, or septicemia; and with inadequate nutrition, uterine torsion, and trauma.[27] Serum calcium should be measured in affected bitches. Prognosis is based on etiology, and may range from guarded to good.

Secondary Uterine Inertia. Secondary uterine inertia occurs after prolonged uterine contractions fail to expel a fetus obstructing the birth canal, or all pups in a large litter.[27] In both primary and secondary inertia, the uter-

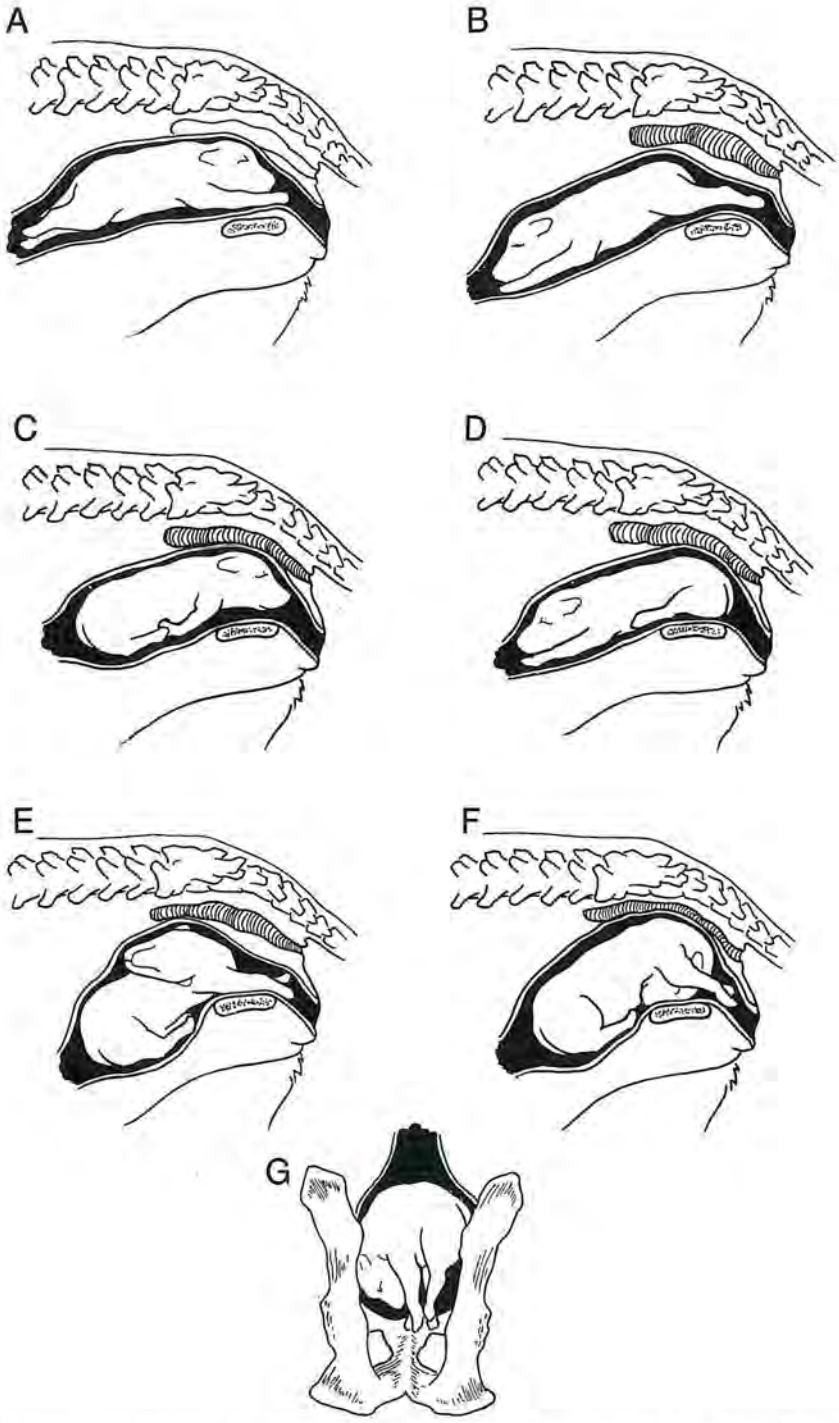


Figure 6–6. Fetal presentations, postures, and positions. **A:** A normal cranial presentation. **B:** A normal caudal presentation. **C:** Front limbs are retained under the body. **D:** Rear limbs are retained under the body (breech posture). **E:** Lateral deviation of the neck. **F:** Ventral deviation of the neck. **G:** Transverse presentation.

Table 6–2. Types of Abnormal Presentation, Posture, or Position in 28 Bitches with Fetal Dystocia

Type	Number of Fetuses	Percentage of Bitches
Transverse presentation	9	32.3
Breech posture	7	25.0
Neck presentation	4	14.3
Ventral presentation	3	10.7
Head flexion	2	7.1
Double bent fetus	2	7.1
Two fetuses presented at the same time	1	3.6
Total	28	100.0

Data from Darvelid AW, Linde-Forsberg C: Dystocia in the bitch: A retrospective study of 182 cases. J Small Anim Pract 35:402–407, 1994.

ine musculature fails to respond to the administration of oxytocin and the bitch fails to strain when pressure is applied per vagina to the pelvic canal (lack of the Ferguson reflex).

Breed. Breed of the bitch may predispose to dystocia. Brachycephalic breeds, such as bulldogs and Boston terriers, are prone to obstructive dystocia because individuals of these breeds may be selected for broad head and narrow pelvis. Elective cesarean section often is requested by owners of these breeds. Other breeds reported at risk for dystocia include the Sealyham terrier (large fetal head:maternal pelvis ratio), Scottish terrier (large fetal head:maternal pelvis ratio, uterine inertia, short vertical pelvic dimension), greyhound (fetal death), dachshund (anatomically abnormal pelvis, uterine inertia), Welsh corgi (fetal oversize), Border terrier (primary uterine inertia), Aberdeen terrier (primary uterine inertia), and cocker spaniel (nervous primigravid bitches that fail to develop early maternal instincts).[21,27] Breeds of dogs with increased ($p < 0.01$) incidence of dystocia include the Chihuahua, dachshund, Pekingese, Yorkshire terrier, miniature poodle, and Pomeranian.[22]

Conformation. Conformation of the bitch can influence parturition dramatically. Congenital and acquired abnormalities of the bony birth canal, such as old healed pelvic fractures, can make normal vaginal delivery impossible. Vaginal and vulvar soft tissue abnormalities, such as vaginal anomalies (hymenal remnants, annular strictures), vaginal prolapse, and vaginal neoplasia may prevent normal delivery. Bands of vaginal tissue preventing birth may vary in size from a few millimeters in diameter (Fig. 6–9) to a wall that bifurcates the vagina completely.

Other Maternal Factors. Other maternal factors that predispose to dystocia are shown in Figure 6–8. Normal expulsion of pups can be impeded through nonreproductive causes, such as excessive perivaginal fat, ruptured maternal diaphragm, pain, or fear.

FETAL FACTORS

Presentation, Position, or Posture. Presentation, position, or posture of the fetus during whelping can predispose to dystocia. Sixty per cent of all puppies in normal parturition are born in a cranial longitudinal presentation with front limbs first; the remaining 40 per cent of pups are born in a caudal longitudinal presentation with rear limbs first. Although the caudal longitudinal presentation is considered a normal variant of whelping in the dog, prolonged first-stage labor has been associated with this presentation. It is theorized that failure of the head to engage the pelvis results in diminished uterine stimulation and abdominal contractions.[28] Transverse presentations are rare, and usually occur only with bicornual pregnancy with a single fetus (Fig. 6–6G). A transverse presentation often results in pelvic canal obstruction.[19,29]

The fetus plays an important role in engaging the pelvic inlet with its head and limbs extended. A fetus that dies before reaching the pelvic inlet may remain unrotated,[21] resulting in an obstructive dystocia. Although the position of the fetal limbs is not as important in predisposing polytocous species to a dystocia as monotocous species, the breech position has

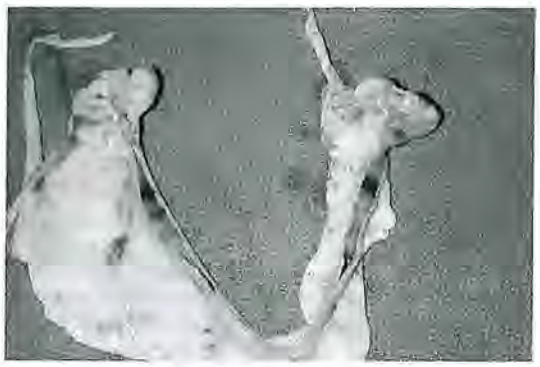


Figure 6–7. Note the dark areas in the endometrium of a uterus from an anestrous bitch, reflecting previous placental sites.

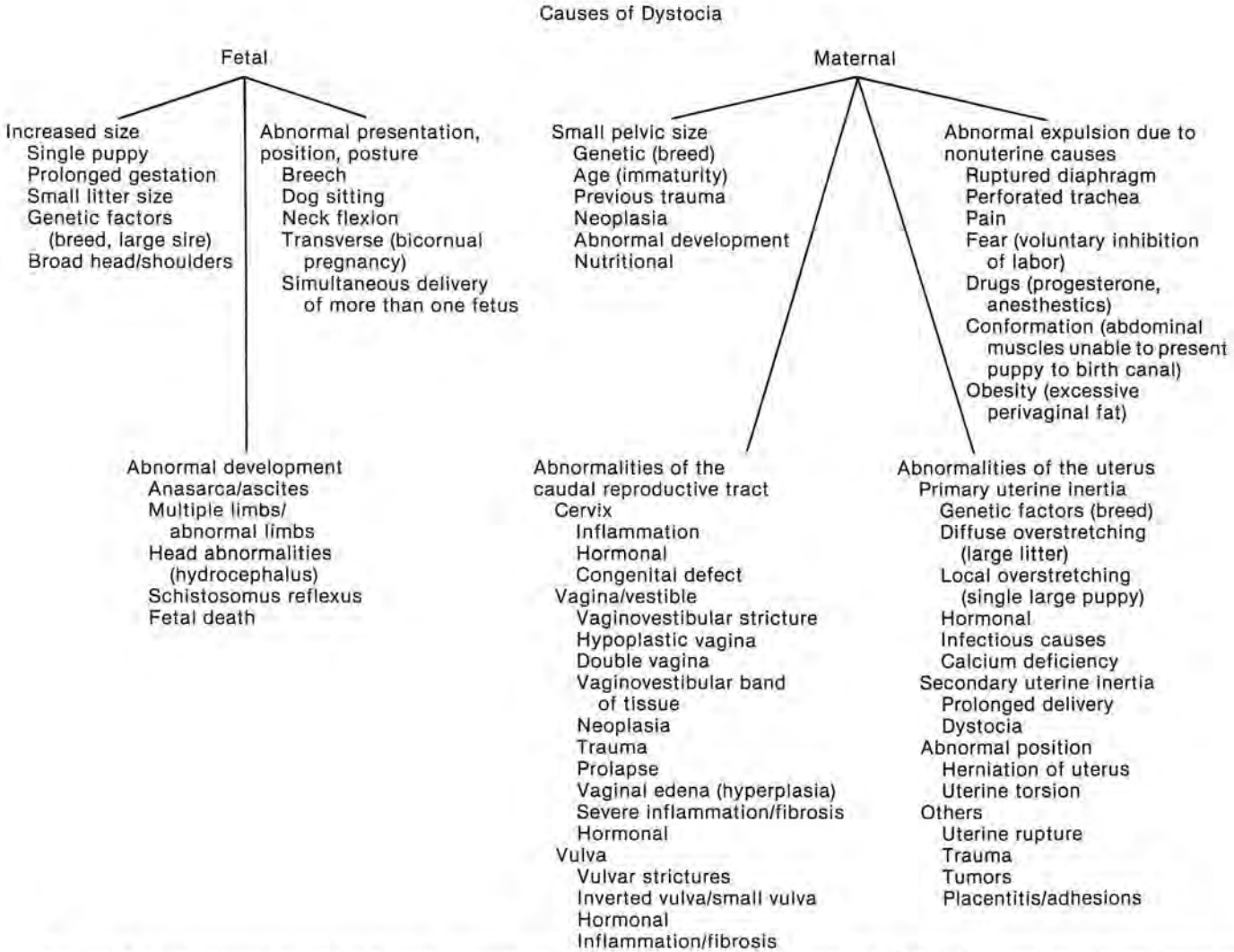


Figure 6–8. Classification of dystocia in the bitch, including maternal and fetal factors. (From Wykes PM, Olson PN: Normal and abnormal parturition. *In* Slatter D [ed]: The Textbook of Small Animal Surgery, 2nd ed. Philadelphia, WB Saunders, 1993, p 1320, with permission.)

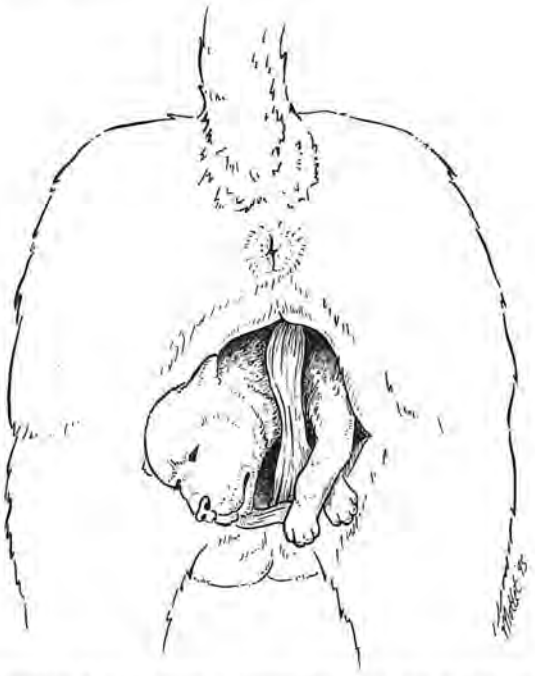


Figure 6–9. A schematic diagram illustrating how a dystocia can result from residual vaginal bands of tissue (hymen).

been associated with canine dystocia. The breech position occurs when a pup in the caudal longitudinal presentation has hind limbs retained or extended beneath the body (coxofemoral posture). Other abnormal positions include cranial presentation with the rear limbs extended beneath the fetus ("dog sitting" posture) (Fig. 6–6C), lateral deviation of the head (Fig. 6–6E), and cranial presentation with the head flexed beneath the neck ("poll" posture) (Fig. 6–6F).

Abnormal Fetal Development. Abnormal fetal development can result in dystocia. Fetal monsters and hydrocephalic or edematous (anasarca) pups predispose to obstructive dystocia. Pups with anasarca ("water pups") may occur as a result of a genetic defect in the English bulldog and beagle.[30,31] Other defects associated with dystocia in the dog include presence of multiple limbs and abdominal and/or thoracic hernia (Fig. 6–10).

Other Fetal Factors. Other fetal factors that result in dystocia are summarized in Figure 6–8 and have been discussed previously. Pituitary or adrenal malformations may result in pups that are unable to initiate parturition. Total body oversize and relative oversize of fetal head in relation to maternal pelvis also contribute to dystocia.

Criteria for Diagnosing Canine Dystocia

ABNORMALLY PROLONGED GESTATION LENGTH

Parameters useful in evaluating possible prolonged gestation, or primary uterine inertia in the overdue bitch, are listed in Table 6–1. In general, breeding dates, which are the most common parameter used by owners to predict delivery date, are the least reliable, because normal canine gestations may range from 57 to 72 days following a single breeding.[32] This variability is due to long gamete (egg or sperm) survival in the female tract, waiting for the arrival of the other gamete in order to initiate fertilization. Therefore, although all pregnant bitches should be considered overdue after 72 days following breeding, many are overdue much earlier if they are more than 65 days from ovulation. Most, but not all, owners of the bitch know breeding dates of the pregnant bitch.

Gestation length timed from day of ovulation instead of day of breeding ranges from 61 to 64 days; bitches more than 65 days from estimated day of ovulation should be considered overdue, as should bitches more than 60 days after onset of cytologic diestrus. The rectal temperature drop is an indicator of luteolysis, and an indirect indicator of ovulation day; the pregnant bitch that is more than 24 hours beyond the rectal temperature drop is overdue.

Gestation length from the day of a single serum progesterone concentration between 1.0 and 10.0 ng/ml during estrus averages 65 days if progesterone was between 1.0 and 1.9, 64 days if between 2.0 and 3.9 ng/ml, and 62.4 if between 4.0 and 10.0 ng/ml.[33] Although concentrations of progesterone can vary within and among bitches and reportedly are affected by diurnal variation in pregnant bitches,[34] a single serum sample from an estrous bitch assayed for progesterone appears to provide important information in predicting onset of parturition.

Some bitches presented to the veterinarian for prolonged gestation may not be pregnant, because the nonpregnant diestrous bitch develops mammary tissue, lactates near the end of diestrus, and often shows nesting behavior as her serum progesterone concentration declines. Confirmation of the nonpregnant status

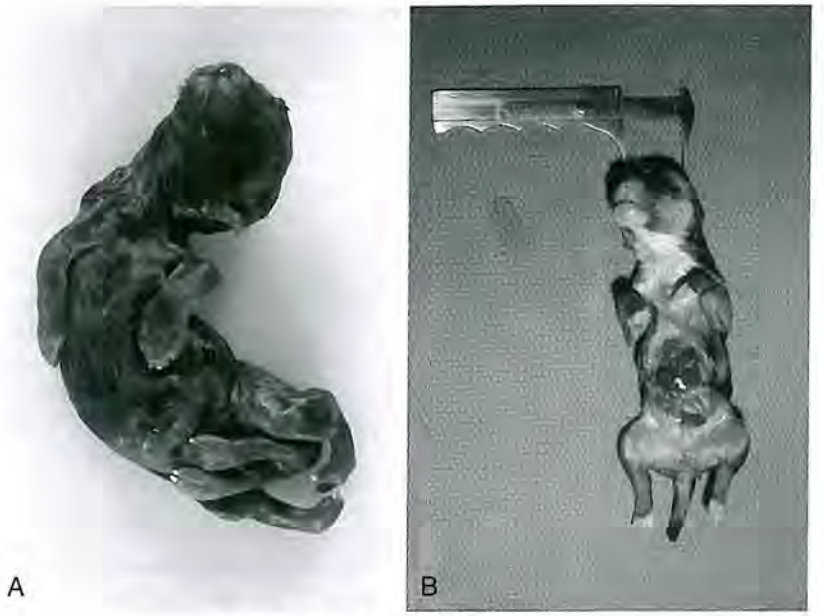


Figure 6–10. Examples of fetal monsters.

at term relies on abdominal palpation and/or radiography.

Influence of litter size on gestation length has been discussed by several investigators, because of the concern that a single pup or small number of puppies may not provide adequate hormonal stimulation for the initiation of parturition in this species. Small litter size has been associated with dystocia in the canine; in two reports, 59 of 100 (59 per cent) and 82 of 172 (48 per cent) bitches with dystocia had litter sizes of one to three puppies (Fig. 6–11).[19,35] Gestation length has been considered prolonged and fetal size larger when a litter contained only one or a few pups. Gestation length was reported prolonged in 15 beagle bitches that carried only one pup. However, in a study of 49 pregnant bitches, mean gestation length, determined from ovulation day on the basis of a serum progesterone concentration, was not prolonged with small litter size.[33] Another explanation for the association of small litter size with prolonged gestation is that number of insemination days before ovulation has been shown to be associated with reduced conception rate and smaller litter size.[8] Therefore, small litter size predisposes the bitch to dystocia, and may be associated with prolonged gestation from day of breeding, but not from day of ovulation.

Primary uterine inertia is diagnosed if gestation is prolonged using predictors of due date described above. Treatment is cesarean section.

SYSTEMIC ILLNESS AT THE END OF PREGNANCY: MATERNAL COMPROMISE

Signs of sepsis, pregnancy toxemia, uterine torsion, uterine rupture, and strangulation of a uterine horn in an inguinal hernia may be remarkably similar, with acute anorexia, abdominal pain, collapse, and shock. Clinical management of such patients includes emergency examination, stat hemogram and serum chemistry profile, abdominal radiography and ultrasonography, fluid and antibiotic administration, and rapid decision about surgical exploration of the abdomen.

Septic Metritis. Septic metritis and toxemia in the bitch at term may be evident by 48 to 72 hours after intrauterine fetal death. If a dystocia goes undiagnosed or untreated, the entire litter may die within 24 hours and serve as a substrate for infection with ascending vaginal bacteria. Diagnosis of fetal death is based on history and on ultrasonography, if available. Sepsis usually is associated with elevated rectal temperature and a regenerative or degenerative left shift in the white cell component of the hemogram. Recommended treatment is aggressive fluid and antibiotic therapy with hysterectomy.

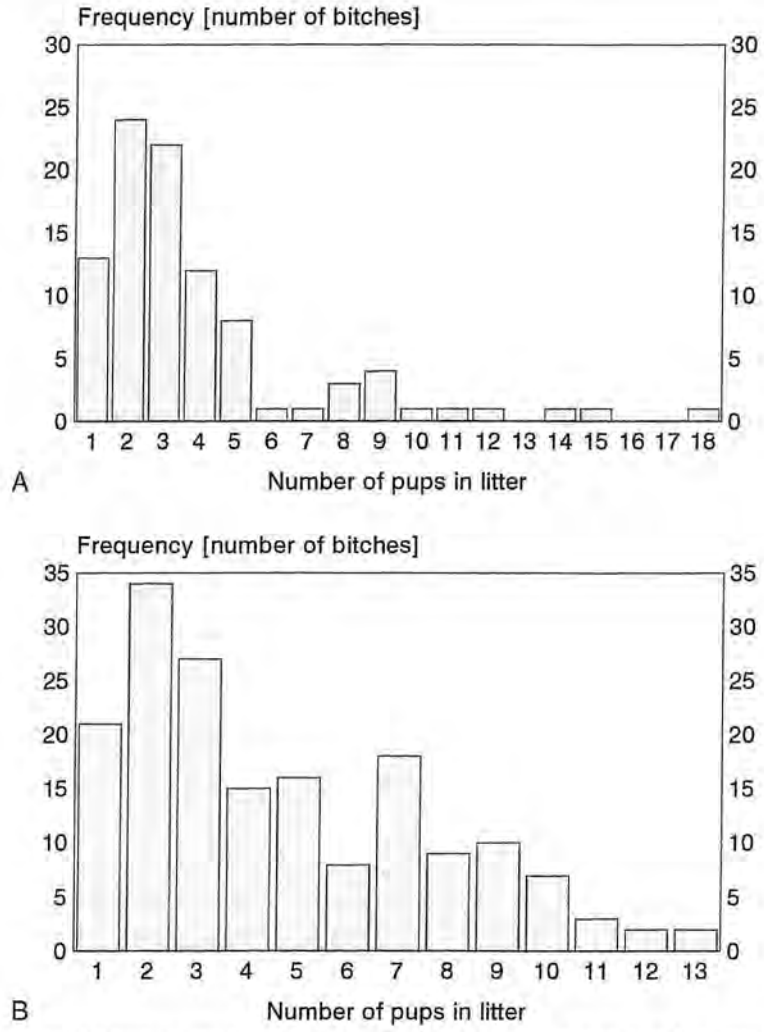


Figure 6–11. A: Litter size in 100 bitches with dystocia. (From Gaudet DA: Retrospective study of 128 cases of canine dystocia. J Am Anim Hosp Assoc 21:813–818, 1985, with permission.) **B:** Litter size in 172 bitches with dystocia. (From Darvelid AW, Linde-Forsberg C: Dystocia in the bitch: A retrospective study of 182 cases. J Small Anim Pract 35:402–407, 1994, with permission.)

Pregnancy Toxemia. Pregnancy toxemia with ketosis occurs in bitches on inadequate nutrition, or in bitches with large litters that do not meet energy demands with nutritional intake (see Chapter 5). Gluconeogenesis and ketogenesis are accelerated during the last third of pregnancy in normal bitches, and can become further exacerbated when energy demands are high because of size or number of fetuses or inadequate nutrient intake by the dam.[36] Although pregnancy toxemia can occur days to weeks before parturition, it also has been associated with prolonged gestation and dystocia.

Ketonuria without glucosuria is a hallmark of prepartum pregnancy toxemia in the bitch. Bitches with reduced stomach capacity due to a large litter size, or those with ketonemia, will develop anorexia, which further accelerates the ketogenesis. Hypoglycemia also may be present, resulting in weakness, inability to stand, seizures, and coma.[37,38] Hepatic lipidosis may accompany this disorder (see Chapter 5). Diagnosis is based on presence of urine ketones in the absence of urine glucose, and, in some bitches, on hypoglycemia. Recommended treatment is supplemental nutrition, intravenous (IV) dextrose administration, or medical induction of parturition with glucocorticoids (see below).

Uterine Torsion. Uterine torsion of both pregnant and nonpregnant uteri have been re-

ported in the dog. Despite the fact that dogs have relatively long and freely movable uterine horns, torsion of these structures is relatively uncommon. One or both horns can twist along the long axis or around the opposite horn, or the entire uterine body can rotate.[39,40] Magnitude of torsion and clinical outcome in eight affected bitches are listed in Table 6–3.[41] Bitches with uterine torsion may have severe abdominal pain with abdominal distention, hemorrhagic vulvar discharge, tachycardia and signs of shock ("acute abdomen"), and dystocia, or may have relatively few clinical signs.[41,42] Severe torsions can cause obstruction of the blood supply to the uterus, with resulting thrombosis or rupture of uterine vessels, congestion, shock, and fetal and/or maternal death. Rupture of the torsed uterus may occur at parturition. Diagnosis is based on clinical signs, ultrasonographic examination of the abdomen, and exploratory laparotomy. Treatment is immediate surgical correction, which may include hysterotomy to remove fetuses or hysterectomy if thrombosis and gangrene are present.[40,43]

Uterine Rupture. Uterine rupture in the pregnant bitch can occur following uterine torsion or trauma. This condition is rare in the bitch. If fetal circulation is not compromised, the condition may go undiagnosed until dystocia results when the puppies fail to enter the birth canal. Fetuses expelled into the peritoneal cavity may die immediately and be resorbed if fetal calcification has not yet occurred, or be retained as mummified fetuses. Peritonitis is a possible sequel.[40]

Inguinal Hernia. Inguinal hernia of pregnant uterine horns through the inguinal ring occurs occasionally in the bitch, and can result in dystocia. Surgical repair of the hernia should be accomplished as soon as possible in order to prevent ischemic compromise of the growing fetus. Cesarean section may be required to deliver term pups that have herniated. Inguinal hernias have been reported as congenital defects in the basset hound, cairn terrier, basenji, Pekingese, and West Highland white terrier.[44]

STRONG AND FREQUENT STAGE II ABDOMINAL STRAINING THAT FAILS TO PRODUCE A PUP WITHIN 30 MINUTES

Strong abdominal straining or tenesmus during stage II of labor suggests that a pup is present in the birth canal. If the bitch does not deliver the pup within 30 minutes, assistance may be necessary to deliver the obstructing pup. A pup lodged in the birth canal can die if complete placental separation is not followed by delivery; obstruction also compromises other pups remaining in the uterus. Active straining and labor will eventually subside as secondary uterine inertia develops.

Table 6–3. Summary of 8 Canine Uterine Torsions

Signalment	Pregnant?	Torsion	Outcome
Dandie Dinmont terrier, 6 yr	Yes Term	Body of uterus twisted >1440°	Peritonitis; 3 dead pups; dam euthanized
Setter type	Yes Term	Body of uterus twisted 720°	4 dead pups; dam euthanized
Terrier type	Yes Term	Body of uterus twisted 360°	3 dead pups; dam died
Collie, 7 yr	No Estrus	Right horn twisted 2160°	Ovariohysterectomy; dam survived
German shepherd, 3 yr	Yes Term	Left horn twisted 180°	Whelped 7 pups; 5 pups removed at cesarean section (all survived)
Papillon, 1 yr	Yes 6 wk	Right horn twisted, adhered to other abdominal organs	Ovariohysterectomy; dam survived
Siberian husky, 1 yr	No Diestrus	Left horn twisted counter clockwise around right horn	Ovariohysterectomy; bitch survived
Toy poodle, 13.5 yr	No Anestrus	Right horn twisted 1080° around left; left horn twisted 270° on long axis	Ovariohysterectomy; bitch survived

Data from Shull RM, Johnston SD, Johnston GR, et al: Bilateral torsion of uterine horns in a non-gravid bitch. J Am Vet Med Assoc 172:601–603, 1978.

WEAK OR INTERMITTENT STAGE II ABDOMINAL STRAINING THAT FAILS TO PRODUCE A PUP WITHIN 4 HOURS (FIRST PUP) OR 2 HOURS (BETWEEN PUPS)

Weak, intermittent, or absent abdominal straining without the delivery of a pup by 4 hours after the onset of stage II labor (first pup) or 2 hours (between pups) suggests that uterine contractions are not effective in advancing the fetus through the birth canal. These bitches frequently respond to medical management with oxytocin or calcium (see below). Although some bitches can deliver a live pup after 2 to 4 hours have lapsed during stage II labor, the incidence of stillbirths rises as the time interval between delivered pups increases.

PARTIAL DELIVERY OF A PUPPY

An owner may observe fetal limbs protruding from the vulvar cleft (Fig. 6–12) without immediate delivery of the entire puppy. Such animals should be examined immediately if owners cannot be coached to deliver the pup in a manner that is safe for both pup and dam.

PRESENCE OF VULVAR DISCHARGE

The presence of lochia or uteroverdin (greenish-blackish vulvar discharge) indicates that placental separation has occurred for at least one pup, and is a reliable sign that whelping should begin within a few hours in a term bitch (Fig. 6–5). Sometimes lochia will pass when a caudal fetus dies in utero several days before term and the remaining pups are born normally at term. However, the passage of lochia from a term bitch usually signifies that whelping should ensue within 1 to 2 hours, and failure to do so signifies a potential dystocia. The entire litter of pups may die within 24 hours if the dystocia is not relieved.

A copious amount of a clear, water-like vulvar discharge suggests that the allantoic or amnionic fluids have passed. Owners may sometimes confuse the passage of placental fluids with urination. Owners may observe a sac or bubble protruding from the vulvar lips, which is the caudal portion of the allantochorion filled with fluid. This signifies the presence of a pup in the birth canal (Fig. 6–3).

Sanguineous vulvar discharge near term may be caused by a traumatic birth, uterine torsion, or inadequate or dysfunctional clotting factors. Sanguineous vulvar discharge earlier in gestation usually is associated with pregnancy loss or abortion. Although some blood is passed during a normal delivery, the amount usually is minimal. The typical characteristic of a normal vulvar discharge at whelping is the greenish-blackish discharge associated with the passage of uteroverdin.

Diagnostic Evaluation of the Dystocia Patient

The goals of diagnostic evaluation of the dystocia patient are to confirm that pregnancy is present, to confirm that parturition is not proceeding normally, to diagnose cause of the dystocia if possible, and to detect maternal and/or fetal compromise if present.

The bitch presented for possible dystocia or systemic illness at term should be evaluated for evidence of maternal compromise. A complete history should be obtained on the bitch's general health and prior illnesses, if any. Information on previous reproductive performance, including breeding dates, past whelpings and dystocias, and rectal temperature measurements at this pregnancy, should be recorded. If possible, estimated time of ovulation based on serum progesterone or luteinizing hormone concentrations or the diestrous vaginal smear should be noted.

The dystocic bitch may appear normal on physical examination, or may be severely compromised and near death as soon as 24 to 48 hours after starting stage II labor. The physical examination should include temperature, pulse and respiratory rates, capillary refill time, and hydration status. Thoracic auscultation to assess cardiopulmonary function, and abdominal palpation of the uterus to confirm fetal presence, should be performed. Palpation of fetal movement and auscultation of fetal heartbeats per abdomen are evidence of fetal viability for at least part of the litter, but their absence does not confirm fetal death. Mammary glands should be inspected for presence of normal or abnormal secretions or milk. The vulva should be examined for presence of discharge, such as lochia or blood. A digital examination of the vestibule and vagina should be performed using a sterile glove to determine presence of relaxation of the birth canal, presence or absence of fetuses in the birth canal, and whether soft or bony tissue impingement on the birth canal is present.

Survey abdominal radiographs should be taken to determine fetal number, size, and position; in addition, radiographic signs of fetal

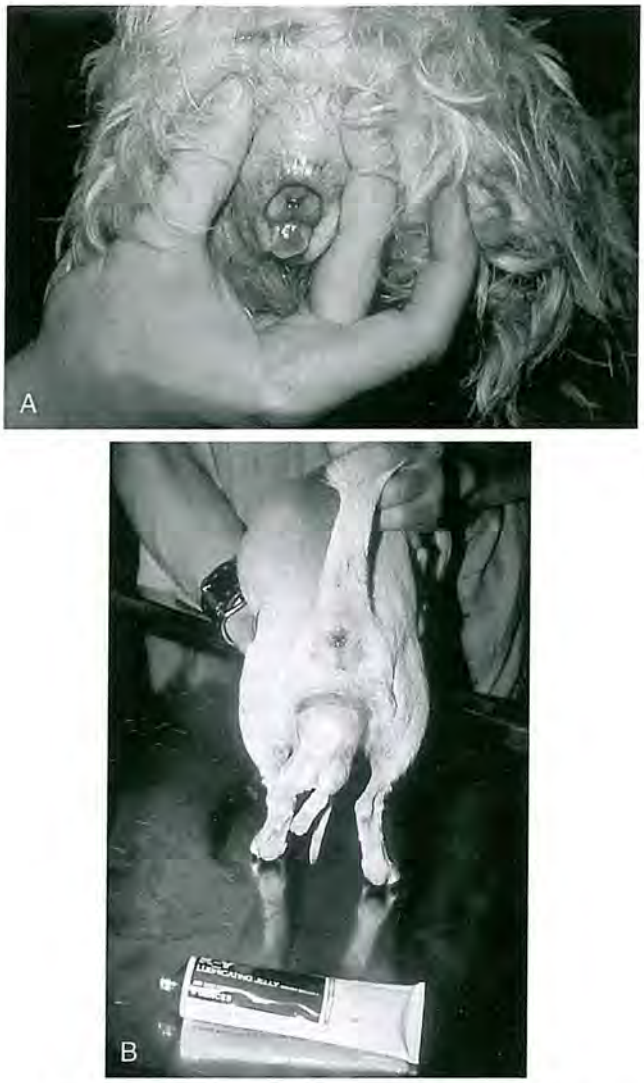


Figure 6–12. Examples of bitches with obstructive dystocias.

death may be observed. Radiographic signs of fetal death depend on the time since death and the extent of fetal maceration; absence of such signs does not imply fetal viability. Radiographic signs of fetal death include presence of gas within fetal body cavities or blood vessels, overlap of fetal cranial bones, alteration in the spatial relationships between bones of the axial skeleton, and failure of the skeleton to calcify or continue to grow.[45]

Ultrasonographic examination of the bitch's abdomen, if available, will confirm presence or absence of intrauterine fetal viability. When oxygen delivery is impaired experimentally to canine fetuses between 7 and 9 weeks of age, fetal heart rate slows (Fig. 6–13); this is unlike the increased heart rate observed in adult dogs with hypoxemia.[46] Experimental induction of hypoxia leads to decrease in fetal pO_2, blood pH, and tissue pH and increase in fetal pCO_2. When fetal tissue pH reaches 7.05, mean fetal heart rate is 40 to 130 beats per minute, suggesting that fetal distress can be predicted using ultrasonographic observation of bradycardia.[46]

External monitoring devices and hand-held Doppler units, which allow assessment of uterine contractile activity and fetal heart rate, are commercially available (WhelpWise). A nonproductive pattern of weak or infrequent uterine contractions can be diagnosed by interpretation of a trace generated by an external monitor that measures intrauterine pressure changes. Fetal distress is assessed by measure-

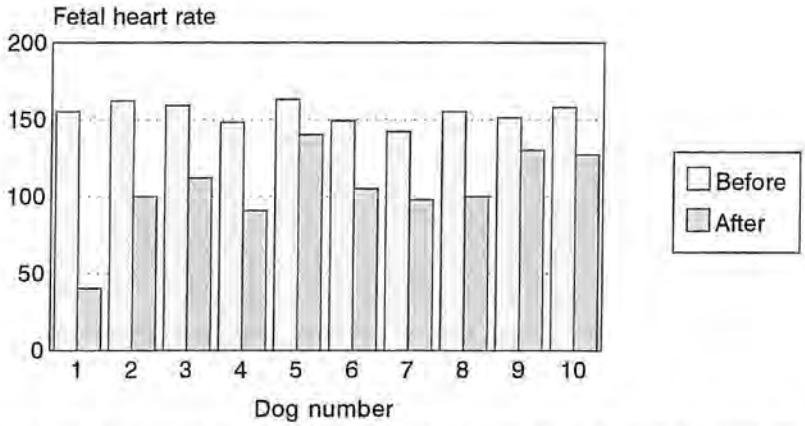


Figure 6–13. Fetal heart rates before and after occlusion of the maternal aorta. (From Monheit AG, Stone ML, Abitbol MM: Fetal heart rate and transcutaneous monitoring during experimentally induced hypoxia in the fetal dog. Pediatr Res 23:548–552, 1988, with permission.)

ment of fetal heart rate; fetal heart rates of less than 130 beats per minute suggest poor viability of pups not delivered within the next 2 to 3 hours, and fetal heart rates of less than 100 beats per minute indicate that immediate veterinary intervention is required.[47]

Laboratory evaluation of the dystocic bitch should include a complete blood count, serum chemistry panel (including glucose and total and ionized calcium, if possible), and urinalysis. Blood gases may be indicated if the bitch is systemically compromised.

Treatment of Dystocia

MANIPULATIVE TREATMENT

Manipulative management of mild dystocia with normal-sized puppies occasionally resolves the problem with minimal risk to the bitch. In these cases, extraction of a malpositioned or slightly oversized fetus may relieve an obstructive dystocia. Instruments that have been used to extract canine fetuses include the Snook ovariohook, sponge forceps, and clamshell forceps. Because instruments can easily injure a puppy or traumatize maternal tissues, we do not recommend their use unless the fetus to be extracted has already died and there is adequate room for the instruments in the birth canal. Forceps applied to the head of the fetus may cause crushing of the skull. Excessive twisting of the fetus may result in dislocated vertebrae.

A puppy that protrudes, in part, from the vulva, often can be delivered vaginally by lubricating the birth canal with a sterile lubricating jelly (applied via syringe or urinary catheter into the vaginal lumen), grasping the body of the puppy with a gauze sponge, and gently extracting the puppy by drawing it up and over the ischial arch. Grasping only a tail or limb is not recommended, since applied pressure to so small an organ may result in tearing off of these tissues. Pups obstructing the birth canal that are only barely protruding may best be removed via cesarean section.

MEDICAL TREATMENT

Medical treatment is indicated for relieving dystocia if the bitch is in good health, labor has not been unduly protracted, the cervix is dilated, and fetal size is consistent with likelihood of vaginal delivery. If protracted labor has resulted in delivery of only one or two pups, and many remain in the uterus, cesarean section may be faster and safer for the mother and puppies than medical management. Sometimes medical treatment results in slow delivery, with 1 to 2 hours between puppies. If only one or two puppies remain in utero, a slow response may be adequate in relieving the dystocia without unduly stressing the bitch. However, when medical treatment produces a slow response and many pups remain in utero, cesarean section should be considered.

Medical treatment involves administration of one or more ecbolic drugs. An ecbolic drug causes uterine contractions and results in the puppy moving into and through the birth canal. Ecbolic drugs should not be used if ob-

structive dystocia is present, because uterine rupture may ensue.

Oxytocin. Oxytocin is an octapeptide hormone formed by the neuronal cells of the hypothalamus and stored in the posterior pituitary gland. Oxytocin has uterine-contracting and milk-ejecting actions. Synthetic oxytocin is the most commonly administered ecbolic drug for treating canine dystocia. The half-life of oxytocin in animals is 1 to 2 minutes. Although generally considered safe for both the bitch and the fetus, some authors have concerns that high and repeated doses of oxytocin may result in uterine hyperstimulation or fetal distress. There are no published studies that have critically evaluated the most appropriate dose and dose frequency of oxytocin to use for maximal efficacy and safety in the bitch, and studies in humans report conflicting results.[48-53] In the bitch, doses of 5 to 20 units per dog, administered intramuscularly (IM) at 30- to 40-minute intervals, are most commonly recommended, although occasional authors report use of total doses of 1 to 5 U IM or subcutaneously (SC)[25,54-60] Intramuscular administration of oxytocin coincident with monitoring of uterine contractions was advocated by one author, starting with doses as low as 0.25 U, to a maximum dose of 4 U.[47] Higher doses of oxytocin caused uterine tetany and may have compromised fetal oxygen supply.

Oxytocin administration to the pregnant bitch may cause placental separation, constriction of umbilical vessels, and/or maternal vasodilation and hypotension.

Calcium. Calcium administration often is added to oxytocin administration, or used alone when concentrations of total or ionized calcium are low. Administration of oxytocin increases frequency of uterine contractions, whereas administration of calcium increases their strength.[47] Calcium ions are necessary for myometrial contraction. Although uterine inertia may be treated initially with oxytocin alone, many patients that do not respond to this treatment do respond to the additional administration of calcium.[35] It is not uncommon to see a dystocic bitch that responds to calcium therapy with explosive delivery of a puppy even when pretreatment total serum calcium concentration is normal. When extracellular calcium concentrations are low, a poor response to oxytocin administration would not be surprising, because oxytocin has a direct action on the rate of calcium ion influx into the myometrial cell.[61] Myometrial activity is suppressed during parturition when dogs are given a calcium channel antagonist.[62]

Calcium therapy for dystocia should be administered IV and slowly over 3 to 5 minutes, while auscultating the thorax for cardiac arrhythmia. Recommended doses of 10% calcium gluconate are 0.2 ml/kg IV or 1 to 5 ml/dog SC, coincident with external monitoring of strength of uterine contractions[47,54,60] Administration should be discontinued if the bitch becomes restless or changes in heart rate and rhythm occur.

Ergonovine. Ergonovine is an ergot alkaloid that can cause very strong uterine contractions when administered to the bitch; it should not be used as an ecbolic agent to treat canine dystocia, but it can be used to cause uterine contraction and vasoconstriction in bitches with postpartum hemorrhage. The canine dose for ergonovine is 10 to 30 μg/kg per os or IM. Women treated with oxytocin and ergonovine had less postpartum blood loss than those treated with oxytocin alone, but suffered side effects of nausea, vomiting, and increased blood pressure.[63]

Glucose. Glucose administration has been recommended by some authors for use in clinical management of canine dystocia, but hypoglycemia is very uncommon in the dystocic bitch. If hypoglycemia is diagnosed in association with pregnancy toxemia, oral glucose or a 5% to 10% glucose solution administered IV is indicated until euglycemia is achieved.

Tranquilizers. Tranquilizers sometimes are administered to a dystocic bitch to overcome voluntary inhibition of parturition or to facilitate clinical and vaginal examination in an extremely nervous animal. In general, their use is discouraged, because most will pass the placenta and depress puppies that remain in utero. In addition, barbiturates and promazine derivatives are poorly metabolized by the fetal liver. Following completion of parturition, tranquilization may be indicated occasionally; dopamine antagonists such as acepromazine and phenothiazine block dopamine receptors, leading to increased pituitary release of prolactin by the mother.[64] However, they still may depress puppies if secreted in the milk.

Medical Therapy Protocol. A suggested protocol summarizing medical therapy of bitches with dystocia is presented in Table 6–4. Despite the common occurrence of dystocia in the bitch, there are no controlled data on the

Table 6-4. Summary of Medical Approaches for Treating Canine Dystocia

History	Approach
Bitch has a 1- or 2-pup litter or fetal oversize	Cesarean section
Bitch has 5 or more pups remaining in utero	Cesarean section
Bitch has 4 or less pups remaining in utero and a nonobstructed birth canal	1. Give 0.1–2.0 IU/kg oxytocin IM; not to exceed 20 IU. 2. If a pup is born within 30 min, repeat the oxytocin at 30-min intervals until all pups are delivered. If delivery slows (>30 min between pups), add calcium as described below. 3. If no pup is born within 30 min following oxytocin therapy, give 10% calcium gluconate (0.2 ml/kg) *slowly* IV, not to exceed 5 ml. Repeat oxytocin after calcium treatment is given. If no pup is born after 30 min, perform cesarean section.

safest and most effective medical regimen in this species.

SURGICAL TREATMENT

Cesarean section should be performed before the dystocic bitch fatigues or distress occurs in the pups. In one review of 113 canine dystocia patients, 39 per cent were managed with manipulative or medical therapy alone, and 61 per cent required cesarean section with or without previous medical management.[35] In a later study, 65.7 per cent of 182 dystocic bitches were treated with cesarean section, and 44 of 145 (30.3 per cent) bitches treated with oxytocin and/or calcium whelped without surgical intervention.[19]

Prompt intervention is necessary for minimizing fetal death. The percentage of puppy death in bitches treated within 1 to 4.5 hours after the beginning of stage II labor was 5.8; percentage deaths rose to 13.7 in bitches treated 5 to 24 hours after onset of stage II labor.[19]

Bitches that have been in prolonged labor, those with a uterus containing dead or decaying fetuses or a friable uterus, or those showing signs of endotoxemia or septicemia should have cesarean sections. The toxic bitch requires supportive fluid and antibiotic therapy prior to, during, and following surgery. Culture of the uterine lumen is indicated at surgery if infection is suspected. Ovariohysterectomy at time of cesarean section occasionally is indicated when fetal death, putrefaction, gangrene, and/or toxemia are present.

Anesthesia. Anesthetic protocols recommended for use in cesarean section of the bitch are listed in Table 6–5.[65–67] The best protocol is one that is safest for the individual bitch and pups treated, that takes into account the physiologic changes that occur during pregnancy

Table 6-5. Anesthetic Protocols Recommended for Cesarean Section in the Bitch

General Anesthetic Protocols

Protocol 1[65]
1. Premedicate with atropine (0.04 mg/kg IM)
2. Induce with diazepam (0.2 mg/kg IV) and thiopental (8–12 mg/kg IV); intubate
3. Maintain with isofluorane or halothane

Protocol 2[65]
1. Induce with ketamine (5–10 mg/kg IV) and diazepam (0.2 mg/kg IV); intubate
2. Maintain with isofluorane or halothane

Protocol 3[65]
1. Premedicate with atropine (0.04 mg/kg IM)
2. Induce with diazepam (0.2 mg/kg IV) and oxymorphone (0.05–1.0 mg/kg IV); intubate
3. Maintain with isofluorane or halothane

Protocol 4[66]
1. Premedicate with atropine (0.04 mg/kg IM) or glycopyrrolate (0.011 mg/kg IM)
2. Induce with oxymorphone (0.1 mg/kg IV) and intubate gently; if bitch resists intubation, add thiopental (4–8 mg/kg IV)
3. Maintain with methoxyflurane (1% early; decrease to less than 0.3% if possible and add 50% nitrous oxide)

Protocol 5[67]
1. Premedicate with atropine (0.04 mg/kg IM) or glycopyrrolate (0.011 mg/kg IM)
2. Administer oxygen 3–5 minutes by mask prior to induction
3. Induce with *one* of the following:
   Thiamylal sodium (6–8 mg/kg IV)
   Thiopental sodium (6–8 mg/kg IV)
   Methohexital sodium (5 mg/kg IV)
   Droperidol-fentanyl (1 mg/20–30 kg IV)
   Diazepam (0.2–0.5 mg/kg IV) with ketamine HCl (4–8 mg/kg IV)
   Acepromazine (0.1 mg/kg IM or IV, not to exceed 4 mg) with oxymorphone (0.11 mg/kg IV)
4. Intubate; maintain with isoflurane, halothane, enflurane or methoxyflurane

Regional Anesthetic Protocol[65,66]
1. Premedicate with atropine (0.04 mg/kg IM)
2. Administer bupivacaine (1 ml/3.5 kg) epidurally
3. Administer oxymorphone (0.1 mg/kg IV)

and that is most familiar to the anesthetist.[66–68] Local, regional and general anesthetic protocols have been advocated.[65–67] General anesthesia usually is preferred for complete analgesia and immobilization and for bitches such as those of brachycephalic breeds with risk of upper airway obstruction. Disadvantages of general anesthesia include depression of the fetuses and the bitch. Disadvantages of regional or local anesthesia are the need for assistants to control the patient's movement and regional vasodilation, which can result in hypotension, fetal hypoxemia, and exacerbation of surgical bleeding. Neuromuscular blocking agents, such as succinylcholine, gallamine, pancuronium bromide, and dimethyltubocurarine, have been used occasionally for cesarean sections in the bitch. Gallamine crosses the placenta, but adverse side effects on the fetus are minimal if appropriate doses are used.[69] The other agents do not cross the placenta in significant amounts, and therefore can be used as adjunctive agents to quiet the bitch and to permit use of lower (analgesic, not anesthetic) doses of the more depressant agents. Disadvantages of muscle relaxants include the need for controlled ventilation and increased technical help. Intravenous fluid therapy is indicated with all anesthetic protocols in order to manage vasodilation and hypotension. Administration of oxygen to the bitch by mask prior to induction is indicated in all dystocia patients to help prevent fetal hypoxemia.[65]

Physiologic changes affecting anesthesia that occur with advancing pregnancy include increased maternal blood volume and cardiac output, decreased vascular resistance, decreased functional residual capacity and expiratory reserve volume, increased inspiratory capacity and inspiratory reserve capacity, and increased respiratory rate, tidal volume, minute volume, and alveolar ventilation.[66] Increased alveolar ventilation may shorten induction time with gas anesthesia.

Tilting bitches 10 to 15 degrees to the side may prevent supine hypotension resulting from weight of the gravid uterus on the caudal vena cava in bitches greater than 30 kg; tilting appears unnecessary in bitches less than 30 kg. In one study of pregnant beagles anesthetized with thiamylal sodium and halothane/nitrous oxide for cesarean section, tilting 10 to 15 degrees toward right or left lateral recumbency or complete lateral recumbency (either side) was not better than dorsal recumbency.[68] Maternal position had no effect on direct arterial blood pressure, arterial blood gases, pH, base excess, heart rate, respiratory rate, or the electrocardiogram. However, pregnancy itself had a significant effect on every parameter measured. Pregnant bitches had lower systolic blood pressure, lower pO_2, lower hematocrit, higher pCO_2, higher respiratory rate, and more severe acidosis than they did after parturition.

Because the bitch requiring cesarean section may not have been fasted, and because the gravid uterus compromises stomach capacity, vomiting and aspiration of vomitus are risked during this procedure. Induction of anesthesia and tracheal intubation should be performed quickly to avoid these risks.[67]

Technique. Surgical protocol recommended for use in cesarean section of the bitch starts with a ventral midline incision from the umbilicus to the pubis. The linea alba often is stretched thin, so care must be taken not to incise underlying organs.[65] The uterine horns are exteriorized and isolated with saline-moistened laparotomy sponges. A dorsal midline incision is made in the uterine body, avoiding inadvertant laceration of an underlying pup. The fetus in the uterine body is removed first, followed by those in the horns through the same surgical incision. After each delivery the surgeon breaks the fetal membranes covering the pup's head with a gauze sponge and wipes the nasal area prior to separating the placenta from the uterine wall. Alternatively, in cases of emergency, pups may be removed rapidly from one or multiple uterine incisions, and the pups handed to attendants for cleaning and resuscitation.

Placentae may be difficult to separate from the uterine wall in preterm bitches. If so, the umbilical cord is clamped and cut 2 to 3 cm from the fetal abdomen, and the pup handed to the attendant. Because of the zonary pattern of the canine placenta, retained placentae may impede passage of the next pup through the uterine incision, necessitating multiple incisions. Generally, the placentae separate from the uterus easily, and are removed along with the pups (Fig. 6–14).

After delivery of the last pup, the uterine lumen is inspected for hemorrhage and evidence of infection; bacterial culture of the uterine lumen or full-thickness uterine biopsy may be performed if indicated. If hemorrhage is excessive, the bitch should be evaluated for coagulopathy.[70,71] The hysterotomy incision is closed with 3-0 or 4-0 absorbable suture material on a taper-point needle. A simple continuous pattern can be used for the first layer, care-

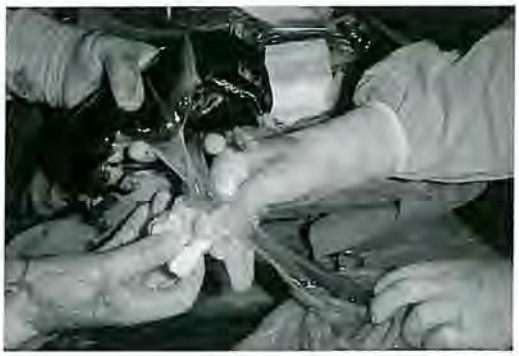


Figure 6–14. Demonstrating the gentle removal of a placenta during a cesarean section. The placenta is left attached to the pup, with both being given to an attendant.

fully avoiding penetration of the uterine lumen, followed by a continuous Cushing oversew pattern for the second layer. If sutures are placed carefully and knots buried, adhesion formation will be minimal.[65] Following closure of the uterus, oxytocin (0.1 to 2 IU/kg IM or intrauterine) may be administered to promote uterine involution.

If the abdomen has been contaminated by fetal fluids, it should be lavaged with 100 to 200 ml/kg warm saline or balanced electrolyte solution. The abdomen and skin are closed routinely. Ovariohysterectomies can be performed at the time of cesarean section without complicating milk production or milk release.

En bloc ovariohysterectomy before hysterotomy and removal of the fetuses has been described in the bitch.[72] Although the authors report survival of 75 per cent of the pups delivered from 37 bitches using this technique, it is possible that survival would have been greater if pups had been removed from the uterus prior to ligating blood vessels and impeding oxygen delivery to the pups.

Cesarean section does not mandate repeat cesarean section in the bitch unless cause(s) of the dystocia recur. The authors are aware of many vaginal deliveries from bitches with previous cesarean sections. However, previous dystocia with cesarean section may increase risk for recurrence of dystocia, so it is advisable to predict anticipated whelping date accurately and to monitor such patients aggressively.

Care of Neonates. Care of neonates delivered by cesarean section includes removal of fetal membranes and removal of fluid in the mouth and nose using gauze sponges or gentle suction. Although some breeders advocate gentle swinging of the puppy downward (head down with head cupped in hands) to promote oronasal fluid expulsion, this procedure may result in trauma to the pup. The pups should be dried with a soft towel, wrapped in a warm cloth, and kept near hot water bottles or on a circulating warm water blanket.

If delivered pups are depressed or not breathing, 1 to 2 drops of doxapram, a respiratory analeptic, should be administered to the pup's tongue. Doxapram selectively stimulates respiration and increases tidal volume by activating carotid chemoreceptors.[54] If spontaneous respiration does not follow within a minute, doxapram can be administered a second time, and gentle chest massage begun. Oxygen can be administered via face mask or a catheter placed in the pup's trachea. Care must be taken not to overinflate the pup's chest. It may take several minutes of treatment before the pup begins to breathe spontaneously, and exhibit bright pinkish mucous membrane color (Fig. 6–15). At this time, the attendant can ligate the umbilical stalk 2 to 3 cm from the body wall, remove the placenta, and disinfect the umbilicus with dilute iodine solution. Importance of permitting placental blood to flow into the neonatal puppy is unknown; in some species, umbilical blood flow is not present in the first 2 minutes of life.[73] It also is unknown whether additional blood would be beneficial or harmful to pups. Experimental induction of polycythemia in puppies has been shown to predispose to necrotizing enterocolitis.[74]

When the pups are breathing normally and fetal membranes have been removed, they should be examined for presence of birth defects such as cleft palate (Fig. 6–16). Pups should be weighed and identified by sex and coat markings, or marked with a noncaustic permanent marker such as fingernail polish for identification. They should then be placed

Figure 6–15. A healthy newborn pup after successful resuscitation.

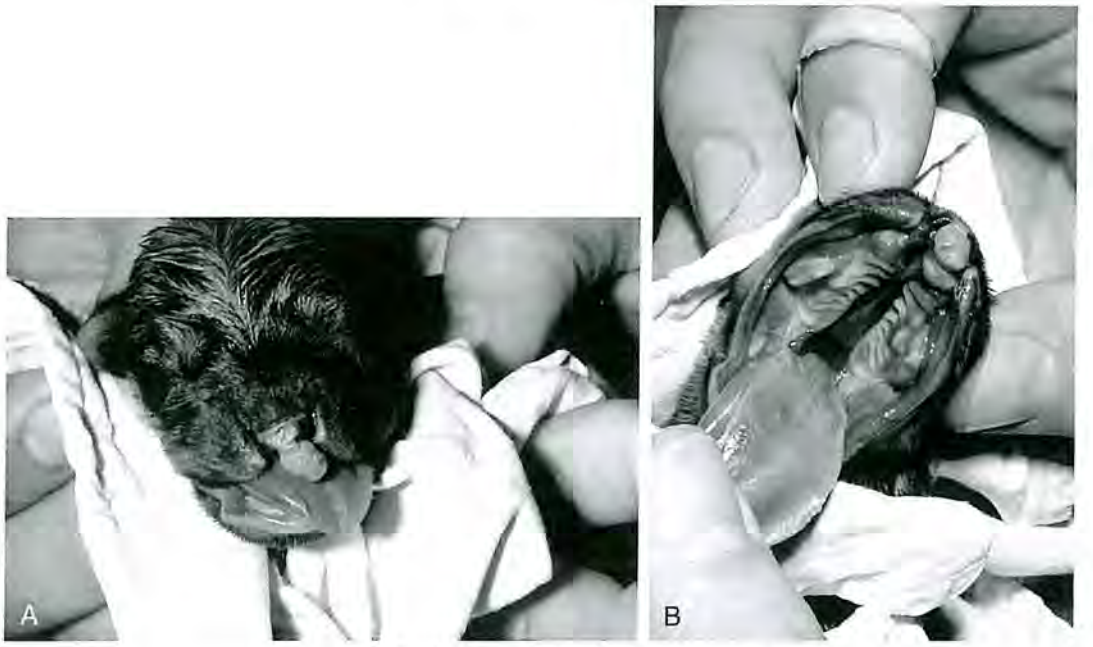


Figure 6–16. Fetal defects noted at the time of resuscitation.

in a prewarmed box until the bitch is able to care for them, and should receive colostrum from their mother or another bitch within 24 hours (see Chapter 8).

Medical Prevention of Parturition in the Bitch

Preterm labor in women and other species is treated with tocolytic drugs, which are those that interfere with uterine contractions.[75–78] In the bitch, preterm labor is not a well-documented phenomenon. Even if preterm labor is diagnosed in the bitch based on knowledge of ovulation day, pregnancy status, and evidence of uterine contractions, use of tocolytic drugs should be strongly discouraged, because they may prevent uterine evacuation in the presence of infection or serious fetal defect. Progesterone therapy has been suggested as a tocolytic therapy for dogs with insufficient luteal function, since serum concentrations above 1 to 2 ng/ml appear to inhibit normal parturition. Dose levels and dose frequency that could maintain pregnancy and yet permit normal parturition are not known.

Ally-trenbolone is a progestational agent shown to maintain pregnancy in bitches ovariectomized on days 34 to 42 of gestation.[79] The drug was given orally at 0.088 mg/kg/d until 2 days before anticipated whelping date, as determined using the diestrous vaginal smear. Bitches whelped between 0 and 2 days after ally-trenbolone therapy was discontinued. All pups born to one bitch the day that treatment was discontinued died following obstructive dystocia with oversized pups. Four of 6 pups born to one bitch 1 day after treatment was discontinued survived, and all 12 pups born to two bitches 2 days after treatment was discontinued survived. Milk production was minimal at parturition but increased within 2 days of whelping.

Because calcium ions are essential for myometrial contraction, calcium channel agonists have been proposed as tocolytic agents. Oral administration of the calcium channel agonist nifedipine (Adalat; Bayer AG, Germany; 10 mg/d) to beagle bitches resulted in suppression of myometrial activity for 60 to 90 minutes during pregnancy.[62] Although no serious clinical side effects were noted in the treated bitches, long-term safety and efficacy were not established for use in spontaneous preterm labor.

Medical Induction of Parturition

Medical induction of parturition may be indicated in the bitch if the pregnancy is compromising maternal health, such as with pregnancy toxemia (Chapter 5). Drugs used to

terminate unwanted pregnancy (Chapter 9) may be used for induction of parturition near term. Preterm induction of parturition in the healthy bitch has not been well studied, however, and, because immaturity of the fetus and hyaline membrane disease have been documented in the dog, this procedure is not recommended for owner convenience. In addition, drugs such as prostaglandins may require 3 to 5 days to induce parturition, so cesarean section or ovariohysterectomy are recommended if need for delivery is urgent.

Glucocorticoids

Dexamethasone was reported effective in terminating pregnancy in 20 bitches 2 to 16 days after initiation of treatment.[80] One dose regimen used was 0.2 mg/kg per os three times a day for 5 days followed by progressively decreasing doses of 0.16 to 0.02 mg/kg per os three times a day for 5 days. The second dose regimen was a 7.5-day course of twice-daily therapy, with dose increasing from 0.1 to 0.2 mg/kg over the first three doses, then 0.2 mg/kg for days 2 through 5, then decreasing from 0.16 to 0.02 mg/kg over the last five treatments. Side effects of polydipsia and polyuria disappeared when therapy stopped. All bitches were treated prior to day 51 of pregnancy, and all fetuses were born alive and died within a few hours.

Dexamethasone also has been used to terminate 57- to 58-day canine pregnancies at a dose of 0.4 mg/kg administered parenterally one time (W.R. Threlfall, Ohio State University, personal communication, June 1995). Parturition started within 1 to 2 days. Late-term glucocorticoids may enhance viability of pups by enhancing maturation of the fetal lung, as they are known to do in humans.[81]

Increased incidence of retained fetal membranes in the dam and decreased intestinal absorption of immunoglobulins by the calf occur when parturition is induced with dexamethasone in cows.[82,83] Although fetal membrane retention is rare in dogs, further studies are indicated to determine whether this may be an adverse sequel to glucocorticoid treatment. Pups may have lower absorption of immunoglobulins when bitches receive cortisol or ACTH prior to parturition.[84]

Prostaglandins

PGF is reported to terminate pregnancy in the bitch 3 to 5 days after SC or IM doses of 20 μg/kg every 8 hours, or 30 μg/kg every 12 hours for 72 hours, or 250 μg/kg every 8 hours for 4 days.[85] Not all bitches treated with the lower dose abort. The majority of fetuses expelled in PGF abortions are live with fetal membranes intact. Similar regimens probably would be effective in inducing parturition in late-term bitches, but PGF effect on delivered pups is unknown.

Oxytocin

Oxytocin is reported effective at inducing parturition in the mare, but not in the cow, ewe, doe, or sow.[12] Administration of oxytocin increases intrauterine pressure in the nonpregnant bitch, and may change uterine activity in the last week of pregnancy when serum progesterone concentrations are declining.[86,87] It is unknown whether oxytocin can induce normal parturition in the term bitch or the bitch with elevated serum progesterone concentrations. Oxytocin infusion into normal dogs is associated with increased plasma levels of glucose, insulin, and glucagon.[88]

Mifepristone (RU 486)

Progesterone antagonists have been reported to induce premature delivery in the bitch.[89] Bitches treated with mifepristone starting on day 32 of pregnancy expelled dead fetuses or dark mucoid vulvar discharge. Whether this drug can induce parturition safely at term in unknown.

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Chapter 7

Peripartum Disorders in the Bitch

Care of the Postpartum Bitch

Early detection of peripartum disorders is important, and may save the life of the bitch and pups. Peripartum disorders include a wide variety of diseases, ranging from those that resolve without medical or surgical treatment to those that are life threatening and require vigorous therapy to ensure a positive outcome for the bitch and pups. Peripartum disorders may occur before parturition (e.g., hypocalcemia) or up to several weeks after parturition (e.g., subinvolution of placental sites). The peripartum period extends from late gestation through several weeks after parturition; the postpartum period refers to the period following parturition and also includes the periods of lactation and weaning (see Chapter 8).

Occasionally, owners request that oxytocin or "clean-out" shots be given to the postpartum bitch. Oxytocin need not be given routinely if the mother is allowed to nurse the neonates; suckling by neonatal pups will induce oxytocin release from the posterior pituitary gland. It may be desirable to give oxytocin to promote uterine involution if the pups are born dead or removed from the mother at birth.

The dam can be allowed to eat soon after parturition is complete. As lactation progresses, the bitch may consume two to three times her normal food intake to provide energy for sustained milk production. Even with this additional consumption of food, the bitch should be evaluated carefully for excessive weight loss. In most cases, the lactating bitch should be fed ad libidum or at least three to four meals each day.

Rectal temperatures should be taken daily for 1 to 2 weeks following parturition. Temperatures exceeding 103.5°F may indicate the presence of metritis, mastitis, or hypocalcemia. Mammary glands and vulvar discharge should be inspected daily for evidence of purulent discharge or odor. Owners should be instructed on the normal appearance of milk and lochia in the postpartum bitch. Milk may be yellowish or white at the time of whelping (see below). If owners are instructed to express milk from each gland for visual inspection, they must adhere to strict hygiene so as not to introduce infectious agents into the teat orifices. Normal lochia will discharge from the uterus, passing through the vulva, for up to 3 weeks postpartum.

Weaning of pups should be accomplished gradually. Pups can be started on some soft foods as early as 3 weeks of age. By 5 to 7 weeks postpartum, the pups can be weaned completely from the bitch. Owners should be encouraged to wean the pups gradually; inflammation of the mammary glands and anxiety in the bitch may accompany acute weaning.

Metritis

Metritis is inflammation of the endometrium and myometrium (unlike endometritis, which is inflammation of only the endometrium or inner mucous membrane of the uterus).[1] Acute puerperal metritis, a disease of the immediate postpartum period (i.e., 0 to 7 days postwhelping), is severe inflammation of the endometrium and myometrium that causes systemic illness in the bitch. Acute puerperal metritis may occur in association with retained placentas, retained pups, macerated or decomposed pups that are delivered per vagina eventually, or prolonged delivery. Although bacteria usually are not present in the uterus of the

mature bitch,[2] they have ready access to the uterus at the time of parturition when the cervix is dilated. Bacteria can thrive in retained or devitalized tissues, resulting in inflammation of the endometrium and myometrium. If the condition is untreated, septicemia or toxemia follows.

Bitches with metritis may be depressed, have high rectal temperatures (103.5° to 105°F), and demonstrate little interest in their pups. A malodorous or putrid, reddish brown uterine discharge may be observed passing from the vulva. While normal lochia can pass for up to 3 weeks following parturition, it is nonodorous and greenish black in color, and not associated with systemic signs of illness.

Cytologic evaluation of uterine discharge samples obtained when passing from the vulva may be helpful when diagnosing acute metritis in the postpartum bitch. Neutrophils are frequently present in the uterine discharge of bitches with acute metritis, but may go unidentified because of severe degenerative changes (Fig. 7–1). Bacteria often are present in smears of uterine discharge from metritic bitches, either free or within white blood cells. Erythrocytes, endometrial cells, and muscle fibers from decomposing fetuses may be observed in smears of uterine discharge (Fig. 7–2). Although neutrophils, erythrocytes, and bacteria may be observed in smears made from discharge passing from the vulvas of normal postpartum bitches, the bacteria and white blood cells are fewer in number, and the neutrophils do not appear degenerative.[3]

An immature neutrophilic leukocytosis frequently accompanies acute metritis, although a normal hemogram may be found occasionally. Leukopenia with immature neutrophils may be present in severe cases of metritis.

Bitches with metritis may be in shock from severe dehydration (i.e., hypovolemic shock), septicemia, or endotoxemia. Treatment of acute metritis consists of treating shock, replacing fluid deficits, initiating broad-spectrum antibiotic therapy, and giving dextrose intravenously (IV) if septicemia or toxemia has resulted in hypoglycemia. Once a bitch is stabilized, it may be necessary to consider surgery to remove remaining placentae and/or devitalized fetal or uterine tissues that are present. If surgery is performed, the uterine contents and uterine tissues should be cultured for aerobic and anaerobic bacteria. In cases where only medical therapy is adequate (i.e., surgery is not necessary), cultures of the cranial vagina should be obtained to identify the bacterial component of the disorder and determine the microbial sensitivity.

Although a catheter may be passed through the postpartum cervix, the merits of infusing the uterus with antibiotics or draining uterine contents are unknown. The uterus may be friable, and manipulation could result in bacteremia or uterine rupture. Although antibiotic infusions have been used to treat metritis in other species for several decades, several studies have suggested that such treatments may be contraindicated.[4–7] Antimicrobial agents infused directly into the uterine lumen may impede the phagocytic function of uterine neutrophils. Even antiseptic solutions infused into the uterine lumen, such as iodine, may damage uterine neutrophils. Additionally, many antimicrobial or antiseptic agents may be irritating to the endometrium, resulting in future infer-

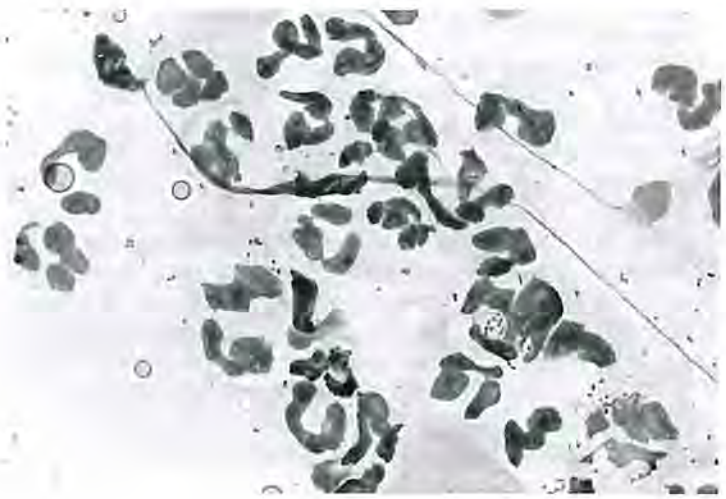


Figure 7–1. Degenerating neutrophils in a vaginal smear from a bitch with necrotizing metritis. Magnification: 1000×.

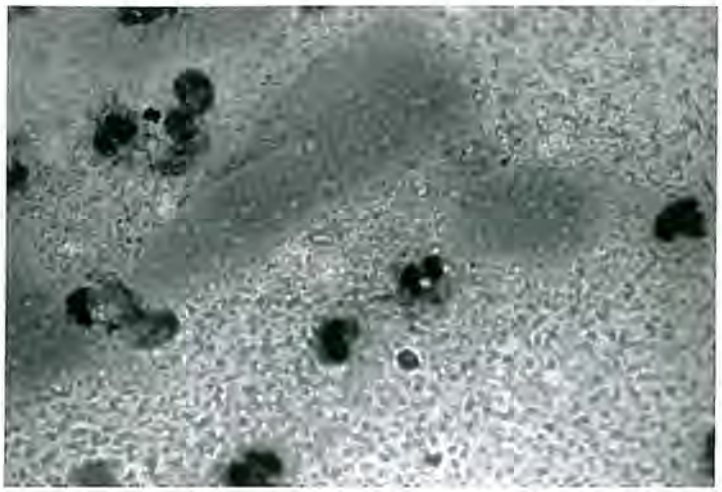


Figure 7–2. Skeletal muscle fibers and neutrophils in a vaginal smear from a bitch with a decomposing pup remaining in the uterus. Magnification 400×.

tility. The presence of purulent material and tissue debris reduces the efficacy of some antimicrobial agents that have been used historically for infusions, such as sulfonamides, aminoglycosides, and nitrofurazone.[8] Although similar information is lacking for the bitch, it seems prudent to consider the bovine studies prior to making a decision to infuse the uterus of a postpartum bitch.

The role of various ecbolic agents in treating canine metritis remains uncertain. Although ecbolics could be used in an attempt to evacuate the uterus, they must be used with caution if a devitalized uterus is prone to rupture if made to contract. Oxytocin has a short half-life, making it fairly safe as an ecbolic agent. The effect of oxytocin on causing uterine contractions has been reported to diminish soon after parturition as estrogen:progesterone ratios change.[9] However, both oxytocin and prostaglandin $F_{2\alpha}$ cause an increase in intrauterine pressures when given to bitches at times other than solely at parturition.[10] The effect of suckling, or the administration of oxytocin, on uterine activity at various times postpartum in the bitch is unknown.[9,11] Use of ergonovine to treat metritis in the bitch is not recommended because uterine rupture may result.[12]

The efficacy and safety of using prostaglandin $F_{2\alpha}$ in treating canine metritis have not been studied critically. Prostaglandins have been advocated as useful treatments for bovine metritis, and possibly are superior to antibacterial drugs in some cases.[4,8] The rationale for the use of prostaglandin $F_{2\alpha}$ includes stimulation of uterine contractions to expel purulent materials and debris, and the possible stimulation of phagocytosis by leukocytes. However, because tissue levels of prostaglandins may already be increased in cases of uterine disease in the bitch, the enhancement of leukocyte-phagocytosis from pharmacologic doses of prostaglandins remains to be determined. In fact, the capacity for phagocytosis of blood neutrophils was actually decreased in bitches with pyometra, even though prostaglandin metabolites were increased in uterine exudate, blood, and serum.[13,14] The efficacy and risk of using prostaglandins to treat canine metritis may vary, depending on the integrity of the myometrium and uterine wall.

Mastitis

Mastitis, like acute metritis, is a disease that primarily affects the postpartum bitch. Rarely, mastitis may occur in the lactating, pseudopregnant bitch. Mastitis may involve one or more sections of a mammary gland or one or more mammary glands. The number of duct orifices opening on a teat varies from 7 to 22[15] (Fig. 7–3). The teat orifice, teat canal, teat sinus, and gland sinus are part of the sinus system (Fig. 7–4). Each gland sinus is separated from surrounding sinuses by connective tissue septa. Thus mastitis can be diffuse within or among glands, or can be localized within a gland. Mastitis can be acute and life threatening, with the bitch displaying signs of systemic illness. In chronic cases the bitch may be asymptomatic but is brought to the veterinarian because her pups are failing to thrive.

ACUTE MASTITIS

In severe cases, the affected glands are hot and painful and the bitch is systemically ill (i.e.,

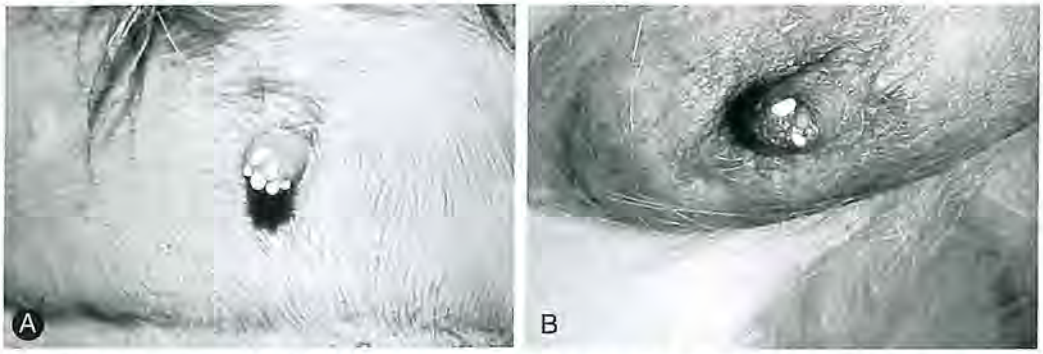


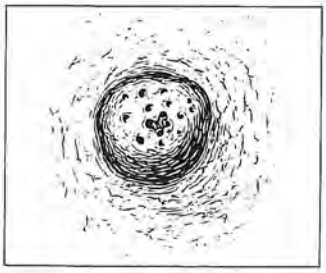
Figure 7–3. A: Milk drops that formed after a bitch's mammary gland was gently expressed. Note the normal-appearing milk and the hemorrhagic discharge from one teat orifice (right). **B:** Normal-appearing milk from two teat orifices and a purulent-appearing discharge from a third orifice (center and right). (From Wheeler SL, Magne ML, Kaufman J, et al: Postpartum disorders in the bitch. Compend Contin Educ Pract Vet 6:495, 1984, with permission.) *See Color Plate*

depression, lethargy, anorexia, fever, failure to care for pups). Bacteria commonly isolated from the milk of bitches with septic mastitis include *Escherichia coli*, staphylococci, and streptococci.[16] Bacteria and degenerative neutrophils usually are abundant in smears prepared from the milk of the involved gland or glands (Fig. 7–5). Caution in interpreting milk smears is necessary, because normal canine milk also may contain large numbers of neutrophils and macrophages[17] (Table 7–1). However, abundant numbers of free and engulfed bacteria are not observed in normal milk unless introduced by contamination or improper storage of samples.

Normal canine milk expressed from the mammary glands at the time of whelping is yellowish to white. A yellowish color at the time of whelping may reflect the high concentration of immunoglobulins in colostral milk. The mammary secretions at whelping also may appear white, or become white from several hours to a few days postpartum. Milk from mastitic bitches may appear normal, have a characteristic purulent appearance (greenish yellow), or appear reddish brown as a result of the presence of erythrocytes and/or leukocytes. A hemorrhagic secretion also has been observed in asymptomatic pseudopregnant and postweaning bitches,[17] but blood generally is absent in milk samples from normal bitches at parturition and during lactation. If milk is expressed gently, drops may form from the various teat orifices, allowing abnormal secretions to be identified if the infection is localized.

Quantitative bacteriologic evaluation may reveal a large number of bacteria in milk from mastitic bitches. Bacteria are isolated frequently from milk samples of normal bitches. Whether these microorganisms are present in the normal sinus system of the mammary gland or enter expressed milk as skin contaminants is unknown. In one study,[18] the milk of 44 clinically healthy postpartum bitches was cultured. Small numbers of microorganisms were isolated from most of the milk samples; 67.4 per cent of the samples had 10^4 bacteria/ml or less. *Staphylococcus aureus* was the most commonly isolated microbe. The role of anaerobic bacteria or mycoplasma in canine mastitis has yet to be determined.

Bitches with septic mastitis should be treated on the basis of antimicrobial sensitiv-


Teat with orifices

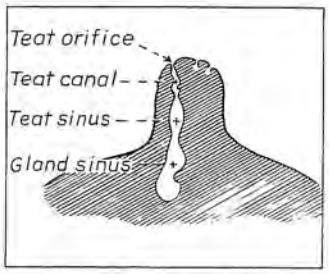


Diagram of sinus system

Figure 7–4. Teat and sinus structure of the dog.(From Evans HE, Christensen GC: The urogenital system. *In* Evans HE [ed]: Miller's Anatomy of the Dog, 3rd ed. Philadelphia, WB Saunders, 1993, p 552, with permission.)

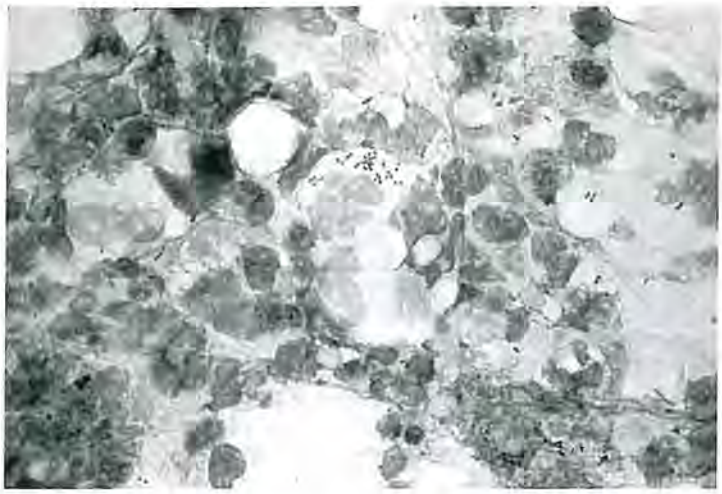


Figure 7–5. Bacteria and degenerating neutrophils in a milk smear from a bitch with acute mastitis. Magnification: 1000×.

ity, pharmacokinetics of selected antibiotics, and whether neonates will continue to nurse from the treated bitch. Pups should be allowed to nurse unless abscessation or gangrene is present, as nursing encourages drainage of the gland and provides antibiotics to the neonates. If pups are allowed to continue nursing, antibiotics should be selected on the basis of safety for both the mother and neonate, especially if the selected therapeutic agent is one that concentrates in milk. Although human infants are allowed to continue nursing from mastitic glands, there is one report of mother-to-infant transmission of *Streptococcus agalactiae* from ingestion of infected mother's milk.[19] Although blood and tissue cultures of a neonate and cultures of the mother's milk may verify that similar microorganisms are associated with mastitis and neonatal septicemia, it may be difficult to determine the original source of either infection. For example, the mouth of a nursing neonate may be the source of bacteria introduced into the mother's mammary gland. One of the authors (P.N.O.) is familiar with two clinical cases in which the same type of bacteria was isolated from the milk of the bitch and the tissues of her septicemic pups. The mode of transmission, however, was not determined.

The decision on whether to hand-rear pups of mastitic dams or allow them to continue nursing is a difficult one that may depend on available nursing care and age of the pups. During the first 2 weeks of a pup's life, when most cases of mastitis occur, pups may need to be fed every 2 to 4 hours. Therefore, owners

Table 7–1. Cell Numbers and Types in Canine Milk

	Total Cells	Macrophages	Polymorphonuclear Cells	Unidentified Mononuclear* Cells
Normal bitches with nursing pups (n = 13)	33–14,548†	0–14,088	0–1418	0–1942
Normal bitches postweaning (n = 3)	13,750–67,654	8054–8869	5303–54,402	1577–4875
Pseudopregnant bitches (n = 3)	7302–38,233	5448–27,211	844–8808	0–910
Abnormal bitches‡ (n = 6)	4302–363,000	157–76,230	2352–283,400	751–24,861

* Unidentified mononuclear cells probably are degenerative nuclei of fat cells.
† Range of means: cells per microliter of milk.
‡ Abnormal bitches include those with mastitis, mammary duct ectasia, and galactostasis and those having septicemic puppies.
Table derived from clinical study data in Olson and Olson.[17] The authors thank A.L. Olson for technical assistance in collecting data for this table.
From Olson JD, Olson PN: Disorders of the canine mammary gland. *In* Morrow DA (ed): Current Therapy in Theriogenology: Diagnosis, Treatment, and Prevention of Reproductive Diseases in Small and Large Animals, 2nd ed. Philadelphia, WB Saunders, 1986, p 507, with permission.

must be available to spend considerable time bottle or tube feeding hand-reared pups during the first 2 weeks of life. If mastitis is diagnosed after 2 weeks postpartum, and a decision is made to hand-rear the pups, it is possible to feed pups a replacement formula every 4 to 6 hours. By 3 weeks of age, pups can be offered a moist dog food and the weaning process can be initiated.

Because the majority of passive immunity in pups is obtained through colostral antibodies, pups should receive a bitch's milk within the first 24 hours of life. Colostral immunoglobulins decrease rapidly during the first few days postpartum; colostrum contains 1500 mg of immunoglobulin/100 ml at whelping, but this concentration decreases to less than 300 mg/100 ml by day 2 after whelping.[20] The ability of the newborn pup to absorb immunoglobulins from the intestinal tract is maximal at 8 hours after birth, with limited absorption after 15 to 24 hours postpartum.[21,22] Although adult pooled serum can be given to neonatal pups that fail to receive adequate colostrum, the beneficial effects of colostrum are difficult to replace.[23,24] Pups allowed to receive colostrum had significantly higher blood levels of immunoglobulins A, G, and M than those deprived of colostrum but receiving pooled adult dog serum (22 ml/kg orally or subcutaneously).[23] Even when pups are no longer able to absorb immunoglobulins through the intestinal tract, a bitch's milk can continue to provide important factors for protecting against potential pathogens: lymphocytes, neutrophils, macrophages, locally secreted immunoglobulin A, proteins, and epidermal growth factors. In one study, weight gains and body measurements were greater in beagle pups who were normal at birth and nursed from their dams than in littermates fed one of two homemade replacement formulas* (M.L. Chandler et al., unpublished data, Colorado State University, 1989) (Fig. 7–6). Occurrence of diarrhea in the formula-fed pups was 85 per cent compared to 52 per cent in the control pups. However, bitches with severely inflamed glands may not be able to provide adequate quantities of milk or milk with appropriate nutrients to meet the needs of growing pups, necessitating the use of well-balanced homemade or commercial formulas.

* Formula 1 contained 240 ml whole milk, 2 egg yolks, 5 ml vegetable oil, and 1 drop of Poly Visol infant vitamins (Mead Johnson Division, Bristol Myers, Evansville, IN). Formula 2 was the same as Formula 1 except it also contained 1.2 g dicalcium phosphate and 0.3 g calcium carbonate.

If pups are hand-reared, the veterinarian can select a therapeutic regimen that is most appropriate for treating the mastitis without being concerned about the potential effects of various antibiotics on the nursing neonate (i.e., altered intestinal flora, staining of neonatal teeth, inability of fetal livers to metabolize the drug). If the pups are allowed to nurse while the bitch is treated, a broad-spectrum bactericidal antibiotic should be selected that lacks toxicity for the bitch and neonates (Tables 7–2 through 7–4).[25–31] In the presence of acute inflammation, many antibiotics will be able to pass through the plasma-milk barrier. Wallace and Davidson[32] suggested that first-generation cephalosporins and β-lactamase–resistant penicillins be used to treat mastitis (cephalexin, 2.5 to 7.5 mg/lb [5.0 to 15 mg/kg] given orally every 8 hours; clavulinic acid–potentiated amoxicillin, 7 mg/lb [14 mg/kg] given orally every 8 to 12 hours) until the results of antimicrobial susceptibility from the cultured milk are available. This recommendation is a good one, especially if a gram-positive infection is suspected. If a gram-negative or mycoplasmal infection is suspected, other choices might also be considered (Table 7–4).

Although antimicrobial agents have been the mainstay of treating canine mastitis, future therapies may add immune stimulators. Homologous recombinant cytokines are effective immunomodulators that augment natural defensive mechanisms and may have a future role in treating mastitis. Interleukin-1β and interleukin-2 have been used experimentally to treat *St. aureus* mastitis in cows,[33] but have not been evaluated in the treatment of canine mastitis.

Acute mastitis may progress to abscessation or gangrenous mastitis, conditions in which the pups should not be allowed to nurse (Fig. 7–7). Gangrenous mastitis is recognized by the presence of black mammary tissue on the surface of the gland. This tissue is dead and should be incised for drainage and débridement. Surgical drainage and flushing with 1% providone-iodine (Betadine) solution are indicated to relieve the inflammation. Bitches with acute or gangrenous mastitis may be able to provide sufficient milk to pups in a subsequent lactation, providing that diffuse inflammation and scarring have not occurred.

INFLAMMATORY MAMMARY ADENOCARCINOMA

Inflammatory adenocarcinoma is a highly malignant neoplasm of the canine mammary

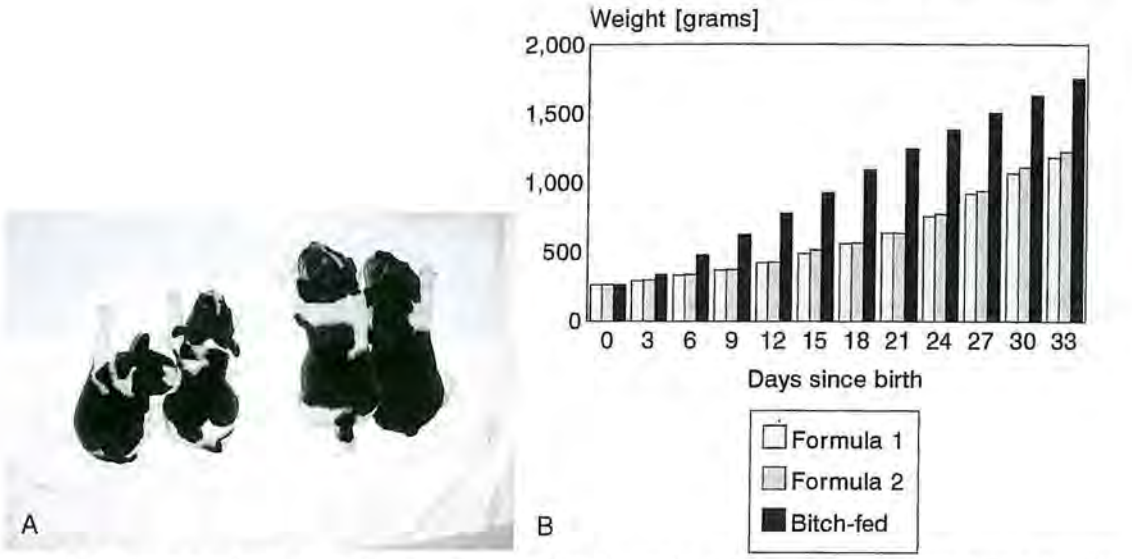


Figure 7–6. A: Beagle pups from the same litter. Smaller pups on the left were fed a homemade formula. Larger pups on the right were allowed to remain nursing from the dam. Following weaning, all pups reached similar weights. (Courtesy of M.L. Chandler, Colorado State University, 1989.) **B:** Mean body weights of beagle pups (homemade formulas versus bitch's milk). (Courtesy of M.L. Chandler, Colorado State University, 1989.)

gland that can mimic mastitis. Bitches with inflammatory adenocarcinoma are generally older bitches that are not necessarily postpartum or even sexually intact, but who have severely inflamed mammary glands. Thrombocytopenia and disseminated intravascular coagulopathy often occur in bitches with inflammatory mammary adenocarcinoma. The prognosis in these cases is extremely guarded. Although mammary tumors will be discussed in Chapter 13, inflammatory mammary adenocarcinoma is mentioned here to alert the veterinarian to a serious disorder that can mimic acute mastitis. It is especially important that the veterinarian evaluate the atypical bitch (i.e., nonpostpartum or neutered older animal) for coagulation factors prior to performing any surgical procedure for treating intensely inflamed mammae.

CHRONIC OR SUBCLINICAL MASTITIS

Chronic mastitis often is suspected when nursing pups fail to thrive. If mastitis is demon-

Table 7–2. Pharmacologic Properties of Certain Antimicrobial Drugs

Drug	Lipid Solubility	Acid/Base	pK_a	Able to Exceed Serum Concentration in Milk?
Ampicillin	Moderate	Acid	2.8, 7.2	No
Amoxicillin	Moderate	Acid	2.8, 7.2	No
Cephalothin	Low	Acid	2.5	No
Chloramphenicol	High	Alcohol	n/a	Equal
Ciprofloxacin	High between pH 6 and 8	Amphoteric	6.4, 8.7	Yes
Clindamycin	High	Base	7.6	Yes
Enrofloxacin	High between pH 6 and 8	Amphoteric	6.4, 8.7	Possibly
Erythromycin	High	Base	8.8	Yes
Gentamicin	Low	Base	8.0	No
Lincomycin	High	Base	7.6	Yes
Norfloxacin	High between pH 6 and 8	Amphoteric	6.4, 8.7	Yes
Penicillin G	Moderate	Acid	2.8	No
Sulfamethoxazole	Moderate	Acid	5.6	No
Sulfadiazine	Moderate to high	Acid	6.5	No
Tetracycline	Moderate	Amphoteric	3.3, 7.7, 9.7	Equal
Tylosin	High	Base	7.1	Yes

Data from Olson and Olson,[25] Ziv,[26] Baggot,[27] Budsberg et al.,[28] and Gasser et al.[29]

Table 7–3. Antimicrobials That Affect Colonization Resistance

Antimicrobials that suppress colonization resistance with resultant increases in Enterobacteriaceae colonization in the pup
Ampicillin
Cloxacillin sodium
Furazolidone
Metronidazole
Antimicrobials with a moderate effect on colonization resistance
Amoxicillin
Chloramphenicol
Tetracycline
Antimicrobials with no adverse effect on colonization resistance
Aminoglycosides
Cephalosporins
Doxycycline
Erythromycin
Penicillin (parenteral)
Sulfonamides
Trimethoprim
Quinolones

Data from Jones[30] and Poffenbarger et al.[31]

Figure 7–7. Gangrenous mastitis in a bitch. Although normal pigment is present on several glands, one gland was acutely inflamed, containing an area of dark and devitalized tissue with a soft center that was about to rupture. (From Wheeler SL, Magn ML, Kaufman, J, et al: Postpartum disorders in the bitch. Compend Contin Educ Pract Vet 6:494, 1984, with permission.) *See Color Plate*

Table 7–4. Therapeutic Guide for Treating Canine Mastitis

Acute Mastitis (blood-milk barrier not intact)	Select bacterocidal drugs when possible
Bitch without nursing pups	Select antimicrobial on the basis of efficacy and safety for the bitch
Aerobic bacteria	
Gram-negative infection	Broad-spectrum cephalosporin (second- or third-generation), quinolones, chloramphenicol
Gram-positive infection	β-Lactamase–resistant panicillins, amoxicillin–clavulanic acid, first-generation cephalosporin, erythromycin, chloramphenicol
Anaerobic bacteria	Penicillin, metronidazole, clindamycin, cefoxitin, chloramphenicol, erythromycin
Mycoplasma	Chloramphenicol, tetracyclines, erythromycin, quinolones
Bitch with nursing pups	Select antimicrobial on the basis of efficacy and safety for the bitch, and safety for the pups
Aerobic bacteria	
Gram-negative infection	Cefoxitin, chloramphanicol
Gram-positive infection	First-generation cephalosporins, erythromycin
Anaerobic bacteria	Cefoxitin, erythromycin, chloramphenicol
Mycoplasma	Erythromycin, chloramphenicol
Chronic Mastitis (blood-milk barrier intact)	Select bacterocidal drugs when possible
Bitch without nursing pups	Select antimicrobial on the basis of efficacy and safety for the bitch and on ability to enter mammary tissue or milk
Aerobic bacteria	
Gram-negative infection	Quinolones, chloramphenicol
Gram-positive infection	Erythromycin, chloramphenicol
Anaerobic bacteria	Clindamycin, chloramphenicol, erythromycin
Mycoplasma	Chloramphenicol, tetracyclines, erythromycin, quinolones
Bitch with nursing pups	Select antimicrobial on the basis of efficacy and safety for the bitch and safety for the pups. The drug's ability to concentrate in mammary tissue and milk is also desirable, providing the drug is deemed safe for the pups.
Aerobic bacteria	
Gram-negative infection	Cefoxitin, chloramphenicol
Gram-positive infection	Erythromycin, first-generation chephalosporin
Anaerobic bacteria	Chloramphenicol, erythromycin, cefoxitin
Mycoplasma	Erythromycin, chloramphenicol

strated to be the cause of such failure, the pups may be hand-reared or allowed to remain nursing after antimicrobial treatment begins. Although subclinical mastitis is well characterized in cows, the incidence and significance in bitches are currently unknown.

Bitches with chronic mastitis should be treated on the basis of antimicrobial sensitivity, the pharmacokinetics of selected antibiotics, and safety for the bitch and pups (if allowed to remain nursing). Some antibiotics may not reach effective concentrations in the milk, especially if the mastitis is chronic rather than acute.[25] In acute mastitis, many antibiotics are able to enter the mammary secretions because milk-plasma barriers are disrupted, local blood vessels are dilated, and tissue temperature is elevated. However, as inflammation subsides and milk-plasma barriers are reestablished, the role of pH partitioning may become important. Antibiotics that are weak bases or weak acids are distributed into body compartment on the basis of pH partitioning. Understanding pH partitioning allows the veterinarian to select a treatment that provides optimal concentrations of the drug to the mammary gland.[26,27] Weak bases tend to concentrate in body compartments that are more acidic than plasma, whereas weak acids tends to concentrate in alkaline environments. Because normal milk is slightly more acidic than normal plasma, weak bases achieve higher concentrations in milk than plasma under normal circumstances. Although milk remains acidic in most types of bovine mastitis, the rate of occurrence of acidic milk in canine mastitis remains to be determined.

In addition to pH partitioning, the lipid solubility of a drug should be considered. Antimicrobials that are poorly lipid soluble will fail to achieve projected concentration advantages because of the inability to cross cell membranes in chronically infected glands. For example, aminoglycosides are basic antibiotics that fail to concentrate in the acidic compartment because of low lipid solubility. Some drugs, such as chloramphenicol, are nonionized and therefore not affected by pH partitioning (Table 7–2). Although chloramphenicol is a broad-spectrum antimicrobial agent that enters the milk, its safety for neonates has been questioned. Although the authors have used chloramphenicol to treat mastitis and neonatal infections without apparent complications to the pups, chloramphenicol has been blamed for cardiovascular effects and "gray baby syndrome" in human infants.[30] Unfortunately, the safety of many antimicrobial agents remains unknown for both neonatal animals and children.

Although quinolones are known to distribute to the mammary gland and milk, and have a favorable spectrum of activity for several types of aerobic bacteria and mycoplasma that might be associated with mastitis in the bitch, they usually are not recommended if pups are to continue to nurse from a treated bitch. Enrofloxacin can cause abnormal cartilage growth in pups and is not recommended for pups of small and medium breeds of dogs until after 8 months of age or for pups of large breeds of dogs until after 18 months of age.[34] The distribution of enrofloxacin in the mammary gland reaches 67 per cent of the simultaneous plasma concentration 2 hours after dogs receive a single oral dose of 2.5 mg/kg. Ciprofloxacin and oflaxacin are known to be distributed in human breast milk. Ofloxacin is highly concentrated in breast milk, reaching 98 per cent of the simultaneous maternal serum concentration within 2 hours of administration.[35] Because of cartilage concerns, quinolones also are not recommended for human mothers with nursing infants.

Tetracyclines also are known to distribute to the mammary gland and milk, but should not be used to treat bitches with nursing pups. Tetracyclines are readily bound to calcium deposited in newly formed bone and teeth. Thus bone deformity and discoloration and dysplasia of tooth enamel can occur if pups receive the drug.[30] Even if pups do not receive large amounts of the drug in milk, the adverse effects of tetracyclines on pups are not always related to dosage or duration of treatment.

If the antibiotic selected for therapy concentrates in milk, it should also be one that minimally suppresses colonization resistance of the pup (i.e., increased colonization of Enterobacteriaceae; see Table 7–3). Antibiotics that suppress colonization resistance are those that decrease anaerobes in the neonatal gastrointestinal tract, resulting in overgrowth of potentially pathogenic bacteria. For example, colonization and nosocomial infections with *Klebsiella* species in both newborn pups[31] and children[36] have been associated with the indiscriminate use of antibiotics such as ampicillin. Whenever pups are allowed to continue nursing from mastitic or antimicrobial-treated bitches, the weights and general health of the pups should be monitored carefully (see Chapter 8).

Because the veterinarian must weigh many factors when treating mastitis, Table 7–4 is offered as a guide for selecting a therapeutic plan. Depending on the results of milk cultures, whether pups remain nursing, and additional information that becomes available on the safety of various drugs, these recommendations may need to be modified by the attending veterinarian.

TOXIC MILK SYNDROME

Although abnormal or "toxic" milk has been associated with neonatal morbidity and mortality, actual definition of "toxic milk" in dogs has not been established.[37] Although "toxic" bitch's milk has been incriminated in causing 3- to 14-day-old pups to become ill, the exact role or composition of milk in neonatal morbidity and mortality remains unknown. Pups, for whatever reason, become uncomfortable, vocalize, and bloat. Although metritis and subinvolution of placental sites (SIPS) have been incriminated in causing the "toxic" milk syndrome,[37] many bitches with SIPS are asymptomatic and have healthy pups (see below). Signs similar to those reported for "toxic" milk syndrome have also been observed in formula-fed pups.[31] Various conditions are likely to cause gastrointestinal ileus that results in bloating after eating. For example, gastrointestinal transit is frequently slowed in hypothermic pups.[38]

Galactostasis

Galactostasis is an abnormal delay in the passage of milk from the mammary glands that can occur during disease, as with mastitis or anatomic abnormalities of the teat, or without concurrent disease, as when pups are abruptly weaned. Galactosis also may occur when pups are not rotated on the mammary glands,[32] when pups are unable to remove milk from all glands (i.e., a small litter, death of the litter), or during pseudopregnancy. Galactostasis becomes problematic for the bitch when inflammation accompanies the accumulation of milk.

Although bitches with galactostasis are not systemically ill unless the milk stasis resulted from mastitis, they may be uncomfortable if the engorged glands become hot and painful. Cytologic evaluation of milk from bitches with galactostasis usually reveals cell counts greater than 3000 cells/μl (range 90 to 136,000/μl), with macrophages and neutrophils as the predominant cell types.[17] Macrophages may be observed to contain milk fat within the cytoplasm. Neutrophil numbers vary with the severity of the inflammation. In some cases, eosinophils are abundant in the milk, suggesting that an allergic component may exist in some forms of the condition.

Treatment is directed at decreasing secretion and reducing inflammation. Cool towels or compresses may be applied to the glands to decrease inflammation. If infection is absent, glucocorticoids can be administered to reduce the inflammation. Diuretics and analgesics have been suggested to be beneficial treatments.[12] Reducing the food intake of the bitch, combined with gradual weaning of pups, may reduce milk production, thereby reducing the severity of the condition. Milking the gland may or may not be beneficial. It is difficult to remove milk from all sections of the canine mammary gland, and stimulation of the gland may result in further milk production.

Agalactia

Agalactia (also called agalactosis) is the absence of milk production or secretion. Primary agalactia occurs from a failure of milk production, resulting from anatomic or physiologic abnormalities, and is extremely rare. Secondary agalactia can result from inadequate nutrition, stress, premature parturition, progestagen therapy, mastitis, metritis, psychological problems, endotoxemia, and systemic illness.

Primary or true agalactia does not respond to therapy if congenital malformations exist in the mammary gland or if the gland is unable to respond to hormonal stimulation. Secondary agalactia should be treated by removing the inciting cause for failure of milk production or letdown. A young or primiparous bitch may be nervous and fearful of nursing. Reassurance from the owner or the use of tranquilizers often is beneficial; phenothiazine tranquilizers promote increased prolactin release from the pituitary[39] and may enhance lactation. Oxytocin nasal sprays can be efficacious in enhancing milk letdown in nervous mothers.

There may be other causes of secondary agalactia still to be identified in the bitch. For example, mycoplasmal infections of the mammary gland have been associated with agalactia, arthritis, and keratoconjunctivitis in goats.[40,41] In swine, agalactia has been associated with mastitis and metritis, and suggested to result from an interaction of endotoxins at lactation. *Escherichia coli* endotoxins given to

sows on day 2 of lactation resulted in marked suppression of prolactin secretion.[42] The administration of drugs (megestrol acetate, progesterone, ally-trenbolone), the feeding of ergot, and the hormonal changes in the periparturient period all have been associated with agalactia in various species.[43–45]

Subinvolution of Placental Sites

Uterine involution, based on histologic observations, is complete by 12 weeks postpartum in normal bitches.[46] Uterine weights return to those of anestrous uteri by 60 to 120 days postpartum.[47] The ultrasonographic appearance of the postpartum uterus is indistinguishable from that of the anestrous uterus by 15 weeks postpartum.[48]

Subinvolution of placental sites (SIPS) occurs when the involution process is delayed. In one study,[49] 20 of 95 reproductive tracts from postpartum bitches were observed to have SIPS; the reproductive tracts were from postpartum spayed bitches and experimental surgical cases. In normal bitches without SIPS, fetal trophoblasts or maternal decidual cells can be observed in the upper loose connective tissue of the lamina propria for the first 2 weeks after whelping.[46] However in bitches with SIPS, these trophoblastic cells do not degenerate and continue to invade the deep glandular layer of the endometrium or even the myometrium. This trophoblastic invasion, concomitant vascular damage to blood vessels, and failure of normal endometrial blood vessel thrombus formation and secondary occlusion are proposed as potential causes of SIPS in dogs.[50] Histologically, large masses of collagen, hemorrhage, and dilated endometrial glands may be observed in the placental sites, which are about twice the size of sites from normal bitches at the same time after parturition. Trophoblast-like cells extend into the myometrium at the base of the collagen masses, and may form a syncytial mass of cells.[49] These syncytial masses of trophoblast-like cells may invade the myometrium and surround the blood vessels in the stratum vasculare. Trophoblast-like cells may be observed in vaginal smears from bitches with clinical signs of SIPS[12,51] but are not observed in smears from normal postpartum bitches. These trophoblast-like (or decidua-like) cells are polynucleated and heavily vacuolated (Fig. 7–8).

The incidence of SIPS is higher in primiparous bitches younger than 3 years of age.[52–55] Dickie and Arbeiter described 20 cases of SIPS (also called metrorrhagia post partum or placentitis postpartum) in bitches between 2 and 6 years of age (average age = 4.5).[51] The disease occurred after the first parturition in 10 of 20 cases, after the second parturition in 6 of 20, and after the third parturition in 4 of 20. Affected animals from various breeds included boxers (n = 3), German shepherds (n = 4), a mop (n = 1), rottweilers (n = 2), dachshunds (n = 2), spaniels (n = 2), deerhounds (n = 2), a Doberman pinscher (n = 1), a Kerry blue terrier (n = 1), and poodles (n = 2).

Historically, bitches with SIPS are normal except for a hemorrhagic uterine discharge that passes from the vulva for several weeks postpartum. The amount of blood passing may range from a few drops each day that subside without therapy to acute, life-threatening metrorrhagia that requires transfusion and/or immediate surgical intervention. In chronic cases, the uterine discharge may pass for 8 weeks or even until the next proestrus onset. SIPS has been diagnosed histologically as late as 9 weeks postpartum in a clinically normal bitch undergoing elective ovariohysterectomy.[51] In our experience, severe postpartum blood loss is rare, and often is accompanied by a coagulopathy or ulceration of the uterus. The chronic form of SIPS, consisting of prolonged hemorrhagic discharge in an otherwise healthy bitch, is far more common. The diagnosis of SIPS, however, is often presumptive rather than confirmatory when minimal blood loss occurs, because biopsied uterine tissues are not usually obtained from animals undergoing spontaneous regression.

In bitches with the chronic form of SIPS, small amounts of hemorrhagic discharge may be obscured by lochia that passes for up to 3 weeks postpartum. As the uteroverdin-containing lochia subsides, owners may become aware of a persistent hemorrhagic discharge and seek veterinary assistance. Owners generally report that the bitch experienced a normal delivery and that pups are doing well. Uterine swellings of different sizes often can be palpated in bitches with SIPS (Fig. 7–9). Radiographic and ultrasonographic examinations confirm the palpation findings. The differential diagnoses for a hemorrhagic discharge passing from the vulva of a postpartum bitch include coagulopathy, metritis, brucellosis, inflammation of the caudal reproductive tract, trauma, genital tract neoplasia, and SIPS.[56]

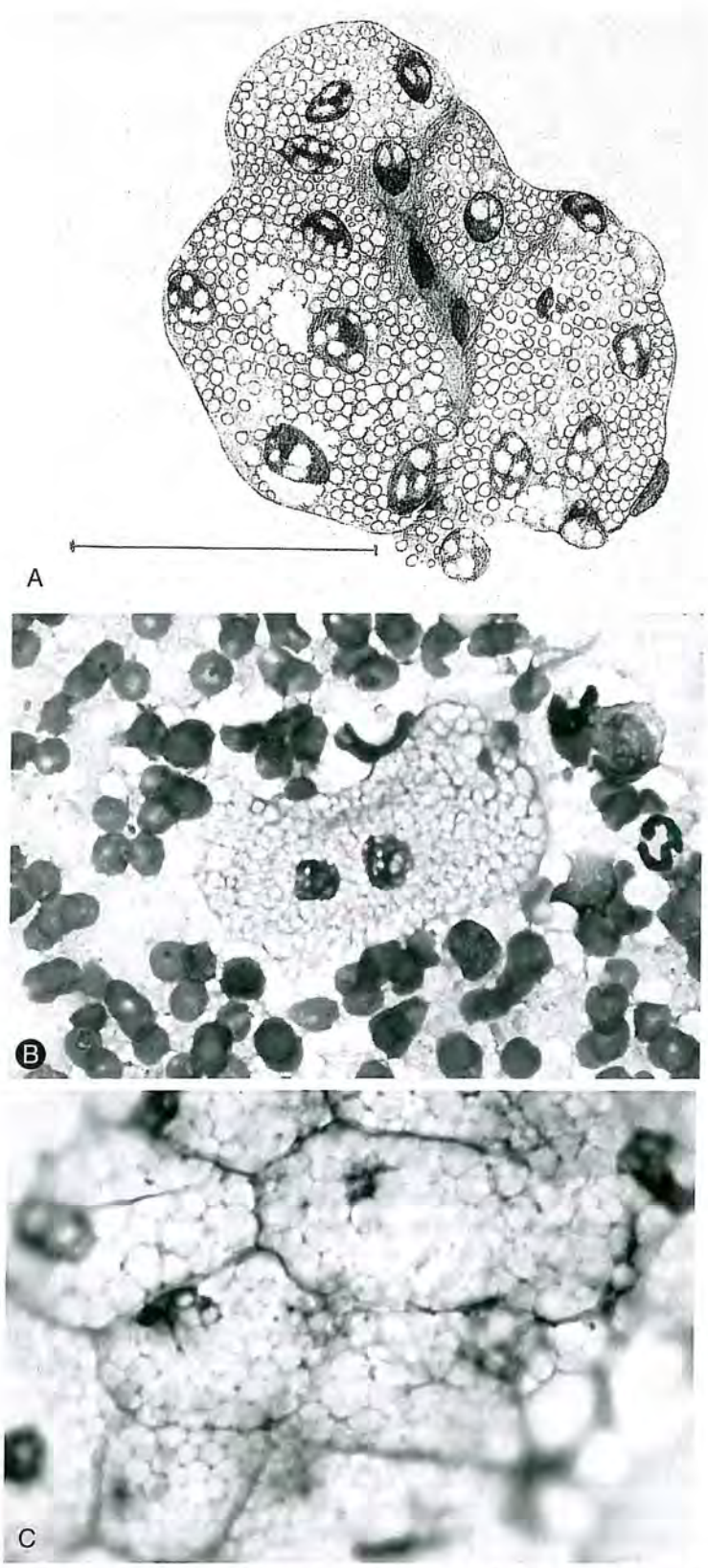


Figure 7–8. A: Schematic diagram of polynucleated, heavily vacuolated giant cell in the vaginal smear of a bitch with prolonged vaginal discharge from subinvolution of placental sites. (From Dickie MB, Arbeiter K: Diagnosis and therapy of the subinvolution of placental sites in the bitch J Reprod Fertil Suppl 47:471–475, 1993, with permission.) **B:** Polynucleated cell observed in a vaginal smear from a bitch with subinvolution of placental sites. Magnification: 1000×. (From Wheeler SL, Magne ML, Kaufman J, et al: Postpartum disorders in the bitch. Compend Contin Educ Pract Vet 6:497, 1984, with permission.) **C:** A syncytium of cells obtained in a vaginal smear from a bitch with subinvolution of placental sites. Magnification: 1000×.

Figure 7–9. Swellings in a uterus removed from a bitch with subinvolution of placental sites. Note the elliptical enlargements that sometimes can be palpated in affected animals.

Because spontaneous remission occurs, bitches with SIPS may not require medical or surgical therapy. Ovariohysterectomy is required if severe hemorrhage or ulceration of the endometrium/myometrium occurs. Although rare, ulceration and perforation of the uterus with subsequent peritonitis has been observed (D. Lein, Cornell University, personal communication, 1986). Ecbolic therapy (i.e., ergonovine, prostaglandins) does not appear to affect the trophoblast-like cells associated with SIPS deep within the myometrium, which are not likely to slough through forced uterine contraction or vasoconstriction.

Progesterone therapy was advocated for SIPS in the past because of its perceived benefit in sloughing residual trophoblast-like cells through endometrial stimulation. However, there has been no substantiated benefit from such therapy. Because progesterone therapy can cause pyometra in the bitch, it should be avoided. In one study, serum progesterone levels were elevated above anticipated postpartum levels in two of four bitches with SIPS, incriminating the hormone in the pathogenesis of the disorder.[49] However, normal placental sites and SIPS can coexist within the same bitch's uterus, which does not support a hormonal etiology.

Although vaginal bacteria have access to the uterus in bitches with SIPS, antibiotic therapy is not recommended unless secondary metritis occurs. Microorganisms that are part of the vaginal flora can develop increased resistance if antibiotics are used indiscriminately, making treatment more difficult if metritis occurs.

Hypocalcemia (Eclampsia, Puerperal Tetany)

Hypocalcemia, referred to as eclampsia or puerperal tetany in the prepartum or postpartum bitch, is associated with a depletion of calcium in the extracellular compartment and is characterized by nervousness, elevated body temperature, dry mouth and sclera, panting, restlessness, whining, tremors, staggering, stiffness, and, finally, collapse, with clonic spasms or seizures, labored breathing, salivation, and death. Although puerperal tetany in the bitch may occur prior to parturition, it is far more common during the first few weeks postpartum when the demand for milk by the pups is great; episodes occurring after 40 days postpartum are rare.[57]

Although the term *eclampsia* has been used to describe prepartum and postpartum hypocalcemia in the bitch, eclampsia in other species may not be associated with hypocalcemia.† Puerperal tetany generally is observed in bitches of small breeds, but dogs of any size may be affected.[16,58–61] Primiparous bitches may be over-represented, perhaps because owners may not breed animals again once they have had the disorder. Litter size has not been demonstrated to predispose animals to the disease.

Initial signs of hypocalcemia (i.e., restlessness, pacing, panting, reluctance to care for the pups, stiffness, and facial pruritus) occur within minutes to hours before the onset of muscle tremors, tetany, and convulsions. Hyperthermia (temperatures >105°F or 40.5°C) is common during subclinical and clinical tetany, resulting from increased muscle activity. The electrocardiogram of the bitch with hypocalcemia has deep, wide T waves, prolonged Q-T interval, and comparatively taller R waves when compared to tracings from unaffected bitches. Both tachycardia[32] and bradycardia[61] have been associated with hypocalcemia in the dog. Pupils of the affected bitch may be dilated and sluggish in response to light.

A total blood calcium level below 7 mg/100 ml (normal = 9 to 11 mg/100 ml) confirms the diagnosis, although most animals are treated on the basis of history and clinical signs before laboratory tests are completed. Phosphorus levels are normal to low in affected patients. Although blood glucose is normal in bitches with eclampsia, the signs of hypoglycemia associated with pregnancy toxemia (see Chapters 5 and 6) can be similar to those of hypocalcemia. Therefore, blood glucose should be evaluated, especially in those cases not responding to calcium treatment. The dif-

† Pre-eclampsia/eclampsia in women is associated with convulsions and coma, but the condition is associated with hemolysis, elevated liver enzymes, low platelets, hypertension, edema, and proteinuria rather than hypocalcemia.

ferential diagnosis of seizures also includes epilepsy, meningoencephalitis, and poisoning (i.e., caffeine, strychnine, lead, metaldehyde).

The pathophysiology of eclampsia in the dog is different from that of postpartum hypocalcemia in the cow. In the cow the transmission of acetylcholine is blocked by decreases in calcium, resulting in a flaccid paralysis. In the bitch, the transmission of acetylcholine is not blocked by hypocalcemia, possibly resulting from differences at the neuromuscular junction between cows and dogs. There is, however, a loss of membrane-bound calcium in the bitch, which results in increased permeability of the muscular membrane to ions, requiring less stimulus for depolarization. Consequently, the signs of spontaneous and repeated depolarization of muscle, or tetany, occur. Although magnesium appears to play a role in certain causes of tetany in cattle, levels of magnesium are within normal limits in dogs with eclampsia. The calcium measured in serum for diagnosis is often total calcium, but it is only the ionized form that is important for normal neuromuscular function. Tests for measuring serum ionized calcium concentrations are not widely available to practicing veterinarians, but total serum calcium should be measured if possible in a bitch that is refractory to treatment. The proportion of calcium that is ionized and available to muscle cells decreases with metabolic or respiratory alkalosis. Dogs with eclampsia often are hyperpneic and may develop an alkalosis, resulting in a further decrease of ionized calcium.

Treatment

Treatment with slow IV administration of calcium should begin as soon as the clinical diagnosis is made. Gradually cooling the bitch, along with the calcium treatment, may be necessary in animals with pyrexia (>107°F).

Because several preparations of calcium are commercially available, the veterinarian should be aware of the relative amounts of elemental calcium and appropriate route of administration of the different products (Table 7–5). One therapeutic regimen is to administer a 10% solution of calcium gluconate (0.22 to 0.44 ml/kg), slowly IV. The response to therapy may vary, so ranges of 1 to 20 ml of the 10% calcium gluconate are suggested,[61] administering the calcium slowly to effect. The amount given must be titrated to the animal's clinical signs and discontinued if abnormal changes in the electrocardiogram occur. Once the neurologic signs have subsided, additional amounts of calcium may be given intramuscularly or subcutaneously for a more prolonged effect. Wallace and Davidson[32] suggested that, once a bitch is stable following IV calcium treatment, a 10% calcium gluconate solution diluted 50 per cent with saline be administered subcutaneously every 8 hours until the bitch can be maintained on oral calcium. One must use only those calcium preparations that are safe for the proposed route of administration. For example, calcium chloride is extremely irritating if given by any route other than IV (Table 7–5).

Calcium preparations vary considerably by milligrams per milliliter of elemental calcium. For example, 10% calcium gluconate solution contains 9.3 mg/ml of elemental calcium and 10% calcium chloride solution contains 27.2 mg/ml of elemental calcium. One preparation commercially available for treating canine eclampsia (Calphosan Solution) 1%; (Glenwood, Tenafly, NJ)[62] contains a 1% solution of calcium glycerophosphate plus calcium lactate, which is only 1.87 mg/ml elemental calcium. Thus it would take approximately five times as much of the 1% Calphosan solution to treat a bitch with eclampsia as the 10% calcium gluconate. Although a 10% Calphosan suspension also is commercially available, it is not approved for use in dogs and cannot be given IV. The Calphosan suspension has been reported to cause the development of aseptic abscesses when given IM to cows.[4]

Although once recommended as therapy, glucocorticoids should not be used to treat bitches with hypocalcemia. Glucocorticoids decrease intestinal absorption and enhance renal excretion of calcium. Once a bitch is stable and able to eat, oral supplementation of calcium should begin; 1 to 3 g of calcium carbonate (Tums E-X, SmithKline Beecham, Pittsburgh, PA; 750 mg calcium carbonate/tablet) or calcium gluconate per day can be given in divided doses to prevent recurrences of hypocalcemia. Vitamin D therapy can be added to the oral calcium therapy, but dogs should be monitored to verify that hypercalcemia does not result.

Pups should be removed from the bitch to reduce the lactational drain on the dam. Bottle feeding may be necessary in some cases, but many pups are near weaning age when eclampsia occurs so they can be started on solid food or gruel. If pups are too young to be weaned, they may be reintroduced gradually (i.e., receive partial supplementation via

Table 7-5. Concentrations of Calcium in Various Commercial Preparations

Form of Calcium	Concentration of Compound (g/100 ml)	Calcium in Compound (%)	Elemental Calcium (mg/ml)	Calcium (mEq/ml)
Calcium gluconate	23*	9.30	21.40	1.07
	10*	9.30	9.30	0.46
	5†	9.30	4.65	0.23
Calcium boro-gluconate	21.5*	9.30	20	1
Calcium glycerophosphate Calcium lactate	0.5 1% solution*†‡	19.07 18.37	1.87	0.09
Calcium glycerophosphate Calcium lactate	5.0 10% suspension‡	19.07 18.37	18.71	0.93
Calcium chloride	5.0*	27.2	13.60	0.68
	10.0*	27.2	27.20	1.36

* Can be given IV.
† Can be given subcutaneously.
‡ Can be given intramuscularly.
Adapted from Wheeler SL, Magne ML, Kaufman J, et al: Postpartum disorders in the bitch. Compend Contin Educ Pract Vet 6:493–500, 1984.

bottle-feeding) to the bitch after she is stable and receiving oral calcium treatment.

Prevention

Balanced diets with calcium:phosphorus ratios ranging from 1:1 to 1.2:1 reportedly are suitable for pregnant bitches and may be useful in preventing eclampsia.[38,60] Recommended diets for pregnant bitches include gestation/lactation/growth diets that meet or exceed the guidelines of the American Association of Feed Control Officers trials. Excessive calcium intake during pregnancy has been associated with an increased incidence of hypocalcemia in postpartum cows, and diets with a high calcium:phosphorus ratio also have been incriminated in predisposing bitches to eclampsia. However, recent work suggests that the dietary cation-anion difference may be more crucial than calcium intake during pregnancy in preventing hypocalcemia. Cows fed a highly anionic (acidic) diet were more responsive to parathyroid hormone, enabling them to more quickly mobilize calcium from bone.[63] Thus feeding a highly anionic diet during the immediate prepartum period reduces the incidence of hypocalcemia in dairy cows. Whether similar dietary manipulations during late pregnancy would be beneficial in the bitch is unknown. Although current recommendations for preventing eclampsia in the bitch include the feeding of diets during *pregnancy* that are not excessive in calcium, the benefit of diets with various calcium:phosphorus ratios remains unknown. During *lactation*, calcium supplementation may be given for preventing eclampsia, especially in bitches that are at risk for developing the disorder.

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Chapter 8

The Neonate—from Birth to Weaning

The newborn puppy may have to overcome numerous obstacles during the first few weeks of life to survive to weaning. Twenty to 30 per cent of all pups die during the first few weeks after birth.[1] This chapter deals primarily with the newborn pup from birth through weaning. The reader also is encouraged to read *Veterinary Pediatrics—Dogs and Cats from Birth to Six Months (Second Edition)*.[2]

Physiologic Differences between the Neonatal and Adult Dog

Pups and adult dogs have significant physiologic differences. It is important that the owner and veterinarian be aware of such differences in order to prevent illness and to formulate therapeutic interventions for sick pups.

Thermoregulation

Newborn pups can lose heat through evaporation, conduction, radiation, and convection.[3] Thus it is important to provide pups with an environment that prevents excessive heat loss. In addition, a neonate can lose heat if wet or if placed next to drafts or cold objects (i.e., metal cages). If pups are orphaned or separated from their dams, environmental temperatures should be kept higher than those necessary for adult animals. Monson[4] suggested that orphaned pups be kept in environments with temperatures of 85° to 90°F during the first week of life (Table 8–1).

Rectal temperatures of newborn pups range from 95° to 99°F for the first week of life and from 97° to 100°F during weeks 2 and 3.[3] In one report,[5] the average rectal temperature of a dry, healthy pup the day after birth was 96°F (± 1.5°F). By weaning, the average rectal temperatures of pups are similar to those of adult dogs[6] (Fig. 8–1).

The shivering reflex and vasoconstrictive mechanisms are not fully operative in the newborn pup. Nonshivering thermogenesis, important for thermoregulation in neonatal pups, occurs in brown adipose (brown fat) tissue.[7,8] Pups are unable to maintain body temperatures in cool environments, especially when separated from their dams. Although pups have reduced ability to maintain body temperatures when chilled, they are able to pant when overheated (M. L. Chandler and S. L. Ralston, unpublished observations, Colorado State University, 1988).

Normal physiologic functions are impaired when a pup becomes chilled. For example, the heart rate in a newborn pup with a rectal temperature of 96°F ranges from 200 to 250 beats per minute. If the rectal temperature drops to 70°F, the heart rate decreases to only 40 to 50 beats per minute. A lowered heart rate may result in failure to suckle, dehydration, and death.[1] However, the hypothermia that leads to decreased cardiovascular function also protects pups from the ischemic brain injury that accompanies cardiovascular failure.[9] In this study, newborn dogs tolerated experimental induction of hypothermic circulatory arrest for up to 1 hour without brain injury. After 1 hour of hypothermic circulatory arrest, graded neuronal injury occurred. Tissue hypoxia and metabolic acidosis also occur with sustained hypothermia.

Bitches may not care for pups with cool skin temperatures, pushing the pups away and not responding to their cries.[3] As rectal temperatures drop below 94°F, gastrointestinal ileus develops, and pups refuse to nurse.[10–12] If chilled pups with ileus are forced to eat, regurgitation followed by aspiration pneumonia can occur.

Table 8–1. Environmental Temperature Guidelines for Orphan Puppies

Age (days)	Temperature (°F)
0–7	85–90
8–14	80
15–28	80
29–35	70–75
35+	70

From Monson WJ: Orphan rearing of puppies and kittens. Vet Clin North Am Small Anim Pract 17:567–576, 1987, with permission.

Figure 8–2. A warm-water bottle wrapped in a towel provides an adequate external heat source for neonatal puppies; the water must be changed as it cools. (From Polfenbarger EM, Olson PN, Ralston SL, et al: Canine neonatology. Part II. Disorders of the neonate. Compend Contin Educ Pract Vet 13:25–37, 1991, with permission.) *See Color Plate*

Chilled pups may have increased susceptibility to infection because of decreased lymphocyte transformation that accompanies chilling.[3]

Owners should provide a safe source of heat for pups if the dam is unavailable or the environment is cold. A warm-water bottle wrapped in a towel or placed inside a cloth cover (i.e., flannel pillowcase) can provide an adequate source of external heat for healthy orphaned pups (Fig. 8–2). Water should be changed as it cools, so as to avoid chilling of the pups from a cold bottle. The cloth covering prevents direct contact of the pup's delicate skin with the water bottle and decreases the risk of contact burns. Heat lamps can be used, but must be carefully placed and monitored to prevent excessive heat, burns, or dehydration (Fig. 8–3).

Pediatric incubators with climate control (Fig. 8–4) can be used for rearing orphaned or sick pups. Climate control also should address humidity, so that a pup's mucous membranes do not become excessively dry. Mosier[3] reported that an environmental relative humidity of 55 to 65 per cent is adequate for preventing drying of the skin. For immature, low-birth-weight pups, a relative humidity of 85 to 90 per cent is more effective in maintaining deep body temperature and normal hydration. Care should be exercised to ensure that the ambient temperature does not exceed 90°F when high humidity is provided. Temperatures of 95°F or greater, coupled with relative humidities of 95 per cent or greater, can lead to respiratory distress in neonatal pups.[3]

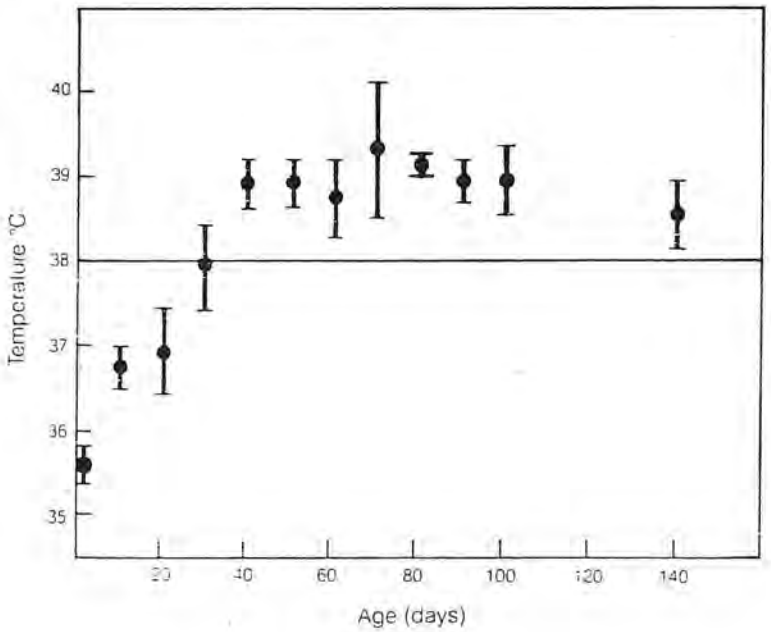

Figure 8–1. Changes in rectal temperature in newborn puppies. (From Mueggler PA, Peterson JS, Koler RD, et al: Post-natal regulation of oxygen delivery: Hematologic parameters of postnatal dogs. Am J Physiol 237:H71–H75, 1979, with permission.)

Figure 8–3. Neonatal pups kept warm with a heat lamp. It is important that such lamps are securely fastened and placed at appropriate distances so as to prevent burns.

Although a variety of incubators can be used for rearing orphaned pups, many orphaned pups that are otherwise healthy can be reared by using a box, towels, and warm-water bottles. If a choice of environmental temperatures is offered to healthy orphaned pups, many are able to select a temperature zone that is comfortable. Ill or comatose pups may not be able to move away from excessive heat sources, and must be monitored carefully for overheating and dehydration. Even healthy pups may be unable to move away from excessive heat (i.e., heating pads) during the first week of life, because neuromuscular reflexes are not fully developed at birth.[13]

Carbohydrate Regulation

Neonatal pups differ from adult dogs in their ability to maintain normal blood levels of glucose (i.e., euglycemia). In some cases, the ability to regulate blood glucose in the neonatal period may relate to the dam's nutritional status during pregnancy. Maternal starvation can reduce fetal birth weight, cause a significant decrease in fetal blood glucose concentrations, and cause an increase in fetal ketone levels. Maternal starvation reduced the birth weight of pups by 23 per cent and caused significantly lower fasting blood glucose levels in pups at 3 hours of age when compared to controls.[14] Bitches fed a low-carbohydrate diet during pregnancy had fewer pups born alive (63 per cent compared to 96 per cent of the pups born to the control bitches) and fewer pups alive at 3 days of age.[15]

Normal pups, whelped by healthy and properly fed bitches, may be able to maintain stable blood glucose levels for several hours following a fast. However, because neonatal livers contain minimal glycogen stores,[16–20] even minimal fasting can result in hypoglycemia in some newborn pups. Factors other than fasting may contribute to the rapidity of the development of hypoglycemia (see below). Toy-breed pups may be more predisposed to fasting hypoglycemia than large-breed pups, possibly from limited body energy reserves, immature mechanisms for gluconeogenesis and glycogenolysis, and higher metabolic demands.[21] A condition referred to as transient juvenile hypoglycemia occurs in young pups, often those of toy breeds. If pups with transient juvenile hypoglycemia are treated appropriately with glucose therapy, and are able to maintain euglycemia through frequent feedings, the condition usually resolves by the time the pup reaches puberty. Yorkshire terriers are over-represented as a breed whose pups de-

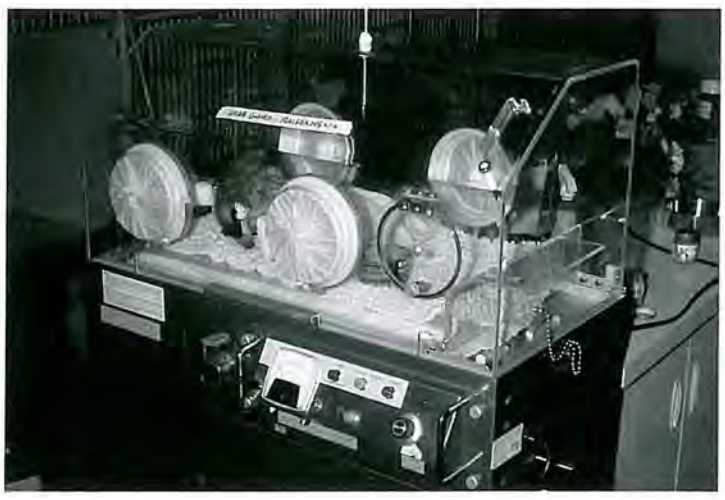

Figure 8–4. A human pediatric incubator used for rearing neonatal pups and kittens. Such units frequently can be obtained from human hospitals when they upgrade equipment for pediatric units.

velop fasting hypoglycemia, a condition that has been associated with fatty liver syndrome.[22] Mosier reported hypoglycemia and hepatic lipidosis in pups of other toy breeds.[23] Depending on the severity, duration, and predisposing factors of hypoglycemia in fasting pups, ketonuria may or may not be present.

Because pups lack the feedback mechanism between hepatic gluconeogenesis and blood glucose concentrations, regulation of blood glucose is more difficult than for the adult dog. Pups are relatively insensitive to insulin and have immature gluconeogenic responses to counter-regulatory hormones. Additionally, the newborn dog has a lower rate of glucose metabolism and reduced glucose clearance.[24] Thus, prolonged hyperglycemia can result from glucose administration because of immature regulatory and metabolic mechanisms. Glucosuria is common in neonates until 2 to 8 weeks of age.[25,26]

OTHER FACTORS LEADING TO DEVELOPMENT OF HYPOGLYCEMIA IN PUPS

Factors other than fasting play a role in the development of hypoglycemia in newborn dogs. (Table 8–2).[1,10,27–29] Although chilling does not seem to enhance the development of hypoglycemia in newborn dogs,[30] disorders such as endotoxemia, sepsis, portosystemic shunts, and glycogen storage abnormalities can cause a profound decline in blood sugar concentrations.[31]

Inborn errors in carbohydrate or amino acid metabolism, inadequate glycogen or protein substrate stores, and immature or deficient enzyme systems should be considered in pups with recurrent hypoglycemia that continues in the absence of identifiable disease (i.e., septicemia/endotoxemia) and in the presence of adequate nutrition and appropriate glucose therapy. Glycogen storage disease may be suspected if recurrent or persistent hypoglycemia is diagnosed in a pup that also has hepatomegaly, acidosis, and ketosis.[21] Glucose-6-phosphatase deficiency (von Gierke's disease) can cause severe hypoglycemia and has been suspected in pups of toy breeds.[32,33] Amylo-1,6-glucosidase deficiencies (Cori's disease or "limit dextrinosis"), a cause of moderate hypoglycemia after a long fast, has been confirmed in some female German shepherds with signs beginning at around 2 months of age.[34,35]

Other causes of hypoglycemia include vascular anomalies (i.e., portosystemic shunts) or other severe hepatic diseases. If more that 70 per cent of the normal functioning liver mass is impaired, inadequate hepatic glycogen stores and insufficiencies of hepatic enzymes result.[27] Hypoglycemia in pups initiated by ingestion of food could be caused by glucagon deficiency, fructose intolerance, galactosemia, or leucine hypersensitivity.[36]

DIAGNOSIS AND TREATMENT OF HYPOGLYCEMIA

Blood glucose concentrations less than 30 mg/dl in newborn pups and less than 40 mg/dl in pups 2 weeks to 6 months of age should be considered abnormal, especially when accompanied by clinical signs.[37] Center et al.[29] reported that concentrations of blood glucose ranged from 52 to 127 mg/dl for neonatal pups (n = 30) between the ages of 1 to 3 days, from 111 to 146 mg/dl at 2 weeks of age (n = 14), and from 86 to 115 mg/dl at 4 weeks of age (n = 7). The normal adult range for blood glucose concentrations given by these authors was 65 to 110 mg/dl (Table 8–3).

Clinical signs of hypoglycemia in neonatal pups include lethargy, failure to nurse, mental dullness, depression or stupor, and seizures. Other signs that may occur include tremor, nervousness, vocalization, irritability and intense hunger.[38]

Treatment should be initiated after the diagnosis of hypoglycemia has been made. Dextrose, 0.2 to 0.5 g/lb (0.5 to 1 g/kg)[39] can be administered slowly (over several minutes) intravenously through the jugular veins of most pups. Ultrafine pediatric catheters are available that can be inserted into cephalic or femoral veins. Five to 10% dextrose solutions are recommended for intravenous administration. Higher concentrations of dextrose may be directly applied to mucous membranes, providing that circulation is adequate for absorption to occur, but should not be given intravenously because of the risk of causing phlebitis (Table 8–4). Because of the immature regulatory and metabolic mechanisms in neonatal pups, and the potential for hyperglycemia following dextrose treatment, blood glucose levels should be measured prior to administering more dextrose to a pup that fails to respond to therapy.

Renal and Hepatic Function

The immature kidneys and livers of neonates contribute to the differences between pups and adults in drug metabolism and excretion, and

Table 8-2. Suspected or Reported Factors Associated with Hypoglycemia in Neonatal Pups

Factor	Incidence in Dogs
Fasting or starvation (pup or pregnant bitch)	Common
Transient juvenile hypoglycemia	Common
Carnitine deficiency	Reported
Deficiency/insensitivity of counter-regulatory hormones/immature feedback mechanisms	
Glucagon deficiency	
Cortisol/adrenocorticotropic hormone deficiency	Reported
Growth hormone deficiency	
Thyroid hormone deficiency	Reported
Catecholamine deficiency	
Low hepatic glycogen stores/inborn errors of metabolism	
Fasting/starvation	Common
Glycogen storage diseases	Reported
Glycogen synthetase deficiency	
Branched-chain ketoacid dehydrogenase deficiency	
Fructose 1-phosphate aldolase deficiency	
Gluconeogenetic enzyme deficiency	
Argininosuccinic acid synthetase deficiency	
Amylo-1,6-glucosidase deficiency	Reported
Hepatic phosphorylase deficiency	
Phosphorylase kinase deficiency	
Glucose-6-phosphatase deficiency	
Drug induced	Reported
Infectious diseases	Reported
Severe hepatic disease	
Portosystemic venous anomalies	Reported
Hepatitis	
Postprandial hypoglycemia	
Hyperinsulinism	
Leucine hypersensitivity	
Deficiency of counter-regulatory hormones	
Glucagon deficiency	
Hepatic enzyme deficiency	
Galactosemia	
Fructose intolerance	

Data from Poffenbarger et al.,[1,10] Atkins,[27] Chastain,[28] and Center et al.[29]

therefore are important for veterinarians to understand.[40] Nephrogenesis is not complete until the third week of life.[41] As maturation of nephrons and cortical blood flow change, predisposition of various parts of the kidney to drug toxicity changes.

Concentrations of protein, glucose, and amino acids are higher in the urine of neonatal pups than in adult dogs, but urine specific gravity usually is lower.[25,42] Urine specific gravity varies during the first 8 weeks of life, ranging from 1.006 to 1.017. After 8 weeks of age, the urine specific gravity approximates that of the adult.[26] Micturition begins soon after birth. A pup frequently will urinate after the vulva or prepuce is stimulated through the licking actions of the dam or following vulvar or preputial stimulation with gentle massage from a piece of cotton that has been moistened with warm water. Urine specimens are relatively easy to obtain from a neonatal pup.

Many metabolic functions of the canine liver are incompletely developed at birth.[29] In a study on the metabolism of salicylate in pups,[43] maximum development of the hepatic microsomal enzymes necessary for drug metabolism occurred within 30 days after birth. However, other enzyme systems such as cytochrome P-450, demethylation, reduction, and hydroxylation may not be fully mature until pups are 5 months old.[44] Drugs that must be metabolized by liver enzyme systems should be avoided or administered according to modified dose schedules. Unfortunately, such modified dose schedules are rarely available for pups, or are designed without the support of data collected from scientific studies in neonatal pups.

Cardiopulmonary Function

The mucous membranes of newborn pups are normally a deep pink or red color. Cutaneous

Table 8–3. Normal Values for Routine Biochemical Indicators of Hepatobiliary Disorders in Young Dogs and Cats (Median and Range)

	Puppies					Kittens		
Test*	1–3 Days (N = 30)	2 Weeks (N = 14)	4 Weeks (N = 7)	8 Weeks (N = 8)	Normal Adult Range	2 Weeks (N = 24)	4 Weeks (N = 8)	Normal Adult Range
BSP % 30 min	<5	<5	<5	<5	0–5	ND†	ND	0–3
Bile acids (μM/L)	<15	<15	<15	<15	0–15	ND	<10	0–10
Total bilirubin (mg/dl)	0.5 (0.2–1.0)	0.3 (0.1–0.5)	0 (0–0.1)	0.1 (0.1–0.2)	0–0.4	0.3 (0.1–1.0)	0.2 (0.1–0.2)	0–0.2
ALT (IU/L)	69 (17–337)	15 (10–21)	21 (20–22)	21 (9–24)	12–94	18 (11–24)	16 (14–26)	28–91
AST (IU/L)	108 (45–194)	20 (10–40)	18 (14–23)	22 (10–32)	13–56	18 (8–48)	17 (12–24)	9–42
ALP (IU/L)	3845 (618–8760)	236 (176–541)	144 (135–201)	158 (144–177)	4–107	123 (68–269)	111 (90–135)	10–77
GGTP (IU/L)	1111 (163–3558)	24 (4–77)	3 (2–7)	1 (0–7)	0–7	1 (0–3)	2 (0–3)	0–4)
Total Protein (g/dl)	4.1 (3.4–5.2)	3.9 (3.6–4.4)	4.1 (3.9–4.2)	4.6 (3.9–4.8)	5.4–7.4	4.4 (4.0–5.2)	4.8 (4.6–5.2)	5.8–8.0
Albumin (g/dl)	2.1 (1.5–2.8)	1.8 (1.7–2.0)	1.8 (1.0–2.0)	2.5 (2.1–2.7)	2.1–2.3	2.1 (2.0–2.4)	2.3 (2.2–2.4)	2.3–3.0
Cholesterol (mg/dl)	136 (112–204)	282 (223–344)	328 (266–352)	155 (111–258)	103–299	229 (164–443)	361 (222–434)	150–270
Glucose (mg/dl)	88 (52–127)	129 (111–146)	109 (86–115)	145 (134–272)	65–110	117 (76–129)	110 (99–112)	63–144

Abbreviations: BSP, sulfobromophthalein; ALT, alanine aminotransferase; AST, asparate aminotransferase; ALP, alkaline phosphatase; GGTP, γ-glutamyltranspeptidase.
ND = Not determined.
Data from Center SA, Hornbuckle WE, New York State College of Veterinary Medicine, Cornell University, 1987; reprinted from Center SA, Hornbuckle WE, Hoskins JD: The liver and pancreas. *In* Hoskins JD (ed): Veterinary Pediatrics—Dogs and Cats from Birth to Six Months, 2nd ed. Philadelphia, WB Saunders, 1995, pp 189–225.

stimulation results in reflex respiration in pups, a response that may be linked to survival of the pup when the bitch licks the newborn pup, or when an attendant rubs a pup following delivery via cesarean section. Immediately after a pup is delivered by cesarean section, the mucous membranes may appear cyanotic or gray, but they become the normal deep pink or red color when the pup begins to breathe on its own. During the first week of life, respiratory rates of 10 to 18 breaths per minute are normal; the rate increases to 18 to 36 breaths per minute during the second week of life and becomes similar to the adult rate of 16 to 32 breaths per minute by the third week of life.[45] Newborn pups can reflexively increase bronchomotor tone in response to hypercapnia. The pulmonary response to hypoxia, however, is minimal.[46,47] Bradycardia can occur in pups as oxygen partial pressure drops, unlike the tachycardia expected in response to hypoxemia in adult dogs. The veterinarian should be aware that neonatal pups who are severely hypoxemic may have heart rates in the range of rates expected for normal adult dogs (i.e., normal heart rates in newborn pups are 200 to 250 beats per minute).

Baroreceptors are functional by the fourth day of life. Therefore, the heart rate of pups varies in response to changes in blood pressure as it does in adults.[45] The mean arterial blood pressure is considerably lower in 1- to 4-week-old pups than in adults, ranging from 30 to 70 mm Hg.[48,49] Many variables, including body temperature and blood glucose concentrations, affect blood pressure. In one study,[50] mean arterial blood pressure dropped 50 per cent in pups with severe hypoglycemia. After pups have reached 1 week of age, mean arterial blood pressures increase with age, reaching adult values between 6 weeks and several months of age.[51]

The electrocardiogram in neonatal pups differs from that of adult dogs. In one study of 36 normal pups (16 randomly selected normal

Table 8–4. Treatment of Acute Hypoglycemia in Neonatal Pups

Concentration of Dextrose	Amount To Administer/Ounce of Pup's Weight
5%	0.25–0.625 ml/ounce IV
10%	0.125–0.31 ml/ounce IV
50%	0.25–0.625 ml/ounce orally (apply directly to mucous membranes)

pups and 20 normal pups with a familial history of congenital heart disease),[52] the QRS modal axis of the electrocardiogram changed from a right, cranioventral direction during the first week of life to a left, caudoventral orientation by week 12 after birth. This change in the cardiac axis may be related to the change in the ratio of right ventricular to left ventricular mass; the mass of the right ventricle relative to the left ventricle is 1:1 in newborn pups and 1:2 to 1:3 in adult dogs.[46]

Nervous System Function

The eyelids and external ear canals of a neonatal pup are closed until approximately 2 weeks of age. However, a bright light directed through the eyelid does elicit a slow blink response, and newborns can respond to noise stimuli.[53] Flexion is the predominant posture at birth in the newborn pup.[38] At 4 to 5 days after birth, the extensor muscles become dominant. The newborn pup can raise its head at birth and propel itself by sliding along its abdomen and ventral thorax. Normal newborn pups should have a sucking response and be able to nurse within an hour or so following birth. The onset of other reflexes varies, as shown in Table 8–5.

Neonatal pups have an immature blood-brain barrier that is permeable to lactic acid,[54,55] which can be utilized as a cerebral metabolic fuel during prolonged starvation or hypoglycemia. The immature blood-brain barrier of the pup also allows for greater access of drugs into the central nervous system. Antibiotics normally excluded from the central nervous system in adult dogs may enter the brain of the neonatal pup. Penicillins, tetracyclines, sulfonamides, and other substances may achieve marked cerebrospinal fluid concentrations, resulting in greater drug effects or toxicity.[56]

Gastrointestinal Function

The gastrointestinal tract of pups is well developed at birth. The stool of normal nursing pups is yellowish tan and semiformed (Fig. 8–5). Because the bitch cleans the pups by licking and ingesting the pup's feces, the stools of neonatal pups are not always observed unless diarrhea occurs. Pups that are orphaned and fed formulas frequently develop diarrhea. This may result from overfeeding, from feeding a formula that is hyperosmolar (i.e., insufficient water has been added), or because formulas, as good as they are, may not replace all the important substances in a bitch's milk.

The gastrointestinal tract of the pup at birth is sterile,[56] and then becomes rapidly colonized with numerous types of microorganisms within a few days of life. Environment, diet,

Table 8–5. Reflexes in Neonatal Puppies of Various Ages[40a]

Reflex	Puppy Age in Days		
	Strong	Weak or Variable	Absent or Adultlike
Motor Responses			
Crossed extensor reflex	1–16	16–18	18+ (absent)
Magnus reflex	1–17	17–21	21+ (absent)
Neck extension posture	1–4 flexion	4–21 hyperextension	21+ normotonia (adultlike)
Forelimb placing	4+	2–4	0–2 (absent)
Hindlimb placing	8+	6–8	0–6 (absent)
Forelimb supporting	10+	6–10	0–6 (absent)
Hindlimb supporting	15+	11–15	0–11 (absent)
Standing on all fours	21+	18–21	1–18 (absent)
Body righting	1+	0–1	—
Auditory and Visual Responses			
Blink response to light	16+	4–16	0–4 (absent)
Visual orientation	25+	20–25	0–20 (absent)
Auditory startle response	24+	15–24	0–15 (absent)
Sound orientation	25+	18–25	0–18 (absent)
Sensory Responses			—
Rooting reflex	0–14	14–25	25+ (absent)
Nociceptive withdrawal	23+	19–23	0–19 (adultlike)
Panniculus reflex	25+	19–25	0–19 (adultlike)
Reflex urination	0–22	22–25	25+ (adultlike)

From Poffenbarger EM, Ralston SL, Chandler ML, et al: Canine neonatology. Part I. Physiologic differences between puppies and adults. Compend Contin Educ Pract Vet 12:1604, 1990, with permission.

Figure 8–5. The appearance of a stool from a normal nursing pup. *See Color Plate*

antimicrobial therapy, and disease all can alter colonization.[1]

The age of the pup can be estimated by determining the eruption dates of deciduous and permanent teeth (Table 8–6).[1,57] Estimating the age of a pup based on tooth eruption dates is useful, especially when attempting to recommend appropriate vaccination schedules for pups with unknown birthdates. The dam begins to encourage weaning when the pups' deciduous teeth erupt, beginning at 3 to 6 weeks of age.

Immune Function

Newborn pups have an immune system that is not fully functional when compared to that of the adult dog.[58,59] Pups rely on passive immunity received postpartum from the dam's colostrum, because they acquire little passive immunity in utero. Passive immunity is an important factor in fighting diseases that occur in pups from birth to several weeks of age. Passive immunity is acquired from colostral antibodies ingested during the first 24 hours of life. After 24 hours, relatively little absorption of antibodies occurs across the gastrointestinal tract of the dog. However, even after the colostrum phase ends, the pup continues to receive some protection from the bitch's milk. Although not readily absorbed into the pup's bloodstream after the first 24 hours, antibodies in the milk (immunoglobulins G and A [IgG and IgA]), continue to protect against infections that begin on surfaces of the pup's oral and intestinal mucous membranes. The IgG antibodies work preferentially in the mouth, oropharynx, and esophagus, and the IgA antibodies work preferentially in the stomach and intestines.[60,61] The concentrations of maternally derived antibodies vary among pups, decreasing to levels that no longer afford protection from various infectious agents by 6 to 20 weeks of age. Although newborn pups can mount an antibody response to various antigens at birth, the response consists primarily of immunoglobulin M. T-helper cells are integral for antibody production by B cells. It is unknown whether the humoral response of pups is less than that of adults as a result of B- or T-lymphocyte immaturity.

The proliferation of T cells induced by mitogens is poor at birth, which may partially explain the susceptibility of neonates to infectious diseases and the lack of response to early vaccination.[1] The thymus is the site of T-lymphocyte differentiation and maturation, increasing 200-fold during the first 12 weeks of life in the dog.[62]

Additional factors, besides B and T lymphocytes, are important for a fully functional immune system. Polymorphonuclear cells, complement components, and enzymes all are necessary for maintaining the health of an animal. In some newborns, phagocytic cells are defective in engulfing and killing microorganisms; complement components are deficient in some newborns, resulting in poor opsonization of bacteria and decreased phagocytosis.[59,63]

Table 8–6. Ages of Tooth Eruption in Pups

Age	Deciduous Teeth*†
3–4 wk	Canine (C)
4–5 wk	Incisor (I) 1, incisor 2, premolar (P) 2, premolar 3
5–6 wk	Incisor 3, premolar 4
	Permanent Teeth‡
4–5 mo	Incisor 1, incisor 2, incisor 3, premolar 1, molar (M) 1
5–6 mo	Canine, premolar 2, premolar 3, premolar 4, molar 2
6–7 mo	Molar 3

* Some adult dogs may lack first and second premolars; last molars may be absent in brachycephalic breeds.
† Dental formula for deciduous teeth: 2(I 3/3; C 1/1; P 3/3) = 28.
‡ Dental formula for permanent teeth: 2(I 3/3; C 1/1; P 4/4; M 2/3) = 42.
Data from Poffenbarger et al.[1] and Jones.[56]

Laboratory Profiles of Normal Neonatal Pups

Hematocrits

The mucous membrane color of newborn pups is normally bright pink or red, possibly result-

ing from the high hematocrits present at birth (Fig. 8–6). Hematocrit values as high as 63 per cent have been reported in healthy newborn to 3-day-old beagle pups.[64] Hematocrits decline after 3 days of age, reaching their lowest values at approximately 6 weeks of age, and then increase to those of adult dogs.[65] Lower hematocrits also may be observed in heavily parasitized pups. Therefore, it is important to differentiate a normal physiologic anemia from a pathologic loss of blood resulting from parasitism. The hemoglobin-oxygen saturation curve of the canine fetus falls to the left of the adult curve[66] but shifts to the right after birth. An increase in 2,3-diphosphoglycerate in erythrocytes parallels the shift to the right of the oxygen saturation curve, allowing oxygen to become more available to tissues. Therefore, oxygen delivery to tissues is facilitated in spite of the pup's physiologic anemia.

White Blood Cell Counts

Beagle pups between birth and 3 days of age have higher white blood cell (WBC) counts that do adult beagles.[64] Total WBC and neutrophil counts decrease slowly and reach a minimum at approximately 3 weeks of age (Fig. 8–7). Although lymphocyte numbers are similar between newborn and adult beagle dogs, lymphocyte numbers are higher in the 6-week-old pup than in the adult. The increase in lymphocytes may occur as the result of numerous antigenic stimuli that the newborn pup encounters.

Serum Chemistry Concentrations

Serum chemistry concentrations differ between pups and adults (Table 8–7). Understanding the differences not only allows the veterinarian to accurately diagnose diseases, but provides important prognostic information. For example, profound increases in serum γ-glutamyltransferase (GGT) and alkaline phosphatase (ALP) occur in association with the ingestion of colostrum in newborn pups.[67] Measuring increased serum levels of GGT and ALP in a neonatal pup can serve as an indicator that colostrum was ingested, and that passive immunity was acquired. If ALP and GGT levels are low, alternative sources of immunoglobulins can be administered to the neonate.[70,71]

Nephrogenesis is not complete in the newborn pup, and renal function continues to develop after birth.[41,68] Serum creatinine concentrations are lower in neonatal pups than in adult dogs, probably because of the relatively lower muscle mass in pups. Because healthy neonates are in an anabolic state, more dietary nitrogen is converted to protein and less urea is produced. Therefore, concentrations of blood urea nitrogen (BUN) also are lower in neonatal pups. A BUN of 20 mg/dl could indicate a significant reduction in glomerular filtration rate in pups (Table 8–7), whereas a similar BUN might be considered normal for adult dogs.[69] Conversely, serum levels of phosphorus are higher in young dogs because of bone growth and metabolism, and should not be

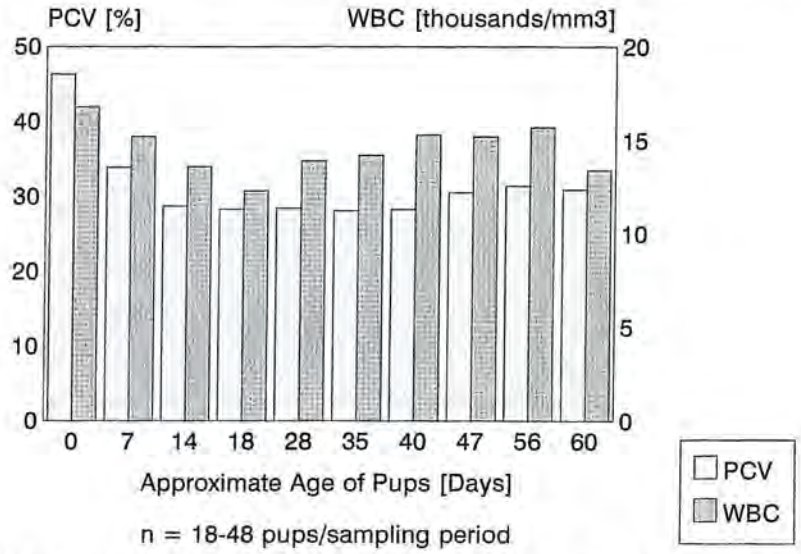

Figure 8–6. Blood values in normal beagle pups from birth to 2 months of age. (From Shifrine M, Munn SL, Rosenblatt LS, et al: Hematologic changes to 60 days of age in clinically normal beagles. Lab Anim Sci 23:894–898, 1973, with permission.)

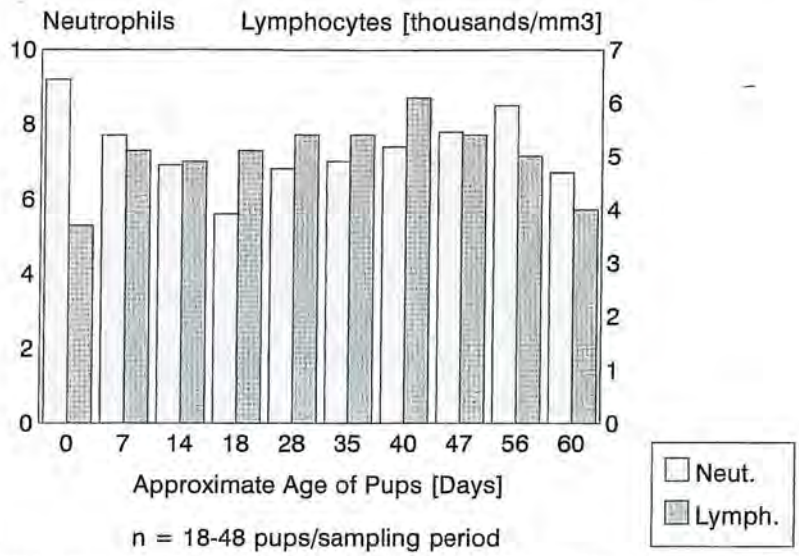

Figure 8–7. Differential leukocytes counts in normal beagle pups. (From Shifrine M, Munn SL, Rosenblatt LS, et al: Hematologic changes to 60 days in clinically normal beagles. Lab Anim Sci 23:894–898, 1973, with permission.)

Table 8–7. Mean Serum Chemistry Values for Neonatal Pups

Parameter	Value	Age (wk)
Glucose (mg/dl)	88	<1
	126	6
Blood urea nitrogen (mg/dl)	8.9	6
Creatinine (mg/dl)	0.4	6
Amylase (U/L)	319	6
Total bilirubin (mg/dl)	0.5	<1
	0.3	6
Uric acid (mg/dl)	0.8	6
Lactate dehydrogenase (U/L)	103	6
Serum glutamic-oxaloacetic transaminase (asparatate aminotransferase) (U/L)	108	<1
	18	6
Serurm glutamate-pyruvate transaminase (alanine aminotransferase) (U/L)	69	<1
	17	6
Serum alkaline phosphatase (U/L)	3845	<1
	132	6
Serum γ-glutamyltransferase (IU/L)	1111	<1
Total protein (g/dl)	4.1	<1
	4.5	6
Albumin (g/dl)	2.1	<1
	2.6	6
Cholesterol (mg/dl)	136	<1
	157	6
	399, 429*	5
Triglycerides (mg/dl)	73	6
Calcium (mg/dl)	10.9	6
Phosphorus (mg/dl)	9.2	6
Magnesium (mEq/L)	2.1	6
Sodium (nmol/L)	148	6
Potassium (nmol/L)	5.3	6
Chloride (nmol/L)	105	6
Ammonium (g/dl)	82	6
Creatinine phosphokinase (U/L)	210	6

* Higher cholesterol values (means of 399 and 429 mg/dl) were in two groups of pups fed homemade formulas containing whole milk, egg yolk, and vegetable oil (M.L. Chandler et al., unpublished data, Colorado State University, 1989).
Data from Center et al.,[29,67] Jezyk,[68] Lawler,[6] and M.L. Chandler et al. (unpublished data, Colorado State University, 1989).

confused with the increases observed in older animals with renal disease.[72]

Serum lipid profiles vary considerably between nursing pups and formula-fed pups. In one study of 54 beagle pups, those receiving homemade formulas consisting of whole milk, egg yolks, and vegetable oil had considerably higher serum concentrations of cholesterol and low density lipoproteins than did nursing pups. Serum concentrations of triglycerides and high-density lipoproteins were not different among groups (M. L. Chandler et al., unpublished data, Colorado State University, 1989).

Paternity Testing of Pups

Owners sometimes are uncertain of the paternity of pups and request that testing be performed to ascertain the identity of the sire. Because estrus can extend over several days, and multiple matings can result, pups within a single litter may have different sires.

Phenotype

The phenotype of a pup can be used to estimate paternity. For example, paternity might be questioned when pups thought to be of one breed have the shape, color, and hair coat qualities of another breed. Understanding the inheritance of certain traits is useful when determining if a pup has the predicted phenotype. For example, if both a Labrador bitch and sire are yellow, all pups born to these parents should be yellow because the color yellow is recessive in this breed. The incidence of other colors in Labrador pups can be used to predict whether the proposed sire is the likely sire (Table 8–8, Fig. 8–8).

Blood Typing

Polymorphisms of blood groups, enzymes, and serum proteins have been the next order of genetic systems used to substantiate a pedigree.[73] These systems are genetically informative, and usually consist of single, unlinked loci that have some degree of polymorphism. Although sample collection is simple because only a blood sample is necessary for testing, it often is difficult to obtain samples from all potential sires. Differences of polymorphism within the dog world, and especially within a single breed, are not extensive. For example, the polymorphism in the red blood cell system is insufficient to be of significant value in proving the paternity of pups, although it may exclude a sire. Although the dog lymphocyte antigen is better in predicting parentage, it also has weaknesses. Similarities among related males make it difficult to exclude potential fathers within a breed because many dogs are line bred or inbred and relatedness is common.

DNA Fingerprinting

Deoxyribonucleic acid (DNA) fingerprinting is a technique that can detect differences at many highly variable genetic loci in a single test. DNA can be obtained from any nucleated cell in the body for the test. Although each cell expresses different genes, all cells contain the total genetic information of an individual dog. Through subjecting extracted DNA to various tests, a unique "bar code" or "fingerprint" can be developed for each animal.[74,75]

Table 8–8. Various Matings Involving *B*- and *E*-Loci Alleles in the Labrador Retriever

Parents				Offspring						
Genotype	Phenotype	Genotype	Phenotype	No. litters	No. black obs.	No. black exp.	No. choc. obs.	No. choc. exp.	No. yellow obs.	No. yellow exp.
1. *B/B, E/E*	black	X *B/B, e/e*	yellow	9	68	68	0	0	0	0
2. *b/b, E/E*	choc.	X *B/B, e/e*	yellow	7	61	61	0	0	0	0
3. *B/B, E/e*	black	X *B/B, E/E*	black	3	14	14	0	0	0	0
4. *B/b, E/E*	black	X *B/B, e/e*	yellow	4	30	30	0	0	0	0
5. *B/B, E/e*	black	X *B/B, e/e*	yellow	7	21	18	0	0	15	18
6. *B/b, E/e*	black	X *B/B, e/e*	yellow	3	13	14	0	0	15	14
7. *B/b, E/e*	black	X *B/b, E/e*	black	7	26	28.7	14	9.6	11	12.8
8. *b/b, E/E*	choc.	X *b/b, e/e*	yellow	2	0	0	12	12	0	0

From Templeton et al: J Hered 68:134–136, 1968, with permission.

Figure 8–8. A: Yellow coat-colored Labrador with genotype *B/B, e/e* has black nose and lips. **B:** Yellow coat-colored Labrador with genotype *b/b, e/e* has the lighter pigment in the nose and lips. (From Templeton JW, Stewart AP, Fletcher WS: Coat color genetics in the Labrador retriever. J Hered 68:134–136, 1977, with permission.) *See Color Plate*

In one report,[74] high-molecular-weight DNA was extracted from blood and used in three cases of disputed paternity involving a Rhodesian Ridgeback litter, an Afghan hound litter, and a Border collie litter. Two DNA fingerprinting probes were used with various restriction enzymes to give positive and negative proof of paternity. The approach was considered useful by the authors, even when the putative sires were related or half-siblings. These authors concluded that, unless the sires were identical twins, DNA fingerprinting could establish parentage. Because the technology is expensive, it has not been widely employed in clinical theriogenology involving companion animals, but the source is commercially available to veterinarians and dogs owners (Synbiotics Corporation, San Diego, CA).

DNA fingerprinting technology is not always able to infer phylogenetic relationships in domestic and wild canids. Individual breeds of dogs cannot be identified by the use of DNA fingerprinting. For example, it may be difficult to determine a "pit bull" dog because of the similarity of genetic profiles between breeds. In one report,[75] the average proportion of shared DNA restriction fragments among 28 individual dogs representing 20 distinct dog breeds was 38 per cent. Among seven dog breeds that were evaluated, individuals among breeds were often as similar as individuals within a breed. One of two American Staffordshire terriers was more similar in its restriction fragment profile to a whippet than to the other American Staffordshire terrier.

Disorders of the Canine Neonate

Genetic Defects

Newborn pups should receive a complete physical examination. The examination should include investigation of the oral cavity to verify that no cleft palate exists and that the oronasal cavity is normal (Fig. 8–9A). Pups with severe genetic defects may need to be euthanized (Fig. 8–9B). Omphalocele and absence of the right forelimb distal to the scapula was observed in a stillborn pup conceived with frozen semen.[76] Triploidy (117,XXX) was also found in the severely deformed pup. Karyotyping can be a useful diagnostic tool for evaluating pups with suspected genetic defects.

Congenital disorders, those present at birth, are not always hereditary, or genetically determined. Hundreds of breed-specific, hereditary disorders have been identified in the dog. Lists

Figure 8–9. A: A pup with abnormal oronasal cavity. **B:** A pup born with extra limbs.

of disorders specific to a given breed, available information about mode of inheritance, and availability of testing for affected and carrier animals reportedly is available at several sites on the Internet. Texts containing canine genetic information include *Medical and Genetic Aspects of Purebred Dogs*,[77] *Abnormalities of Companion Animals: Analysis and Heritability*,[78] and *The Genetic Connection: A Guide to Health Problems of Purebred Dogs*.[79]

Failure to Thrive

The weight of a newborn pup may decrease slightly during the first day of life, but thereafter should increase steadily. Pups should gain 10 to 15 per cent of their birth weight each day, doubling their birth weight by 10 to 14 days of age.[11] Pups can be weighed using postage or food scales (Fig. 8–10). Pups that fail to gain weight should be evaluated for physical disorders and diseases. The bitch should be examined, and the quality and quantity of her milk assessed. Because pups may give owners the appearance of nursing, even when they are weak and ineffectual nursers, monitoring weight gain can be an important and sensitive prognostic indicator. Failure to thrive can be an early warning sign for neonatal septicemia, genetic diseases, inadequate nutrients, or abnormal milk (see Chapter 7).

Swimmer Pups

If newborn pups are raised in whelping areas with smooth-surfaced flooring, the pups may splay their legs and be unable to begin walking. Although smooth surfaces often are deemed desirable by owners for ease of cleaning, they can be troublesome when pups need traction to begin walking. If the pup is unable to walk, it may attempt to move across the floor with "paddling" movements, hence the term *swimmer pup*. If uncorrected, the condition can become irreversible (Fig. 8–11). Even when the condition is irreversible and severe muscle atrophy occurs, pain perception in the limbs may be normal.

Overly fat pups or those with illness (i.e., severe cardiovascular disease) may be reluctant to elevate themselves. If the underlying

Figure 8–10. A food scale that is used to weigh a neonatal pup.

Figure 8–11. A neonatal pup with splayed forelegs. The condition was treated with physical therapy and hobbles, but did not improve.

condition is not corrected, and the pup is unable to use any limb, the pup may begin to flatten as the thoracic cavity widens laterally and compresses dorsoventrally. As the cavity changes in contour, the thoracic organs may also change shape. Swimmer pups may experience regurgitation if the esophagus deviates or is compressed. Rarely, only the forelimbs may be affected.

To prevent splaying, pups should be placed on a surface that prevents slippage. Towels or carpet remnants can be added to the whelping box. Towels should be washed frequently, and carpet cleaned or exchanged with clean remnants. The legs of the pup may be hobbled together to prevent further splaying, and physical therapy may improve muscle function. Owners should consider a genetic cause if environmental factors have been eliminated; the condition has been reported to occur in more than one litter from some bitches.[80] The swimmer pup condition has been likened to a myofibrillar hypoplasia syndrome in piglets. Differential diagnosis of the swimmer pup condition should include osteopetrosis, a rare inherited disease of dachshunds and Australian shepherds that results in a failure to resorb calcified cartilage.[81] In cases of osteopetrosis, the limbs also spread laterally and pups rest on their sternebrae.

Pectus Excavatum

Pectus excavatum describes a congenital deformity of the sternum and costocartilages resulting in dorsal-to-ventral narrowing of the thorax.[82] The deformity, and subsequent thoracic constriction, may occasionally be confused with "swimmer pups" (see above). The lateral widening and dorsal compression of swimmer pups is generally more diffuse and likely to affect multiple pups within a litter; the narrowing in pectus excavatum is generally more localized and may affect single pups in a litter. In pectus excavatum, the sternum appears to "intrude" or "project" into the thorax, rather than the more diffuse flattening (and absence of sternal curvature) that is more likely observed in swimmer pups. Because of the thoracic malformation, both respiratory and cardiovascular abnormalities may exist in pups with pectus excavatum. Associated clinical signs in pups include dyspnea, cyanosis, vomiting, and mild inspiratory stridor. In one study,[82] all pups became symptomatic at birth or shortly thereafter.

Neonatal Septicemia

Neonatal septicemia has long been associated with deaths in pups, sometimes referred to as the "fading puppy syndrome" because of the rapidity of death after signs are first noticed. The incidence of neonatal septicemia in pups is unknown, partly because of the lack of a confirmatory diagnosis in many cases. Even when a necropsy is performed on dead pups, the role of bacteria as a contributing cause of death may be uncertain. Some organisms that are implicated in neonatal septicemia may also be isolated as postmortem invaders without pathologic significance. Because death may be rapid in septic pups, gross postmortem changes may be minimal. Thus confirmation of a septic process may require histologic evaluation, which delays the diagnosis. In the sick pup, diagnosis may be complicated by the rapidity of the disease. The small blood volume in pups, and the necessity to obtain a blood sample of sufficient size to optimize the isolation of a pathogen with blood cultures, further hampers the diagnostic effort. Because sepsis results in acute death, pups suspected of sepsis must be treated immediately (before cultures have been analyzed; see below).

FACTORS THAT PREDISPOSE TO SEPTICEMIA

Pups may be predisposed to septicemia if endometritis or metritis was present in the bitch, or if delivery was prolonged, possibly resulting from prolonged exposure to microorganisms in the uterus or birth canal. Pups also are predisposed to sepsis if placed in contaminated environments, especially since the pup's immunity is not fully developed at birth. Even

when pups are placed in excellent critical care facilities at veterinary hospitals, they may be at risk for developing septicemia from nosocomial infections.[10] Factors that were speculated to contribute to *Klebsiella* infections at one veterinary teaching hospital included the use of antimicrobial drugs for treating other conditions; the feeding of hyperosmolar replacement formulas; the transmission of organisms from adult dogs to neonates via animal caretakers, students, and doctors; hypoxemia; and lack of immunity in the hospitalized pups. Whenever pups are treated with antimicrobial drugs, those drugs should not suppress colonization resistance or result in increased colonization of Enterobacteriaceae. Ampicillin, frequently utilized in the past to treat a variety of conditions in neonatal pups, suppresses colonization resistance by decreasing the numbers of anaerobic bacteria, and thereby allowing the potentially pathogenic Enterobacteriaceae to increase in number. Ampicillin is not the only drug that affects colonization resistance; any antimicrobial drug that reduces the number of anaerobic bacteria in the gastrointestinal tract may suppress colonization resistance.

In children, necrotizing enterocolitis may result after direct intestinal mucosal injury is induced by excessive osmolarity of the formulas being fed.[83] Although commercial formulas are available that are suitable for rearing orphaned pups, it is important that the formulas are properly diluted to protect against intestinal mucosal injury. Whenever possible, the bitch's milk should be fed, because crucial factors in the milk, such as IgA, may protect the pups against gastrointestinal infection and sepsis.

Stress has been associated with the development of neonatal septicemia. Anecdotal reports have suggested that pups may be predisposed to neonatal septicemia following tail docking or removal of dewclaws. Although such procedures are generally performed without complication, it is prudent to delay these surgeries in pups who are failing to thrive or may be predisposed to developing septicemia.

Chilled pups may be predisposed to infection as a result of decreased lymphocyte transformation that accompanies chilling.[3]

CLINICAL SIGNS OF SEPTICEMIA

Clinical signs observed in septic pups vary. Death may be so acute that signs were not noted by the owner. If owners are weighing pups daily, they may observe a decrease in weight early in the course of the disease. Complete failure to suckle generally occurs later in the course of the disease. Other clinical signs include cyanosis, hematuria, diarrhea, vocalization, and coma. One clinical sign that may accompany neonatal septicemia is the sloughing of extremities (i.e., digits, tails, ears, and nose) (Fig. 8–12). The cause of the extremity sloughing in pups is unknown, but a similar condition has been reported in children. Possible explanations for the sloughing include reduced blood flow to the extremities of septic and hypovolemic neonates; infarction secondary to disseminated intravascular coagulation, hypoxemia, or vasculitis; and a direct effect of the organism or toxin.

TREATMENT OF NEONATAL SEPTICEMIA

Because of the rapidity of the disease, treatment must begin before the etiologic organism has been identified. Antimicrobial agents should be selected that are safe for pups and have a wide spectrum of activity. *Escherichia coli*, β-hemolytic streptococci, *Staphylococcus intermedius*, *Klebsiella pneumoniae*, and *Enterocloacae* species are the microorganisms most frequently implicated in neonatal septicemia.[84] Cephalosporins have been used safely and effectively in septic pups, and are considered the first choice for treating bacteremia and septicemia in children.[85] Dosages and intervals for treatment for various antimicrobial agents have not been established for neonatal pups. Jones[56] recommended that minimal adjustment be made when using cephalosporins, possibly lengthening the dose interval and increasing the initial dose over those considered for adult dogs (Table 8–9). Even if the half-life is prolonged, accounting for the recommenda-

Figure 8–12. Sloughed extremities in a pup suspected of developing septicemia in utero.

Table 8-9. Recommended Modification of Antimicrobial Administration to Puppies and Kittens

Drug Group	Dosage Compared with Standard Adult Dosage	Comments
Penicillins	Minimal adjustment, may lengthen dose interval	Increase initial dose
Cephalosporins	Same as above	
Aminoglycosides	Lengthen dose interval	Avoid use in first weeks of life
Tetracyclines	Minimal adjustment	Try to avoid use
Chloramphenicol	Reduce dose	Try to avoid use in puppies; do not use in kittens
Sulfonamides	Reduce dose	Avoid use
Trimethoprim	Reduce dose and lengthen dose interval	Try to avoid use
Macrolides	No change	
Lincosamides	No change	
Metronidazole	No change	

From Jones RL: Special considerations for appropriate antimicrobial therapy in neonates. Vet Clin North Am Small Anim Pract 17:581, 1987, with permission.

tion to lengthen the dose interval, cephalosporins tend to be safe because of a wide therapeutic index. If the etiologic agent is later identified, and susceptibility testing suggests the use of a different drug, antimicrobial selection may be modified. Antimicrobial therapy also may be modified if the response to therapy appears inadequate. Although it always is desirable to select the safest drugs possible, drugs with potential side effects sometimes are necessary to treat drug-resistant bacteria or microorganisms necessitating specific treatment (i.e., mycoplasma, ureaplasma, *Pseudomonas* species, anaerobes) (Table 8–10).[10,56,86,87] Gentamicin nephrotoxicity has been induced experimentally in pups;[88] gentamicin should be avoided if less toxic drugs are deemed efficacious in treating the septicemia. Available information on the toxicity of other aminoglycosides in neonatal pups is limited. Although chloramphenicol at a reduced dosage (10 mg/lb every 8 hours for up to 7 days) has been used successfully to treat neonatal septicemia in pups,[10] it should be avoided whenever possible, because cardiovascular compromise has been reported to occur in chloramphenicol-treated children and neonatal pigs, and possibly in neonatal calves.[10,89,90] Tetracyclines may not be overly toxic to pups and kittens but may cause chelation of calcium. The calcium

Table 8-10. Antimicrobial Considerations for Neonatal Pups with Septicemia

Drug	Considerations
Aminoglycosides	
Amikacin	Safer than gentamicin
Gentamicin	Nephrotoxicity has been reported in pups
Aminopenicillins	
Amoxicillin	Less adverse effect on colonization resistance than ampicillin
Ampicillin	Suppresses colonization resistance, thereby promoting the growth of potentially pathogenic intestinal bacteria; effective against anaerobes
Anti-*Pseudomonas* penicillins	No effective oral formulations available for pups
Cephalosporins	Safe; fewer oral formulations available as the antibacterial spectrum increases; no adverse effect on colonization resistance
Chloramphenicol	Cardiovascular toxicity reported in some species; moderate effect on colonization resistance; effective against mycoplasma and ureaplasma
Fluoroquinolones	Abnormal development of articular cartilage; avoid using before 18 mo of age in large and giant breeds
Macrolides (Erythromycin, Tylosin)	Safe; gastrointestinal disturbances; may consider for treating mycoplasma and ureaplasma; no adverse effect on colonization resistance
Metronidazole	Suppresses colonization resistance; may consider for treating anaerobic infections and *Giardia*
Penicillins	Safe; oral formulations suppress colonization resistance; effective against anaerobes
Tetracyclines	Chelates calcium; abnormal bone and tooth development
Trimethoprim-sulfonamide	Hepatitis, anemia, keratoconjunctivitis, polyarthritis reported; no adverse affect on colonization resistance

Data from Poffenbarger et al.,[10] Jones,[56] Boothe and Hoskins,[86] and Papich.[87]

chelation results in enamel dysplasia and discoloration of teeth, inhibition of growth, and skeletal deformities. Fluoroquinolones should be avoided in large- to giant-breed pups younger than 18 months of age because of destructive lesions in the cartilage of long bones.

Pups with septicemia should receive fluid therapy for dehydration, oyxgen to counter tissue hypoxemia, glucose if hypoglycemia is present, and plasma or serum to support immune function. Care must be taken when administering oxygen over several days because oxygen can result in retrolental fibroplasia, leading to blindness. Oxygen therapy does not usually reach a level of excess unless oxygen concentrations of greater than 40 per cent are used continuously for several days.[10]

Pooled serum from healthy adult dogs can be administered (22 ml/kg) subcutaneously to septic pups, especially if there is concern that the pups did not receive adequate colostrum at birth. Concentrations of serum alkaline phosphatase and γ-glutamyltransferase in 1- to 3-day old pups receiving colostrum at birth are 30 and 100 times higher, respectively, than those in normal adult dogs.[67] Therefore, the likelihood of colostrum ingestion may be predicted by measuring a small quantity of serum obtained from a neonatal pup. Although IgG concentrations are higher in the serum of neonatal pups that nurse immediately following birth, the administration of pooled serum to pups who failed to receive bitch's colostrum results in increased IgG concentrations.[69] Subcutaneous administration of serum is superior to oral administration, resulting in higher serum concentrations of immunoglobulins in neonatal pups.[29,69] The infusion of normal plasma (20 ml/kg) significantly improved mean arterial blood pressure in adult dogs after receiving an intravenous injection of *E. coli* when compared to dogs receiving saline.[91]

Sick pups who fail to nurse may need to be tube-fed (Fig. 8–13) bitch's milk or formula (see below). Care must be taken not to overfeed, especially since gastrointestinal ileus may be present in septicemic pups.

Viral Diseases

CANINE HERPESVIRUS INFECTION

Although canine herpesvirus (CHV) infection generally causes only a mild respiratory disease in adult dogs, infection of newborn pups without protective immunity results in high morbidity and mortality (see also Chapter 4). Pups are especially susceptible to the fatal form of the disease during the first 3 weeks of life, possibly because of their lower body temperatures, which favor the replication of the virus. Pups with CHV infection often are those born to young bitches who have not yet been exposed, or developed immunity, to the virus. Fortunately, the condition does not generally recur in subsequent litters born to the same bitch, possibly because of the development of immunity in previously exposed bitches that can be transferred to newborn pups. Clinical signs of herpesvirus infection include depression, diminished suckling response, persistent crying, diarrhea, rhinitis, severe abdominal pain, and incoordination. Neurologic signs may occur immediately prior to death.[29] Death frequently occurs within 24 to 48 hours after the onset of clinical signs.

Definitive diagnosis is made by viral isolation of affected tissues (i.e., kidney, liver). Gross pathologic findings include disseminated multifocal ecchymotic hemorrhages in the kidneys, liver, and lungs.

Prevention of CHV infection includes isolating the pregnant bitch during the last 3 weeks of pregnancy, and isolating the bitch and her pups during the first 3 weeks following parturition. Because most adult dogs have been exposed to CHV, it is desirable to expose young bitches to older animals prior to pregnancy, but definitely not during the last 3 weeks of pregnancy. Because CHV induces a weak humoral immune response with serum antibody titers that rise and fall quickly (4 to 8 weeks), it may be difficult to determine if a bitch has had prior exposure. Thus isolation during the "6-week critical period" (last 3 weeks of pregnancy plus first 3 weeks postpartum) offers the best protection in preventing the fatal form of CHV in neonatal pups.

Treatment of herpesvirus usually is unrewarding because of the acute nature of the disease. Pooled serum from older adult dogs (i.e., likely to have had prior exposure to CHV) may be administered. Increasing the environmental temperature to 100°F at 45 to 55 per cent humidity may be beneficial. Owners should be warned that permanent nervous, renal, or lymphoid tissue damage can occur in survivors.[92]

MINUTE VIRUS OF CANINES

Minute virus of canines (MVC) is a cause of embryonic resorption from transplacental in-

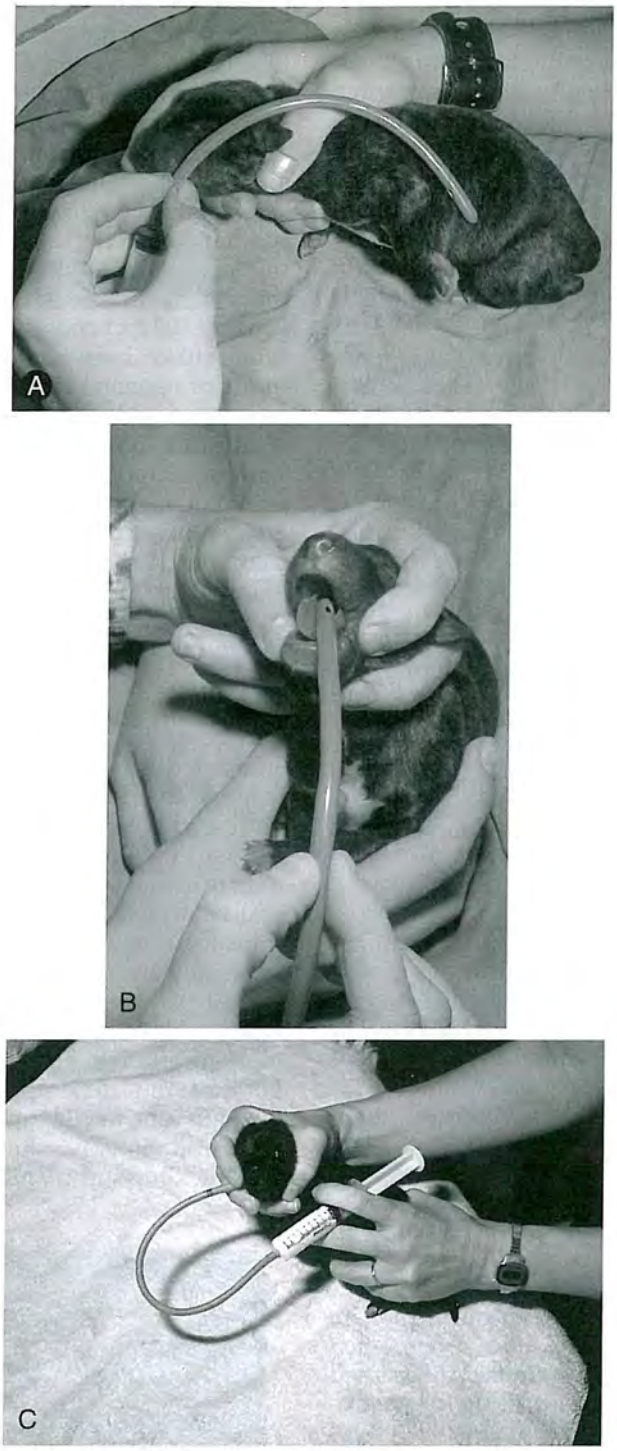

Figure 8–13. Demonstrating the method of tube feeling a pup. **A:** The tube is measured to the last rib of the pup to assure proper placement when feeding the pup. The tube should be soft and have the largest diameter that passes easily. **B:** The tube should easily pass into the stomach, and should pass to the predetermined length. **C:** After the tube is placed, the formula can be slowly delivered into the stomach. Excessive amounts of formula, or formula delivered too quickly, may predispose to regurgitation and aspiration of the formula into the lungs.

fection, and a cause of illness in neonatal pups. As with CHV infection, many adult dogs have had prior exposure to MVC. The prevalence of MVC hemagglutinin-inhibiting antibodies was high (approximately 50 per cent) in serum samples obtained from adult dogs in various geographical areas in the United States.[93]

Clinical signs of MVC infection in neonatal pups include vomiting, diarrhea, dyspnea, crying, and sudden death.[94] Among 13 pups with suspected MVC infection, the age of pups ranged from 5 to 21 days. At necropsy, all 13 pups had large intranuclear epithelial inclusions at the tips of the villi in the jejunum. Other intestinal changes included crypt epithelial hyperplasia and single-cell necrosis of crypt epithelial cells. These changes appeared to be limited to the duodenum and jejunum. Moderate to marked depletion and/or necrosis of the lymphoid cells of Peyer's patches were observed in all specimens. Lesions in other tissues included pneumonitis and myocarditis. When 4-day- and 8-week-old pups were given MVC experimentally, the pups remained clinically normal even though virus was isolated from various tissues and necropsy revealed thymic atrophy and edema.[95] Thus, the exact role of MVC, when acting alone or synergistically with other microorganisms, in causing clinical disease in neonatal pups remains unknown.

CANINE PARVOVIRUS

Generalized canine parvovirus (CPV) can occur in neonatal pups and usually is associated with vomiting and diarrhea. The disease can also manifest in the form of sudden death from cardiovascular failure in pups 3 to 8 weeks of age.[96] Myocarditis, which was associated with cardiovascular collapse, was observed when CPV infection became an epizootic in the late 1970s, and occurred in very young pups who had received no maternally derived immunity. Although myocarditis is no longer a common manifestation of CPV infection in very young pups, generalized CPV in the form of gastrointestinal disease remains a serious problem. Ancedotal reports suggest that, even when bitches are properly immunized, increased numbers of pups may be acquiring the gastrointestinal form of the disease at very early ages (before 8 weeks of age).

Rearing the Orphaned Pup

Successful rearing of orphaned pups requires excellent sanitation, an appropriate environment free from drafts and cold, regular monitoring of the pups' weight gains, and frequent physical examinations of the pups for signs of infection. Although it is always desirable that pups be reared by their dams, this sometimes is impossible. When a bitch is ill or dies, hand-rearing often becomes necessary, especially if a suitable foster mother cannot be identified.

Several commercial formulas are available for rearing motherless pups. Most of these formulas provide 1 to 1.2 kcal/ml of formula. The daily caloric need for most pups is 22 to 26 kcal/100 g of body weight. Commercial formulas have been developed to address the needs of neonatal pups. Essential amino acids have been added to prevent the development of nutritional cataracts, a condition associated with homemade and earlier commercial formulas. It is essential that the formula be properly prepared, with strict attention to proper dilution.

Pups can be fed either with a bottle that contains a soft nipple (Fig. 8–14) or by tube feeding. Although it takes longer to bottle-feed than tube-feed, pups usually consume more formula at one feeding when bottle fed. This may result from the gradual distention of the stomach during bottle feeding, allowing more formula to be consumed by the pup. Because bottle-fed pups consume more formula at each feeding, they require fewer feedings per day than tube-fed pups. Healthy neonates are generally bottle-fed four times per day. Tube-fed pups may require feedings every 2 to 4 hours, especially during the first week of life. It is crucial that the stomach is not overdistended when tube feeding. Overdistention can result in regurgitation and aspiration pneumonia. Because the suckling response is bypassed through tube feeding, tube-fed pups often will suckle on the vulvas and prepuces of siblings. This suckling stimulates the siblings to urinate,

Figure 8–14. Bottle feeding a pup.

Figure 8–15. *Moist pyoderma in orphaned pups resulting from suckling on the genitalia of littermates. See Color Plate*

providing warm fluid to the suckling pup. Thus the suckling may continue and frequently results in a moist pyoderma (Fig. 8–15).

The mean weight of bitch-fed beagle pups increased 3.5 times by day 15 of age, whereas the mean weight of formula-fed pups (22 kcal/100 g body weight), doubled during a comparable time period (M. L. Chandler et al., unpublished data, Colorado State University, 1989). After weaning and receiving the same growth diets, the formula-fed pups and bitch-fed pups reached comparable weights. As a general rule, formula-fed pups should increase their weight by at least 10 to 15 per cent each day during the first 2 to 3 weeks of life.

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Chapter 9

Prevention and Termination of Canine Pregnancy

Methods for controlling canine reproduction include those for preventing reproduction permanently (sterilants), preventing conception or pregnancy (contraceptives) (Table 9–1), and terminating pregnancy (abortifacients).

There are many reasons for controlling reproduction in the bitch. Owners may want a safe and efficacious method for preventing estrus temporarily. Such reversible contraception may be desired when an animal accompanies the family on a vacation or participates in conformation, obedience, or other show events. Reversible contraception should only be considered if one is fairly certain that future offspring are desired from the bitch. If the pedigree, animal's health or disposition, or owner's lifestyle argue against a future litter of puppies, the bitch should be spayed. Because the health benefits for bitches neutered early are now well recognized, controlling reproduction with any contraceptive or abortifacient that permits continued exposure to steroid hormones should only be considered if future reproductive function is desired and warranted. Mammary tumors, ovarian tumors and cysts, and pyometra are examples of conditions that can be reduced in incidence or eliminated if bitches are neutered early in life.

An additional reason for controlling reproduction in the bitch is to alleviate the "surplus animal problem" or "pet overpopulation" in the United States. According to data collected by the National Council on Pet Population Study and Policy (NCPPSP),[1] millions of healthy dogs and cats are euthanized each year in U.S. animal care and control facilities (Table 9–2). Many animals are relinquished to shelters as young adults because of a variety of reasons, including animal behavior (Table 9–3). When various animal behaviors were combined, undesirable behavior was the largest reason for relinquishment of dogs and the third largest reason for relinquishment of cats. Dogs and cats are relinquished or abandoned because pet population control is still not occurring at a magnitude to offset fecundity. Many well-meaning agencies attempt to find homes for the multitude of surplus animals, often without the luxury of time to adequately address the tremendous demands of pet ownership. According to the NCPPSP, the majority of relinquished dogs (47.7 per cent) and cats (40.3 per cent) are between 5 months and 3 years of age. Approximately half of the pets surrendered are not neutered. As young animals enter puberty, owners may soon appreciate additional problems associated with pet ownership. Estrous bitches have physiologic manifestations (bloody discharge passing from the vulva) and behaviors that can be problematic for the new owner. Intact male dogs may become aggressive and territorial and seek to roam. Therefore, population control remains extremely important when addressing the complex web of inter-related problems associated with "pet overpopulation."

Prevention of Canine Pregnancy

Surgical Control of Reproduction

Surgical removal of the ovaries and uterus in female dogs for control of reproduction has been performed for decades.[2] Removal of the ovaries not only controls reproduction in the dog but stops behavioral and physiologic changes characteristic of estrus that may be distressing to the owner, such as exudation of serosanguineous discharge from the bitch's vulva and attraction of male dogs. Less com-

Table 9–1. Approaches and Methods for Regulation of Fertility in the Bitch

Approach and Method	Efficacy and Safety, Concern, or Duration of Effect
Prevention of estrous cycles or ovulation	
Surgical	
Ovariohysterectomy	Permanent
Steroid treatment with progestogens or androgens	
Oral administration	
Megestrol acetate	
Proestrus treatment*,†	1 or 2 estrous cycles
Anestrus treatment*,†	1 or 2 estrous cycles
Combined treatments*,†	2 estrous cycles
Mibolerone	
Medium-term*	2–9 mo
Long-term*	1–2 yr
Very long-term‡	3–5 yr
Depo injections	
Medroxyprogesterone acetate†,‡	6–18 mo
Proligestone†,‡	6–18 mo
Subcutaneous implants	
Progesterone‡	12 mo
Testosterone‡	15 mo
Testosterone in racing dogs	
Serial injections of testosterone propionate	No reports
Orally administered testosterone	No reports
Nonsteroid hormone treatment	
Gonadotropins‡	
GnRH agonists‡	1–2 yr
Immunization	
Against gonadotropins‡	Variable
Against GnRH‡	Variable
Against zona pellucida‡	Variable
Prevention of copulation	
Isolation of bitch	
Intravaginal devices‡	
Antiinidatory estrogen treatment§	Questionable safety and efficacy
DES orally administered	
Repositol DES injection‡	
Mestranol orally administered	
Estradiol cypionate	
Estradiol valerate	
Estradiol benzoate	
Estrone	
Postestrus prostaglandin‡	Dose and timing
GnRH antagonist‡	Dose and efficacy
Postestrus epostane‡	Dose and efficacy
Experimental termination of pregnancy	
Prostaglandin $F_{2\alpha}$‡,§	Side effects
Bromocriptine‡,§	Side effects
Corticosteroids‡	Efficacy
GnRH antagonists‡	Timing
Epostane‡	
Progesterone antagonists‡	Timing
Isoquinolones‡,§	Toxicity

* Method is available, marketed, and approved in the United States.
† Method is available, marketed, or approved outside the United States.
‡ Method is not marketed, or approved, or experimental in the United States.
§ The method's availability, approval, or indication is limited in the United States.
GnRH, gonadotropin-releasing hormone; DES, diethylstilbestrol.
From Concannon PW, Meyers-Wallen PN: Current and proposed methods for contraception and termination of pregnancy in dogs and cats. J Am Vet Med Assoc 198:1215, 1991, with permission.

monly employed surgical sterilization techniques described in the bitch include ligation of the uterine tubes and salpingectomy. After these surgeries, bitches will be unable to conceive but will show normal estrous cycles and still be predisposed to uterine and mammary disease.[2,3] Intraovarian injection of cadmium chloride also has been described for sterilization of bitches. Ovaries injected with 0.5 to 2.0 mg/kg cadmium chloride dissolved in water were devoid of germ layers and glandular structures when examined 30 days later.[4]

Table 9–2. Disposition of animals entering reporting U.S. shelters, 1994–1996

	1994	1995	1996
Total number of dogs and cats entering U.S. shelters	4,131,831	3,761,550	3,916,977
Adopted (%)	23.6	23.5	24.0
Reclaimed by owner (%)	9.6	9.8	9.9
Euthanized (%)	63.6	63.5	63.7
Others (%)	3.1	2.5	2.1
Unknown (%)	NA	0.7	0.3

Data based on 1100 shelters reporting in 1994, 1054 in 1995, and 1038 in 1996; this represents approximately 20 per cent of U.S. shelters.[1]

Table 9–3. Top 10 Reasons for Relinquishment of Dogs and Cats to U.S. Shelters

Dogs	Cats
1. Moving	1. Moving
2. Landlord not allowing pet	2. Landlord not allowing pet
3. Too many animals in household	3. Too many animals in household
4. Cost of pet maintenance	4. Cost of pet maintenance
5. Owner having personal problems	5. Owner having personal problems
6. Inadequate facilities	6. Inadequate facilities
7. No homes available for litter mates	7. No homes available for litter mates
8. Have no time for pet	8. Allergies in family
9. Pet illness(es)	9. House soiling
10. Biting	10. Incompatibility with other pets

OVARIECTOMY AND OVARIOHYSTERECTOMY

Both bilateral ovariectomy (OV; oophorectomy, or removal of both ovaries) and ovariohysterectomy (OHE; oophorohysterectomy [spay], or removal of both ovaries and the uterus) have been described as surgical methods of controlling reproduction in female dogs. Bilateral OV may be the preferred technique. Surgery and anesthesia times are reduced, the incision is smaller, and the degree of abdominal trauma is less than in animals undergoing OHE.[5,6] In a retrospective survey comparing 138 dogs that had undergone OHE and 126 dogs that had undergone OV 8 to 11 years previously, no significant differences in short- or long-term complications of surgery were noted.[5] Uterine disease was not diagnosed in any of the 126 dogs undergoing only OV in that study or in 72 female dogs evaluated 6 to 10 years after OV via a flank approach.[6] Despite this, OHE is the surgery most commonly performed for control of reproduction in the dog in the United States.

Techniques for OHE via laparotomy are well described and are not reviewed here. Laparoscopic OHE also has been described in the dog.[7]

Reproductive behavior and physical manifestations of estrus cease in female dogs after OHE. Bitches spayed while in proestrus or estrus stop showing copulatory behaviors within several days of surgery.[3] Temperament of the bitch is not altered by OHE; similarly, temperament is not altered by allowing bitches to go through estrus or parturition.[8] After OHE, ovarian and uterine disease cannot occur, nor can disorders related to pregnancy and parturition. Spayed bitches are no longer susceptible to disorders mediated by estrogen, such as vaginal hyperplasia.

A significant benefit of early OHE is reduction in incidence of mammary neoplasia. Bitches ovariohysterectomized before the pubertal estrus have 0.5 per cent the risk, those spayed after 1 estrous cycle 8.0 per cent the risk, and those spayed after two estrous cycles 26.0 per cent the risk of developing mammary neoplasia of intact females.[9] The sparing effect of OHE on development of mammary neoplasia is lost after the bitch cycles more than two times or reaches 2.5 years of age.[10]

Complications. Ovariohysterectomy is the most commonly performed surgery on companion animals in the United States, and the vast majority of these surgeries are performed with no anesthetic or surgical complications. Studies reporting such complications described in this section are intended to represent the range of complications which can occur, and which have been observed in small retrospective or prospective studies. Anesthetic complications are reported to occur in 4.1 per cent of bitches undergoing elective OHE (n = 73).[11] Overall intra- and postoperative complication rates after elective OHE have been reported at 7.3 per cent (n = 550)[12] and 27.4 per cent (n = 73).[11] No correlation was demonstrated between incidence of complications and age of the bitch, skill level of the surgeon (veterinary student or graduate veterinarian), or presence of disease of the reproductive tract, such as pyometra, or disease outside of the reproductive tract, such as idiopathic epilepsy.[11] No specific breed predisposition exists,[11] but an increased incidence of complications is seen in miniature- and small-breed dogs compared to female dogs of medium, large, and giant breeds and mixed-breed dogs.[12]

Intraoperative and immediate postoperative complications of OHE reported include hemorrhage of the ovarian or uterine pedicle(s) as a result of incomplete ligation or rupture of ovarian or uterine vessels (4.1 per cent [n = 73]),[2,3,11] dehiscence,[2,3] infection of the suture line (13.7 per cent [n = 73] to 20 per cent [n = 109]),[3,11,13] peritonitis,[2] and evisceration.[2] Hemorrhage of the ovarian or uterine pedicle(s) may be more likely if the surgery is performed when the bitch is in estrus (Fig. 9–1). Long-term surgical complications reported include formation of a fistula over nonabsorbable suture material (2.7 per cent [n = 73] to 20 per

Figure 9–1. Uterine tissue may hemorrhage easily when removed from a bitch in estrus. Note the large follicles on the ovary. (From Olson PN, Nett TM, Bowen RA, et al: A need for sterilization, contraceptives, and abortifacients: Abandoned and unwanted pets. Part I. Current methods of sterilizing pets. Compend Contin Educ Pract Vet 8:87–92, 1986, with permission.)

cent [n = 109]),[5,11,13,14] retention of a cotton surgical sponge,[15] ureteral ligation with secondary hydronephrosis,[3,16,17] formation of a ureterovaginal fistula with secondary urinary incontinence,[3,18–20] formation of a vesicovaginal fistula with secondary hydroureter,[21] ovarian remnant syndrome with or without secondary uterine stump inflammation/infection (9.5 per cent [n = 42] to 50 per cent [n = 102]),[3,11,13,22–25] and granulomas of the ovarian pedicle(s) or uterine stump.[26]

Obesity is a commonly reported sequel to OHE in dogs. Obesity is more common in female than in male dogs.[27] Predisposing factors in intact and neutered dogs include increasing age and feeding of a homemade diet.[27,28] Breeds predisposed to obesity include the Labrador retriever, cocker spaniel, collie, dachshund, and cairn, West Highland white, and Scottish terriers.[27–30]

Neutering also has been identified as a risk factor for obesity. In a prospective study comparing weight gain and food intake 90 days after surgery between dogs undergoing OHE and dogs undergoing laparotomy without OHE, the spayed females had greater food intake and gained significantly more weight than sham-operated controls.[31] In three retrospective surveys, obesity was reported in 21.4 per cent (n = 8268),[30] 26.2 per cent (n = 42),[11] and about 50 per cent (n = 320)[24] of dogs, with incidence two times higher in spayed females than in intact females.[30] Female dogs were reported to have an increase in indiscriminate appetite 6 months after OHE, with an increase to at least 1.5 times presurgical body weight in 10.9 per cent of 95 dogs evaluated.[32,33]

Obesity is the most common nutritional disease of dogs and is multifactorial in pathogenesis. However, three prospective studies evaluating the effect of OV[34] or OHE[35,36] on body weight in dogs did not demonstrate an increase in body weight after surgery, perhaps due to feeding regimens used, or short durations of the studies. Other causes of obesity that must be considered include endocrinopathies, such as hypothyroidism and hyperadrenocorticism; drug-induced or psychogenic polyphagia; and inactivity with increasing age.[29] Although obesity is a common sequel to OHE, it is less likely to occur in spayed female dogs that are fed and exercised properly.

Estrogen-responsive urinary incontinence is the term applied to urinary incontinence in ovariohysterectomized dogs. The classical presentation is pooling of normal urine in areas where the spayed female dog has been sleeping. Estrogen-responsive urinary incontinence has been reported to occur in 3.0 per cent (n = 320)[24] to 20.1 per cent (n = 412)[37] of spayed female dogs. It may occur immediately after OHE or as late as 10 years after surgery, with a mean interval to onset of 2.9 years.[37] It has been reported to have higher incidence in the boxer breed, and to be more common in dogs weighing 20 kg or more (30.9 per cent incidence) than in dogs weighing less than 20 kg (9.3 per cent incidence).[37]

Exact pathogenesis of urinary incontinence in spayed female dogs is unknown. Urine leakage occurs as a result of incompetence of the urethral sphincter.[38] Estrogen increases affinity of α-adrenergic receptors for sympathetic neurotransmitters in the urethral sphincter, and increases sphincter tone.[39] However, not all spayed female dogs with urethral sphincter incompetence respond to estrogen treatment. In one survey, 73.7 per cent of affected spayed female dogs recovered after treatment with ephedrine, a sympathomimetic, and only 64.7 per cent recovered after treatment with oral estrogen.[37] In a study in which oral estrogen

(stilbestrol, 1 mg once daily per os for 3 weeks) was administered to normal and incontinent ovariohysterectomized female dogs, and urethral pressures were measured after treatment with the sympathomimetic phenylephrine, normal dogs showed a greater elevation in urethral pressure than did incontinent dogs. Serum estrogen concentrations do not differ between ovariohysterectomized bitches and intact bitches in anestrus,[38] suggesting that prolonged estrogen deprivation may be a required part of development of this form of urinary incontinence, but occasional onset of clinical signs immediately after OHE refutes this supposition. Current recommendations for treatment of estrogen-responsive urinary incontinence include diethylstilbestrol (DES; 0.5 to 1 mg once daily per os for 5 days, then 0.5 to 1 mg per os every 4 to 7 days, as needed) with or without concurrent phenylpropanolamine (1.1 mg/kg three times daily per os). Treatment with a human conjugated estrogen product (Premarin; Wyeth-Ayerst Laboratories, Philadelphia, PA) at a dose of 20 μg/kg per os every 4 days also may reduce clinical signs, although anecdotal reports suggest it is less effective than treatment with oral DES. Dogs with urinary incontinence of a type that is not characteristic of estrogen-responsive urinary incontinence, and dogs that are not responsive to the therapies described above, should undergo a complete evaluation for urinary incontinence (Table 9–4).

Table 9–4. Routine Work-up of Bitches with Urinary Incontinence

Examinations	Rule Out
Performed in all dogs	
Physical examination	Polyuria/polydispsia, urinary tract infection
Blood biochemistry	Urinary tract infection
Urinalysis	Neurologic causes of incontinence
Bacterial culture of urine	
Neurologic examination	
Performed in young bitches	
Intravenous pyelogram	Genitourinary abnormality (i.e., ectopic ureters)
Performed in bitches with incontinence <1 mo after ovariohysterectomy	
Intravenous pyelogram	Iatrogenic ureterovaginal fistula

From Arnold S: Relationship of incontinence to neutering. *In* Kirk RW, Bonagura JD (eds): Curr Vet Ther Small Anim Pract 11. Philadelphia, WB Saunders, 1992, p 876, with permission.

Miscellaneous long-term effects of OHE reported in the dog include bilaterally symmetrical nonpruritic alopecia[33,40] and increased rate of bone remodeling.[41,42] In women and female dogs, bone turnover is increased after OHE, with significantly greater bone resorption than formation.[41,43] Loss of up to 15 per cent of trabecular bone in vertebrae was demonstrated in adult female beagles 11 months after OHE.[42] Osteoporosis and bone fragility are not generally recognized problems in the spayed female dog. There is one report of gastroesophageal and enteroenteric intussusceptions developing in a dog with prior megaesophagus after OHE; these complications were likely due to anesthesia and relaxation of the esophageal sphincter and were therefore not complications of the OHE itself.[44]

Ovarian Autotransplantation. Ovarian autografts were reported to prevent complications of OHE by the implanting of minced ovary or slices of ovarian tissue in pockets in the serosa of the greater curvature of the stomach or intestine in areas drained by the portal vein. In one study of 15 working dogs, no difference in body weight or working ability was demonstrated in dogs that had undergone ovarian autotransplantation when compared to intact dogs.[34] However, body weight and working ability also were comparable in those dogs with ovarian autotransplants and those undergoing only ovariectomy, and the dogs with ovarian autotransplants did show short periods of proestrus, with attraction of male dogs.[34] A retrospective study of 66 dogs that had undergone ovarian autotransplantation at least 5 years earlier reported signs of estrus in 14 per cent, and urinary incontinence in 20 per cent of the dogs. Two dogs (3 per cent) died as a result of complications of the ovarian autotransplant itself; one dog died of metastatic granulosa cell tumor arising from the implanted ovarian tissue, and the other from a bleeding ulcer that developed over the ovarian implant.[45] Ovarian autotransplantation cannot be recommended because of the poor efficacy in preventing complications of OHE and the relatively high mortality resulting from the procedure itself.

EARLY SPAY/NEUTER

Early spay and neuter, or prepuberal gonadectomy, refers to the surgical sterilization of sexually immature animals. This topic has received increasing attention because it has been

regarded as a tool to help control pet overpopulation in the United States. In 1992, the American Humane Association (AHA) put forth a resolution supporting further research in early spay and neuter, and stated that "the AHA believes that no dog or cat adopted from a shelter should be allowed to reproduce."[46] The American Veterinary Medical Association also encouraged additional research to ensure provision of more long-term data to better define safety of the procedure and its possible effect on pet overpopulation.[47] Early spay/neuter has been endorsed by the American Animal Hospital Association, British Veterinary Association, British Small Animal Veterinary Association, and American College of Theriogenologists. A reader survey in *Pet Veterinarian* showed that 67.5 per cent of those responding thought preadoption pediatric neutering was a good idea for animal shelters and humane societies but that only 40.0 per cent of those practitioners responding had performed pediatric (6- to 12-week) spay/neuter surgeries.[48] Of 18 veterinary colleges responding to a survey by the Arizona Humane Society about early spay/neuter training, only 8 (44 per cent) performed early spay/neuter surgeries on client animals (B. Bean, personal communication, June 27, 1995).

Proponents of early spay/neuter stress its worth for animal humane facilities, where gonadectomy before adoption or sale may facilitate placement of animals and alleviate problems of unwanted litters.[49,50] At this time, early spay and neuter surgeries are more commonly performed in animal shelters and humane societies than in general small animal practices. This reflects the fact that, although most puppies are released from a shelter with a spay/neuter contract, many of those animals are left intact once adopted. If animals in shelters were neutered before adoption, they would be unable to reproduce and repopulate animal shelters. Practitioners may be asked to provide early spay and neuter surgeries for breeder clients, who wish to control reproductive potential of purebred animals they wish to place as pets.

Anesthesia Considerations. Anesthesia of pediatric animals requires limitation of preanesthetic excitement, knowledge about possible altered pharmacokinetics of anesthetic agents, and enhanced monitoring of the patient. Anesthetic concerns unique to pediatric animals include (1) altered metabolism and excretion of drugs as a result of immature hepatic enzyme systems, decreased protein binding of drugs, and decreased glomerular filtration rate; (2) predisposition to hypoglycemia with fasting as a result of decreased glycogen stores secondary to smaller liver size and skeletal muscle mass; and (3) decreased ability to maintain body temperature because of a low percentage of body fat and decreased ability to shiver.[51]

Preoperative excitement of pediatric animals should be minimized by housing the animals in groups in a quiet location, and not handling them excessively before anesthetic induction.[52,53] Animals that are excited are more likely to resist handling and show inadequate sedation.[52] Administration of anesthetic agents by the intramuscular (IM) route, rather than the intravenous (IV) route, is preferred, to decrease the amount of restraint required for anesthetic induction.[52]

Pediatric animals should be fasted for no more than 8 hours before anesthetic induction to minimize hypoglycemia.[52,53] Animals less than 10 weeks of age may be fasted for as little as 3 to 4 hours.[52,54] A small meal should be offered to the animals within an hour after anesthetic recovery[52,53]; kittens with apparent prolonged recovery after prepuberal gonadectomy surgery showed increased alertness and responsiveness when given 50 per cent dextrose solution orally.[52]

To minimize hypothermia, pediatric animals should be placed on a supplemental heat source during surgery, such as a circulating warm water blanket.[52] Clipping of hair at the surgery site should be minimized, and warmed surgical scrub solutions used.[52] Rectal temperature should be checked immediately after surgery. Hypothermic animals may have prolonged anesthetic recovery as a result of decreased metabolism of injectable anesthetic agents.[52] In a survey of 142 puppies neutered at either 6 to 10 weeks of age or 7 months of age, anesthesia recovery time was significantly shorter in those animals undergoing surgery at 6 to 10 weeks of age.[36]

Anesthetic regimens that have been shown to provide the smoothest induction, fastest recovery times, and best intraoperative analgesia in pediatric dogs are:

Female puppies—atropine (0.04 mg/kg IM) and oxymorphone (0.11 mg/kg IM), followed 15 minutes later by propofol (3.4 mg/kg IV) with inhalant isoflurane for mainentance[54]

Male puppies—atropine (0.04 mg/kg IM) and oxymorphone (0.22 mg/kg IM), followed 15 minutes later by propofol (6.5 mg/kg IV).[54]

Intubation is recommended in female puppies whenever possible to ensure a patent airway, allow ready assistance of ventilation if needed, and decrease the gas exposure to surgical personnel that may occur with mask administration of anesthetic gases.[52]

Anesthetic complications reported in pediatric animals undergoing early spay/neuter surgeries vary. None were reported in surveys of 98 kittens[52] and 98 puppies,[54] ranging in age from 6 to 14 weeks. Cardiac dysrhythmia, gastric dilation, and drug overdosage were reported in a survey of 775 cats and 1213 dogs that had undergone OHE or castration, ranging in age from less than 12 weeks to greater than 23 weeks.[55]

Surgical Technique. Basic principles of pediatric surgery include minimization of surgery time and tissue handling, and controlling intraoperative bleeding meticulously. Routine techniques for OHE or castration are employed. Castration techniques involving tying of the spermatic cord to itself, or tying the vas deferens to the spermatic artery, are not recommended because of the fragility of the tissues.[53] Hemoclips or fine suture material may be used for ligation.[53,54] Polydioxanone (PDA) suture material may cause calcinosis circumscripta and should be avoided.[53]

Complications. Postsurgical complications of pediatric neutering are rare. Among 98 six- to 14-week-old puppies, 10 per cent developed signs of inflammation at the surgical site within 1 to 2 days of surgery, all of which responded to conservative treatment.[54] In a large survey comparing short-term postoperative complication rates in dogs and cats gonadectomized at less than 12 weeks of age, 12 to 23 weeks of age, or more than 23 weeks of age, those gonadectomized at less than 12 weeks of age had a significantly lower postoperative complication rate (6.5 per cent) than did those animals gonadectomized at greater than 23 weeks of age (10.8 per cent).[55] Most of the complications reported were minor and required little or no treatment; inflammation at the surgical incision site was the most common complaint.[55]

Potential complications of prepuberal gonadectomy include immaturity of the penis and prepuce, obesity, urinary incontinence, stunted growth, behavioral abnormalities, and possible increased incidence of adrenal disease. Male dogs gonadectomized at 7 weeks of age had smaller penile diameters, decreased radiodensity and size of the os penis, and immature preputial development compared to male dogs gonadectomized at 7 months of age or left intact.[35]

Obesity is a multifactorial problem (see Ovariectomy and Ovariohysterectomy above). No difference was shown in food intake, weight gain, or back-fat depth in dogs that had been gonadectomized at 7 weeks or 7 months of age or left intact.[35] In a study of 142 puppies matched with a littermate, in which one underwent gonadectomy at 6 to 10 weeks of age and the other at 7 months of age, there was no significant difference in body weight between the groups at 18 months of age.[36] If a physiologic predisposition to obesity resulting from gonadectomy does exist, there is no evidence that age at time of gonadectomy would change this effect.

Older ovariohysterectomized dogs may develop urinary incontinence secondary to decreased circulating serum estrogen concentrations and subsequent laxity of the external urethral sphincter (see Ovariectomy and Ovariohysterectomy above). Female dogs gonadectomized at 7 weeks of age have immature vulvar development at 15 months of age compared to female dogs gonadectomized at 7 months of age or left intact.[35] Older ovariohysterectomized dogs may undergo vulvar atrophy as a result of hypoestrogenism. If infolding of the vulva occurs concurrently with estrogen-responsive urinary incontinence, perivulvar dermatitis may result, especially in obese dogs. At present, there is no evidence suggesting that dogs ovariohysterectomized prepuberally are more predisposed to any of these conditions than are females ovariohysterectomized later in life.

An argument against early spay and neuter has been that of stunted growth and poor muscle development in prepuberally gonadectomized animals. Gonadal steroids stimulate growth of cartilage and maturation of the physes of the long bones. Prepuberally gonadectomized animals are therefore more likely to show delay in closure of the physes, and increased long bone length than decreased bone length. This delay in physeal closure and subsequent increase in mature bone length has been demonstrated in pre- and postpuberally gonadectomized dogs.[35] Whether this predisposes these animals to Salter-type fractures at open physes is unknown.

Owners of dogs may request surgical sterilization to decrease undesirable sexual behaviors in their pet, such as roaming, urine marking, mounting, or the vaginal discharge and

unique behaviors of estrus. Although good correlation has been shown between castration and decrease in sexual behaviors in dogs in one study,[56] it also has been shown that prior sexual experience does not affect retention of sexual behaviors after castration,[57] suggesting that these behavioral problems may occur later in the life of animals gonadectomized prepuberally.

Conversely, some owners prefer that animals remain intact until after puberty, in an effort to control undesirable juvenile behaviors of dogs, such as excitability. Male and female dogs gonadectomized at either 7 weeks or 7 months of age were judged to be more active than age-matched intact dogs in one study, and those gonadectomized at 7 weeks were more excitable than those left intact.[35]

Certain strains of mice, when gonadectomized within days of birth, are predisposed to adrenocortical nodular hyperplasia and carcinomas. An increase in adrenocortical tumors and nodular hyperplasia has been reported in ferrets.[58,59] Most ferrets raised commercially are gonadectomized prepuberally. Therefore, the reported incidence of 99 per cent of these tumors occurring in neutered male and female ferrets is not necessarily indicative of a cause-effect relationship.[59] Of 100 cases of proliferative adrenal lesions in ferrets archived at the Armed Forces Institute of Pathology, almost 30 per cent were in intact females.[60] To date, no studies evaluating incidence of adrenal disease relative to early spay/neuter have been published in the dog.

Pediatric gonadectomy is quick, with minimal bleeding, and the animals recover quickly.[55,61] Currently available information indicates that early spay and neuter are safe procedure, although further research and the passage of time are indicated to understand long-term effects. Whether the adoption of early spay and neuter programs by humane organizations will significantly impact pet overpopulation remains to be seen, but it is likely that early spay and neuter, coupled with increased enforcement of animal control ordinances, enhanced public education, and a change in attitudes concerning responsible pet ownership will help reduce the pet overpopulation problem.

Nonsurgical Control of Reproduction

Enforced sexual abstinence, or strict control of bitches when in estrus, is the least invasive method of reproduction control. Confinement indoors and supervision when on a leash when the estrous bitch is outdoors are required from early proestrus to the onset of diestrus.

Nonsurgical methods for sterilizing an animal have been sought for decades. With newer technologies, such as biotechnology and genetic engineering, researchers are again attempting to develop alternatives to surgical sterilization. Nonsurgical methods must be safe, effective, easy and convenient to administer, and inexpensive.[62] Their primary advantages are that nonsurgical methods are less invasive than surgical methods and are potentially reversible.

MECHANICAL METHODS

Intravaginal devices have been marketed that were reported to work by interfering with copulation (Fig. 9–2). A device of appropriate size for a given bitch was placed in the cranial vagina. Reported complications included difficulty in fitting the device to bitches of all sizes, persistent vaginal infection, and perforation of the vaginal wall.[62] Intravaginal contraceptive devices for the bitch are no longer commercially available.

PHARMACOLOGIC AGENTS

Progestins. Progestins administered in anestrus prevent onset of proestrus and estrus by suppressing the increases in frequency, magnitude, and duration of gonadotropin secretion necessary for induction of a new cycle.[63] Progestins such as megestrol acetate, administered early in proestrus, prevent development of ovarian follicles and the luteinizing hormone (LH) surge and ovulation.[62,64] Efficacy and side effects vary with drug administered, dose, time within the estrous cycle during which the progestin is administered, treatment regimen, and age and health of the bitch.[65]

Megestrol acetate (Ovaban; Schering Corporation, Kenilworth, NJ) is the only progestin approved for use as an estrus-suppressing drug in the United States. The drug can be administered either in anestrus starting (at least 1 week prior to the next proestrus) at a dose of 0.55 mg/kg (0.25 mg/lb) once daily per os for 32 days, or in early proestrus (within the first 3 days of proestrus, characterized by vulvar swelling and exudation of serosanguineous vaginal discharge) at a dose of 2.2 mg/kg (1 mg/lb) once daily per os for 8 days.[62] The drug is 92 per cent effective at suppressing

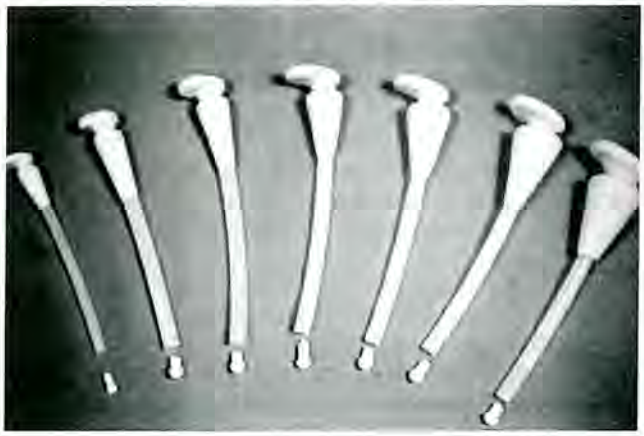

Figure 9–2. Intravaginal devices were once used to prevent pregnancy in bitches. (From Olson PN, Nett TM, Bowen RA, *et al*: A need for sterilization, contraceptives, and abortifacients: Abandoned and unwanted pets. Part II. Contraceptives. Compend Contin Educ Pract Vet 8:173–177, 1986, with permission.)

the expected estrous cycle when given in anestrus, and is 97 per cent effective when given in proestrus.[66] Treatment in early proestrus is less effective in bitches with proestrous periods of less than 4 days or greater than 20 days in length.[67] Treatment in early proestrus will decrease vulvar swelling and serosanguineous vaginal discharge within 3 to 8 days.[13,64] In that period of time, the bitch still will be attractive to male dogs and mating may occur.[62] If the bitch has received treatment with megestrol acetate for at least 3 consecutive days prior to mating, conception will not occur.[64]

Return to estrus after treatment is variable, averaging 4 to 6 months with a reported range of 1 to 9 months.[62,63] Fertility, litter size, and sex ratio are reported normal after treatment.[64] The manufacturer does not recommend that bitches be bred if return to estrus occurs less than 1 month after cessation of treatment.[62] Treatment at the puberal estrus or for more than two consecutive estrus cycles is not recommended.

Transient side effects of increased appetite with weight gain, lethargy or restlessness, and mammary development with minimal lactation are reported to occur in up to 40 per cent of treated bitches (n = 700).[64,66] Megestrol acetate is contraindicated in bitches with a history of mammary neoplasia, diabetes mellitus, liver disease, or uterine disease[13,62,64,66]; in a survey of 700 bitches treated with megestrol acetate at the manufacturer's recommended dose in anestrus, 0.6 per cent developed pyometra.[64] No increase in incidence of mammary neoplasia was reported in that study.[64] The drug is contraindicated in potentially pregnant dogs, in which it may cause masculinization of female fetuses. Adrenal suppression was reported after treatment with megestrol acetate (2 mg/kg once daily per os for 14 days, followed by 1 mg/kg once daily per os for 14 days). Mean cortisol concentrations at baseline and after stimulation with adrenocorticotropic hormone (ACTH) were decreased 14 and 28 days after treatment.[68] Clinical manifestations of adrenal suppression have not been reported after treatment with megestrol acetate in bitches.

Medroxyprogesterone acetate (MPA) is a long-acting progestin. Administration of MPA in a subcutaneous implant has been described for estrus suppression in the bitch,[69] but the drug is more commonly administered as a periodic injection.[63] It is recommended that it be used only in anestrus.[66] Minimal effective doses are 2 mg/kg IM every 3 months or 3 mg/kg IM every 4 months.[13] Return to estrus is variable, ranging from 1.5 to 26 months.[66] Side effects are common with use of this drug, ranging from mild lethargy, polyphagia, and weight gain to adrenal suppression, acromegaly, uterine disease, and mammary neoplasia.[13,66,70] Uterine disease is reported to occur in 4 to 10 per cent of bitches treated, with increased incidence in bitches with irregular estrous cycles.[66] Mammary tumors were diagnosed in 30 per cent of 358 bitches receiving MPA at 5-month intervals, compared to 16 per cent of 192 bitches in a control group.[70] The authors do not recommend the use of MPA for estrus suppression.

Chlormadinone acetate (CA) and delmadinone acetate are long-acting synthetic progestins. They are not commercially available in the United States; CA is the one best described. Return to estrus after treatment with CA may be protracted, but fertility at the subsequent estrus is apparently normal.[66] Subcutaneous Silastic implants containing CA reportedly are

effective for estrus suppression in bitches; in one study, estrus was suppressed in 17 of 19 bitches with CA implants containing 5.4 to 10.9 mg/kg CA.[71] At doses of greater than 5 mg/kg, ovaries were devoid of corpora lutea (CL) 2 years after placement of the implant.[72] Although dose-dependent development of cystic endometrial hyperplasia, uterine enlargement, and mucometra have been described in studies using doses as high as 25.0 mg/kg, only 1 of 19 female dogs treated with up to 10.9 mg/kg CA developed pyometra.[71,72] No clinical, hematologic, or biochemical abnormalities were noted in bitches treated with implanted doses of at least 10 mg/kg CA.[73]

Proligestone is a compound with antigonadotropic properties that exerts minimal progestational effect.[66] It is not commercially available in the United States. It can be used safely at any stage of the estrous cycle.[13] It is administered as a subcutaneous (SC) injection at a dose of 10 to 30 μg/kg repeated 3 and 7 months later, and then every 5 months.[13] Efficacy as an estrus-suppressing agent in dogs is reported as 95 to 99.9 per cent.[66,74,75] Return to estrus after treatment averages 6 months, with a range of 3 to 9 months.[66] Proligestone must be given SC to ensure absorption, but the side effect of pain at the injection site has been reported to occur in 5 per cent of animals treated (n = 160).[75] Discoloration of hair also may occur at the injection site.[66] These side effects may be minimized by warming the injection and giving it in the fold of the flank instead of the scruff of the neck.[66] Pyometra was reported to occur in 1 of 60 bitches treated with proligestone.[74]

Androgens. Mibolerone (Cheque drops; Pharmacia & Upjohn, Peapack, NJ), is the only androgen approved for estrus suppression in bitches in the United States. Mibolerone is not recommended for use in breeding animals by the manufacturer, although this drug has not been shown to adversely affect reproduction after it is discontinued. Androgens exert negative feedback on the pituitary, decreasing gonadotropin secretion. The ovaries of dogs treated with mibolerone contain primary and secondary follicles but few that mature to ovulatory size.[62]

The dose of mibolerone administered is based on weight and breed of the dog (Table 9–5). The highest dose, 180 μg daily per os, should be administered to German shepherds and German shepherd crosses, regardless of body weight.[13,62] Treatment with mibolerone must be instituted 30 days or more before onset of the next proestrus or it may not prevent the next estrus.[62] The manufacturer recommends that mibolerone not be administered to bitches before the puberal estrus so as not to cause premature closure of physes. The drug is given once daily, orally, as long as estrus suppression is desired. Treatment for up to 2 years is approved, but the drug has been demonstrated to be safe and effective even if used for up to 5 years.[13] Return to estrus averages about 70 days, with a range of 7 to 200 days.[62] Bitches have been reported to exhibit normal fertility after estrus suppression with mibolerone.[76]

Table 9–5. Dosages of Mibolerone Recommended for Long-Term Estrus Prevention in Anestrous Bitches*

Body Weight Range		Mibolerone Dosage (μg/d)
lb	kg	
1–25	0.5–12	30
26–50	12–23	60
51–100	23–45	120
101+	>45	180
Any German shepherd dog or any Alsatian-derived mixed breed		180

* Return to estrus after end of treatment expected in 1 to 7 months; average, 2.5 months. Up to 24 months continuous treatment approved. Efficacy to 5 years demonstrated.
From Concannon PW, Meyers-Wallen PN: Current and proposed methods for contraception and termination of pregnancy in dogs and cats. J Am Vet Med Assoc 198:1218, 1991, with permission.

The most common side effect reported with mibolerone treatment is clitoral hypertrophy, which occurs to some degree in 15 to 20 per cent of dogs treated with mibolerone.[13,62,66] Other reported side effects include creamy vaginal discharge, vaginitis, increased mounting and aggressive behaviors, anal gland inspissation, musky body odor, obesity, and epiphora.[62,66] Mibolerone is contraindicated in potentially pregnant bitches, in which it may cause masculinization of female fetuses; in prepuberal bitches, in which it may precipitate premature physeal closure; and in dogs with renal or hepatic diseases.[62] Presence of intranuclear hyaline bodies in hepatic cells, elevation in serum liver enzyme concentrations, and, rarely, changes in liver function tests have been described in dogs after treatment with mibolerone; clinical significance of hepatocellular changes is unknown.[77] The manufacturer recommends that mibolerone not be administered to Bedlington terriers or other breeds with familial liver disease.

Testosterone also has been described for estrus suppression in bitches. Successful regi-

mens reported include injection of 100 mg testosterone propionate once weekly, oral treatment with 25 to 50 mg methyltestosterone twice weekly, and SC implantation of at least 759 μg/kg.[64,78,79] Side effects reported include clitoral hypertrophy and vaginal discharge.[64]

Miscellaneous Pharmacologic Agents. Gonadotropin-releasing hormone (GnRH) agonists have been reported that act by down-regulating pituitary GnRH receptors and suppressing release of follicle-stimulating hormone and LH.[63] These compounds are not commercially available.

IMMUNOSTERILIZATION

Induction of antibodies against endogenous hormones or reproductive tissues could be a noninvasive, reversible form of estrus suppression and contraception in dogs.

The zone pellucida (ZP) is an extracellular matrix of glycoproteins secreted by oocytes during folliculogenesis that is important in differentiation of follicular cells and that regulates adhesion and penetration of spermatozoa (Fig. 9–3).[80] These glycoproteins are unique and cross-react between species.[80] One expected effect of ZP immunization is for antibodies to develop and coat the ZP of ovulated oocytes, preventing their fertilization by spermatozoa (contraception). Female dogs that developed high antibody titers after injection with porcine ZP had demonstrable antibody coating of the surfaces of their ZPs in vivo and in vitro, and in vitro evidence of decreased penetration of spermatozoa.[81,82] None of three dogs with high antibody titers to porcine ZP were successfully bred.[81]

A second expected effect of ZP immunization is for antibody binding to alter oocyte-follicle interaction and eventually deplete ovaries of fertile follicles (sterilization). Rabbits immunized with ZP proteins had virtually no maturing follicles in their ovaries by 23 weeks after treatment, and many of the follicles present contained no oocytes (Fig. 9–4).[80] Bitches immunized with porcine ZP are infertile, with no active follicular maturation, lack of primordial follicles, and follicular cysts lined with granulosa cells or luteinized cells as demonstrated by ovarian histopathology.[83–85]

Unfortunately, dogs immunized with porcine ZP often have abnormal estrous cycles.[81,84] Although bitches do not conceive if antibody titers are high,[86] the desirable lack of estrous cycling does not occur reliably with ZP immunization. Immunization with canine ZP stimulates only low titers that are not contraceptive.[81] Both porcine and canine ZP must be injected with adjuvant to stimulate an immune response; commonly used adjuvants, such as Freund's complete, cause an unacceptable number of sterile abscesses at the injection site.[84] Immunization with ZP proteins is not currently an accepted form of reproductive control in the dog, although studies are ongoing.

Genetically engineered *Salmonella* with ZP antigen is currently being evaluated as a potential oral sterilant or contraceptive for stray cat

Figure 9–3. Canine ovum with zona pellucida investment. (From Olson PN, Nett TM, Bowen RA, et al: A need for sterilization, contraceptives, and abortifacients: Abandoned and unwanted pets. Part IV. Potential methods of controlling reproduction. Compend Contin Educ Pract Vet 8:303–308, 1986, with permission.)

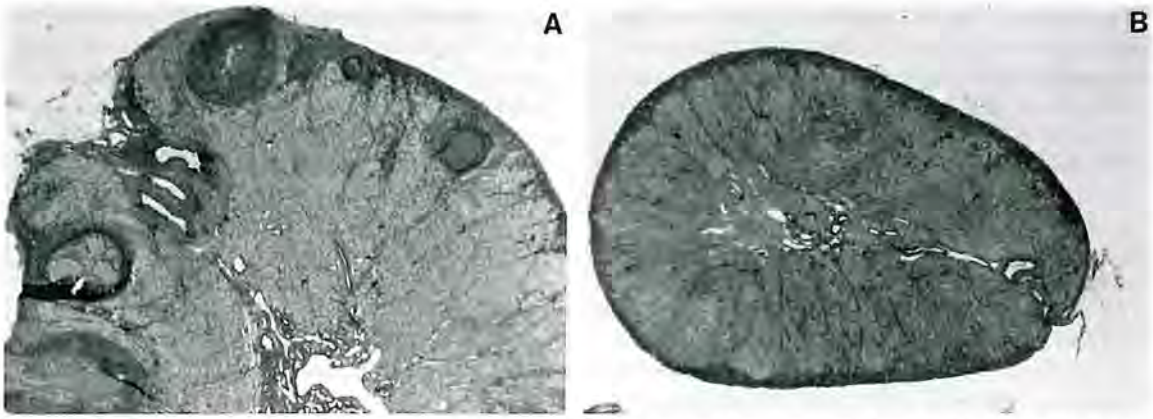

Figure 9–4. Light micrographs illustrating changes in ovaries. **A:** Cross section of mature ovary from a rabbit immunized with adjuvant only. Notice the active cortex, with numerous follicles at various stages of development, and the ovulation site. Periodic acid–Schiff stain; 12×. **B:** Cross section of ovary from rabbit immunized with porcine zona pellucida glycoprotein. Notice the complete absence of growing follicles. Animals so immunized have the same serum hormonal profile as that in long-term castrated animals. Periodic acid–Schiff stain; 12× (From Dunbar BS, Schwoebel E: Fertility studies for the benefit of animals and human beings: Development of improved sterilization and contraceptive methods. J Am Vet Med Assoc 193;1165–1170, 1988, with permission.)

populations, and might also have applicability for dogs.[87] Through use of recombinant DNA, ZP proteins have been isolated and subcloned into a plasmid vector designed to express in an attenuated *Salmonella* organism. Obviously, such sterilants/contraceptives would have to be carefully administered to avoid the inadvertent sterilization of nontargeted populations of animals (or humans).

A secretory glycoprotein from canine oviductal epithelial cells recently has been isolated.[88] This protein is associated with the ZP and may facilitate capacitation or adhesion of spermatozoa. No studies regarding induction of antibodies against this protein have been reported to date.

GnRH is a decapeptide and so must be conjugated with a larger protein, such as albumin, to be antigenic. Beagles injected with GnRH bound to a large protein three times 4 weeks apart developed moderate anti-GnRH antibody titers.[89] Three of eight bitches cycled, and two of those three had serum progesterone concentrations indicative of ovulation.[89]

Immunization with ovine pituitary gonadotropins caused delayed puberty in bitches in one study, presumably via stimulation of formation of antibodies against LH.[90] Immunization of dogs with human chorionic gonadotropin, which has LH activity, stimulated formation of antibodies but did not induce sterility because the antibodies were not cross-reactive with canine gonadotropins.[90] High antibody titers against constantly produced molecules such as GnRH and LH may cause formation of antigen-antibody complexes that may cause glomerulonephritis if deposited in renal glomeruli.[90] Similarly, development of nonspecific antibodies to LH, a glycoprotein that has an α subunit identical to that of thyrotropin-releasing hormone, may precipitate development of iatrogenic hypothyroidism.[90] Immunization with endogenous hypothalamic or pituitary hormones has not proven to be an effective form of contraception or sterilization in dogs.

Termination of Canine Pregnancy

Pregnancy termination, or mismating, may be requested of veterinarians for bitches bred to undesirable males of their own or other breeds, or bred when too young or too old. Occasionally, pregnancy termination is indicated in pregnant bitches with unrelated serious illness or disease. The bitch should be confirmed pregnant prior to initiation of pregnancy termination treatment. In two surveys, only 30 of 48 (62 per cent)[91] and 35 of 50 (70 per cent)[92] bitches presented for pregnancy termination were actually pregnant. Questions to be asked of the owner include (1) Was a breeding witnessed? and (2) At what stage is the bitch in her estrous cycle?

Many bitches present for pregnancy termination after roaming free for a time in estrus, with no breeding witnessed. Although some place great emphasis on the greater likelihood

of pregnancy in bitches witnessed to have been bred with a copulatory lock (tie), the copulatory lock need not occur for a breeding to be successful. Behavioral estrus is not well correlated with the bitch's fertile period, and fertile matings may occur from 5 days before to 5 days after ovulation.[93] However, bitches in early proestrus or in diestrus are less likely to have conceived than those known to be in late proestrus or estrus.

Physical examination of the bitch should be performed as soon after the unplanned breeding as possible. Collection of a vaginal cytology specimen and measurement of serum progesterone concentration allow assessment of stage of the cycle and fertility of the bitch (see Chapter 4). In one study, spermatozoa heads and occasional intact spermatozoa were seen in about 65 per cent of vaginal cytology specimens collected within the first 24 hours after known breedings, and in about 50 per cent of vaginal cytology specimens collected 48 hours after natural service.[94] A technique has been described that enhances the chance of recovery of spermatozoa from the vaginas of bred bitches.[95] A cotton-tipped applicator is inserted into the vagina as for collection of a vaginal cytology specimen (see Chapter 3). After 1 minute, the swab is withdrawn and placed in a tube containing 0.4 ml physiologic saline for 10 minutes, with occasional gentle agitation of the tube. The swab is squeezed against the side of the tube and removed. The saline is centrifuged and the pellet examined under 400× magnification for spermatozoa. With this technique, spermatozoa were identified in 100 per cent of specimens up to 24 hours, and in 75 per cent of specimens up to 48 hours after mating ($n = 16$).[95]

After a successful breeding, fertilization occurs in the uterine tube. The zygote develops to a morula and then a blastocyst in the uterine tube. The uterotubal junction opens about 8 days after ovulation, and the blastocyst hatches and implants into the endometrium 16 to 17 days after ovulation.[93] Progesterone is required throughout pregnancy. In the bitch all progesterone is secreted from the CL on the ovary; ovariectomy at any stage of gestation causes resorption or abortion of fetuses unless the bitch is supported with exogenous progesterone.[96,97] The CL are autonomous early in gestation and rely on two luteotropic hormones, LH and prolactin, after midgestation.[98–100] Hypophysectomy on days 4 or 10 of pregnancy did not cause a prolonged decline in serum progesterone concentrations, but after hypophysectomy on days 18 to 50 of pregnancy,[98,99] or administration of equine anti-LH serum on day 42 of pregnancy,[100] serum progesterone concentrations declined to basal levels within 1 to 4 days and remained low. Similarly, treatment with the prolactin inhibitor bromocriptine on days 8 to 22 of pregnancy did not cause a prolonged decline in serum progesterone concentrations, whereas treatment after day 42 of pregnancy caused a permanent, significant fall in serum progesterone concentrations.[100]

The surgical treatment best described for pregnancy termination in dogs is OHE. Abortifacients described include drugs that prevent movement of the ova within the uterine tubes, agents that cause fetal death, drugs that cause lysis of the CL either directly or by suppressing luteotropic agents in late gestation, and drugs that block progesterone receptors or production.

Ovariohysterectomy

Ovariohysterectomy terminates pregnancy in the dog by physical removal of the ovaries and gravid uterus. The primary advantage of this technique is its effectiveness. This is the preferred technique for bitches with no need for future reproduction. Because of its irreversibility, OHE may be contraindicated in valuable breeding bitches. Hysterotomy with removal of fetal tissues can be performed instead of complete OHE, but effect on subsequent fertility is unknown, and less invasive options for pregnancy termination are available.[101] Although it is generally considered that anesthesia and surgery are prolonged in OHE of pregnant bitches, no postoperative complications were reported in five pregnant bitches ovariohysterectomized in one study.[12] Short- and long-term complications of OHE have been described (see Ovariectomy and Ovariohysterectomy above).

Estrogen

Estrogen has been recommended in the veterinary literature as a "mis-mate shot," to be given soon after the observation of an undesired mating in order to prevent pregnancy. In humans, estrogen has been administered to rape victims as a "morning after pill."[102,103] Estrogen acts as a contraceptive and abortifacient. Movement of ova through the uterine tubes is impaired by estrogens, perhaps by

alteration of the estrogen:progesterone ratio after ovulation.[102,103] Degeneration of the ova and abnormalities of implantation also are seen after estrogen administration in pregnant female dogs and women.[94,102,103]

Types of estrogen described for pregnancy termination in dogs include crystalline estradiol in Silastic implants,[104] oral DES,[94] and injectable estradiol benzoate[105] and estradiol cypionate.[94] In a group of 358 bitches treated with estradiol benzoate (0.01 mg/kg IM or SC on days 3, 5, and sometimes 7 after mating), the pregnancy rate was 3.4 per cent.[105]

Estradiol cypionate is the most effective estrogenic compound described for pregnancy termination in the dog. In one report, groups of four bitches each were treated with single IM injections of estradiol cypionate, at doses of either 22 or 44 μg/kg, in late proestrus, on day 4 of behavioral estrus, or on day 2 of cytologic diestrus (Table 9–6). Bitches were bred at each cycle, and pregnancy determined. The drug was most effective at the higher dose and when administered in estrus or diestrus.[94] Oral DES administration did not prevent pregnancy (Table 9–6).

After ovulation, canine oocytes remain in the uterine tubes, susceptible to the effect of estrogen, until 1 to 3 days after the onset of cytologic diestrus.[106,107] To be used to best effect, therefore, estradiol cypionate must be administered after ovulation but before the dog progresses well into diestrus. The belief that estradiol cypionate must be administered for pregnancy termination within a day after breeding has no scientific basis.

Side effects of estrogen may be severe and may make use of estrogen for pregnancy termination counterproductive in valuable breeding bitches. One reported side effect is bone marrow suppression, with subsequent aplastic anemia, leukopenia, and thrombocytopenia, which may be clinically evident as petechial or ecchymotic hemorrhages or bleeding into body cavities.[13,108,109] Uterine diseases, described as endometritis, cystic endometrial hyperplasia, and/or pyometra, also have been described.[13,94,110,111] In surveys of 400 and 164 dogs with uterine disease, 25 and 56 per cent, respectively, had been treated with an estrogenic compound for pregnancy termination within the previous 6 months.[110,111] In the study using estradiol cypionate reported in Table 9–6, one of four dogs in each group treated with estradiol cypionate in diestrus developed pyometra.[94] The pathogenesis of cystic endometrial hyperplasia–pyometra is described in Chapter 11.

Tamoxifen citrate is a compound with estrogenic activity in dogs. It acts as a contraceptive and abortifacient, by altering transit time of ova within the uterine tubes and interfering with implantation. Bitches treated with 1 mg/kg per os twice daily for 10 days beginning in late proestrus, on day 4 of behavioral estrus, or on day 2 of cytologic diestrus, however, maintained pregnancy.[112] Two of four bitches treated with the same dose beginning on day 15 of diestrus resorbed their pups, and two of four bitches treated beginning day 30 of diestrus aborted all pups.[112] Of the 20 bitches treated with tamoxifen citrate, 4 (20 per cent) developed ovarian cysts and 5 (25 per cent) developed uterine disease.[112] The authors do not recommend use of tamoxifen for this purpose.

Neither estradiol cypionate nor tamoxifen citrate are approved for use as pregnancy ter-

Table 9–6. Efficacy of Estrogens to Terminate Canine Pregnancy

Times of Drug Administration	Drug Dosage	No. of Dogs	Pregnancy Rate
Proestrus	Diethylstilbestrol: 75 μg/kg daily for 7 days	4	100%
Estrus		4	100%
Diestrus (day 2)		4	75%
Proestrus	Estradiol cypionate: 22 μg/kg IM once	4	50%
Estrus		4	50%
Diestrus (day 2)		4	25%*
Proestrus	Estradiol cypionate: 44 μg/kg IM once	4	100%
Estrus		4	0%
Diestrus (day 2)		4	0%*

* One pyometra in each group.
From Bowen RA, Olson PN, Behrendt MD, et al: Efficacy and toxicity of estrogens commonly used to terminate canine pregnancy. J Am Vet Med Assoc 186:783–788, 1985, with permission.

mination drugs in the dog in the United States. Safe and effective treatment with estradiol cypionate requires verification that the bitch has ovulated and is not yet in diestrus. The authors do not recommend use of estradiol for pregnancy prevention/termination in the dog because of its side effects.

Prostaglandins

PROSTAGLANDIN $F_{2\alpha}$

Prostaglandins are modified long-chain fatty acids found in most body tissues.[113] The specific prostaglandin of interest for canine pregnancy termination is prostaglandin $F_{2\alpha}$, ($PGF_{2\alpha}$) which is luteolytic and which causes contraction of smooth muscle. The native acid of $PGF_{2\alpha}$ is waxy and difficult to administer; the compound commonly used is the tham salt, dinoprost tromethamine (Lutalyse; Pharmacia & Upjohn, Peapack, NJ).[113]

$PGF_{2\alpha}$ acts as an abortifacient in the dog by lysing the CL, with subsequent decline in serum progesterone concentrations, and by causing contraction of uterine smooth muscle, physically breaking down the pregnancy. The CL are resistant to the luteolytic effect of $PGF_{2\alpha}$ in early diestrus. Dogs treated with high doses of $PGF_{2\alpha}$ (250 μg/kg twice daily SC for 4 days) on days 1 to 5 of diestrus maintained serum progesterone concentrations similar to untreated control bitches, and maintained pregnancy.[114] Four of five bitches treated with the same dose of $PGF_{2\alpha}$ on days 5 to 8 of diestrus after multiple matings had a decline in serum progesterone concentrations to baseline levels, and did not whelp.[115] Serum progesterone concentrations must be less than 2 ng/ml for at least 48 hours to reliably induce pregnancy termination.[91,113,114,116,117] The occasional bitch will maintain pregnancy despite a decline in serum progesterone concentrations,[118] and the occasional bitch will not respond to a 4 to 5 day treatment with $PGF_{2\alpha}$, maintaining high serum progesterone concentrations, although refractory bitches usually respond to repeated, or continued treatment (Fig. 9–5).[115] There is a report of one bitch treated with $PGF_{2\alpha}$ early in pregnancy that aborted three fetuses and carried two pups to term.[113] It is recommended that serum progesterone concentrations be measured in all bitches at the end of treatment to document a fall in serum progesterone, and that the bitch be reevaluated to ensure termination of pregnancy 1 to 2 weeks after treatment is completed; treatment may be repeated if necessary. Ultrasound is the preferred diagnostic technique to assess completion of pregnancy loss; palpation is unreliable for accurate monitoring of pregnancy loss after $PGF_{2\alpha}$ treatment.[91,119]

Depending on the stage of gestation in which treatment is begun, abortion may or may not be clinically evident. In general, treatment with $PGF_{2\alpha}$ prior to days 35 to 40 after breeding will cause resorption with few clinical signs, if any, whereas treatment after day 40 of pregnancy will cause overt vaginal discharge and abortion.[120,121] Signs associated with abortion include mucoid to serosanguineous vaginal discharge, passage of fetal tissues or fetuses, abdominal contractions, restlessness, trembling and muscle fasciculation, and nesting.[91,119] Aborted fetuses may be alive.

Success of treatment with $PGF_{2\alpha}$ is dependent on dose used, duration of treatment, and stage of gestation in which treatment is instituted (Table 9–7).[91,113–116,118,119,121–125] In general, higher doses are required in early to midgestation. The advantage of treatment early in pregnancy is that puppies will be resorbed. Late in gestation, lower doses of $PGF_{2\alpha}$ may be used, minimizing side effects associated with the drug, and overt abortion will occur. Bitches treated very late in gestation will abort premature live pups. It has been demonstrated that a dose of 200 μg/kg of $PGF_{2\alpha}$ will not cause stronger uterine contractions than will a dose of 50 μg/kg when administered IM, but that the duration of effect is greater at the higher dose.[126] The median lethal dosage of $PGF_{2\alpha}$ is 5.13 mg/kg.[127]

The side effects of treatment with $PGF_{2\alpha}$ are primarily referable to its action on smooth muscle. Reported side effects and their frequencies include hyperpnea (78 per cent, n = 18), hypersalivation (73 per cent, n = 18), vomiting (67 per cent, n = 18), defecation (56 per cent, n = 18), micturition (17 per cent, n = 18), tachycardia (17 per cent, n = 18), lactation (50 per cent, n = 6), nesting, and transient hypothermia.[91,113,114,116,120,123,128–130] The severity of side effects is dose dependent, and severity, frequency, and duration of side effects decrease over the course of treatment.[113,123,126,128,131] Side effects begin within 5 to 15 minutes of injection and subside within 60 minutes.[113–115,123,129] Frequency and severity of side effects may be decreased by concurrent treatment with atropine (500 mg/kg IM)[132] or chlorpromazine (0.25 mg/kg IM).[125] Concurrent treatment with $PGF_{2\alpha}$ and other abortifacients may allow use of lower doses of $PGF_{2\alpha}$, and a subsequent de-

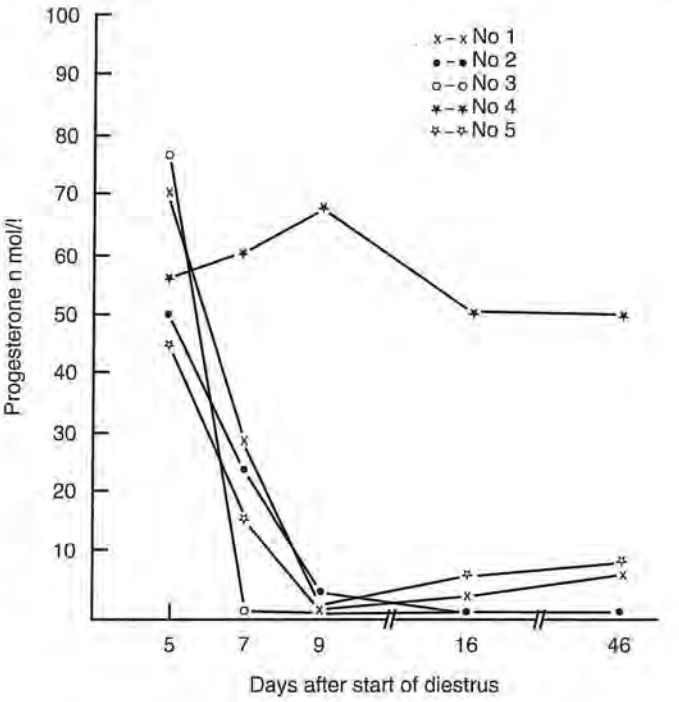

Figure 9–5. Plasma progesterone concentrations of five bitches treated with prostaglandin $F_{2\alpha}$ (250 μg/kg SC twice daily for 4 days) from days 5 to 8 of diestrus. Note the lack of response in dog number 4. (From Oettlé EE, Bertschinger HJ, Botha AE, et al: Luteolysis in early diestrous beagle bitches. Theriogenology 29:757–763, 1988, with permission.)

crease in side effects may be seen (Table 9–7).[115,119,125]

Whelpings of bitches after failed attempts with $PGF_{2\alpha}$ for pregnancy termination are reportedly normal, as are the pups that are born.[113,115] Bitches are reported to cycle normally and exhibit normal fertility after pregnancy termination with $PGF_{2\alpha}$.[91,113,119]

Interestrous intervals may be decreased in bitches receiving $PGF_{2\alpha}$ in mid-diestrus, with the shortening of the interestrous interval comparable to the length of diestrus obviated by premature luteolysis. An average decrease in interestrous interval length of 71 days (range 40 to 111.5 days) has been reported.[117,128,130,133] $PGF_{2\alpha}$ is not approved for pregnancy termination or control of the estrous cycle by shortening of the interestrous interval in dogs in the United States. At time of this writing, the authors recommend extralabel use of this product as the best available medical treatment for terminating pregnancy in this species. Recommended dose depends on stage of pregnancy at which treatment is initiated (Table 9–7), and treatment should be continued until pregnancy termination is completed.

In general, it is recommended that bitches known or suspected to have been bred be examined for pregnancy using ultrasonography at 21 to 24 days after suspected ovulation and one week later if not observed pregnant. This prevents nonpregnant bitches from being treated unnecessarily. If pregnant, treatment with $PGF_{2\alpha}$ should be initiated immediately so as to promote resorption of fetuses rather than abortion. Recommended protocol is 100 μg/kg SC every 8 hours for two days, followed by 200 μg/kg SC every 8 hours until pregnancy is terminated. Treated bitches are monitored ultrasonographically and with serum progesterone concentrations twice weekly starting one week after initiation of treatment.

PROSTAGLANDIN ANALOGS

Prostaglandin analogs that have been described for pregnancy termination in the dog include fluprostenol, cloprostenol, and α-prostol (Table 9–8).[63,92,113,134,135] Fluprostenol has limited efficacy with the doses and routes investigated.[113,134] α-Prostol, in combination with a prolactin inhibitor (cabergoline), was completely effective in one study.[135]

Cloprostenol is the prostaglandin analog best described and is the most efficacious of the analogs described (Table 9–8). The minimal effective dose is 2.5 μg/kg SC administered three times at 48-hour intervals.[136] Side effects are as described for $PGF_{2\alpha}$; no side effects were noted when cloprostenol was given at a low dose (1 μg/kg) in combination with cabergoline.[135] Side effects may be minimized by pre-

Table 9-7. Efficacy of Various Regimens with Prostaglandin $F_{2\alpha}$ for Pregnancy Termination in the Dog

Dose*(SC Unless Specified)	Duration of Treatment (d)	Days of Pregnancy When Treatment Started	Efficacy	Days after Start of Treatment Abortion Seen	Side Effects	Study
20 μg/kg TID	—	20–21	5/5	—	Fetal resorption associated with endometritia	Lange et al.[122]
20 μg/kg TID	4	5–8 of diestrus	3/5	—	—	Lange et al.[122]
20 μg/kg TID or 30 μg/kg BID	3	25–58	4/7	—	Emesis, hypersalivation, hyperpnea, hypothermia	Concannon and Hansel[116]
30 μg/kg BID	3–9	21–23	1/1	—	—	Oettlé[125]
30 μg/kg BID	3–9	40–42	1/1	—	—	Oettlé[123]
30 μg/kg BID followed by 250 μg/kg BID	3–9 followed by 4 at higher dose	27–30; 49–52 at higher dose	1/1	—	—	Oettlé[123]
50 μg/kg BID IM	4–10	26–55	5/6	2–9	Emesis, hypersalivation, tachycardia	Lein et al.[113]
50 μg/kg BID, then 62.5 μg/kg SID, then 100 μg/kg SID–BID, then 50–100 μg/kg SID	2, then 3, then 4, then 3–7	27–41	6/6	7–11	Emesis, hypersalivation, lactation, nesting	Lein et al.[113]
50–250 μg/kg BID	4–8	21–55	28/28	—	—	Lein et al.[113]
50 μg/kg TID	7	5–11 days of diestrus	5/5	—	—	Lange et al.[122]
60 μg/kg BID	2	6–7	0/1	—	—	Oettlé[123]
125 μg/kg BID	6	43–45	4/4	—	—	Wichtel et al.[124]
100 μg/kg TID or 100 μg/kg TID followed by 200 μg/kg TID or 250 μg/kg TID	—	30–35	18/18	—	Hyperpnea, hypersalivation, emesis, defecation, urination, tachycardia	Feldman et al.[91]
150–200 μg/kg BID	4	8–19 days of diestrus	11/12	—	—	Romagnoli et al.[118]
250 μg/kg SID	6	22–27	1/1	—	—	Oettlé[123]
250 μg/kg BID	4	> 5 days of diestrus	6/6	3–5	Emesis, hypersalivation, diarrhea	Johnston[121]
250 μg/kg BID	4	5–8 days of diestrus	4/5	—	Emesis, diarrhea	Oettlé et al.[115]
250 μg/kg BID	4	1–5 days of diestrus	0/5	—	—	Paradis et al.[114]
250 μg/kg BID	4	31–35 days of diestrus	5/5	3–5	Emesis, hypersalivation, hypothermia	Paradis et al.[114]
100 μg/kg TID with 1–3 μg/kg misoprostol (PGE) intravaginally	—	30–43	9/9	2.5–3.5	Emesis, hypersalivation, hyperpnea	Davidson[119]
100 μg/kg with bromocriptine (10 μg/kg TID PO)	3–7	30–45	—	3–5	Emesis	Purswell[125]
250 μg/kg with bromocriptine (20 μg/kg SID PO)	4	5–8 days of diestrus	4/5	—	Emesis, diarrhea	Oettlé et al.[115]
250 μg/kg with dexamethasone (0.1 mg/kg)	4	5–8 days of diestrus	4/5	—	Emesis, diarrhea	Oettlé et al.[115]

* SC, subcutaneously; TID, three times a day; BID, twice daily; IM, intramuscularly; SID, once daily; PGE, prostaglandin E; PO, per os.

Table 9–8. Efficacy of Various Regimens with Prostaglandin Analogs for Pregnancy Termination in the Dog

Dose*	Duration of Treatment (d)	Days of Pregnancy When Treatment Started	Efficacy	Days after Start of Treatment Abortion Seen	Side Effects	Study
Cloprostenol						
10 μg/kg intravaginally	—	27–28	4/5	—	—	Jackson et al.[134]
10 μg/kg (aqueous form)	—	14	1/3	—	Severe emesis, diarrhea	Jackson et al.[134]
10 μg/kg (aqueous form)	—	28	1/2	—	Severe emesis, diarrhea	Jackson et al.[134]
1 μg/kg SID with cabergoline (1.65 μg/kg SC SID)	5	>25	5/5	—	None	Onclin et al.[135]
2.5 μg/kg IM or SC BID	4	30–40	—	—	—	Concannon[63]
2.5 μg/kg SC 3 times at 48-h intervals	—	—	16/16	6	Emesis, hypersalivation, anxiety, hypothermia	Fieni et al.[92]
10 μg/kg	—	14	1/3	—	Emesis, diarrhea	Jackson et al.[134]
10 μg/kg	—	28	5/5	—	Emesis, diarrhea	Jackson et al.[134]
20 μg/kg	—	14	1/3	—	Emesis, diarrhea	Jackson et al.[134]
40 μg/kg	—	14	2/3	—	Severe emesis, diarrhea	Jackson et al.[134]
2.5 μg/kg SC with cabergoline (1.65 μg/kg SC SID)	5	>25	5/5	—	None	Onclin et al.[135]
Fluprostenol						
25 μg/kg intravaginally	—	25	0/1	—	—	Jackson et al.[134]
2.5 μg/kg BID	2	~40	0/1	—	—	Paradis et al.[113]
10 mg/kg	—	25–26	1/2	—	—	Jackson et al.[134]
12.5 mg/kg	—	4	0/2	—	—	Jackson et al.[134]
12.5 mg/kg	—	7	0/2	—	—	Jackson et al.[134]
12.5 mg/kg	—	14	0/2	—	—	Jackson et al.[134]
12.5 mg/kg	—	35	1/1	—	—	Jackson et al.[134]
15 mg/kg	—	14	1/4	—	—	Jackson et al.[134]
20 mg/kg	—	28	1/1	—	—	Jackson et al.[134]
25 mg/kg	—	25–26	3/3	—	Emesis, diarrhea	Jackson et al.[134]
α-Prostol						
20 mg/kg SC SID with cabergoline (1.65 mg/kg SC SID)	5	32 days after LH peak	5/5	—	Hypersalivation, prostration, emesis, diarrhea, scratching at injection site	Onclin et al.[135]

*SID, once daily, SC, subcutaneously; IM, intramuscularly; BID, twice daily.

treatment 15 minutes before administration of cloprostenol with atropine (0.025 mg/kg), prifinium bromide (antispasmodic; 0.1 ml/kg), and metopimazine (antiemetic; 0.5 mg/kg); side effects were decreased in 58.2 per cent of 67 bitches receiving this combination of drugs before treatment with 2.5 μg/kg cloprostenol.[136] Prostaglandin analogs are not approved for use in dogs in the United States.

Glucocorticoids

Dexamethasone is the glucocorticoid best described for pregnancy termination in dogs. The mode of action as an abortifacient is unknown. One author hypothesized that administration of exogenous glucocorticoids may cause release of endogenous $PGF_{2\alpha}$,[65] but serum progesterone concentrations have not been demonstrated to decline in all dogs that resorbed or aborted puppies after treatment with dexamethasone.[137] Fetal death has been demonstrated to occur within 5 to 13 days after treatment with dexamethasone is instituted, with expulsion of fetuses 7 to 15 days after start of treatment.[138]

Injectable dexamethasone (5 mg twice daily IM for 10 days)[137] and oral dexamethasone (0.1 to 0.2 mg/kg twice to three times daily at a decreasing dose for 5 to 10 days) have been reported for pregnancy termination in dogs.[138,139] Reported treatment failures have occurred in bitches treated for short duration on days 30 to 35 of pregnancy.[138] Efficacy is best in bitches treated for up to 10 days after day 35 of gestation.[138,139] Concurrent treatment with dexamethasone (0.1 mg/kg once daily IM for 4 days) with $PGF_{2\alpha}$ (250 μg/kg twice daily SC for 4 days) was not more effective than treatment with $PGF_{2\alpha}$ alone.[115] Although dexamethasone is reported to be teratogenic in laboratory animals, puppies born live to bitches that are nonresponsive to dexamethasone as an abortifacient usually are normal and show no abnormalities as adults (M. Wanke, personal communication, February 15, 1999).

The primary side effect of dexamethasone treatment for pregnancy termination in dogs is polyuria/polydipsia (PU/PD), reported to occur in all dogs treated.[138,139] PU/PD resolves after withdrawal of the drug. Vaginal discharge and lethargy have been described at the time of abortion.[139]

Prolactin Inhibitors

Prolactin secretion from the pituitary is stimulated by serotonin and inhibited by dopamine. Treatment with dopamine agonists, which stimulate dopamine release and, therefore, increase dopamine's inhibitory effect on prolactin secretion, decreases serum prolactin concentrations in dogs. Prolactin is a luteotropic agent; inhibition of prolactin secretion after midgestation causes luteolysis, decreased progesterone concentrations in serum, and pregnancy loss.[100] Two ergot alkaloids that act as dopamine agonists have been described, bromocriptine and cabergoline (Table 9–9).[63,124,125,135,140–143] Both drugs must be given after day 30 of pregnancy to be effective as abortifacients.[13]

Bromocriptine is stable as a tablet, but tablets are only available at a dose of 2.5 mg (Parlodel; Novartis Pharmaceuticals Corporation, East Hanover, NJ). These tablets can be crushed and dissolved in water, but the resulting solution is unstable and must be protected from light and heat.[125] Duration of efficacy of the liquid formulation is unknown.

Bromocriptine is reported to be effective at doses from 20 to 100 μg/kg per os once to twice daily for 4 to 7 days.[63,140] Concurrent treatment with $PGF_{2\alpha}$ has been described[125,135]; treatment with both allows use of low doses (bromocriptine 10 μg/kg per os three times daily until abortion is clinically evident; $PGF_{2\alpha}$ 100 μg/kg SC three times daily until 2 days after abortion begins), which minimizes side effects.[125]

Side effects of bromocriptine are vomiting (60 per cent, $n = 5$) and anorexia (20 per cent, $n = 5$).[63,125,140] Side effects may be minimized by pretreatment with the antiemetic chlorpromazine at a dose of 0.25 mg/kg IM.[125] Bromocriptine is not approved for use in dogs in the United States.

Cabergoline, which decreases serum prolactin concentrations, is reported effective as an abortifacient at doses of 1.65 to 5 μg/kg once daily per os or SC for 5 days (Table 9–9).[141–143] Concurrent treatment with the prostaglandin analogs cloprostenol and α-prostol has been reported.[135] Side effects are negligible. In one survey of 11 dogs, all treated bitches went on to cycle normally, and all those that were bred had normal pregnancies after treatment with cabergoline.[142] Cabergoline is not commercially available in the United States at this time.

Miscellaneous Pregnancy Termination Agents

EPOSTANE

Epostane acts by inhibiting the 3β-hydroxysteroid dehydrogenase/$\Delta^{4,5}$ isomerase enzyme

Table 9–9. Efficacy of Various Regimens with Prolactin Inhibitors for Pregnancy Termination in the Dog

Dose*	Duration of Treatment (d)	Days of Pregnancy When Treatment Started	Efficacy	Days after Start of Treatment Abortion Seen	Side Effects	Study
Bromocriptine						
20–30 mg/kg SID	4	≥42	5/5	3–5	Emesis, anorexia	Conley and Evans[140]
62.5 mg/kg BID	≤6	43–45	2/4	—	Emesis, hypersalivation	Wichtel et al.[124]
100 mg/kg SID–BID	4–7	> 30	—	—	Emesis, anorexia	Concannon[63]
10 mg/kg with $PGF_{2\alpha}$ (100 mg/kg) TID	3–7	>30–45	—	3–5	Emesis	Purswell
Cabergoline						
1.65 mg/kg SID SC	5	≥40	3/5	—	Vaginal discharge	Onclin et al.[142]
5 mg/kg SID PO	5	≥49	8/8	3–5	—	Jöchle et al.[141]
5 mg/kg SID	28	1	0/6	—	—	Post et al.[143]
5 mg/kg SID	5	~30	0/6	—	—	Post et al.[143]
5 mg/kg SID	5	42–49	8/8	3–5	None	Post et al.[143]
15 mg/kg SID	5	~30	0/6	—	—	Post et al.[143]
1.65 mg/kg SC SID with cloprostenol or α-prostol	5	25–32 days after LH peak	15/15	—	As for prostaglandins	Onclin et al.[135]

* SID, once daily; BID, twice daily; TID, three times a day; SC, subcutaneously; PO, per os.

system, which catalyzes the formation of progesterone from pregnenolone.[63,144] The drug is effective as an abortifacient any time after diestrus begins.[63] The reported minimum effective dose is 2.5 to 5.0 mg/kg,[144] but efficacy improves as the dose nears 10 mg/kg (25 per cent pregnancy rate, $n = 12$).[145] No side effects are noted at doses up to 10 mg/kg; at higher doses, sterile abscesses may form at the site of injection.[145] Vomiting also has been reported as a side effect of epostane. Dogs treated with epostane showed a normal return to estrus and normal fertility.[145] Research suggests that, although epostane is an effective abortifacient, reported incidence and severity of side effects in experimental dogs preclude its use as a humane alternative to other forms of pregnancy termination in dogs.

MIFEPRISTONE

Mifepristone, also called RU486, is used as a "morning after" pill along with prostaglandin injections for termination of pregnancy in women. Mifepristone competitively binds progesterone and glucocorticoid receptors.[146] In dogs, treatment with 2.5 mg/kg per os twice daily for 4.5 days beginning on day 32 of pregnancy caused abortion within 3 to 5 days.[147] Single treatments with higher doses (10 to 20 mg/kg) have been reported to successfully terminate pregnancy as early as day 11 to 26, however, pregnancy status before treatment was not well described in all cases and length of time from treatment to abortion was prolonged, with two of five dogs aborting 11 days after treatment.[90,148] No side effects have been reported with mifepristone.[147] Despite binding of glucocorticoid receptors, plasma ACTH and cortisol concentrations are not increased after treatment with mifepristone at doses of less than 20 mg/kg once daily per os for 10 days.[146] One bitch was successfully rebred after treatment with mifepristone; no other animals have been described.[149] This drug or a related drug, is expected by the authors, to become the treatment of choice for pregnancy termination in the dog if it becomes commercially available in the United States.

GnRH ANTAGONISTS

GnRH antagonists act by decreasing concentrations of circulating gonadotropins, including luteotropic LH, causing luteolysis, decline in serum progesterone concentrations, and

subsequent pregnancy loss. These drugs therefore are not effective in dogs early in diestrus when the CL is not dependent on support from luteotropic agents. GnRH antagonists are effective after day 20 of diestrus, and have not been shown to be effective earlier, even at very high doses.[150] Detirelix has been reported to be effective as an abortifacient in dogs at a dose of 2 mg/kg; its use is currently limited by lack of commercial availability and expense.[65,150]

NONHORMONAL COMPOUNDS

The nonhormonal triazole derivatives, the phenyltriazole isoindoles and isoquinolones, induce degeneration of the canine conceptus at the time of implantation.[89] Treatment with these drugs, which are very potent and slowly released from the injection site, is most effective from day 15 to 20 after mating, near the time of implantation.[151,152] The mechanism of action of these drugs is unknown. The most potent, lotrifen (L12717) was 80 per cent effective as an abortifacient with a single dose of 0.5 to 1.0 mg/kg about day 20 of pregnancy ($n = 5$).[152] Reported side effects include anorexia, weight loss, vomiting, and blood loss from the gastrointestinal tract.[151] These drugs are not commercially available in the United States.

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Chapter 10

Disorders of the Canine Ovary

Congenital Abnormalities

The embryonal "indifferent" gonad of the dog differentiates into ovarian tissue in the absence of a functional Y chromosome. Cortical sex cords formed in the indifferent gonad split into clusters near the surface, with each cluster surrounding one or more germ cells.[1] Reported congenital abnormalities of the canine ovary include agenesis, ovarian hypoplasia resulting from abnormal chromosome number, and presence of atypical gonads in pseudo- or true hermaphrodites.

Ovarian agenesis—complete absence of one or both ovaries—has been reported to occur in the bitch[2–4]; character of reproductive cycles was not reported in these cases. Ovarian agenesis may be associated with other abnormalities of the reproductive tract, such as uterus unicornus.[3]

Ovarian hypoplasia has been reported in female dogs with abnormal chromosome number. A Doberman pinscher with X chromosomal monosomy (77,XO) presented with stunted growth. Her ovaries were small and consisted of interstitial-type cells and solid epithelial sex cords. Her dam, sire, and littermate were karyotypically normal.[5] An American Eskimo bitch with X chromosomal monosomy (77,XO) presented with persistent proestrus of 8 months' duration. She too had stunted growth and small, fibrous ovaries containing no follicles or corpora lutea. The cause of the apparent persistent reproductive cycling was not defined.[6] An Airedale terrier with X chromosomal trisomy (79,XXX) presented with primary anestrus. The dog's ovaries were inactive and contained large masses of interstitial cells and solid epithelial sex cords. Concentrations of serum luteinizing hormone (LH; 670 ng/ml) and follicle-stimulating hormone (11,210 ng/ml) were markedly elevated compared to those in karyotypically normal intact dogs.[7] None of these dogs had grossly abnormal external genitalia. Definitive diagnosis of ovarian hypoplasia requires assessment of the karyotype and gonadal histology. Female dogs with anomalous numbers of sex chromosomes and abnormal ovaries are usually infertile.

True hermaphrodites have both ovarian and testicular tissue, and may have portions of both male and female tubular reproductive tracts and external genitalia. Fourteen- and 16-month-old cocker spaniels, a German shorthaired pointer and mixed breed dog of unreported age with normal female karyotypes (78,XX) have been described with bilateral ovotestes, normal female tubular reproductive tracts, and enlarged clitorides containing an os penis.[8–11] A 3-year-old, phenotypically female weimeraner with a normal female karyotype has been described with lateral hermaphroditism, in which one gonad was an ovary and the other was a testis. A normal female tubular tract was present, and the dog exhibited irregular estrous cycles.[12] There is one report of a canine true hermaphrodite with an XX karyotype that reproduced successfully.[13] Testicular differentiation in the absence of the Y chromosome requires either presence of multiple cell lines, as in mosaicism and chimerism, or translocation of portions of the Y chromosome onto an autosome or X chromosome during meiosis, and is therefore very uncommon.[8]

Pseudohermaphrodites are animals with gonads that do not match their phenotypic sexual characteristics. They are named by the gender of the gonads, not the secondary sexual characteristics.

Male pseudohermaphrodites are dogs with bilateral testes, which usually are retained, and

female external genitalia or presence of a uterus masculinis. These dogs usually have a normal male karyotype (78,XY). Differentiation of the female tubular tract (müllerian ducts) and external genitalia, and concurrent lack of development of the male tubular tract (wolffian ducts) and external genitalia, occurs when anti-müllerian hormone and testosterone normally produced by the embryonic testicles are either not produced or not recognized because of lack of or abnormal function of receptors for these hormones in target tissues.

Female pseudohermaphrodites are karyotypically normal females (78,XX)[9] with ovaries and male external genitalia. Female pseudohermaphrodites are rare in all species, and usually occur as a result of masculinization of female fetuses with androgens in utero, causing varying degrees of virilization.[9,14,15] Three female greyhound littermates were reported with normal ovaries and female tubular tracts and with prepuces containing a penis; these dogs' dam had received testosterone propionate for estrus prevention prior to gestation.[14] Presumptive diagnosis of female pseudohermaphroditism is by visual inspection to verify abnormal external genitalia. Definitive diagnosis requires histology of gonadal tissue and/or karyotype. Reproductive cycling in these dogs has not been characterized, and androgen exposure may be expected to suppress ovarian function. Abnormalities of the external genitalia generally preclude breeding, making ovariohysterectomy the treatment of choice.

How To Evaluate Karyotype

The karyotype is the chromosomal complement, usually expressed as total number of chromosomes followed by abbreviations for the number of X and Y sex chromosomes present. Normal female dogs, therefore, have a 78,XX karyotype, and normal male dogs have a 78,XY karyotype. Karyotypes usually are determined by culturing lymphocytes from a peripheral venous blood sample or fibroblasts from a skin biopsy. Blood (≥ 5 ml) is collected, into a tube containing heparin (green-topped tube). For skin biopsies, a nonhaired region is selected and cleansed as in preparation for surgery. A punch biopsy is used to collect the sample, which is placed in a red-topped evacuated glass tube. The tube is labeled with identification of the animal and date of sample collection. The sample should be maintained at room temperature, with avoidance of temperature extremes, and shipped to the laboratory performing the analysis within 24 hours. Samples should be collected and shipped so as to arrive Monday through Thursday. (One laboratory at which karyotype analysis is performed is the University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN; 651-625-8787.)

Lymphocytes or fibroblasts are cultured and then induced to undergo mitosis with mitogens. Cell division is arrested in metaphase of mitosis, and the chromatin within the cells is squashed, fixed, and stained. Photographs are taken of at least 30 individual metaphase spreads, and the chromosomes in individual spreads are rearranged using "cut and paste" computer technology, in homologous pairs for assessment.[16]

Normal dogs have 76 autosomes and two sex chromosomes; normal males have a karyotype of 78,XY and normal females 78,XX. Abnormalities that have been reported in dogs include

Trisomy: presence of an extra member of a monologous pair (e.g., 79,XXX) (Fig. 10–1)
Monosomy: lack of one member of a homologous pair (e.g., 77,XO)
Presence of multiple cell lines (e.g., 78,XX/79,XXY): *mosaic* (presence of more than one cell line, arising from one zygote) or *chimera* (presence of more than one cell line, acquired in utero from a twin)

Figure 10–1. The karyotype from a dog with three x-chromosomes (arrows). (From Johnston SD, Buoen LC, Weber AF, et al: X trisomy in an Airdale bitch with ovarian dysplasia and primary anestrus. Theriogenology 24:597–607, 1985, with permission.)

Translocation: transfer of all or a segment of one chromosome onto a nonhomologous chromosome (this may be apparent if the characteristic banding pattern present on the chromosomes after staining is abnormal)

Polyploidy: presence of more than two multiples of the haploid chromosome number; triploidy reported in the bitch, is 3n = 117,XXX.

Ovarian Cysts

Ovarian cysts are fluid-filled structures with a distinct wall that develop within the ovary. Parovarian cysts are similar in morphology to ovarian cysts, but lie next to the ovary. In a survey of 126 bitches with ovarian disease, 101 had ovarian or parovarian cysts.[17] Cystic ovarian structures that have been described in female dogs include follicular cysts,[17–28] luteal cysts,[17,18,28] germinal cysts,[17,18,21] cystic corpora lutea,[18,29] cystic rete ovarii,[17,18,21] cystic atretic follicles,[18] cystic granulosa cell tumors,[18] and parovarian cysts.[18,20,30]

Follicular Cysts

Follicular cysts are thin-walled structures containing clear, serous fluid.[17] Follicular cysts may be single or multiple; if multiple cysts are present on one ovary, the cysts do not communicate.[17] Ultrasonographically, follicular cysts appear as focal hypoechoic to anechoic structures that may show far enhancement.[31] Normal mature ovarian follicles in dogs average 5 to 8 mm in diameter.[32] Ovarian follicular structures greater than 8 mm in diameter present during proestrus or estrus prior to ovulation, or follicles of any size present during late estrus (postovulation), diestrus, or anestrus, are defined as follicular cysts.

Reported incidence of follicular cystic disease in the dog varies widely. Reports of follicular cysts as a percentage of ovarian cystic disorders have included reports of 3 per cent,[18] 23.5 per cent (n = 34),[21] and 62 per cent (n = 101).[17]

Canine follicular cysts may be single (37.5 to 65 per cent)[17,21,23,26] or multiple (35 to 62.5 per cent).[17,19,21,24,25,30] Multiple follicular cysts may be unilateral or bilateral; incidence of bilateral follicular cysts in one study was reported at 32 per cent.[17] Reported diameters vary from 0.5 to 19 cm,[17,19,23,25–27] with single cysts most often reported at 1 to 1.5 cm and multiple cysts at 10 cm or less in diameter.[17] One large follicular cyst, measuring 32.5 × 23.2 cm and containing 4100 ml of serous fluid, has been reported in the bitch.[27] Follicular cysts contain up to 250 to 750 ml of clear, serous fluid on average.[23,26] In one report, this fluid contained 3.5 g/100 ml protein, 52,400 red blood cells (RBCs)/mm^3, 9200 white blood cells (WBCs)/mm^3, and cellular debris, with pH of 6.5 and specific gravity of 1.024,[26] and variable concentrations of estrogen, progesterone, and testosterone (Table 10–1).[27,33] Follicular cysts are lined primarily with granulosa cells, and remnants of degenerating cumulus oophorus may be present.[17,18] No oocytes are present (Fig. 10–2).[17,25] Rarely follicular cysts contain some luteal cells.[18]

Pathogenesis of follicular cystic disease in the dog ovary is unknown. A 5-year-old intact mixed breed dog and a 2.5-year-old intact golden retriever with follicular cysts had historically received parenteral estrogen for pregnancy termination, and a 12-year-old intact German shepherd had been treated historically with an undefined estrus-suppressing drug years prior to the diagnosis of follicular cystic disease.[24,27,33,34] In one study, ovarian cysts were reported to develop after treatment with tamoxifen for pregnancy termination.[35]

Granulosa cells lining follicular cysts may be productive, secreting estrogen, with subsequent estrogen-mediated effects on the dog's reproductive tract and extrareproductive systems. Reported serum concentrations of estrogen in dogs with follicular cysts vary from 3 to 143 pg/ml.[19,20,25,33,36] Concurrent diseases reported in dogs with follicular cysts include cystic endometrial hyperplasia–pyometra complex, reported in 57 per cent of dogs with follicular cysts[17,19,20,25,26,28]; mammary neoplasia[22]; ovarian neoplasia[28]; uterine neoplasia[22]; skin changes characteristic of hyperestrogenism, including bilaterally symmetrical alopecia of the trunk, lichenification, and hyperkeratosis[19,20,22]; and prostate disease (squamous metaplasia and prostatic abscessation) in a female pseudohermaphrodite.[20]

Mean age at diagnosis of bitches with follicular cysts is 8.0 years (n = 70).[17,19,20,22–27] Reported age range at time of diagnosis is 1 to 16 years.[17,19,20,22–27] Dogs have been reported to develop clinical signs of single follicular cysts at a younger age than those with multiple follicular cysts; reported mean ages are 8.7 years and 10.5 years, respectively.[17] Although no breed predisposition has been identified, the majority of dogs reported with follicular cysts are of large breeds. The most commonly reported breeds are the malamute, German shepherd, golden retriever, Bouvier des Flan-

Table 10–1. Characteristics of Bitches in Persistent Estrus with Ovarian Cysts

Animal No. and Breed	Age	Pertinent History	Treatment*	Results	Concentrations of Hormones In Serum			Concentrations of Hormones in Follicular Fluid			Cyst(s) Observed with Ultrasonography
					Estradiol (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)	
1. Malamute	7 mo	In estrus 7 wk	GnRH × 2	Did not resolve	6	1.0	0.8	48,830	2	12	Yes
			GnRH × 3	Resolved + surgical drainage							
2. Husky X	1.5 yr	In estrus 8 wk	GnRH × 1	Resolved but recurred next cycle, then spayed	24	1.9	0.13	1379	1133	1.9	Yes
3. Malamute	10 mo	In estrus 4 mo	hCG × 1	Did not resolve	38	1.0	0.01	—	—	—	Yes
			GnRH × 2	Resolved							
4. Golden retriever	15 mo	In estrus 4 mo	hCG × 3	Did not resolve	16	6.0	2.8	—	—	—	Yes
			GnRH × 1	Resolved							
5. Yorkshire terrier	3.5 yr	In estrus 3 mo	GnRH × 2	Did not resolve	9	0.8	0.39	—	—	—	Yes
			hCG × 1	Did not resolve							
			GnRH × 3	Resolved but pyometra occurred and bitch was spayed							
6. Chesapeake retriever	16 mo	In estrus 4 wk, out 1 wk, back in estrus	hCG × 1	Resolved	9	1.0	—	—	—	—	—
7. Golden retriever	2.5 yr	In and out of estrus for 2 mo after estrogen treatment for terminating pregnancy	hCG × 2	Resolved; was bred and delivered a single pup	3	2.0	—	—	—	—	ND
8. Greyhound	9.5 yr	Estrous vaginal smear for 2 mo	hCG × 3	Resolved but recurred	143	0.04	0.07	—	—	—	Yes
			GnRH × 3 surgically drained	Did not resolve	19	0.88	ND	138,000	193	35	Yes
			Tamoxifen citrate	Resolved but recurred							
9. Golden retriever	8 mo	In estrus for 8 wk	GnRH × 2	Resolved	26	4.8	0.13	—	—	—	ND
10. Malamute	9 mo	In and out of estrus for 3 mo	$PGF_{2\alpha}$	Did not resolve	ND	1.9	0.06	—	—	—	Yes
			GnRH × 1	Resolved							
11. Scottish terrier	1 yr	In estrus 8 wk	Spayed	Resolved	—	0.39	0.10	—	233	50	—
12. Malamute	3 yr	In estrus 6 wk	Spayed	Resolved	—	0.6	ND	92,000	7392	2.6	—
13. St. Bernard	4 yr	In estrus 4 wk	GnRH × 2	Did not resolve; developed pyometra	24	0.03	0.19	—	—	—	Yes

GnRH, gonadotrophin-releasing hormone; hCG, human chorionic gonadotrophin; ND, not detectable;—, not determined; X, mixed-breed dog.

* Multiple treatments (two or three) were usually separated by 24 to 48 hours.

From Olson PN, Wrigley RH, Husted PW, et al: Persistent estrus in the bitch. *In* Ettinger SJ, Feldman EC (eds): Textbook of Veterinary Internal Medicine, 3rd ed. Philadelphia, WB Saunders, 1989, p 1793, with permission.

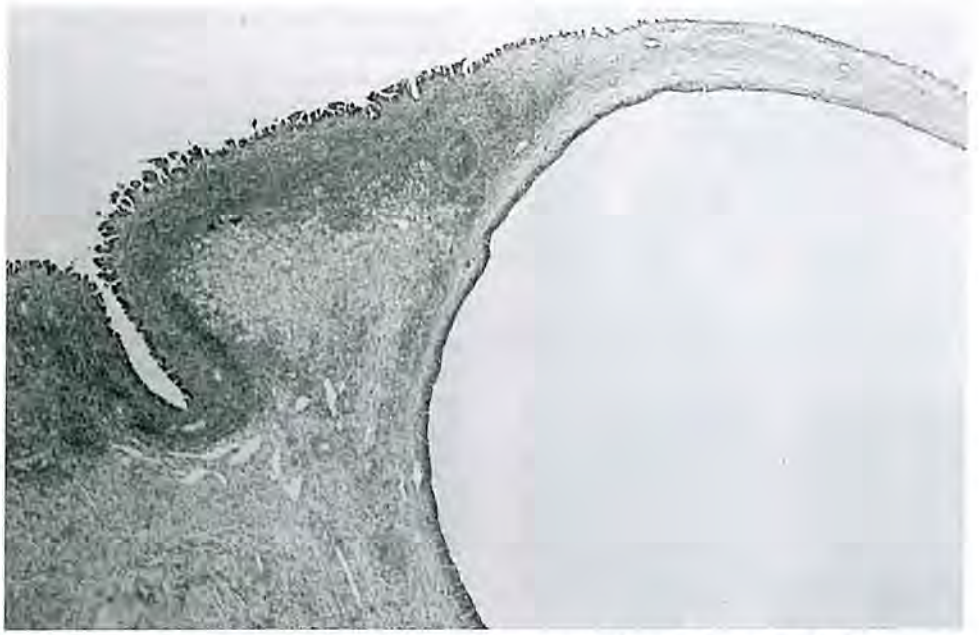

Figure 10–2. Photomicrograph of a follicular cyst. This large cystic space in the ovarian cortex is lined by a narrow layer of granulosa cells and contains no ovum. Hematoxylin and eosin stain; 10×). (From Shille VM, Calderwood-Mays MB, Thatcher MJ: Infertility in a bitch associated with short interestrous intervals and cystic follicles: A case report. J Am Anim Hosp Assoc 20:171–176, 1984, with permission.)

dres, and Labrador retriever.[19,22,23,25–27,33] Heritability of follicular cysts in the dog is unknown. In one study, 75 per cent of dogs with follicular cystic disease ($n = 63$) were reported to be nulliparous.[17]

Clinical signs of cystic ovarian follicular disease in dogs are referable to increased serum estrogen concentrations. The most common presenting complaint is estrous cycle irregularity, most often exhibited as prolonged proestrus or estrus.[18,19,22,23,25,26,28] Physical changes characteristic of proestrus and estrus, such as vulvar swelling and presence of serosanguineous vulvar discharge, are present.[18,19,23,26,28] The affected female dog may attract male dogs, but may not show normal breeding behavior, such as flagging or allowing the male to mount.[24] One report describes a dog failing to whelp by 71 days from the last breeding that was actually in a prolonged estrus with minimal estrual signs.[25] Other estrous cycle irregularities observed include irregular interestrous intervals, excessive serosanguineous vulvar discharge, and variability in physical changes and breeding behavior during estrus.[22,24,26] In dogs with large follicular cysts, abdominal distention may be present.[27] Progressive, nonpruritic, bilaterally symmetrical alopecia of the neck, trunk, and perineum, with associated lichenification and hyperkeratosis, may be present (Fig. 10–3).[19,20,22] Prolonged exposure of the endometrium to elevated serum estrogen concentrations may induce cystic endometrial hyperplasia–pyometra.

The primary differential diagnosis for follicular ovarian cysts in the female dog is functional ovarian neoplasia (granulosa cell tumor [GCT]). Presumptive diagnosis of ovarian follicular cystic disease is based on signalment, with mean age at diagnosis with follicular cysts younger than with ovarian neoplasia, and diagnosis is based on clinical signs suggestive of prolonged hyperestrogenism. Response to medical treatment, described below, may allow differentiation of follicular cysts from ovarian neoplasia. Definitive diagnosis requires histopathology of excised ovarian tissue.

Elevated serum estrogen concentrations may be verified by radioimmunoassay, but radioimmunoassays for estrogen are not always readily available and normal concentrations may vary greatly by laboratory.

Assessment of vaginal cytology specimens, with presence of cornified vaginal epithelial cells indicative of elevated serum estrogen concentrations, is readily available and inexpensive, and of adequate accuracy to detect presence of serum estrogen in the bitch. Dogs with follicular cysts have a predominantly cornified

Figure 10–3. Prominent alopecia and hyperpigmentation secondary to hyperestrogenism in a canine female pseudohermaphrodite with an ovarian follicular cyst. (From Nemzek JA, Homco LD, Wheaton LG, et al: Cystic ovaries and hyperestrogenism in a canine female pseudohermaphrodite. J Am Anim Hosp Assoc 28:402–406, 1992, with permission.)

population of vaginal epithelial cells. Cellular debris, RBCs, polymorphonuclear leukocytes, and bacteria may be present.[19,25] Vaginoscopic appearance of the vaginal mucosa in affected bitches is characteristic of either proestrus (rosy pink, edematous, billows of mucosa) or estrus (blanched, crenated, longitudinal folds of mucosa).[19] Concentrations of serum progesterone, testosterone, and cortisol have been demonstrated within normal limits in dogs with follicular cysts.[19,20]

Imaging techniques may be used to try to differentiate follicular cysts from ovarian neoplasms. Large follicular cysts may appear on abdominal radiographs as soft tissue–density structures caudal to the kidney that may displace the kidney cranially.[28] Ultrasonography may be more valuable, because it may allow visualization of cystic structures caudal to the kidney, and therefore presumably within the ovary.[20,28,33] The cysts appear as focal hypoechoic to anechoic structures with far enhancement.[19,20,28] Small cysts may not be visible. Granulosa cell tumors often appear polylocular on ultrasonography. The ovary may be difficult to visualize in large- or giant-breed dogs, especially deep-chested breeds. Inability to definitively identify cystic ovaries by ultrasound or radiography does not rule out cystic ovarian disease.

The treatment of choice for canine ovarian follicular cysts is ovariohysterectomy (OHE).[20,23–27] For dogs that have valuable reproductive potential, induction of luteinization of the cystic follicle(s) with gonadotropin-releasing hormone (GnRH; 50 μg intramuscularly [IM]), human chorionic gonadotropin (hCG; 220 IU/kg intravenously [IV] once *or* 500 IU IM, two doses with 48-hour interval *or* 1000 IU, half IV, half IM), or pituitary LH (50 mg IM) may be attempted.[19,25,33] Limited success has been reported with these hormonal therapies. Rate of recurrence has not been reported. There is one report, however, of surgical aspiration and flushing of follicular cysts as a treatment in the dog; the cysts did not recur, but the dog had concurrent cystic endometrial hyperplasia–pyometra that necessitated later OHE.[19] There is one report of resection of wedges from bilateral polycystic ovaries; the bitch went into heat 6 weeks after surgery and was bred successfully.[24]

Other Ovarian Cysts

Luteal cysts, also called luteinized follicles, are luteinized anovulatory follicles.[18] Secretion of LH from the pituitary is sufficient for luteinization of granulosa cells in these cases, but not sufficient to cause ovulation.[18] In a survey of 101 bitches with cystic ovarian disease, 9 (9 per cent) had luteal cysts.[17] Mean age at diagnosis was 8.6 years, with a range of 2 to 13 years.[17] Luteal cysts vary in diameter from 1.5 to 5 cm.[17,28] The cyst wall is thicker and more opaque than that of a follicular cyst, but radiographic and ultrasonographic appearance are

similar.[17,28] Clinical significance of luteal cysts in the dog is unknown.

Germinal cysts are also called cysts of subsurface epithelial structures.[18] These form as infoldings of the continuously growing peritoneal covering of the ovary.[18] Reported incidence among ovarian disorders is 20 to 38 per cent.[17,21] These microscopic cystic structures lie within the ovarian cortex and do not impair ovarian function.[17,18]

Cystic corpora lutea are reported to occur rarely in the dog.[18] A 4-year-old intact mixed breed dog was reported to have an ovarian remnant containing 2-cm-diameter cysts filled with viscous fluid that were diagnosed as cystic corpora lutea.[29]

Cystic rete ovarii, or rete cysts, are small masses of irregular, anastomosing tubules with cystic changes in the hilar region of the ovary.[17] They are reported to occur in 9 to 35 per cent of dogs with cystic ovarian disease.[17,21] Although they do not cause clinical disease, they may replace the surrounding normal ovarian tissue.[18]

Parovarian cysts are cystic structures in remnants of the mesonephric and paramesonephric tubules surrounding the ovary.[18] Parovarian cysts may be single or multiple and are greater than 1 cm in diameter.[18] A 9-year-old intact West Highland white terrier was asymptomatic despite presence of a parovarian cyst large enough to be radiographically visible as a soft tissue–density mass caudal to the kidney, displacing the colon medially.[30] Parovarian cysts are not reported to impair ovarian function.[18,30]

Ovarian Remnant Syndrome

Ovarian remnant syndrome refers to clinical signs indicating presence of functional ovarian tissue in a previously ovariohysterectomized female dog.[36,37] It is not a pathologic change, but is instead a complication of OHE.[36] In one survey of 27 dogs with complications after OHE, 12 (17 per cent) had an ovarian remnant.[38]

Ovarian remnant syndrome occurs when a retained piece of ovarian tissue revascularizes and becomes functional. Revascularization of ovarian tissue in the dog has been demonstrated experimentally; in female dogs in which minced ovarian tissue was placed in subserosal pouches on the stomach or under the splenic capsule after OHE, cyclic signs of proestrus and estrus were exhibited.[39] Retention of ovarian tissue after OHE may be due to surgeon error (e.g., improper placement of clamps) or may be due to presence of anomalous accessory ovarian tissue in the broad ligament.

Women are more likely to develop ovarian remnant syndrome after OHE than are companion animals, because women undergoing this surgery more often have inflammatory periovarian disease causing fibrous adhesions of the ovary to surrounding tissues that complicates complete removal.[37] Ovarian remnant syndrome is less common in dogs than in cats.[40] In dogs, no correlation has been shown between occurrence of ovarian remnant syndrome and age of the dog at OHE, breed, difficulty of surgery (elective vs. nonelective), physical condition of the bitch (normal weight vs. obese), or status of the veterinarian (new graduate vs. experienced practitioner).[36,40]

The most common presentation of ovarian remnant syndrome is recurrent estrus after OHE.[29,36,38,40,41] Interval from OHE to signs of estrus averages 15.5 months, and ranges from 3 months to 5 years (n = 48).[29,40,41] Once cyclic estrous activity returns, most dogs with ovarian remnant syndrome exhibit normal periodicity of estrous cycles.[36] In a survey of 46 dogs with ovarian remnant syndrome, mean interestrous interval was 8.8 months.[40]

Dogs with ovarian remnant syndrome exhibit physical changes characteristic of proestrus and estrus, with vulvar swelling and variable amounts of mucoid to serosanguineous vulvar discharge, and behavioral signs of estrus, such as flagging and attracting and standing to be mounted by male dogs.[29,37,38,41] Dogs with ovarian remnant syndrome also may exhibit signs of false pregnancy weeks to months after estrous signs and behavior, or, recurrent false pregnancy in the absence of observed estrus.[36,38] There is one report of a toy poodle with ovarian remnant syndrome that presented with chronic vaginitis.[41]

Differential diagnoses for ovarian remnant syndrome are those conditions that cause bloody vulvar discharge in spayed female dogs, including vaginal neoplasia, vaginitis, uterine stump pyometra, trauma, exogenous estrogen therapy, and coagulopathy.[36] Abdominal radiographs or ultrasound usually are noncontributory to the diagnosis. Normal ovaries are not readily demonstrable on radiographs or sonograms,[42] and ovarian remnants, unless large and cystic, are smaller and even less likely to be visualized with these techniques.[41]

Presumptive diagnosis of ovarian remnant syndrome requires demonstration of a cornified vaginal epithelial specimen in a spayed bitch.[36] Estrogen radioimmunoassay is not always readily available, and may not be as accurate an interpretation of vaginal cytology specimens, as is a bioassay. The vaginal cytology specimen should be collected when the bitch is in estrus, defined by behavioral changes noted by the owner and physical changes characteristic of proestrus. Total or predominant cornification of vaginal epithelial cells is indicative of elevated serum estrogen concentrations. Estrogen also can be produced by the adrenal glands, but adrenal diseases associated with excessive estrogen production are much less common than ovarian remnant syndrome as a cause of vaginal cornification in spayed female dogs.

Once the presence of estrogen has been verified by vaginal cytology, serum progesterone concentrations in blood collected one to two weeks later should be evaluated to determine if luteinization or ovulation of the follicle has occurred, with a serum progesterone concentration of greater than 2 ng/ml indicative of functional luteal tissue.[36] If luteinization of the follicle has not yet occurred, the dog may be allowed to ovulate spontaneously, with recheck of serum progesterone concentration in 7 to 10 days for verification, or luteinization of the follicle may be induced with GnRH (50 μg IM) or hCG (400 IU IV or 1000 IU, half IV, half IM),[41] with recheck of serum progesterone concentration in 10 to 14 days for verification of luteinization.

Exploratory laparotomy is the treatment of choice. Lifelong medical therapy with estrus-suppressing drugs, such as megestrol acetate (Ovaban; Schering Corporation, Kenilworth, NJ) or mibolerone (Cheque; Pharmacia & Upjohn, Peapack, NJ), is neither a safe nor a practical alternative and is not recommended. Exploratory laparotomy should be performed either when follicles are present on the ovarian remnant or after luteinization of the follicle has occurred, because the remnant will be more readily visible with follicles or corpora lutea present. There is less intraoperative bleeding in dogs that are in the luteal phase of the estrous cycle. Both ovarian pedicles should be examined, because sometimes remnants occur bilaterally. Conflicting reports exist as to whether unilateral ovarian remnants are more common on the right side[38] or equally common on right and left sides.[40] Bilateral ovarian remnants are reported to be present 35 per cent of the time.[40] If no overt functional ovarian tissue is present, scar tissue should be removed at both ovarian pedicles. In a survey of 46 cases of canine ovarian remnant syndrome, all remnants were found at the ovarian pedicle.[40] Therefore, an exhaustive search of the entire abdominal cavity usually is not necessary. All excised tissue should be submitted for histopathology. There is one report of a functional GCT on the ovarian remnant of a dog that had been ovariohysterectomized 7 years before onset of clinical signs.[43]

If surgical correction of ovarian remnant syndrome is undertaken when functional luteal tissue is present, signs of false pregnancy may develop postoperatively. This is a normal consequence of the surgery. Signs generally will subside without treatment in less than 4 weeks (see Chapter 13).

Oophoritis

Oophoritis is diffuse infiltration of the ovary with mononuclear inflammatory cells, with subsequent degeneration of germ cells and fibrosis of surrounding tissues.[2,75,76] An autoimmune pathogenesis is hypothesized. Three cases of canine oophoritis have been reported in the literature; a 5-year-old intact cocker spaniel and an intact beagle of unreported age presented with persistent anestrus,[2,75] and a 3.5-year-old intact Rhodesian Ridgeback presented with persistent estrus and infertility.[76] Ovaries of the cocker spaniel showed collapse and degeneration of ovarian follicles prior to antrum formation. Serum progesterone concentrations were less than 2 ng/ml, indicating absence of luteal function, despite treatment with GnRH in the dog with persistent estrus.[76] Serum estrogen, testosterone, LH, and follicle-stimulating hormone concentrations were within normal limits in the two anestrous bitches.[2,75] All dogs were diagnosed by histopathology of ovarian tissue removed at ovariohysterectomy.

Ovarian Neoplasia

Ovarian neoplasia is relatively uncommon in the dog. Reported incidences of tumors of the ovary in all dogs with neoplasia are 0.5 per cent (n = 2350 tumors),[44] 1 per cent (n = 2361 tumors),[45] and 6 per cent (n = 400 tumors).[45] Incidence of ovarian neoplasia in 269 female dogs with reproductive tract neoplasia was re-

ported to be 3.7 per cent.[46] Of 115 dogs with ovarian disease, 25 (20 per cent) had ovarian neoplasia.[17]

Pathogenesis of ovarian neoplasia is unknown. Induction of malignant ovarian tumors has been demonstrated experimentally in dogs treated with estrogen alone or with estrogen and progesterone, but histologically these tumors more closely resembled germinal cysts than naturally occurring ovarian tumors.[47] A report of increased incidence of ovarian neoplasia in a group of four maned wolves housed together at the National Zoo suggests genetic predisposition or environmental factors as possible contributors to tumor development.[48]

Ovarian mass lesions may be palpable per abdomen, and often are visible on radiographs or sonograms. On abdominal radiographs, ovarian neoplasms are visible as soft tissue–density masses caudal to the kidney, displacing the caudal pole of the kidney ventrally and the colon medially (Fig. 10–4).[42] Ultrasonographically, ovarian tumors are visible as rounded or irregular masses caudal to the kidney with variable or polylocular echotexture.[42] Specifics of diagnosis of the various types of ovarian neoplasms are described below.

The three general categories of primary ovarian neoplasms are those arising from epithelial cells, those tumors of sex cord/stromal origin, and those arising from germ cells.[45,49] Secondary (metastatic) tumors of the canine ovary include lymphosarcoma, mammary carcinoma, intestinal carcinoma, and pancreatic carcinoma.[17,49]

Epithelial Tumors

Ovarian epithelial neoplasms arise from cells of the surface epithelium extending into the ovarian cortex.[49] Tumors of epithelial cell origin account for 20 to 64 per cent of ovarian tumors; mean incidence is 45 per cent.[17,45,46,49,50] Types of ovarian epithelial cell tumors reported include adenocarcinoma,[17,45,50,51] papillary cystadenocarcinoma,[49,52,53] serous cystoadenocarcinoma,[54] papillary adenoma,[45,49] serous and pseudomucinous cystadenomas,[17] fibromas,[17,55] and undifferentiated carcinoma.[49] Adenocarcinomas are more common than are adenomas.[50]

Mean age at diagnosis of ovarian cystadenocarcinoma in the dog is 6.5 years, with a range of 3.5 to 12 years ($n = 4$).[51–54] Mean age at diagnosis of ovarian cystadenoma in the dog is 8.8 years, with a range of 6 to 13 years ($n = 10$).[17]

Ovarian epithelial tumors may be cauliflower-like or smooth, and may or may not be cystic.[45,49] Size is variable; tumors as large as 7 to 10 cm in diameter have been reported.[17] Serous cystadenomas and cystadenocarcinomas contain variably sized cysts filled with watery fluid.[54] Pseudomucinous cystadenomas are multilobular, with lobules containing viscous, milky fluid.[17] Both adenomas and adenocarcinomas often occur bilaterally.[45] In a survey of eight dogs with unilateral ovarian serous cystadenomas, the contralateral ovary was inactive in 63 per cent.[17]

The most common clinical signs reported in dogs with ovarian tumors of epithelial origin are ascites and abdominal distention.[45,49,51,52] Ascites may develop as a result of obstruction of diaphragmatic lymphatics by the tumor, or lymphatic obstruction resulting from metastasis.[45,49] Pleural fluid also may be present, confounding auscultation of the heart and lungs.[52] The tumor may be palpable in the cranial abdomen.[45] Dogs with ovarian tumors of epithelial origin may be asymptomatic; ovarian neopla-

Figure 10–4. Lateral abdominal radiograph of a bitch with an ovarian neoplasm. (From Jergens AE, Shaw DP: Tumors of the canine ovary. Compend Contin Educ Pract Vet 9:489–495, 1987, with permission.)

sia was found at OHE in a 3.5-year-old intact German shepherd that presented with vaginal transmissible venereal tumor, and in a 5-year-old intact Labrador retriever that presented with clinical signs of pyometra and mammary neoplasia.[53,54]

Abdominal radiography frequently is unrewarding in dogs with ovarian tumors of epithelial origin, because ascitic fluid obscures abdominal contents.[51,52] Presence of intra-abdominal fluid enhances ultrasonographic images.[51] Epithelial-origin ovarian tumors have been observed at ultrasound as large, discrete, anechoic structures with irregular margins caudal to the kidney.[52] Ascitic fluid, collected by abdominocentesis, has been reported to contain 4.1 g/100 ml protein, 1.34×10^6 RBCs/mm^3 and 3600 WBCs/mm^3, and to have a specific gravity of 1.026.[52] Neoplastic cells may exfoliate into intra-abdominal fluid.[52]

Rate of metastasis of ovarian adenocarcinoma is reported as 48 per cent.[50] Metastases are 1- to 2- mm nodules, diffusely scattered over the peritoneum and serosal surfaces of the abdominal organs.[52] Metastases are unlikely to be visible by radiography or ultrasound.

The treatment of choice for ovarian neoplasia of epithelial origin is surgical removal. Complete OHE is most often performed because of the risk of metastasis.[49,52,54] Metastatic disease was treated with chemotherapeutic agents in one dog.[52] Cyclophosphamide (50 mg/m^2 three times weekly) and chlorambucil (8 mg/m^2 two times weekly) on alternating days were used until the dog developed sterile cystitis. The cyclophosphamide was withdrawn and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosurea (CCNU; 90 mg/m^2 every 6 weeks) was instituted. The dog developed neutropenia and septicemia as side effects of CCNU, which was then withdrawn. At 10 months OHE, the dog was clinically normal on chlorambucil alone.[52]

Sex Cord/Stromal Tumors

Incidence of sex cord/stromal tumors among ovarian tumors in dogs is 34 per cent (n = 71).[50] The three types of ovarian tumors of sex cord/stromal origin in dogs described in the literature are GCT,[17,43–45,56–60] and, much more rarely, thecal cell tumors or thecomas[45,49,61] and luteomas.[45,49,62]

Reported incidence of GCT among ovarian tumors varies from 23 to 52 per cent, with mean reported incidence of 38 per cent.[17,46,49,50,59] Mean age at diagnosis of GCT is 7.2 years (n = 20), with a reported range of 14 months to 15 years.[17,43,44,56–60] Although GCT is most common in intact females, there is one report of a GCT in an ovarian remnant of a spayed dog.[43] In one survey of 13 dogs with GCT, 10 (77 per cent) were nulliparous.[17] The English bulldog is reported to be at increased risk for GCT.[45]

Granulosa cell tumors usually are unilateral,[49] although bilateral masses have been reported.[56] The neoplastic ovary is abnormally firm.[63] Granulosa cell tumors vary from 0.4 cm to greater than 10 cm in diameter.[17,44,49,58] Smaller tumors usually are solid, and larger tumors often are friable and cystic.[17]

Granulosa cell tumors usually are well encapsulated with no local invasion.[17] Metastasis is reported to occur in 10 to 20 per cent of cases.[49] Reported sites of metastasis include the omentum, mesentery, diaphragm, liver, kidneys, urinary bladder, and intra-abdominal lymph nodes.[44,56,57]

Reported clinical signs caused by the tumor mass are abdominal distention and ascites.[44,56,60] Ascites may develop either from leakage of fluid through the ovarian capsule or from the tumor obstructing peritoneal lymphatics.[44] Granulosa cell tumors often are functional; elevations in serum estrogen concentration alone,[43] or in serum estrogen and progesterone,[45,49,58] may occur. Clinical signs referable to increased serum estrogen concentration include persistent or erratic estrus[44,45,49,58]; vulvar swelling and serosanguineous vulvar discharge[43,45,58,59]; purulent vulvar discharge, polyuria/polydipsia, and other signs of concurrent cystic endometrial hyperplasia–pyometra complex, which has been reported to occur in 95 per cent of female dogs with GCT (n = 24)[29,43–45,58,59]; nonregenerative anemia with agranulocytosis and thrombocytopenia resulting from estrogen-induced bone marrow toxicity[43,45,58]; and skin changes characteristic of hyperestrogenism, including bilaterally symmetrical alopecia of the trunk, lichenification, and hyperkeratosis.[43] Other clinical signs may develop with metastatic disease, including anorexia, weight loss, vomiting, and diarrhea.[56,57,59] Clinical signs referable to increased serum progesterone concentrations include those of the cystic endometrial hyperplasia-pyometra complex.

Presumptive diagnosis of GCT is based on presence of a cranial abdominal mass, cornified vaginal epithelium and/or elevated serum progesterone concentrations. The tumor mass may be palpable per abdomen, or may be visualized by radiography as a soft tissue–

density mass caudal to the kidney, or by ultrasonography as a mass with complex echogenicity caudal to the kidney.[43,44] Reported serum estrogen concentrations in functional GCT range from 55 to 166 pg/ml.[43,58] Reported serum progesterone concentrations range from 0.64 to 11.0 ng/ml, with values greater than 2 ng/ml indicative of active progesterone secretion by the tumor.[43,58] Definitive diagnosis requires histopathology of excised ovarian tissue.

Surgical removal of the affected ovary is the recommended treatment, with complete OHE most often indicated.[43,49,57] Although unilateral ovariectomy may be considered in valuable breeding bitches, the high incidence of concurrent cystic endometrial hyperplasia–pyometra in dogs with GCT, and possibility of extension of malignant tumors to the uterus, must be considered as contraindications.[29,43–45,58,59] The contralateral ovary is reported inactive in 31 per cent (n = 13) of bitches with unilateral GCT.[17]

Chemotherapy for treatment of metastatic GCT has not been described. There is one report of immunotherapy for metastatic GCT, with intramuscular injection of increasing doses of mixed bacterial toxins to a final biweekly dose of 0.15 ml. This regimen maintained the bitch clinically free of disease for 2 years after OHE.[44]

Germ Cell Tumors

The fertilized mammalian ovum differentiates into extraembryonic and embryonic tissues. Abnormal growth of embryonic cell lines leads to development of germ cell tumors.[63] Two types of germ cell tumors have been reported in the dog. Dysgerminomas are solid tumors derived from undifferentiated ovarian epithelium.[49,63,64] They are histologically similar to testicular seminomas, and also are called embryonal carcinomas and ovarian seminomas.[65] Teratomas are solid tumors containing differentiated tissue from two to three cell lines.[49,63] Tissues contained within teratomas may arise from ectoderm (hair, sweat and sebaceous glands, nervous tissue), mesoderm (cartilage, bone, teeth, smooth and skeletal muscle), and/or endoderm (respiratory and intestinal epithelium).[45] Dermoid cysts are a form of teratoma containing cysts lined with epidermis.[45] Overall incidence of germ cell tumors among ovarian tumors in dogs is 20 per cent (n = 17).[50] Dysgerminomas and teratomas are reported to occur with equal frequency.[50]

Mean age at diagnosis of bitches with dysgerminomas is 9.5 years, with a range of 7 to 13 years (n = 4).[64–66] Teratomas occur in younger dogs.[45] Mean age at diagnosis is 6.5 years, with a range of 20 months to 13 years (n = 11).[67–72] No breed predisposition has been reported for germ cell tumors.

Dysgerminomas are large (10 to 30 cm diameter), smooth, firm, multilobular, noncystic masses containing areas of hemorrhage and necrosis.[49,64,65] Metastasis is reported to occur in 10 to 20 per cent of cases,[49] with reported sites of metastasis including the intra-abdominal lymph nodes, liver, and kidneys.[73] Teratomas are moderately large (1 to 12 cm diameter), irregular, firm, noncystic masses commonly containing hair, bone, teeth, and other differentiated tissues.[67,70,72,74] Metastasis is reported to occur in 33 to 50 per cent of cases,[45,50] with reported sites of metastasis including bone, intra-abdominal and peripheral lymph nodes, lungs, and omentum.[68,70]

The most common clinical sign of both dysgerminomas and teratomas is abdominal distention.[64–67,71] Peritoneal effusion has not been reported with these tumors. Dogs with germ cell tumors may be asymptomatic.[63,67,69] Germ cell tumors do not impair ovarian function, because dogs with dysgerminomas and teratomas are reported to have normal estrous cycles.[63,65,69,71,74] Clinical signs referable to the site affected may be present in metastatic disease.[68,71]

Large germ cell tumors commonly are palpable per abdomen.[64,65,67,71,74] On abdominal radiographs, germ cell tumors are evident as soft tissue–density masses caudal to the kidney.[65,67,68,74] Mineralization may be visible radiographically in teratomas.[67,68,74]

Surgical removal of the neoplastic ovary is the treatment of choice. Complete OHE is recommended because of risk of metastasis. Unilateral ovariectomy may be considered in valuable breeding bitches. The contralateral ovary and uterus usually are normal in bitches with unilateral teratoma.[67,69,74] A 2-year-old intact rottweiler bitch with ovarian teratoma underwent unilateral ovariectomy and later reproduced successfully; however, she had only a single puppy.[67]

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Chapter 11

Disorders of the Canine Uterus and Uterine Tubes (Oviducts)

Uterine Disorders

Congenital Abnormalities

Unilateral aplasia, partial fusion, and unequal length of uterine horns have been reported in the dog.[1–4] Aplasia of the associated ovary may or may not be present (Fig. 11–1). Three nulliparous bitches with unilateral aplasia of uterine horns had apparently normal estrous cycles; the abnormality was an incidental finding at the time of elective ovariohysterectomy (OHE).[1] Cystic endometrial hyperplasia with associated mucometra or pyometra was reported in the complete horn of three bitches with unilateral uterine horn aplasia.[2,3] Heritability of uterine horn aplasia is unknown.

Abnormal development of the uterus or uterine tubes may occur in intersex animals. Male pseudohermaphrodites are animals with testes and female external genitalia. Three of 26 (12 per cent) male pseudohermaphrodites were reported to have a uterus masculinus. The remainder had grossly normal uteri. Six were reported to have normal uterine tubes.[5,6] True hermaphrodites are animals with both ovarian and testicular tissue in their gonads. Of 14 true hermaphrodites, all were reported to have grossly normal uteri, but one had blind-ended uterine horns, suggesting abnormality of the uterine tubes.[5,6] In lateral true hermaphrodites, with an ovary on one side and a testis on the other, the uterus and uterine tubes may be abnormal only on the side with the testis.[7] Normal uteri are reported in female pseudohermaphrodites, animals with ovaries and male external genitalia, and in XX sex-reversed cocker spaniels, genetic females with male gonads and abnormal male external genitalia.[5,6,8]

Hydrometra/Mucometra/ Hematometra

Hydrometra and mucometra are accumulations of sterile serous or mucoid fluid in the uterus.[4] Incidences of hydrometra and mucometra are unknown, because these conditions usually are incidental findings either at the time of elective OHE or in aged intact female dogs undergoing diagnostic work-ups for unrelated disorders, such as congestive heart failure or mammary neoplasia.[3,9,10] Of 60 uteri with disease in one survey, 8 (13 per cent) were classified as having hydrometra or mucometra.[11]

Cystic endometrial hyperplasia (CEH) frequently is associated with hydrometra and mucometra.[3,10,12,13] Pathogenesis of these conditions therefore is likely to include that of CEH (see below). The secretion of progesterone during diestrus in normal dogs increases secretory activity of the endometrial glands, decreases myometrial contractility, and functionally closes the cervix, allowing fluid to accumulate within the uterine lumen.[9,13]

Presumptive diagnosis of hydrometra and mucometra is based on presence of uterine enlargement, documented by abdominal palpation, radiography or ultrasound, and lack of a systemic inflammatory response (normal white blood cell [WBC] number and differential). The primary differential diagnoses are pyometra and pregnancy. Definitive diagnosis requires cytology and culture of the intrauterine fluid. The fluid varies in character from serous to mucoid and in color from straw colored to serosanguineous.[9,10,12] Volumes of uterine fluid as high as 240 to 500 ml in affected bitches have been reported.[9,10,12] The treatment of choice is OHE, especially in bitches not intended for breeding.

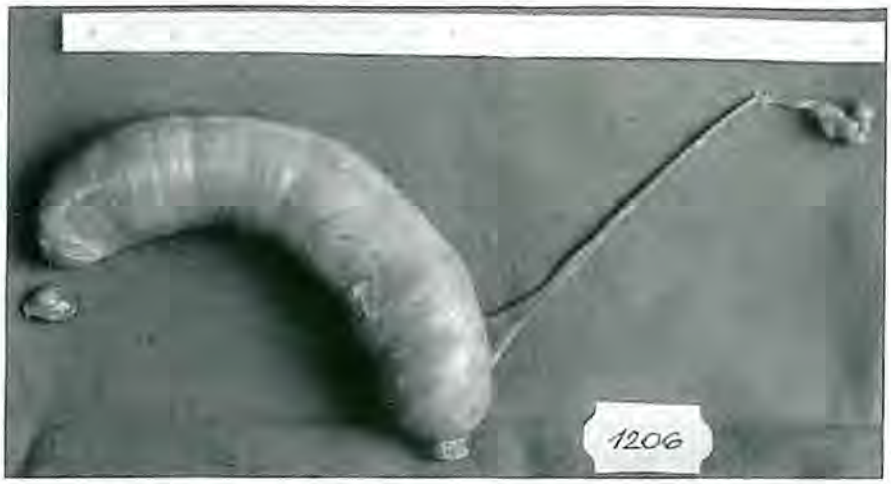

Figure 11–1. Unilateral uterine horn aplasia in a dog. (From Láznička A, Jarešová H, Vitásek R, et al: Segmental aplasia of müllerian ducts in bitches—a case report. Veterinářství 47:410–412, 1997, with permission.)

Hematometra is sterile accumulation of blood within the uterus. Hematometra has been reported in two dogs with uterine torsion[14,15] and in one bitch as a manifestation of anticoagulant rodenticide toxicity.[16] In all three, the distended uterus was visible on radiographs or ultrasound. The intrauterine fluid was of mixed echogenicity but could not be defined absolutely as hemorrhage by ultrasound alone.[16] Concurrent or recent pregnancy should be ruled out in bitches with hematometra. Therapy is dependent on the inciting cause of the hematometra; the three dogs reported here all underwent OHE and supportive therapy and recovered uneventfully.

Cystic Endometrial Hyperplasia–Pyometra Complex

Cystic endometrial hyperplasia–pyometra complex is an acute or chronic postestrual disease of adult intact bitches leading to inflammatory exudate in the uterus that is associated with variable clinical and pathologic signs.[13,17] It also is called pyometritis, pyometra complex, catarrhal endometritis, purulent endometritis, chronic cystic endometritis, and chronic purulent endometritis.[13,18] Prevalence of naturally occurring pyometra is reported as 0.6 per cent, but this figure is misleading, because incidence increases with age in the intact bitch and may approach 66 per cent in bitches over age 9.[19,20] There are few reports in the literature describing incidence; one report described CEH-pyometra in 395 of 4295 (9 per cent) of female dogs admitted over a given period of time.[21]

PATHOGENESIS

Pyometra is differentiated from metritis by time in the cycle during which it occurs and by pathogenesis. Metritis is inflammation of the uterus caused by primary bacterial infection that occurs postpartum when serum progesterone concentration is low. Pyometra is a progesterone-mediated uterine disease that is initiated or occurs during diestrus. The primary pathologic lesion is CEH. Bacterial infection with opportunistic organisms from the vagina occurs secondary to CEH.

CEH is caused by repeated exposure of the endometrium to progesterone. The unique 2-month diestrus of dogs predisposes this species to CEH. In a survey of uterine pathology in 216 female dogs that were age 9 years or more and had histories of normal estrous cycling, two thirds had CEH.[4,20]

Estrogen promotes growth, vascularity, and edema of the normal endometrium; cervical relaxation and dilation; and migration of polymorphonuclear leukocytes into the uterine lumen.[13] Experimental exposure of the endometrium to estrogen alone causes no specific pathologic change.[17,22] Progesterone stimulates proliferation and secretory activity of endometrial glands, maintains functional closure of the cervix, and inhibits myometrial contractility.[13] Experimental exposure of the endometrium to progestogens, including megestrol acetate and medroxyprogesterone acetate, causes superficial epithelial proliferation and increased secretory activity of the endometrial glands (CEH).[17,22–26] Extent of the pathologic effect of progestogens on the endometrium is corre-

lated with dose and duration of treatment.[22,25] The progestogen effect is enhanced by priming of the endometrium with estrogen, especially if the drugs are given cyclically so as to mimic normal estrous cycling in the bitch.[17,22] Of 28 female dogs receiving estrogen (5 mg once daily) followed by progesterone (10 to 50 mg once daily) at 20-day intervals, all developed CEH to the extent seen in spontaneous disease by the fourth artificial cycle.[22] Estrogens may increase the number of progesterone receptors in the endometrium, amplifying the effect of progesterone. The normal down-regulation of estrogen receptor expression in endometrial glands under the influence of rising progesterone may be defective in dogs with CEH, causing a prolongation of estrogen's effect on the endometrium in the luteal phase.[27,28] Development of naturally occurring CEH is not due to abnormally elevated serum concentrations of estrogen or progesterone. When serum collected from 13 dogs with pyometra at the time of OHE was assayed for estrogen and compared with serum from normal bitches at the same stage of the estrous cycle, no difference was found between the groups (Table 11–1).[29] Although 96 of 100 dogs with pyometra were reported to have persistent (nonregressing) corpora lutea (CL) on their ovaries in one study,[30] no difference has been demonstrated in serum progesterone concentrations between dogs with pyometra and normal female dogs at a similar stage of the estrous cycle (Table 11–1).[29,31,32] No correlation has been shown between concentrations of progesterone in diestrous bitches and incidence of pyometra.[33] Although CL are retained histologically for months in normal dogs, these CL are not functional and are not involved in the pathogenesis of CEH.

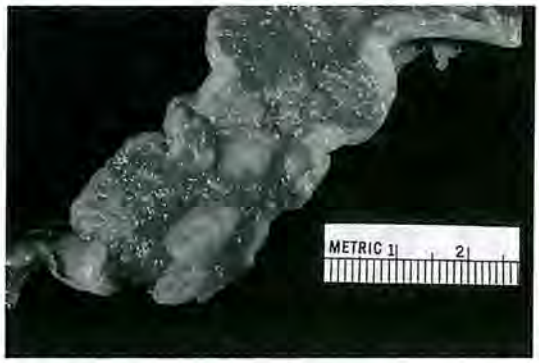

Figure 11–2. Endometrial surface of a canine uterus with cystic endometrial hyperplasia.

CEH also has been induced experimentally by scratching of the endometrium during the luteal phase[34] and by serial uterine biopsies early in the luteal phase.[35] The endometrium is hyper-reactive in the early luteal phase, showing a hyperplastic response to any stimulus.[34]

STAGES OF DISEASE

Dow described four stages of CEH-pyometra.[17,30,36] Type I is uncomplicated CEH. Grossly, the endometrium has a cobblestone appearance, with thickening and many cystic irregular elevations, 4 to 10 mm in diameter, covering the endometrial surface (Fig. 11–2).[13,17]

Table 11–1. Total Unconjugated Estrogen and Progesterone Levels in the Peripheral Blood of Bitches with Pyometra

Bitch	Day of Metestrus When Sampled	Total Unconjugated Estrogens (pg/ml)	Mean Total Unconjugated Estrogen Levels in Normal Bitches ±SD (pg/ml); *n* = Number of Bitches	Progesterone (ng/ml)	Mean Progesterone Levels in Normal Bitches ±SD (ng/ml); *n* = Number of Bitches
I	18	16.2	13.3 ± 10.4 (n = 6)	17.4	16.5 ± 3.8 (n = 6)
II	39	3.7	6.3 ± 5.4 (n = 6)	4.6	6.3 ± 2.0 (n = 6)
III	40	3.4	5.6 ± 3.3 (n = 4)	5.1	4.6 ± 1.2 (n = 5)
IV	41	7.1	4.1 ± 3.3 (n = 6)	2.9	4.3 ± 1.2 (n = 6)
V	42	5.4	5.2 ± 3.8 (n = 5)	4.2	3.4 ± 0.7 (n = 6)
VI	43	Undetectable	6.6 ± 1.8 (n = 4)	3.2	3.9 ± 1.2 (n = 5)
VII	44	1.6	3.9 ± 3.6 (n = 5)	2.7	3.9 ± 1.3 (n = 6)
VIII	46	8.6	4.2 ± 3.6 (n = 5)	2.9	3.5 ± 1.1 (n = 6)
IX	51	Undetectable	4.0 ± 5.1 (n = 5)	3.2	2.5 ± 1.1 (n = 6)
X	56	4.2	5.1 ± 5.7 (n = 6)	1.6	1.3 ± 0.5 (n = 6)
XI	56	1.7	5.1 ± 5.7 (n = 6)	2.8	1.3 ± 0.5 (n = 6)
XII	60	2.3	5.8 ± 4.7 (n = 6)	1.8	1.3 ± 0.9 (n = 4)
XIII	70	Undeterctable	4.7 ± 4.3 (n = 4)	1.0	0.5 ± 0.4 (n = 4)

From Hadley C: Unconjugated oestrogen and progesterone concentrations in the blood of bitches with false pregnancy and pyometra. Vet Rec. 96:546, 1975, with permission.

Histologically, there is an absolute increase in the number of glandular elements throughout the endometrium, with irregular size and configuration of the glands. Translucent cysts usually are evenly distributed over the endometrial surface.[36]

Type II is CEH plus diffuse infiltration of plasma cells.[17,36] No tissue destruction is visible histologically (Fig. 11–3).[17]

Type III is CEH with overlying acute endometritis.[17,36] Areas of endometrial ulceration and hemorrhage may be visible grossly, and intrauterine discharge varying in color from red-brown to yellow-green may be present. The acute inflammatory reaction is characterized by congestion, edema, and superficial and deep infiltration of neutrophils into the endometrium.[36] Myometrial inflammation is present in up to 40 per cent of cases (Fig. 11–4).[36]

Type IV is CEH with chronic endometritis.[17,36] If the cervix is open, allowing drainage of intrauterine fluid, the uterine horns will be narrow in diameter, the walls grossly thickened, and minimal discharge present.[17] The endometrium is atrophied, and infiltration of plasma cells and lymphocytes is present.[36] The myometrium is hypertrophied and fibrotic.[36] If the cervix is closed, the uterine horns are distended with purulent fluid.[17] Marked atrophy of the endometrium and myometrium are present (Fig. 11–5).[36]

Factors affecting patency of the cervix in bitches with pyometra are not well defined. The normal cervix dilates during proestrus, concurrent with peak serum estrogen concentrations.[37] The muscular cervix undergoes maximal epithelial glandular proliferation and muscular hypertrophy during estrus.[38,39] The cervix closes before onset of cytologic diestrus.[37] At parturition, cervical dilation occurs secondary to release of oxytocin and perhaps prostaglandins stimulated by pressure of the fetus at the internal cervical os. It is not known why the increased intrauterine pressure caused by accumulation of purulent fluid in dogs with types III or IV CEH-pyometra does not consistently cause cervical dilation. In a review of 20 cases of canine CEH-pyometra complex, 17 dogs (85 per cent) had an open cervix and 3 dogs (15 per cent) had a closed cervix. There was histopathologic evidence of chronic cervicitis in 11 of 13 cases; no correlation was noted between histopathologic changes and patency of the cervix (A. Mattson, P. Schultheiss and P. Olson, Fort Collins, CO, 1989, unpublished data).

BACTERIOLOGY

The vagina of the normal female dog is not a sterile environment (see Chapter 12). Bacteria cultured from the uteri of normal dogs always are found in the vagina or cervix as well, sug-

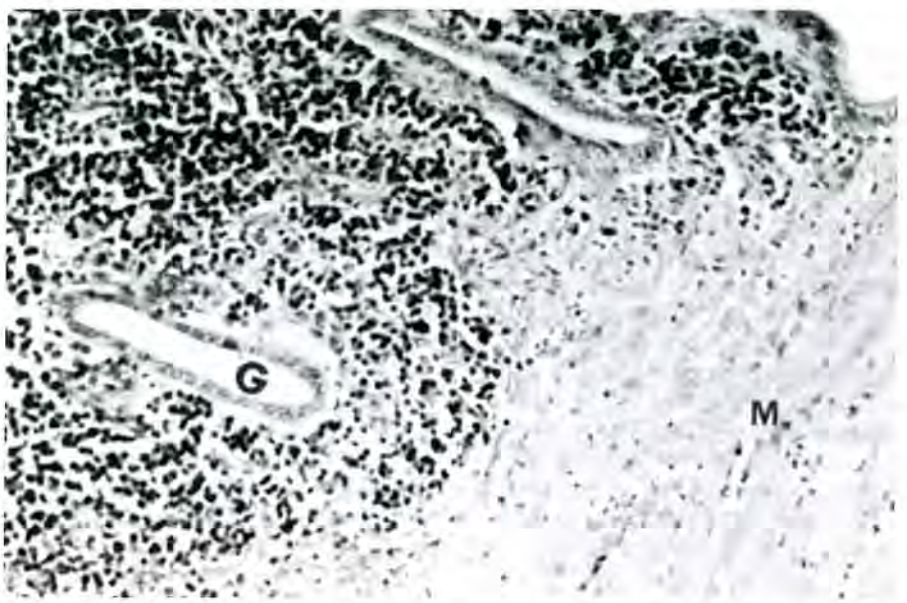

Figure 11–3. Photomicrograph of Dow type II pyometra. Numerous plasma cells and lymphocytes are present in endometrium adjacent to myometrium. G, endometrial gland; M, myometrium. Hematoxylin and eosin stain; 450×. (From Hardy RM, Osborne CA: Canine pyometra: Pathophysiology, diagnosis and treatment of uterine and extra-uterine lesions. J Am Anim Hosp Assoc 10:245–268, 1974, with permission.)

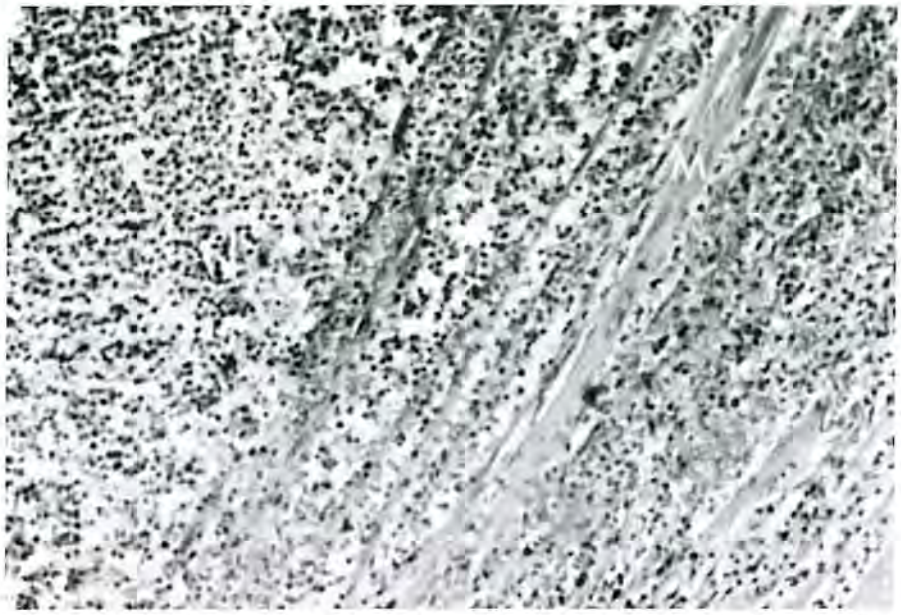

Figure 11–4. Acute endometritis associated with Dow type III pyometra. Large numbers of neutrophils and moderate numbers of mononuclear cells are present in the endometrium and myometrium. I, inflammatory cells in the endometrium; M, myometrium. Hematoxylin and eosin stain; 400×. (From Hardy RM, Osborne CA: Canine pyometra: Pathophysiology, diagnosis and treatment of uterine and extra-uterine lesions. J Am Anim Hosp Assoc 10:245–268, 1974, with permission.)

gesting movement of organisms from the nonsterile vagina through the patent cervix.[40] Bacteria were cultured from the uterus of normal bitches in the following percentages at various stages of the estrous cycle: proestrus/estrus, 18 per cent (n = 11) to 100 per cent (n = 12); diestrus, 0 per cent (n = 14) to 38 per cent (n = 16); and anestrus, 0 per cent (n = 12) to 7 per cent (n = 14).[40,41] The uterus of normal dogs does not harbor bacteria.[42] Although bacteria inoculated into the vagina of healthy dogs were recovered from the uterus in up to 100 per cent of dogs, depending on the stage of the estrous cycle during which inoculation occurred, all dogs spontaneously cleared the uterus of bacteria by 5 days postinoculation.[43]

In dogs with CEH, secondary infection with opportunistic bacteria occurs when organisms that move into the uterus during proestrus and estrus cannot be cleared prior to the luteal phase. Experimental production of pyometra was possible in 50 per cent of bitches inoculated with *Escherichia coli* vaginally during proestrus, and occurred in 100 per cent of bitches inoculated during diestrus.[33] The progesterone-dominated uterus has decreased contractility and movement of neutrophils into the uterine lumen.[13] Aerobic organisms causing the secondary infection of CEH-pyometra are those found in the normal vaginas of dogs (Table 11–2).[11,13,17,36,45–52] Anaerobic organisms were cultured from 15 per cent (n = 20) of dogs with CEH-pyometra in one study; organisms recovered included *Bacteroides* species, *Peptostreptococcus* species, *Clostridium perfringens*, and *Fusobacterium necrophorum*. *Mycoplasma* species were cultured from 3 of 19 (16 per cent) dogs with CEH-pyometra in that study (A. Mattson, P. Schultheiss and P. Olson, Fort Collins, CO, 1989, unpublished data).

The most common organism cultured from the uteri of female dogs with CEH-pyometra is *E. coli*, with mean reported prevalence of 70.3 per cent (n = 270).[11,13,48,49,51,52] (A. Mattson, P. Schultheiss and P. Olson, Fort Collins, CO, 1989, unpublished data). *Escherichia coli* colonizing the uterus affected with CEH in a given bitch ascend from the vagina, with origination on skin or in the bowel.[53] Biochemical fingerprinting of *E. coli* cultured from the uterus of a bitch with pyometra showed that the organisms were identical or very similar to *E. coli* isolated from feces of that same bitch.[54]

Escherichia coli is a gram-negative bacterium containing a chemically stable, biologically active lipopolysaccharide endotoxin in the cell membrane.[55,56] This endotoxin is released as the bacteria die and disintegrate.[55,56] Clinical endotoxemia occurs when serum levels exceed 0.053 ± 0.004 ng/ml.[57] Clinical signs of endotoxemia include hypothermia, disorientation, and signs of septic shock. The lethal concentra-

Figure 11–5. Chronic endometritis associated with Dow type IV pyometra. A layer of inflammatory cells is present in the uterine lumen (*arrows*). The endometrium (E) and myometrium (M) are abnormally thin. L, uterine lumen. Hematoxylin and eosin stain; 125×. (From Hardy RM, Osborne CA: Canine pyometra: Pathophysiology, diagnosis and treatment of uterine and extra-uterine lesions. J Am Anim Hosp Assoc 10:245–268, 1974, with permission.)

tion of endotoxin in serum is 0.7 to 1.0 ng/ml.[58] In a review of 92 female dogs with pyometra, 7 (8 per cent) had endotoxemia.[11] The release of endotoxin may be enhanced and clinical endotoxemia exacerbated by treatment of the bitch with coliform pyometra with antibiotics, because these drugs have no effect on endotoxin concentrations in serum and may increase endotoxin concentrations as bacteria die.[59]

Renal dysfunction is common in bitches with CEH-pyometra with *E. coli*, but renal failure, defined as elimination of greater than 75 per cent of the total renal capacity, is uncommon.[13] Renal abnormalities that may develop secondary to coliform pyometra include (1) prerenal azotemia resulting from dehydration or septic shock with subsequent decreased perfusion of normal glomeruli, (2) primary glomerular disease and decreased glomerular filtration rate (GFR), (3) decreased ability of renal tubules to concentrate urine as a result of endotoxemia, and (4) concomitant renal disease unrelated to pyometra, such as renal calculi or pyelonephritis.[13,18]

The large inflamed epithelial surface of the endometrium in bitches with CEH-pyometra provides continual exposure of *E. coli* antigens to the immune system and allows formation of high concentrations of circulating antibodies against *E. coli*.[13,18,60] Immunofluorescence studies have demonstrated deposition of antigen-antibody complexes in the glomeruli of bitches with coliform pyometra, with secondary membranous glomerulonephritis.[18,61] However, decreased GFR also has been demonstrated in nonazotemic bitches with coliform pyometra, suggesting that a functional decrease in GFR unrelated to glomerular damage also may occur.[49]

Decreased concentrating ability of the renal tubules has been shown to occur in bitches with pyometra caused by *E. coli* infection secondary to endotoxemia.[18] The loops of Henle, distal tubules, and collecting ducts have a decreased capacity to reabsorb water despite adequate circulating concentrations of antidiuretic hormone (renal diabetes insipidus).[13]

Other organ systems that may be affected in female dogs with CEH-pyometra include the bone marrow, in which hyperplasia of myeloid elements has been demonstrated, and the liver and spleen, in which extramedullary myelopoiesis may develop.[13] Unilateral or bilat-

Table 11–2. Aerobic Bacteria Cultured from the Uterus of Dogs with CEH-pyometra, Listed from Most to Least Prevalent

E. coli
Streptococcus species
Streptococcus canis
Staphylococcus species
Staphylococcus aureus
Staphylococcus intermedius
Klebsiella species
Proteus species
Pseudomonas species
Corynebacterium pyogenes
Enterococcus species
Pasteurella species
Serratia species
Haemophilus species
Bacillus species

Data from Fransson et al.,[11] Hardy and Osborne,[13] Dow,[17,36] A. Mattson, P. Schultheiss and P. Olson, Fort Collins, CO, 1989, (unpublished data), Láznička and Nesňalová,[44] Memon and Mickelsen,[45] Gandotra et al.,[46] Prescott et al.,[47] Vandeplassche et al.,[48] Stone et al.,[49] Nelson et al.,[50] Meyers-Wallen et al.,[57] and Wheaton et al.[52]

eral necrosis of the cortices and hemorrhage of the medulla of the adrenal glands also have been reported in female dogs with CEH-pyometra.[13]

SIGNALMENT

CEH-pyometra usually is a disease of adult bitches that have undergone repeated estrous cycling. It is a progressive disorder; mean age of bitches with Dow types I and II CEH-pyometra is 7.5 years, while mean ages of bitches with types III and IV CEH-pyometra are 8.0 years and 11.2 years, respectively.[17,36] Overall mean age reported for bitches diagnosed with pyometra is 7.25 years (n = 1079), with a reported range of 4 months to 16 years.[13,17,21,36,49,50,52,62–65] Occurrence in young dogs may be due to treatment with estrogens or progestins for estrus suppression or induction, contraception, or pregnancy termination. Six of eight dogs 3 years of age or younger diagnosed with pyometra in one study had received estradiol cypionate or megestrol acetate within the 6 months prior to presentation.[52]

Evidence of breed predisposition for CEH-pyometra is equivocal. Two studies (n = 73 and n = 395) demonstrated no breed predisposition.[21,52] However, a review of 487 cases of canine pyometra reported underrepresentation of dachshunds and fox terriers and overrepresentation of rottweilers, Saint Bernards, chow chows and Swedish hounds.[66] In another study, chow chows represented 1.6 per cent of the admitting population of female dogs but 6.4 per cent of the cases of pyometra diagnosed.[67]

DIAGNOSIS

History. Significant events that should be recorded in the history of the bitch with suspected CEH-pyometra include whelping history; prior treatment with estrogenic or progestogenic drugs for estrus suppression or induction, contraception, or pregnancy termination; and length of time elapsed since completion of the most recent estrus. False pregnancy does not predispose to CEH-pyometra; false pregnancy is a normal phenomenon occurring at the end of diestrus in bitches (see Chapter 13), and no correlation has been shown between incidence of false pregnancy and pyometra in the bitch.[62]

Lack of pregnancy in a cycling bitch before middle age may predispose her to pyometra later in life. In reviews of 100 and 172 female dogs with pyometra, 73 and 78 per cent, respectively, of the affected bitches were nulliparous.[17,36] Cause and effect between parity and development of CEH-pyometra is not known.

Treatment with estrogens or progestins may cause or exacerbate CEH-pyometra in female dogs.[50,51,64,68–70] Two of eight dogs treated with estradiol cypionate as a contraceptive while in diestrus developed pyometra in one study.[68] Of 358 dogs treated with estradiol benzoate 3, 5, and sometimes 7 days after mating, 26 (7.3 per cent) developed pyometra within 10 weeks.[69] Conversely, of 40 and 369 dogs with pyometra, 2.5 and 3.0 per cent, respectively, had received estrogens prior to presentation.[64,70] In those same populations of dogs, 7.5 and 12.0 per cent, respectively, had received a progestin, either megestrol acetate or medroxyprogesterone acetate, prior to presentation.[64,70]

The majority of bitches present with CEH-pyometra within 12 weeks of the onset of the previous estrus. Mean reported duration since completion of the most recent estrus is 5.7 weeks (n = 641), with a range of 1 week to 3 years.[13,17,21,52,63] In a review of female dogs with CEH-pyometra, 74 per cent (n = 369) of dogs presented within 8 weeks of completion of the prior estrus.[70] Reported mean duration of illness prior to presentation is 12.7 days, with a range of 1 to 180 days.[13]

Clinical Signs. Clinical signs of pyometra in the bitch vary with patency of the cervix. Bitches with open-cervix pyometra present with vulvar discharge and generally are less systemically ill than bitches with closed-cervix pyometra.[13,17,21,36,52,64,71,72] Vulvar discharge has been reported in 65 per cent (n = 395)[21] to 98 per cent (n = 40)[64] of bitches with CEH-pyometra. The vulvar discharge is copious, mucoid to purulent, varying in color from red-brown to yellow-green, and usually foul smelling.

Bitches with closed-cervix pyometra have minimal vulvar discharge, if any, and are more likely to present with abdominal distention as a result of the progressive enlargement of the uterus. Bitches with closed-cervix pyometra often exhibit more severe systemic signs of illness.

Clinical signs of pyometra in affected bitches include depression (73.1 per cent [n = 116][13] to 100 per cent [n = 49][17]), gastrointestinal signs such as vomiting and diarrhea (64.6 per cent [n = 116][13] to 70.0 per cent [n = 27][49]), and anorexia (65.0 per cent [n = 40][64] to 74.1 per

cent [n = 116][13]). Polyuria (5.0 per cent [n = 40][64] to 33.6 per cent [n = 116][13]) and polydipsia (5.0 per cent [n = 40][64] to 65.0 per cent [n = 49][17]) may occur in dogs with secondary renal disease.

Physical Examination. Physical examination of bitches suspected to have CEH-pyometra should include careful examination of the vulvar region and vagina to assess presence of purulent vulvar discharge, and careful abdominal palpation to attempt to ascertain uterine size and tone. Direct assessment of cervical patency is difficult in the dog. The length of the canine vagina, presence of the dorsal median postcervical fold obscuring the external cervical os, and perpendicular orientation of the cervical canal to the vagina and uterine body at all stages of the estrous cycle combine to effectively prevent routine visualization of the cervix.[38] Recent reports describe visualization of the cervix with a long, narrow-diameter rigid endoscope, and collection of uterine cytology and culture specimens by passage of a 4- to 7-Fr polypropylene urinary catheter through the cervix and infusion and aspiration of several milliliters of sterile saline.[73,74] Most practitioners do not have this technology available and so must rely on presence of vulvar discharge and uterine size to infer cervical patency. The size of the uterus is inversely proportional to the degree of cervical patency.[17] Cervical patency may vary during the course of disease.

The abdomen may be difficult to palpate in obese, tense, or painful dogs, or in dogs with abdominal distention resulting from uterine enlargement. Caution is recommended when attempting to palpate the distended, friable uterus of dogs with closed-cervix pyometra. Palpable uterine enlargement is reported in 28 per cent (n = 40)[64] to 31 per cent (n = 109)[13] of dogs with pyometra. Unilateral uterine horn enlargement was reported in 3 of 100 dogs with pyometra,[17] and unilateral pyometra occasionally is observed in bitches with normal pregnancy in the contralateral horn. The uterine horns may be diffusely enlarged or may contain annular constrictions, mimicking pregnancy (Fig. 11–6).

Dehydration was reported on physical examination of 27.5 per cent of 109 dogs with pyometra.[13] Dogs may be febrile on presentation.[13,21] Severely ill dogs and dogs with endotoxemia are hypothermic; 2.0 per cent (n = 395),[21] 3.4 per cent (n = 109),[13] and 6.0 per cent (n = 49)[17] of bitches with pyometra were reported to have temperatures of 100° F or less at presentation. Severe endotoxemia is associated with high operative mortality at OHE.[17]

Laboratory Findings. Cytology of specimens of vulvar discharge originating in the uterus reveals full fields of degenerative neutrophils and bacteria. Polymorphonuclear cells are the most common leukocyte in the uterus of normal dogs during diestrus, so their presence alone does not indicate inflammatory uterine disease.[71] Specimens for aerobic culture of vaginal discharge originating from the uterus should be collected from as far anteriorly in the vagina as possible to minimize contamination with normal vaginal flora. The secondary infection of CEH-pyometra is caused by organisms from the vaginal flora, most commonly *E. coli* (71.1 per cent; n = 250).[11,13,48,49,51,53] Significance of the culture result depends on extent of growth, with moderate to heavy growth of a pure culture most likely to be significant. Culture and sensitivity always should be performed to allow choice of an appropriate antibiotic for therapy. Antibiotic treatment is indicated whether the CEH-pyometra is resolved medically or surgically.

A hallmark of CEH-pyometra is peripheral leukocytosis. The increase in WBC count is less extreme in open-cervix than closed-cervix pyometra.[46,75] Neutropenia may be present in animals with endotoxemia. Overall reported mean WBC count in dogs with CEH-pyometra is 37,108 cells/mm^3 (n = 531), with a range of 2500 to 196,800 cells/mm^3.[13,21,49] A left shift was reported in 70 per cent (n = 395),[21] 75 per cent (n = 40),[64] and 87 per cent (n = 73)[52] of dogs with CEH-pyometra. Mean number of bands reported is 520 cells/mm^3, with a range of 0 to 4300 cells/mm^3.[13] Toxic change may be visible in polymorphonuclear cells of dogs with pyometra, and degree of toxic change is correlated with severity of disease.[76]

Mild normocytic, normochromic anemia was reported in 25.0 per cent (n = 101)[13] to 25.7 per cent (n = 73)[52] of dogs with CEH-pyometra. Mean packed cell volume reported was 38 per cent, with a range of 21 to 48 per cent.[49]

Abnormalities that may be evident on a serum chemistry profile include azotemia, hypergammaglobulinemia, and hypoalbuminemia. Prerenal azotemia is present in dehydrated animals. Azotemia secondary to renal dysfunction caused by damage resulting from glomerular deposition of immune complexes and endotoxemia in dogs with *E. coli* pyometra

Figure 11–6. A uterus from a 9-year-old Saint Bernard with pyometra. This organ contained 2.6 L of pus. The uterus externally resembles a pregnant uterus because of the regions of distention and constriction. (From Gilbert RO: Diagnosis and treatment of pyometra in bitches and queens. Compend Contin Educ Pract Vet 14:777–784, 1992, with permission.)

also may occur. Blood urea nitrogen (BUN) concentrations are reported to be elevated in 17.7 per cent (n = 79),[13] 26.0 per cent (n = 27),[49] and 27.0 per cent (n = 73)[52] of dogs with pyometra. Mean reported BUN is 33 mg/dl, with a range of 21 to 119 mg/dl (n = 100).[17]

Hypergammaglobulinemia is reported to occur in 27.0 per cent (n = 26) of dogs with CEH-pyometra,[49] and is more severe in dogs with closed-cervix pyometra than in bitches with open-cervix pyometra.[77] Hypergammaglobulinemia may be reflected as increased erythrocyte sedimentation rate.[65] Hypoalbuminemia is reported to occur in 23.0 per cent (n = 26) of dogs with CEH-pyometra.[49] Endotoxemia may be present in dogs with *E. coli* pyometra.[78]

The acid-base abnormality most commonly reported in dogs with CEH-pyometra is metabolic acidosis.[79–81] Both respiratory acidosis and alkalosis are reported.[80,81]

Decreased urine specific gravity is reported in 20.4 per cent (n = 109)[13] of dogs with CEH-pyometra. Proteinuria also may be present.[13,82] Urinary γ-glutamyltransferase, an indicator of proximal renal tubular damage, was elevated in 37 of 75 bitches with CEH-pyometra.[82] Bilirubinuria was identified in 30 per cent (n = 34) of bitches with pyometra; because it was not associated with bilirubinemia, it was attributed to cholestasis, not to hepatic disease.[83]

Diagnostic Imaging. Abdominal radiography of affected bitches is indicated for assessment of uterine size, but radiography does not differentiate uterine enlargement caused by pyometra from pregnancy until after fetal mineralization occurs (42 to 45 days after ovulation or 43 to 54 days after breeding). Uterine enlargement is more easily assessed on lateral than on ventrodorsal radiographic projections.[84] Hysterography, distention of the uterus with contrast medium, may allow definition of CEH, with a corrugated appearance of the endometrium if the cervix is open.[85]

Ultrasonography is the preferred imaging technique for diagnosis of CEH-pyometra. Uncomplicated CEH appears as a fluffy thickening of the endometrium, peppered with hypoechoic areas of varying size (Fig. 11–7). Extensive CEH may nearly obliterate the uterine lumen.[72] Pyometra appears as an enlarged uterus with convoluted, tubular horns filled with anechoic to hypoechoic fluid (Fig. 11-8).[72,86]

Differential Diagnosis. Differential diagnoses for open-cervix pyometra include those for uterine enlargement and/or vulvar discharge (see Chapter 12). Differential diagnoses for either open- or closed-cervix pyometra include those for uterine enlargement, especially pregnancy, and those for polyuria and polydipsia, such as diabetes mellitus, hyperadrenocorticism, renal disease, and diabetes insipidus. Pregnancy can be ruled out by abdominal ultrasound after 25 days postovulation. Causes of polyuria and polydipsia other than pyometra usually are not associated with neutrophilia.

TREATMENT

Surgical Therapy. Ovariohysterectomy is the treatment of choice for CEH-pyometra in the bitch, regardless of cervical patency. Medical treatment of closed-cervix pyometra with ecbolic agents, although effective in some bitches, may cause peritonitis as a result of uterine rupture or leakage of mucopurulent uterine fluid through the uterine tubes.[50,75,87,88] The authors strongly recommend OHE for all

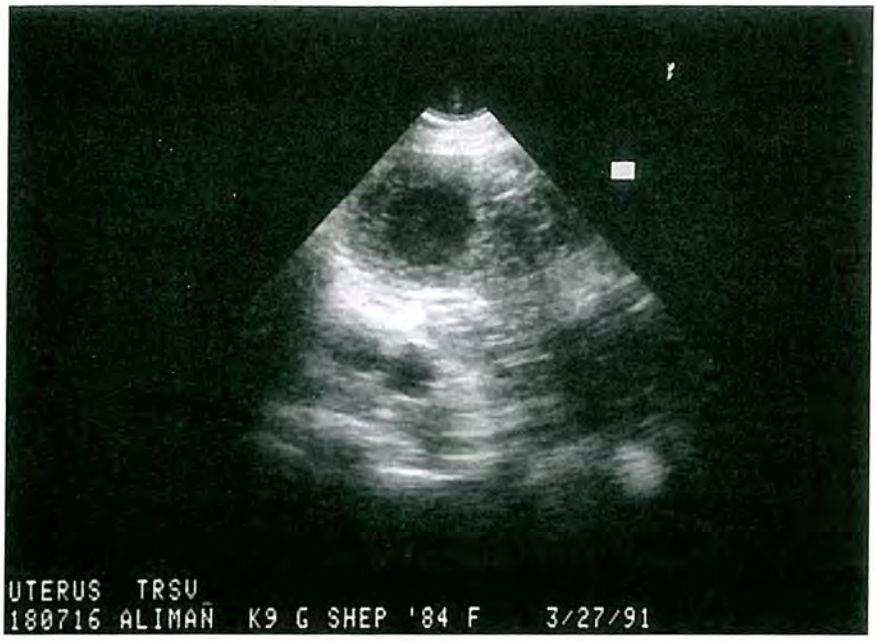

Figure 11–7. Ultrasonograph of transverse plane of uterus with cystic endometrial hyperplasia. Note uniform accentuation of endometrium.

cases of closed-cervix pyometra. Open-cervix pyometra may be managed more safely with medical treatment than closed-cervix pyometra, but the dog with CEH-pyometra may not regain a normal endometrium and normal fertility.[51,64,87,89] OHE is strongly recommended for cases of open-cervix CEH-pyometra as well.

Ovariohysterectomy in dogs with CEH-pyometra should be performed after stabilization of the bitch, if possible. A specimen of

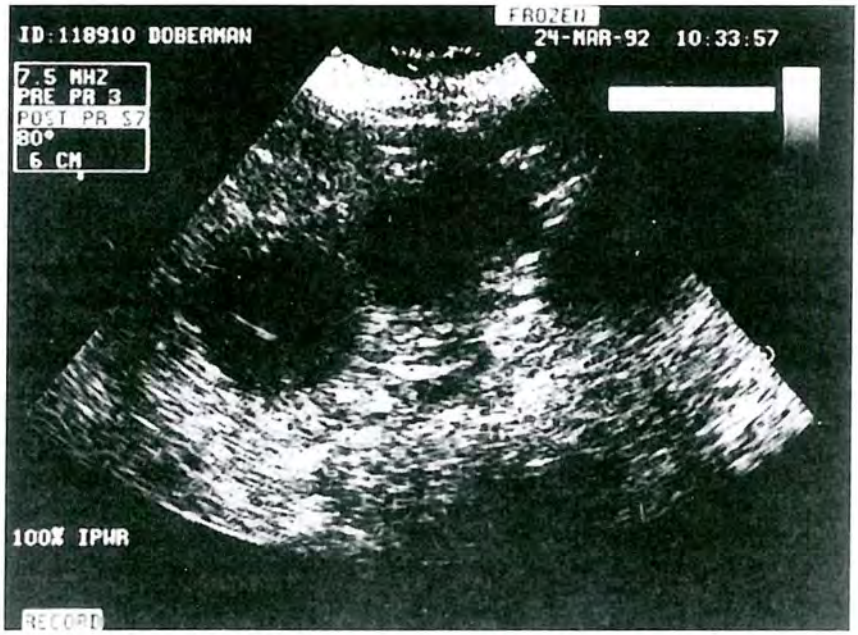

Figure 11–8. Ultrasonograph of the abdominal region of a bitch with an advanced case of pyometra. The coils of uterine horn, filled with fluid, are seen in cross section as anechoic areas. (From Renton JP, Boyd JS, Harvey MJA: Observations on the treatment and diagnosis of open pyometra in the bitch (Canis familiaris). J Reprod Fertil Suppl 47:465–469, 1993, with permission.)

vulvar discharge, if any, should be collected, and empirical treatment with broad-spectrum antibiotics (ampicillin, 10 mg/lb three times daily) instituted, pending culture and sensitivity results.

Surgical technique for OHE in bitches with pyometra is the same as for elective OHE. Caution should be used when handling a distended, friable uterus. Evidence of uterine rupture, such as a visible tear in the uterus or presence of mucopurulent fluid in the abdomen, is a poor prognostic indicator for survival.[17,52,90] A specimen of abdominal fluid should be collected for culture and sensitivity if there is evidence of leakage of uterine content. In a review of 30 dogs with CEH-pyometra, 13 per cent had bacterial growth from abdominal fluid collected at OHE.[48]

Success rates reported for OHE as a treatment for CEH-pyometra are 83 per cent (n = 395),[21] 90 per cent (n = 73),[52] and 100 per cent (n = 10).[91] In a review of 73 dogs ovariohysterectomized as treatment for CEH-pyometra, 4 (5 per cent) died intraoperatively or immediately postoperatively; 1 had a ruptured uterus, 1 had concurrent hyperadrenocorticism, and 2 had concurrent primary renal disease.[52] Long-term complications occurred in three animals. All were related to septicemia in bitches with *E. coli* pyometra and included intracranial emboli and osteomyelitis.[52]

Postoperative management following OHE in bitches with pyometra includes supportive care and pain management as needed. Antibiotic therapy should be continued for 7 to 10 days after OHE.[75] Total WBC count cannot be used to measure response to treatment, because it continues to rise after surgery until myelopoiesis declines.[13]

Other reported surgical options for treatment of CEH-pyometra in bitches include ovariectomy,[92] intrauterine placement of a Foley catheter through the cervix to promote expulsion of intrauterine contents,[93] and laparoscopic OHE.[94]

Medical Therapy. Medical therapy for CEH-pyometra may be appropriate in bitches that (1) are of breeding age; (2) are vital to the breeding program in that kennel; (3) are not systemically ill, with normal BUN and no evidence of endotoxemia, such as hypothermia; and (4) have an open cervix, evidenced by presence of vulvar discharge. If these criteria are not met, medical therapy for CEH-pyometra is discouraged.

Antibiotic therapy is an integral part of treatment for pyometra but usually will not effect a cure by itself unless uterine diameter is normal.[75] A specimen of vulvar discharge for culture of aerobic and anaerobic organisms and mycoplasma should be collected at admission, and empirical therapy with a broad-spectrum antibiotic (ampicillin, 10 mg/lb three times daily) instituted, pending culture and sensitivity results.

Prostaglandin $F_{2\alpha}$. The preferred drug for medical treatment of CEH-pyometra in dogs is prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse, Pharmacia & Upjohn, Peapack, NJ). $PGF_{2\alpha}$ induces luteolysis, increases myometrial contractility, promotes expulsion of uterine contents, and may enhance cervical relaxation. After day 5 of diestrus, $PGF_{2\alpha}$ is luteolytic, with effect dependent on dose and frequency of treatment. Lysis of the CL, which are the site of production of progesterone in the diestrous bitch, decreases serum progesterone concentration and minimizes progesterone-mediated hypertrophy and secretion from the endometrial glands and myometrial quiescence.

Treatment regimens reported for treatment of CEH-pyometra with $PGF_{2\alpha}$ are described in Table 11–3.[50,51,63,64,87–89,95,96] Preferred treatment algorithm of the authors is depicted in Figure 11–9. Serum progesterone concentration should be measured in bitches with CEH-pyometra prior to initiation of $PGF_{2\alpha}$ therapy. If the serum progesterone concentration is greater than 1 ng/ml, a luteolytic dose of $PGF_{2\alpha}$ should be used (100 μg/kg subcutaneously three times daily for 2 days followed by 200 μg/kg subcutaneously three times daily to effect). Doses of 10 to 500 mg/kg with frequency of one to three times daily have been reported. A general trend toward increased recovery rate with higher doses exists, but higher doses may be associated with more severe side effects. Expulsion of uterine contents occurred at doses of $PGF_{2\alpha}$ as low as 20 μg/kg.[89] Clinical response, such as increased appetite and improvement in attitude, occurred on average 48 hours after treatment was instituted.[50] Decrease in WBC count was not evident until 7 days after start of treatment.[51] Duration of treatment ranged from 1 to 26 days, with most protocols at 3 to 5 days of treatment with $PGF_{2\alpha}$.[50,51,63,64,87–89,95,96] Treatment should be continued until a decrease in uterine size to near normal is determined by palpation or ultrasound. Prognosis for future fertility declines if 6 or more days of treatment are required.[88]

Table 11-3. Use of Prostaglandin $F_{2\alpha}$ for Medical Treatment of CEH-pyometra in Dogs

Dose $PGF_{2\alpha}$ (Subcutaneous)	Recovery Rate (%)	Side Effects	Recurrence Rate (%)	Fertility Rate after Treatment (%)	Study
10–50 μg/kg once daily × 4–5 d	90 (n = 10)	Restlessness, hypersalivation, vomiting, diarrhea	11 (n = 9)	71 (n = 7)	Rudd and Kopcha[87]
20 μg/kg three times daily × 8 d	53 (n = 15)	None	—	—	Nolte et al.[95]
20 μg/kg three times daily × 8 d	75 (n = 12)	None	—	86 (n = 7)	Hubler et al.[89]
26.8–258 μg/kg once daily (n = 7), twice daily (n = 32) or three times daily (n = 1) × 2–26 d	83 (n = 40)	—	10 (n = 20)	68 (n = 19)	Gilbert et al.[64]
50–100 μg/kg once to three times daily × 3–10 d	—	—	—	—	Purswell[88]
100–500 μg/kg once daily × 5 d, repeated after 1–2 wk if necessary	46 (n = 15)	Restlessness, hypersalivation, vomiting, diarrhea	11 (n = 15)	—	Nelson et al.[30]
250 μg/kg once daily × 5 d	75 (n = 8)	—	—	100 (n = 1)	Renton et al.[63]
250 μg/kg once daily × 5 d	93 (n = 44)	—	—	90 (n = 42)	Nelson and Feldman[96]
250–500 μg/kg once daily × 3 d	100 (n = 10)	—	40 (n = 9) within 1 yr, 77 (n = 9) by 27 mo after treatment	55 (n = 10)	Meyers-Wallen et al.[51]

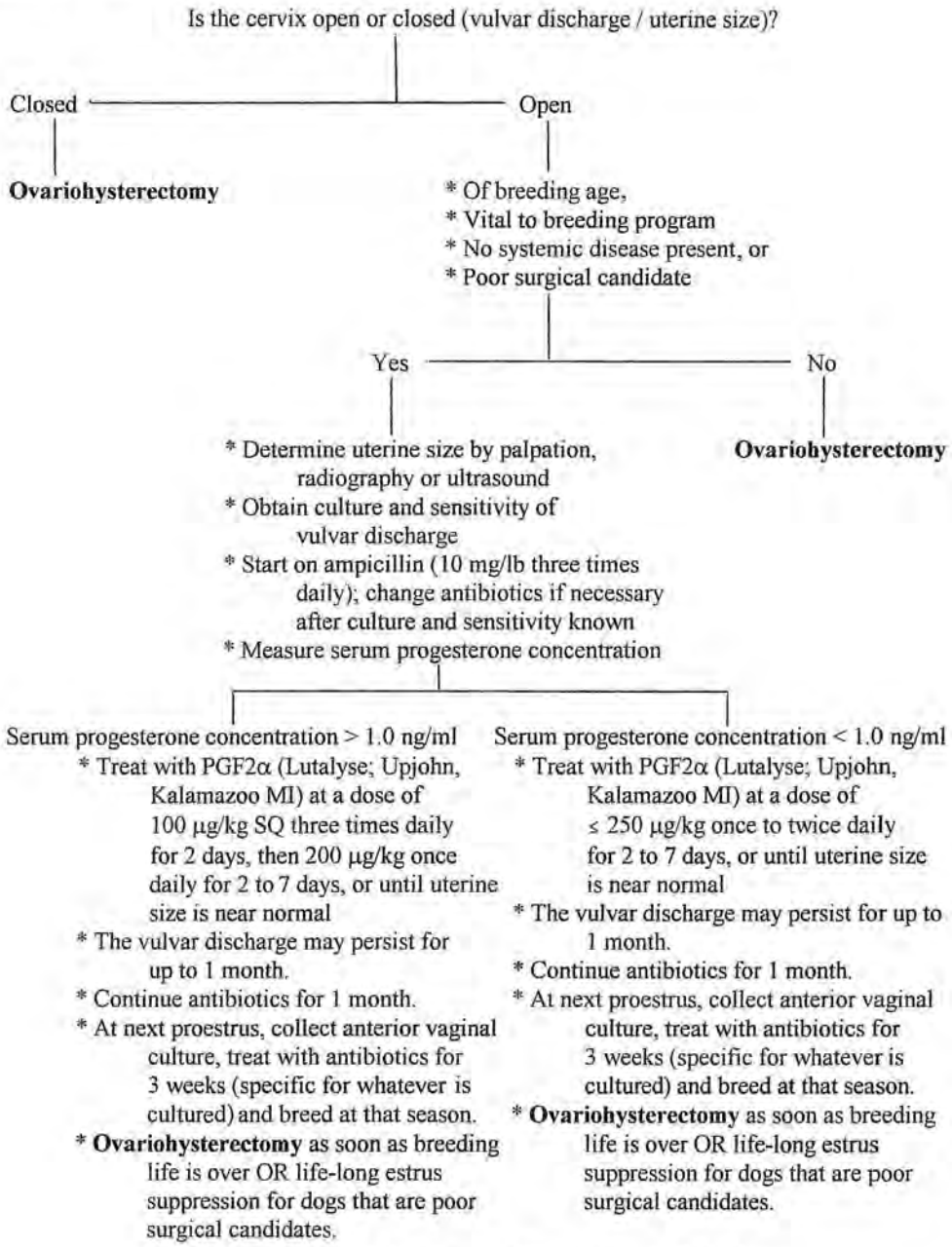

Figure 11–9. Algorithm for treatment of CEH-pyometra in the bitch.

Percent recovery is less than 100 in most reports of treatment of CEH-pyometra with $PGF_{2\alpha}$ in the dog (Table 11–3). Reported reasons for treatment failure include persistent uterine enlargement and deterioration of the bitch's health early in treatment, necessitating OHE.

Side effects of $PGF_{2\alpha}$ in the dog are referable to its causing smooth muscle, contraction and include hypersalivation, restlessness, vomiting, and diarrhea.[45,50,87] Side effects occur within 5 minutes and subside within 60 minutes after administration of $PGF_{2\alpha}$.[45] Severity is dose dependent and decreases with treatment course.[50,87] Side effects may be decreased in occurrence and severity by walking the bitch for 20 to 40 minutes after administration of $PGF_{2\alpha}$.[45,75]

Recurrence is reported in 10 to 77 per cent of bitches with CEH-pyometra treated with

$PGF_{2\alpha}$ (Table 11–3). Recurrence is likely because there is no treatment that will reverse CEH except, perhaps, pregnancy. However, recurrences during pregnancy and in estrous cycles following pregnancy have been reported.[51] Dogs with medical resolution of CEH-pyometra may have a decrease in disease to a subclinical level; in three bitches with recurrent CEH-pyometra, the antibiotic sensitivity patterns to *E. coli* isolated in both episodes were similar, suggesting recrudescence rather than separate episodes of disease.[51]

Fertility after medical treatment for CEH-pyometra is decreased compared to that in normal bitches (Table 11–3). Reported conception rates are altered by the fact that many bitches are not rebred after treatment for pyometra. Hypothesized causes of failure to reproduce in bitches rebred after treatment with $PGF_{2\alpha}$ for CEH-pyometra include failure to conceive, early embryonic death, and abortion. Whether decreased fertility is due to subclinical infection, persistent endometrial pathology, or both, may vary by bitch.

Although OHE is strongly recommended as the treatment for closed-cervix pyometra, there are reports of successful treatment of dogs with closed-cervix pyometra with $PGF_{2\alpha}$. Of seven bitches described, two had a successful resolution. A 4-year-old golden retriever with closed-cervix pyometra, treated with 25 to 250 μg/kg $PGF_{2\alpha}$ subcutaneously twice daily for 7 days, exhibited vulvar discharge by day 2, decreased uterine size by day 5, and clinical improvement by day 6, and whelped eight pups after breeding at the subsequent estrus.[45] One bitch with closed cervix CEH-pyometra responded to $PGF_{2\alpha}$ treatment, but required two 5-day courses of treatment with a break of 1 to 2 weeks between treatments.[50] Of five bitches treated medically for closed-cervix pyometra unsuccessfully, one developed cerebellar signs after 1 day of treatment that resolved after OHE, one did not show a decrease in uterine size even after three 5-day treatment courses, one died with no cause described, and two developed peritonitis.[50,88,96] No uterine tear was evident in either dog with peritonitis, and one had concurrent salpingitis with mucopurulent discharge in one uterine tube and ovarian bursa, suggesting that uterine contractions forced purulent fluid from the uterus through the uterine tube and ovarian bursa into the peritoneal cavity.[50,88]

Combined therapy using $PGF_{2\alpha}$ at an increasing dose of 10 to 25 μg/kg subcutaneously twice to three times daily for 5 to 7 days and a dopamine agonist, either bromocriptine (25 μg/kg twice to three times daily) or cabergoline (5 μg/kg once daily), has been described. (C. Gartley and J. Verstegen, personal communication, 1999). Dopamine agonists inhibit the release of luteotropic prolactin, causing luteolysis, and are therefore best used in bitches being treated for CEH-pyometra in diestrus.

Other Medical Treatment Agents. Cloprostenol, a prostaglandin analog, has been described for medical treatment of dogs with CEH-pyometra.[91,97] The reported dose is 10 μg/kg subcutaneously twice daily for 9 to 15 days.[91] Recovery rate in a study investigating treatment of induced pyometra was 60 per cent (n = 10) with cloprostenol, compared to 100 per cent for OHE.[91] One dog died of shock after 2 days of treatment with cloprostenol. Side effects were similar to those of $PGF_{2\alpha}$, with the addition of restlessness followed by severe depression for 1 to 2 hours.[91] Cloprostenol is not recommended for treatment of pyometra in dogs.

Antiprogestins competitively bind progesterone receptors and decrease intrauterine progesterone concentrations, potentially allowing increased myometrial contractility and cervical relaxation.[70] Two antiprogestins, RU46534 and aglepristone, have been described for treatment of CEH-pyometra in dogs[70,98] (C. Gartley and J. Verstegen, personal communication, 1999). RU46534 caused significant vulvar discharge within 12 to 48 hours, clinical improvement, return to normal circulating WBC count by the sixth day of treatment, and complete uterine evacuation within 12 days in dogs with either open- or closed-cervix pyometra.[70,98] No side effects were noted, and both dogs bred at the next estrus whelped normal litters.[70,98] Aglepristone is used in combination with $PGF_{2\alpha}$ (C. Gartley and J. Verstegen, personal communication, 1999). Antiprogestins are not available for veterinary use in the United States.

Estrogen promotes cervical relaxation and increases myometrial contractility.[13] Testosterone (testosterone propionate, 25 mg twice weekly) was reported to cause resolution of pyometra within 3 weeks in 7 of 10 dogs treated.[99] Estrogens and androgens are neither approved nor recommended as medical treatments for CEH-pyometra in dogs.

Ecbolic agents such as oxytocin and ergot alkaloids are not as effective as $PGF_{2\alpha}$ for treatment of CEH-pyometra.[75] Although degree

and duration of uterine contractility induced by oxytocin is similar to that induced by $PGF_{2\alpha}$ at 30 days postestrus, both parameters decline significantly by 60 days postestrus in dogs treated with oxytocin compared to those treated with $PGF_{2\alpha}$.[100]

There is one report of the use of equine-derived hyperimmune serum to treat endotoxemia in dogs with CEH-pyometra caused by *E. coli* infection.[101] Treatment with hyperimmune serum either intravenously or subcutaneously decreased serum endotoxin concentrations to a greater degree than did conventional therapy alone, consisting of OHE, supportive care, and antibiotic therapy.[101]

PREVENTION

Prevention of CEH-pyometra is not routinely practiced. Estrus suppression, with a subsequent decline in exposure of the endometrium to estrogen followed by progesterone, may slow development of CEH. However, megestrol acetate, one of two pharmaceutial products sold in the United States for canine estrus suppression is a progestin itself and is not recommended for use for more than two consecutive cycles. Mibolerone is not approved for use in breeding dogs, although mibolerone has not been observed or reported to adversely affect reproduction. Once a dog has been treated for pyometra, she is predisposed to its development at each subsequent estrus. Desired breedings for the bitch that has been treated medically for CEH-pyometra should be accomplished as quickly as possible and the bitch spayed as soon as her breeding life is over. If an estrous cycle must be bypassed without breeding, appropriate antibiotic therapy while vulvar discharge is visible, and the cervix open, may prevent development of secondary infection. Dogs that are poor surgical candidates may require estrus suppression with mibolerone for life after medical treatment for CEH-pyometra in order to prevent recurrence.

Uterine Stump Pyometra

Uterine stump pyometra is development of CEH-pyometra in uterine tissue left behind after OHE. Pathogenesis is the same as for CEH-pyometra in intact animals. Functional remnants of ovarian tissue are reported to occur in 58 per cent (n = 19)[102] and 71 per cent (n = 7)[103] of dogs with uterine stump pyometra. Clinical signs include vulvar discharge, depression, and anorexia.[104,105] Total WBC count and total plasma protein usually are elevated.[105] Retrograde vaginography may reveal arborization of contrast medium into the infected uterine stump. Abdominal ultrasound reveals a pattern similar to pyometra in intact bitches, with single or multiple fluid-filled areas adjacent to the urinary bladder.[105] Treatment is surgical resection of the uterine stump and residual ovarian tissue, if any, and appropriate antibiotic therapy.[104,106]

Other Uterine Disorders

ADENOMYOSIS

Adenomyosis is growth of hyperplastic endometrial glands into the myometrium.[4] It is not associated with infection.[11] Clinical significance is unknown.

SEROSAL CYSTS

Serosal cysts are fluid-filled structures protruding from the serosal surface of the uterus.[107] Serosal cysts form as a result of rapid contraction of the myometrium with mesothelial infolding during postpartum involution, and usually are seen in older, pluriparous bitches.[107] These usually are incidental findings at OHE or laparotomy. Clinical significance is unknown.

METRITIS

Metritis is inflammation of the uterus. Endometritis, inflammation of the endometrium or uterine lining, is most commonly described, and usually is due to uterine infection with vaginal organisms ascending through the open cervix after whelping (see Chapter 7).

SUBINVOLUTION OF PLACENTAL SITES

Subinvolution of placental sites is a postpartum disorder characterized by prolonged sanguineous vaginal discharge after whelping (see Chapter 7).

UTERINE PROLAPSE

Prolapse of the uterus most commonly occurs near the time of parturition in dogs. If the tissue is devitalized by the time of diagnosis, hysterectomy is the treatment of choice (see Chapter 7).

UTERINE NEOPLASIA

Uterine neoplasia is uncommon in female dogs, with reported incidences among tumors of the female reproductive tract of 1.0 per cent (n = 266),[108] 2.0 per cent (n = 535),[109] 9.4 per cent (n = 117),[110] 11.0 per cent (n = 96),[111] and 19 per cent (n = 115).[112] It is most common in bitches 10 years of age or older.[110] The boxer breed was over-represented in one review; no breed predisposition has been published in other studies.

Benign uterine tumors that have been reported include leiomyoma (fibroid), fibroma, fibroleiomyoma, fibromyoma, fibroadenoma, adenoma, lipoma, and endometrial polyp.[4,110,112–120] Leiomyoma is the most common uterine tumor in dogs.[111,112] Leiomyomas usually are incidental findings at OHE, and do not appear to interfere with pregnancy.[114]

Endometrial polyps are benign, focal, cystic proliferations of the endometrial glands associated with stromal changes.[119,120] They are broad based or pedunculated and may project into the uterine lumen, appearing ultrasonographically as multilobular masses containing numerous small cysts.[119,120] Affected bitches usually are asymptomatic, although torsion of a pedunculated endometrial polyp was reported to induce rapid hemorrhagic shock and death in a dog in one case report.[4,119]

Malignant uterine tumors reported in the dog include adenocarcinoma, endometrial carcinoma, undifferentiated carcinoma, lymphosarcoma, metastatic transmissible venereal tumor, and metastatic dysgerminoma.[111,118,121–126] Female dogs with malignant uterine adenocarcinoma may present with clinical signs including persistent bloody or purulent vulvar discharge, dysuria, hematuria, lethargy, anorexia, and abdominal distention.[121,122] Uterine adenocarcinoma usually is a solitary, nodular tumor that may resemble CEH grossly. Definitive diagnosis requires excision and histopathology.

Treatment for uterine neoplasia is surgical removal (OHE). Prognosis for benign uterine neoplasia is excellent. Prognosis for malignant uterine neoplasia is dependent on tumor type, degree of local invasion, and extent of metastasis.

UTERINE LITHIASIS

A 7-year-old intact female crossbred dog presenting with lethargy, ataxia, and polydipsia was diagnosed with diabetes mellitus, cardiomyopathy, and palpably enlarged uterine horns. At ovariohysterectomy, CEH and numerous 2- to 3-mm calculi containing calcium, oxalate, phosphate, and magnesium were identified in the uterus.[127]

Disorders of the Uterine Tube (Oviduct)

Abnormalities of the uterine tubes that have been described in the dog include (1) developmental abnormalities, such as remnants of the paramesonephric and mesonephric ducts; (2) uterine tube cysts; (3) salpingitis; and (4) growth disorders, such as hyperplasia and neoplasia.[50,128] Uterine tube cysts vary in size and location and have not been reported to occlude the lumen. The authors are unaware of reports of salpingitis unassociated with pyometra in the dog. Mild suppurative inflammation of the uterine tube was reported in 20 per cent and severe suppurative inflammation of the uterine tube in 5 per cent of 20 dogs with CEH-pyometra in one study (A. Mattson, P. Schultheiss and P. Olson, Fort Collins, CO, 1989, unpublished data). Salpingitis also has been associated with forceful expulsion of mucopurulent fluid through the uterine tube after administration of $PGF_{2\alpha}$ to a dog with closed-cervix pyometra.[50] Neoplasms reported in the canine uterine tube include adenoma, fibroadenoma, adenomyoma, adenomatous papilloma, lipoma, adenocarcinoma, and metastatic granulosa cell tumor.[128,129]

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Chapter 12

Disorders of the Canine Vagina, Vestibule, and Vulva

Vulvar Discharge

Vulvar discharge in dogs may occur during normal physiologic conditions, such as estrus or parturition, or secondary to abnormalities of the ovaries, uterus, cervix, vagina, vestibule, or urinary tract, or coagulopathy. Determination of the origin and etiology of vulvar discharge requires assessment of history and physical examination findings, evaluation of vaginal cytology specimens and imaging of the reproductive tract (Table 12-1).

Increasing cornification of vaginal epithelial cells is indicative of increasing serum estrogen concentrations as occurs during proestrus/estrus or as a manifestation of ovarian remnant syndrome, ovarian cystic disease or functional ovarian tumors. Differentiation of these conditions is based on duration of serosanguineous vulvar discharge; in dogs with ovarian cystic disease or functional ovarian tumors, sanguineous vulvar discharge is present for longer than 6 weeks.

If significant cornification of vaginal epithelial cells is not present, signalment of the bitch and history may allow differentiation of non-estrogen-dependent conditions causing vulvar discharge in bitches. Uterine disorders are seen only in bitches that are intact or in uterine stump infections in spayed bitches, while estrogen-responsive urinary incontinence is most common in ovariohysterectomized bitches. Prepuberal bitches may exhibit the scant, tacky discharge of juvenile vaginitis. Aged bitches are more likely to develop neoplasia than are younger bitches. Specific coagulopathies are more prevalent in some breeds, such as von Willebrand's disease in Doberman pinschers. A history of late-term abortion should raise suspicion of canine brucellosis as a causative agent. Postpartum conditions causing vulvar discharge in bitches include metritis and subinvolution of placental sites. Pyometra is a diestrual disorder; definitive diagnosis requires confirmation of uterine enlargement, purulent vulvar discharge (open cervix), and elevated white blood cell count with a left shift. For a more complete description of these disorders, please refer to the appropriate chapters (Table 12–1).

Inflammatory and infectious disorders of the genitourinary tract cannot be confirmed by direct microbial culture. The canine uterus is virtually inaccessible because of the length of the vagina; the presence of the dorsal median postcervical fold, a nonelastic structure composed of smooth muscle and collagen that extends caudally from the external cervical os on the dorsal midline, effectively preventing visualization and catheterization of the cervix[1]; and the tight closure of the vertically oriented abdominal cervix throughout most of the estrous cycle. Because of the difficulty in obtaining uterine culture specimens, vaginal cultures often are performed to infer presence of uterine infection or diagnose vaginal infection. Unfortunately, the canine vagina is not a sterile environment, so one can never be certain of the origin (uterus or vagina) of an organism isolated.

Normal vaginal flora originate in the bowel and on the skin.[2] The aerobic organisms most commonly cultured from vaginal swabs in healthy bitches are *Escherichia coli*, *Streptococcus* species, *Pasteurella* species, and *Staphylococcus* species (Table 12–2).[3–11] Normal population distribution varies with sexual status (prepuberal versus adult)[5] and may vary with stage of the estrous cycle.[4,5] Anaerobic bacteria also have been cultured from the vaginas of normal bitches.[5,6,11]

Table 12–1. Differential Diagnosis of Vulvar Discharge in the Bitch

Condition	History	Physical Examination Findings	Gross Appearance of Vulvar Discharge	Cytologic Appearance of Vulvar Discharge	Comments	Chapter Reference for Additional Information
NORMAL PHYSIOLOGIC CONDITIONS						
Proestrus	Time correct for onset of estrous cycle	Vulva swollen	Serosanguineous	0-100% cornification, ± PMNs	Intact females—male dogs attracted to bitch	2
Estrus	Time correct for onset of estrous cycle	Vulva swollen	Serosanguineous to straw colored	100% cornification with >50% anuclear squame cells, no PMNs, ± cocci	Intact females—bitch will allow male to mount and breed	2
Diestrus	Has just "gone out of heat"	—	Scant, mucoid to dark red	0% cornification, many healthy PMNs early in stage	—	2
Late pregnancy	Due to whelp within 1 wk	Mammary development ± milk	Scant, mucoid	0% cornification, few healthy PMNs	—	5
Parturition	At term	Mammary development ± milk	Clear to green-black as placentas separate	0% cornification, few healthy PMNs	Restlessness, panting → abdominal contractions	6
Normal lochia	One to 3 wk post partum	Mammary development ± milk	Red to green to brown	% cornification, RBC's, PMNs, degenerating	—	6
PATHOLOGIC CONDITIONS						
Ovarian cystic disease	Combined length of proestrus and estrus >6 wk	Vulva swollen	Serosanguineous	100% cornification with >50% anuclear squame cells, no PMNs, ± cocci	Intact bitch—May respond to therapy for ovulation induction	10
Brucellosis	Late-term abortion or persistent vulvar discharge	—	Mucosanguineous to purulent	0% cornification, many degenerative PMNs	Culture vulvar discharge early in infection, serologic tests accurate 8–12 wk after exposure	11
Metritis	Postpartum—history of retained placentas, dystocia	—	Purulent	0% cornification, many degenerative PMNs	—	11

Pyometra (open cervix)	During or after diestrus—may be systemically ill (PU/PD, lethargic)	Uterus palpable per abdomen	Purulent	0% cornification, many degenerative PMNs	White blood cell count greatly increased; OHE recommended therapy	11
Subinvolution of placental sites	Postpartum—discharge persistent >3 wk after whelping	—	Sanguineous	0% cornification, many RBCs, few healthy PMNs	Usually resolves spontaneously; monitor PCV	7
Neoplasia of uterus, vagina or urinary tract	—	Neoplasm may be palpable per abdomen (uterine) or per rectum (urinary tract), or visible (vaginal)	Sanguineous to mucopurulent	0% cornification, ± PMNs, ± RBCs	More common in older bitches; vaginal and urinary tract neoplasia may occur in spayed bitches	11 (Uterine) 12 (Vaginal)
Vaginitis	Prepuberal or adult onset	—	Mucoid and tacky to mucopurulent	0% cornification, ± healthy PMNs	May resolve spontaneously or after estrus; look for concurrent anatomic abnormalities or urinary tract disease	12
Vestibulitis	—	Scooting, vulvar licking, vulvar discharge	Variable	0% cornification	Diagnose with vaginoscopy	12
Estrogen-responsive urinary incontinence	Urinary leakage where dog sleeps	—	—	0% cornification, ± PMNs	Spayed females, especially those over 20 kg in weight—may respond to treatment with oral estrogens or sympathomimetics	9
Coagulopathy	Bleeding from one or more body orifices or into body cavities; may occur at time of proestrus/estrus and/or parturition	May see pale mucous membranes, petechial and/or ecchymotic hemorrhages	Sanguineous	0% cornification, many RBCs	Diagnose with clotting profile	—

PMN, polymorphonuclear leukocyte; PU/PD, polyuria/polydipsia; RBC, red blood cell; PCV, packed cell volume; OHE, ovariohysterectomy.

Table 12-2. Aerobic Bacteria Cultured from the Vagina of Normal Intact or Spayed Dogs, Listed from Greatest to Least Prevalence

Escherichia coli
Streptococcus species
Pasteurella species
Pasteurella multocida
β-Hemolytic *streptococcus* species
Streptococcus canis
Staphylococcus aureus
Staphylococcus species
Staphylococcus epidermidis
α-Hemolytic *streptococcus* species
Bacillus species
Enterobacter species
Proteus mirabilis
Klebsiella species
Haemophilus species
Moraxella
Flavobacterium
Pseudomonas species
Corynebacterium species
Neisseria

Data from Ling and Ruby,[3] Bjurström and Linde-Forsberg,[4] Olson and Mather,[5] Osbaldiston et al.,[6] Láznička,[7] Schaefer et al.,[8] Platt and Simpson,[9] Hirsh and Wiger,[10] and Van Duijkeren.[11]

Significance of vaginal culture results must be interpreted with regard to location in the vagina from which the sample was collected and extent of bacterial growth in culture. Although significantly fewer organisms are isolated from samples collected from the cranial vagina (0.975 isolates per bitch) than from samples collected from the caudal vagina (2.35 isolates per bitch), only 5.2 per cent ($n = 59$)[4] and 8.6 per cent ($n = 81$)[5] of samples collected from the cranial vagina are reported sterile.

In general, culture of moderate to heavy growth of a single organism is necessary to qualify a culture result as significant. Treatment with antibiotics on the basis of a positive culture alone is not always indicated. In a study comparing vaginal culture results in five healthy bitches during and after treatment with either ampicillin or trimethoprim-sulfamethoxazole (TMP-SMX) to vaginal culture results pretreatment, bacteria were demonstrated to recolonize the vagina within 0 to 4 days after cessation of treatment, with 80 per cent of bitches showing complete recolonization of bacterial flora within 1 day, suggesting treatment with antibiotics to eradicate normal vaginal flora is not effective in the long term.[12] *Escherichia coli* emerged on vaginal cultures of bitches during and after treatment with TMP-SMX, and mycoplasmas emerged in bitches during and after treatment with either ampicillin or TMP-SMX.

Mycoplasmas and ureaplasmas are small, free-living organisms without a rigid cell wall. Although they are reported to be associated with reproductive dysfunction in dogs, no difference in percentage of female dogs with positive mycoplasma or ureaplasma vaginal cultures was demonstrated when comparing normal, fertile female dogs ($n = 26$) to bitches with infertility ($n = 27$) or vaginitis ($n = 22$).[13] Mycoplasmas and ureaplasmas are fastidious, making quantification of growth in a given sample difficult.

Congenital Abnormalities

Embryologically, the paired paramesonephric (müllerian) ducts fuse to form the uterus, cervix and vagina of the female dog. The caudal tip of the paired paramesonephric ducts projects into the urogenital sinus to form the paramesonephric tubercle, which is canalized and fuses to the genital folds to form the vestibule. The genital swellings form the vulvar lips. The hymen forms at the junction of the paramesonephric ducts and urogenital sinus, and usually is open at birth in dogs. Congenital abnormalities of the vagina, vestibule, and vulva may arise from (1) incomplete fusion of the müllerian ducts (elongated vertical vaginal septa or double vaginas); (2) incomplete perforation of the hymen (circumferential vaginovestibular strictures or discrete vaginal septa); or (3) imperfect joining of the genital folds to the genital swellings (vestibulovulvar strictures or hypoplasia).[14,15] Reported congenital abnormalities of the vagina, vestibule, and vulva of the dog include vaginal septa,[14,15] vaginovestibular strictures,[15–18] vestibulovulvar strictures,[15] segmental aplasia of the vagina,[19–21] and vulvar agenesis.[22] Normal bitches have a slight circumferential narrowing at the junction of the vestibule and vagina—the cingulum—that may be more evident during anestrus and that should not be misinterpreted as a vaginal anomaly.

Vaginal septa and circumferential vaginovestibular strictures are the most commonly reported congenital vaginal anomalies of the dog. Incidence of vaginal septa in one study was 0.03 per cent.[14] The true incidence is unknown, because many bitches with a vaginal septum or stricture have no clinical signs until estrus or breeding, and most bitches in the United States are spayed without ever having

been allowed to cycle or breed. Dogs with congenital vaginal abnormalities may be asymptomatic. No breed predisposition has been identified. Age at first diagnosis has been reported as 2.4 years for vaginal septa[14] and 4.6 years for vestibulovaginal strictures.[16] Heritability of vaginal anomalies in dogs is not well defined.

Presenting complaints or clinical signs reported in dogs with congenital vaginal abnormalities include chronic vaginitis (32 per cent, $n = 73$), inability to breed naturally (27 per cent), urinary incontinence (26 per cent), chronic urinary tract infection (8 per cent), dystocia (1 per cent), infertility (1 per cent), and ambiguous external genitalia (1 per cent).[14–18] Four per cent of dogs with vaginal septa or circumferential strictures are asymptomatic.[14–16]

General physical examination findings in affected bitches are nondiagnostic except for the digital vaginal examination. Sanguineous to mucopurulent vulvar discharge may be seen in bitches with chronic or recurrent vaginitis. Concurrent urinary tract infection was reported in 2 of 22[18] and 9 of 18[16] female dogs with circumferential vaginal strictures.

Diagnostic techniques for the detection of vaginal anomalies in the bitch include digital vaginal examination, vaginoscopy, and contrast vaginography. Digital vaginal examination often is diagnostic, because septa and strictures are most common at the vaginovestibular junction, just cranial to the urethral papilla, which is palpable in most female dogs. Vaginal anomalies were palpable in 11 of 15 dogs with vaginal septa[14] and in 18 of 18 bitches with circumferential vaginal strictures.[16] Digital vaginal examination may be impossible in small or very uncomfortable dogs, and may not be diagnostic if the anomaly is too far cranial to be identified on digital palpation. Sedation of bitches may be necessary for digital vaginal examination.[16]

Vaginoscopy allows visualization of septa and strictures, and may better allow assessment of extent of the anomaly. Vaginoscopy was used to identify vaginal anomalies in 6 of 7 dogs with vaginal septa[14] and in 17 of 17 dogs with circumferential vaginal strictures.[16] Sedation may aid diagnosis in small or uncomfortable bitches. An endoscope, vaginoscope, or otoscope can be used; specialized equipment usually is not necessary, because vaginal septa and strictures usually develop just cranial to the urethral papilla.

Contrast vaginography (see side bar, Figs. 12–1 and 12–2) allows definition of the type and extent of the vaginal anomaly present, and may detect multiple or cranial vaginal anomalies, if present. Vaginography was used to identify vaginal anomalies in 12 of 12 dogs with vaginal septa[14] and 9 of 10 dogs with circumferential vaginal strictures.[16] Complete assessment of degree of vaginal involvement with contrast vaginography provides the greatest amount of information to guide treatment recommendations. Vaginal septa are visible as dark bands within the opaque contrast medium (Fig. 12–3). A circumferential vaginal stricture is visible as a stenotic area cranial to the urethral papilla (Fig. 12–4). Contraction of the constrictor vestibulae muscles may mimic a vaginal stricture, so the dog must be completely relaxed during the contrast procedure. Also, the normal area of vaginal narrowing cranial to the urethral papilla (the cingulum) (Fig. 12–1) should not be mistaken for a vaginal stricture.

Manipulative and surgical treatments are described for repair of vaginal anomalies in the bitch. Manipulative treatment is manual dilation of the strictured area in the sedated animal. Manual dilation alone was ineffective in two dogs and manual dilation with concurrent treatment with glucocorticoids was ineffective in two dogs; all four redeveloped the stricture to some degree after treatment.[16] In a review of 21 dogs with circumferential vaginal strictures treated with manual dilation, 7 showed a good response, 1 a fair response, and 13 a poor response to manual dilation.[17]

Vaginal septa may be removed surgically via episiotomy (Fig. 12–5). Some very small vaginal septa may be broken down digitally. Surgical removal of broad-based or elongated septa usually requires episiotomy and ligation. Extensive vaginal septa (double vagina) may not be resectable because of the inaccessibility of most of the canine vagina unless the pelvis is split. Four dogs with vaginal septa showed complete resolution of clinical signs after surgical removal of the septum.[14]

Reported surgical treatment for circumferential vaginal strictures includes resection of the stenotic area, vaginoplasty, and vaginectomy. Resection of the stenotic area was reported to cause complete remission of clinical signs in three of three dogs.[16] Postoperative scarring may cause recurrence of the stricture with nonelastic granulation tissue. T-shaped vaginoplasty was reported in four dogs; it was less successful than the other surgical methods

described in decreasing clinical signs.[16] Vaginectomy has been described as successful in resolving clinical signs associated with circumferential vaginal strictures.[16,17] Of 10 dogs with circumferential vaginal strictures treated with vaginectomy, 7 had a good response, 1 a fair response, and 2 a poor response.[17]

A final surgical alternative is ovariohysterectomy (OHE). Extensive surgical repair is unwarranted in most females not intended for breeding and in asymptomatic dogs. Intact female dogs with no secondary disease may be bred by artificial insemination with planned cesarean section. If artificial insemination is to

How To Perform Vaginography/Hysterography

Food should be withheld for 24 hours, and a cleansing enema performed 2 to 3 hours prior to the procedure. The dog should be sedated (oxymorphone, 0.1 to 0.2 mg/kg intravenously [IV]) or general anesthesia should be induced. The dog is placed in lateral recumbency.

Required equipment includes iodinated contrast medium (iothalamate meglumine 60%, Malinckrodt Medical Inc., St. Louis, MO) diluted with an equal volume of lactated Ringer's solution, a balloon-type (Foley) catheter, and atraumatic tissue forceps. For large dogs, a human barium enema retention catheter may be required to ensure that the bulb, when inflated, will occlude the vaginal lumen.

The lumen of the catheter is filled with contrast medium to prevent infusion of air bubbles into the vaginal vault. The catheter is placed into the vagina so as to allow inflation of the balloon in the vestibule. The vulvar lips may be held closed with atraumatic tissue forceps to prevent backflow of contrast medium through the vulva. One to 5 ml/kg of prepared contrast medium is infused into the vagina. Distention is complete when backpressure is felt on the syringe or the dog attempts to dislodge the catheter by constricting the vaginal musculature. Lateral and ventrodorsal radiographic views are obtained (Fig. 12–1).[14,23] Vaginography permits assessment of the type and extent of congenital vaginal abnormalities and assessment of patency of the tubular reproductive tract.

The normal canine cervix is patent to contrast medium only during proestrus, estrus, and the postpartum period. If vaginography is performed at these times, contrast medium can move through the cervix and into the uterine horns (Fig. 12–2). Hysterography may help diagnose intra-

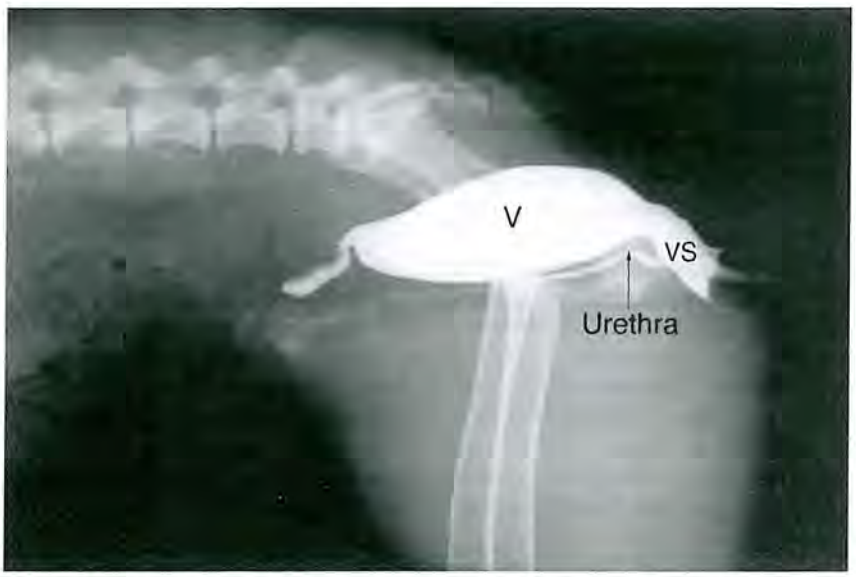

Figure 12–1. Lateral radiographic view of a normal positive-contrast vaginogram in a dog. V, vagina; VS, vestibule.

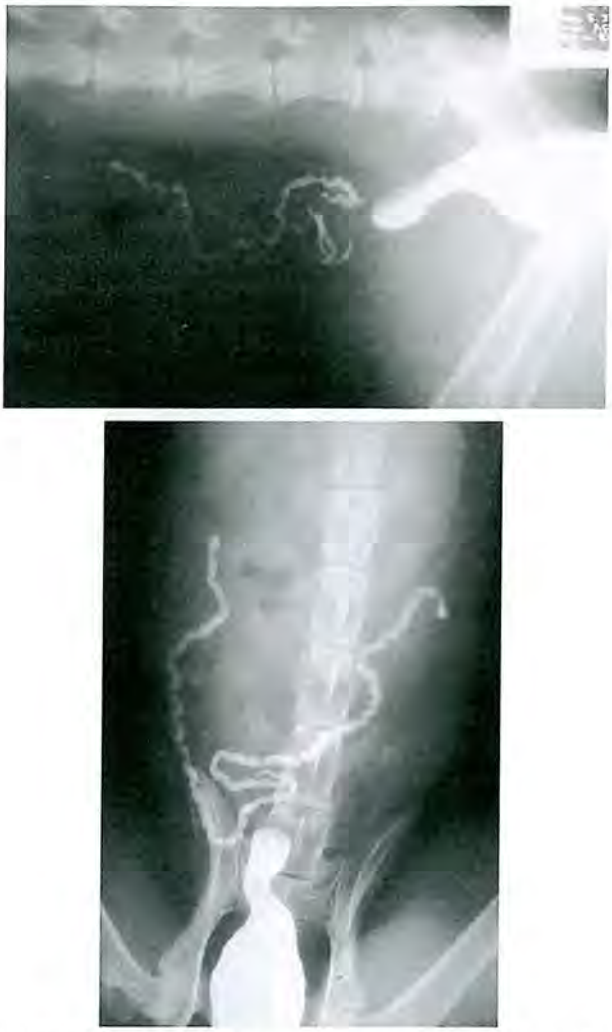

Figure 12–2. Lateral and ventrodorsal radiographic views of a normal positive-contrast hysterogram in a dog.

uterine abnormalities such as subinvolution of placental sites or retained fetal or placental tissue, but ultrasound usually is a better diagnostic tool to assess presence, if any, of fluid or tissue in the uterus. Hysterography is best used to evaluate completeness and patency of the tubular reproductive tract. The effect of vaginography/hysterography on future fertility in dogs is unknown.

be performed, a prebreeding contrast vaginogram is recommended to diagnose anomalies in the cranial vagina and to try to assess if the vagina is patent to the cervix. The authors examined one bitch for infertility after multiple attempts at breeding by artificial insemination that had always been inseminated to the left side of an asymmetrical vaginal septum; vaginography revealed a large, asymmetrical septum and impatency of the left side of the vagina.

Segmental aplasia of the vagina and vulvar agenesis have been described.[19–22] A 14-month-old intact bull-mastiff with cranial vaginal agenesis presented with chronic vaginitis that had begun at the time of her first estrous cycle. A fluid-filled dilation of the vagina just caudal to the cervix was identified on radiographs. Exploratory surgery revealed impatency of a 9-cm length of vagina caudal to the distention, which was filled with blood and tissue debris (hematocolpos) from the dog's previous es-

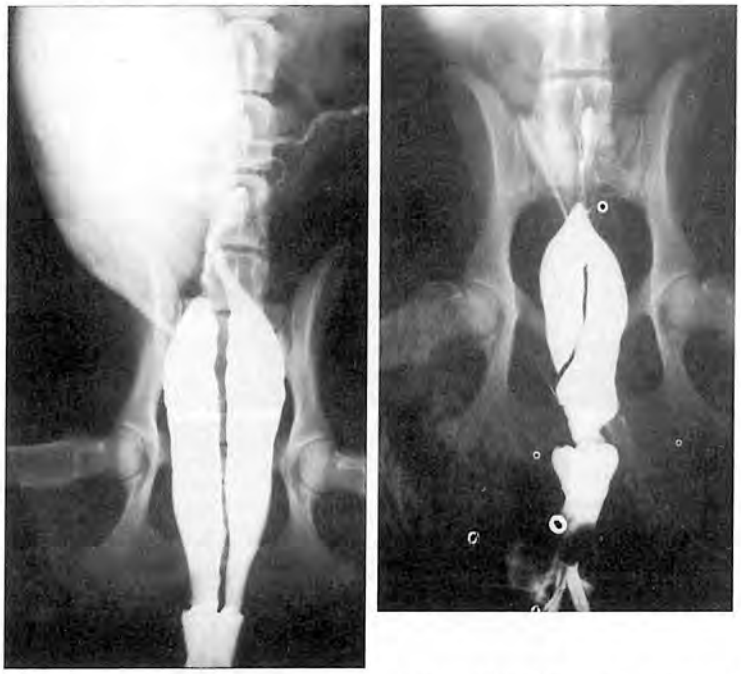

Figure 12–3. Ventrodorsal radiographic views of positive-contrast vaginograms from dogs with vaginal septa. **A:** Note the complete separation of the vagina by a septum. **B:** Note the asymmetrical septum extending from the cranial aspect of the vagina to the caudal right wall. (From Root MV, Johnston SD, Johnston GR: Vaginal septa in dogs: 15 cases (1983–1992). J Am Vet Med Assoc 206:56–58, 1995, with permission.)

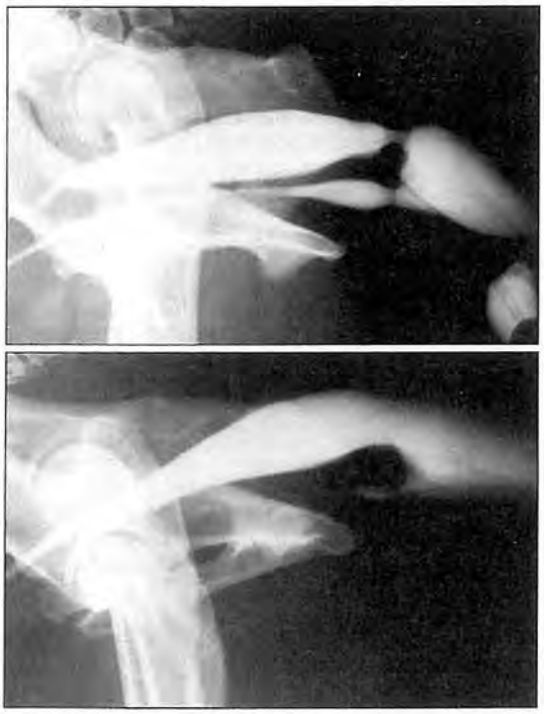

Figure 12–4. Oblique lateral radiographic view of a positive-contrast vaginogram from a dog with a circumferential vaginovestibular stricture. Note the marked narrowing of the vagina cranial to the urethral papilla. (From Kyles AE, Vaden S, Hardie EM, et al: Vestibulovaginal stenosis in dogs: 18 cases (1987–1995). J Am Vet Med Assoc 209:1889–1893, 1996, with permission.)

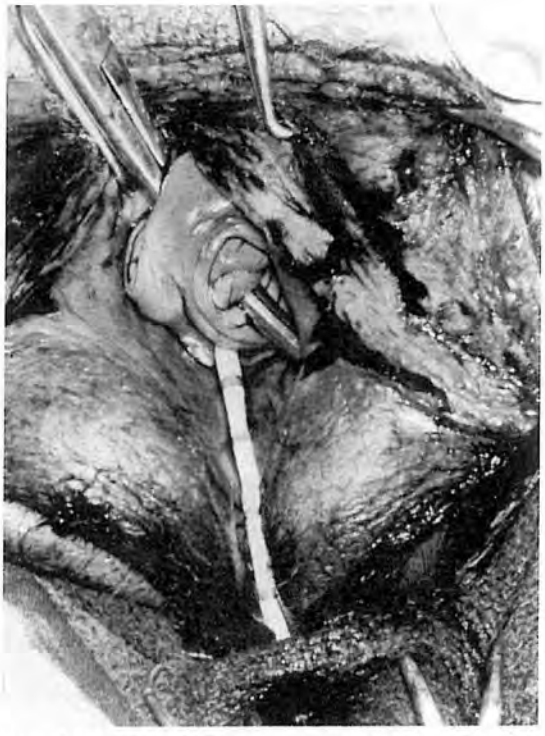

Figure 12–5. A curved instrument is used to draw a large vertical septum into the surgical field. (From Wykes PM, Soderberg SF: Congenital abnormalities of the canine vagina and vulva. J Am Anim Hosp Assoc 19:995–1000, 1983, with permission.)

trous cycle. The obstructed section was resected and vaginal anastomosis performed; the bitch later conceived and whelped eight puppies.[20] Caudal vaginal agenesis was described in a 5-year-old intact Shih Tzu with primary anestrus. Hematocolpos was present in her cranial vagina. The dog was euthanized because of unrelated renal disease.[21] Vulvar agenesis was reported in a 4-month-old intact Maltese that presented with dysuria and urinary incontinence. Reconstructive surgery and formation of a functional urethral papilla caused resolution of clinical signs.[22]

Clitoral Hypertrophy

The clitoris is hormone-dependent tissue that lies within the ventral clitoral fossa of female dogs. Dogs with clitoral hypertrophy may show excessive licking or sensitivity in the area of the vulva, or a visible mass protruding through the vulvar lips. Clitoral hypertrophy may occur secondary to irritation of the clitoral fossa (including licking because of pruritus), hormone-dependent hypertrophy, or intersex states.

Clitoral hypertrophy may be congenital in masculinized female puppies born to a dam treated with progestogens or androgens during pregnancy[24] or in dogs with intersex states that include presence of functional testicular tissue. Fifty-two per cent ($n = 25$) of male pseudohermaphrodites (dogs with female external genitalia and testes) and 100 per cent ($n = 13$) of true hermaphrodites (dogs with both ovarian and testicular tissue in the gonads) had clitoral hypertrophy reported in one study.[25] Clitoral hypertrophy in dogs with intersex states may not become apparent until the dog reaches puberty and testicular testosterone secretion increases.

Clitoral hypertrophy may be present in as many as 20 to 30 per cent of female dogs with hyperadrenocorticism.[26] Bitches treated with androgens, including the estrus-suppressing drug mibolerone, also may develop clitoral hypertrophy[27–29]; varying degrees of clitoral hypertrophy are reported to occur in 15 to 20 per cent of female dogs treated with mibolerone at the recommended dose.[27]

Clitoral hypertrophy often persists even after removal of the inciting stimulus. If the bitch is uncomfortable or is traumatizing the vulvar area excessively, clitoridectomy may be necessary.

Vaginal Prolapse

Vaginal prolapse is also called vaginal hyperplasia, vaginal hypertrophy, estrual hypertrophy, vaginal eversion, and vaginal protrusion;[30] *vaginal prolapse* is the correct term. Vaginal prolapse is protrusion of edematous vaginal tissue into the vaginal lumen and often through the vulvar lips of the female dog.[31] This disorder is due to accentuation of the normal increase in vaginal hyperemia and edema secondary to the estrogen stimulus occurring during proestrus and estrus in the female dog (Fig. 12–6).[31,32] The exact etiopathogenesis of this condition is unknown, because hyperestrogenism is not present, and affected dogs are fertile in the absence of other disorders of the reproductive tract.[33]

Vaginal prolapse occurs most commonly during the time of peak estrogen secretion in

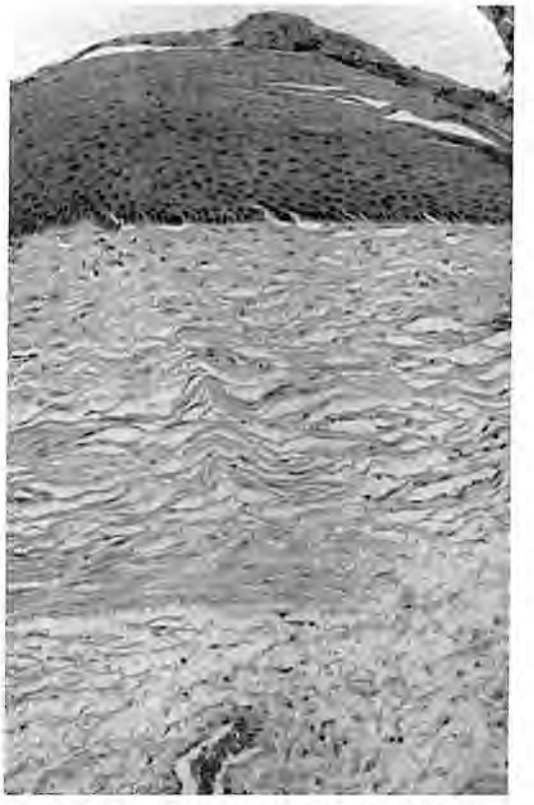

Figure 12–6. Histologic appearance of prolapsed vaginal tissue surgically removed from a dog with type III vaginal prolapse. The tissue is characterized by a stratified squamous epithelium lining the lumen of the vagina, consistent with the estrous status of the bitch; submucosal tissue is characterized by marked edema. Hematoxylin and eosin; 125×. (From Johnston SD: Vaginal prolapse. *In* Kirk RW [ed]: Curr Vet Ther Small Anim Pract 10. Philadelphia, WB Saunders, 1989, pp 1302–1305, with permission.)

intact female dogs. Serum estrogen concentrations peak at 50 to 100 pg/ml in late proestrus, 1 to 2 days before the preovulatory luteinizing hormone surge.[34,35] Vaginal prolapse has been reported to occur during proestrus/estrus in 73 per cent (n = 26),[36] 81 per cent (n = 21),[33] and 86 per cent (n = 66)[31] of affected bitches. It also may occur near parturition, as serum progesterone concentrations decline and serum estrogen concentrations increase. Occurrence at parturition is reported as 8 per cent (n = 66),[31] 10 per cent (n = 21),[33] and 12 per cent (n = 26).[36] Vaginal prolapse occurs less commonly in diestrus,[31,33,37] and normal pregnancy.[38] Serum estrogen concentration was low in a 2-year-old Labrador retriever with recurrent vaginal prolapse throughout gestation; etiology was not defined. In a review of 66 cases of vaginal prolapse, none occurred during anestrus.[31] Vaginal prolapse unrelated to estrogen secretion has been reported in a pregnant dog with increased intra-abdominal pressure secondary to ascites from severe hepatic cirrhosis,[39] and in a dog with altered perineal and vulvar conformation after an injury.[40]

Vaginal prolapse almost always occurs in intact female dogs. Reported mean age at diagnosis varies from 18 months to 4.6 years, with a median of 21.5 months and a range of 6 months to 16 years.[30–33,36,41] On average, vaginal prolapse is first diagnosed at either a bitch's second or third estrous cycle.[33] No breed predisposition has been described, although boxers and boxer crosses have been overrepresented in several studies.[33,36,41] It is generally seen in large-breed dogs, with one study reporting 61 per cent of cases (n = 18) in dogs weighing 50 pounds or more, and only 11 per cent of cases in dogs weighing less than 20 pounds.[30] Increased incidence in some families of large-breed purebred dogs suggests a hereditary influence.[31]

Three degrees of vaginal prolapse are described.[33] Type I is slight to moderate eversion of the vaginal floor with no protrusion of vaginal tissue through the vulvar lips. Type II is prolapse of the cranial floor and lateral walls of the vaginal through the vulvar lips, forming a tongue or pear-shaped mass. Type III is prolapse of the entire vaginal circumference as a "doughnut"-shaped mass with a lumen. Because the vaginal prolapse always originates from the floor of the vagina, cranial to the urethral papilla, the external urethral orifice is on the ventral surface of the prolapsed tissue (Fig. 12–7).

The most common clinical sign in dogs with vaginal prolapse is a visible mass protruding from the vulvar lips.[30] Incidence of type I vaginal prolapse is probably much higher than suspected, because the owners may be unaware of its presence. Vulvar discharge may be present. Dysuria is occasionally seen in dogs with vaginal prolapse.

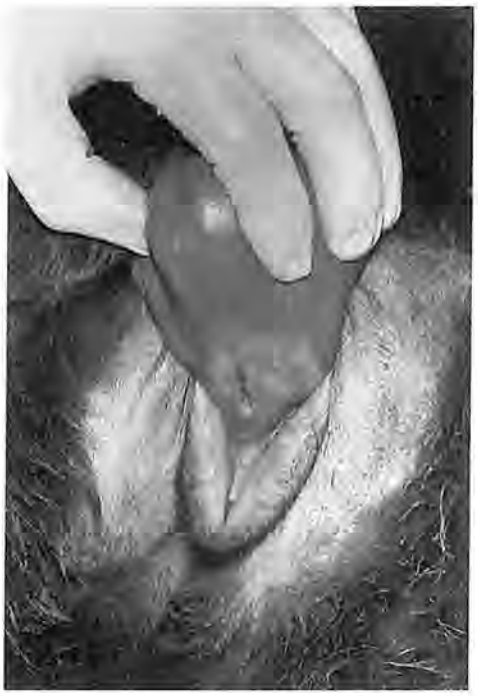

Figure 12–7. Vaginal prolapse in a 4-year-old pregnant Labrador retriever bitch. Note the urethral orifice in the floor of the vagina. (From Memon MA, Pavletic MM, Kumar MSA: Chronic vaginal prolapse during pregnancy in a bitch. J Am Vet Med Assoc 202:295–297, 1993, with permission.)

The primary differential diagnosis in dogs with vaginal prolapse is vaginal neoplasia. Differentiation is based on signalment of the female dog, site of origin of the mass, and changes of the mass with the estrous cycle (Table 12–3).

Treatment of vaginal prolapse involves either decreasing estrogen stimulus in the animal, removing the prolapsed tissue, or both. In affected dogs that have ovulated (indicated by serum progesterone concentration of greater than 4 ng/ml), under decreasing estrogen influence the prolapse probably will regress spontaneously. Exposed vaginal tissue should be kept clean. An Elizabethan collar may be necessary to prevent the bitch from traumatizing the tissue. Dogs affected at parturition have been reported to whelp despite the presence of the prolapsed tissue, although outside assistance is required in some cases.[36] If the vaginal prolapse is allowed to regress

Table 12–3. Differentiation of Vaginal Prolapse and Vaginal Neoplasia in the Bitch

	Vaginal Prolapse	Vaginal Neoplasia
Signalment	Young, intact bitches	Older, intact or spayed dogs (except TVT)
Site of origin of mass	Arises from vaginal floor, cranial to the urethral papilla	Arises from anywhere within the vagina
Change with estrous cycle?	Increases in size in proestrus/estrus; regresses in diestrus, anestrus, or post-OHE	Does not vary with the estrous cycle

spontaneously, recurrence at the subsequent estrous cycle is very common, reported to occur in 66[31] to 100 per cent[36] of cases.

Affected dogs that are not yet periovulatory, as indicated by a serum progesterone concentration of less than 2 ng/ml, may be induced to ovulate with injection of either gonadotropin-releasing hormone (2.2 μg/kg intramuscularly [IM]) or human chorionic gonadotropin (1000 IU IM); regression of the prolapse should follow ovulation induction by about 1 week.[31] Medroxyprogesterone acetate (50 mg subcutaneously) was reported successful in resolving vaginal prolapse within 15 days in 6 of 10 bitches treated, compared to a spontaneous resolution rate of 40 per cent in a control group, but is not recommended by the authors for this purpose.[42]

Surgical treatments for canine vaginal prolapse include purse-string sutures, hysteropexy, circumferential excision of prolapsed tissue, and OHE. Manual reduction of the prolapse with placement of purse-string sutures has been reported as a successful treatment for vaginal prolapse in dogs, but is not recommended because of the discomfort and perivulvar trauma this causes the bitch.[33,38] Hysteropexy via abdominal surgery was described in one case report[38] but is not routinely used as a surgical treatment for vaginal prolapse in dogs. Circumferential excision of prolapsed tissue may be required in dogs with type III vaginal prolapse, which is less likely to resolve completely without surgical intervention than are types I and II,[33] or in bitches with severely inflamed or devitalized prolapsed tissue. Vaginal prolapse has been reported to recur in some bitches at the subsequent estrus despite excision of prolapsed tissue.[31,32,36]

Ovariohysterectomy may hasten resolution of vaginal prolapse by removing the primary endocrine stimulus, the estrogen-secreting ovary. Regression of prolapsed vaginal tissue is reported to occur by 4 to 8 days[36] or within 21 days[33] of OHE. Vaginal prolapse usually does not recur after complete OHE.

Vaginitis

Vaginitis is inflammation of the vagina. Oftentimes, vestibulitis also is included under this general heading. Incidence in one study over a 5-year period was 0.7 per cent ($n = 10{,}000$).[43] Juvenile and adult-onset forms are described.

Juvenile Vaginitis

Juvenile, or puppy, vaginitis is defined as vaginitis in bitches less than 1 year of age, and comprises 40 per cent ($n = 15$)[44] to 52 per cent ($n = 71$)[43] of cases of vaginitis reported. It may be seen in females as young as 8 weeks of age.[44] Juvenile vaginitis may be an incidental finding during physical examination at time of vaccination or may be a complaint in puppies with vulvar discharge. Vulvar discharge is almost always present, and ranges in volume and character from scant and mucoid to copious and mucopurulent. Presence of vaginal irritation is variable. Affected bitches usually are not systemically ill.[43,45] Cytology of the vaginal discharge usually consists of polymorphonuclear leukocytes, with or without bacteria.[43] Significant bacterial growth was not recovered from any of 15 vaginal culture specimens collected from dogs with juvenile vaginitis.[43] For this reason, treatment with antibiotics, either topically or systemically, may not effect a cure.[45] Antibiotic treatment is warranted if the vaginal discharge is cytologically purulent or the bitch is showing signs of discomfort, such as excessive licking of the vulva. Antibiotic choice should be based on culture and sensitivity testing of a sample retrieved from the cranial vagina, and should be continued for 4 weeks.

Conservative treatment is indicated for bitches with juvenile vaginitis that is not causing the bitch discomfort. In a review of 37 dogs with juvenile vaginitis, 84 per cent of the cases resolved with or without treatment.[43] Allowing bitches with juvenile vaginitis to go through

an estrous cycle may hasten resolution. The rationale for waiting to perform OHE until after at least one estrus is based on observation of some bitches in which vaginitis resolved at the first heat, and on knowledge that estrogen secretion in proestrus thickens the vaginal epithelium. Of seven bitches with juvenile vaginitis allowed to go through estrus, three improved after the first cycle and one after a second cycle. The three remaining dogs showed no overt change after heat but all signs resolved by 3 years of age.[43] It is unknown what effect increasing age and maturation of the immune system have on resolution of juvenile vaginitis. The authors disagree on whether spaying bitches with juvenile vaginitis will cause chronic vaginitis to persist to adulthood, and on whether allowing bitches that have juvenile vaginitis to cycle will prevent persistence of vaginitis in adulthood.

Adult-Onset Vaginitis

Vaginitis in adult dogs may be primary or secondary. Primary vaginitis may be caused by infection with *Brucella canis* or canine herpesvirus. Secondary vaginitis may occur subsequent to vaginal atrophy following OHE, to urine or mucus pooling with a congenital vaginal anomaly, to therapy with drugs such as mibolerone, to the presence of a vaginal neoplasm or foreign body, or secondary to urinary tract disease or systemic disease, such as diabetes mellitus.[14–16,20,28,38,43,44,46] The most common factors underlying vaginitis in adult dogs are estrogen deprivation following OHE and congenital vaginal abnormalities. Urinary tract disease and vaginal neoplasia may be mistaken for vaginitis because of the presence of persistent urine dripping or vulvar discharge (Table 12–1).

In reviews of dogs with congenital vaginal abnormalities, 7 per cent ($n = 15$),[14] 11 per cent ($n = 18$),[16] and 46 per cent ($n = 13$)[15] presented with the complaint of chronic vaginitis. Conversely, in reviews of dogs presenting with vaginitis, 20 per cent ($n = 15$)[44] and 35 per cent ($n = 34$)[43] had a congenital anomaly of the reproductive tract. Reported congenital abnormalities included vaginal septa, strictures and hermaphroditism.[43,44]

Urinary tract disease was reported as an underlying or concurrent disorder in 26 per cent[43] to 60 per cent[44] of bitches with vaginitis. Disorders of the urinary tract in bitches with vaginitis include urinary tract infection and urinary incontinence.[43,44]

Adult-onset vaginitis occurs, by definition, in bitches greater than 1 year of age. Reported age at diagnosis varies from 1 to 16 years.[43] Adult-onset vaginitis is equally common in intact and spayed bitches.[44] It is termed chronic if it has been present for greater than 1 month.

Vulvar discharge is the most common presenting complaint in dogs with adult-onset vaginitis.[43] It varies in character from mucoid to purulent, and occasionally is blood tinged.[43,44] Adult dogs with vaginitis also may present with pollakiuria, pain when urinating, and vulvar licking.[43] Other clinical signs that have been reported were dependent on the inciting cause of the vaginitis and included polyuria/polydipsia, urinary incontinence, pruritus, and infertility, none of which are signs of vaginitis but instead reflect concurrent disease.[43]

On physical examination, vulvar discharge usually is present, either dripping from the vulva or caught in the perivulvar hair. Vulvar hyperemia may be present. Vaginoscopic examination often reveals diffuse hyperemia of the vestibular and vaginal mucosa and luminal exudate. Follicular lesions may be present in the vaginal mucosa. Localized erythema at the urethral papilla or within the clitoral fossa may be seen occasionally.[43] Vaginoscopic examination may reveal presence of congenital vaginal abnormalities, vaginal neoplasia, or foreign body.

Cytology of vaginal specimens collected from mature bitches with vaginitis is more often indicative of septic inflammation than are samples from immature dogs.[43] Vaginal culture rarely yields heavy growth of a single organism. Of vaginal culture specimens collected from 78 bitches with vaginitis, 74 per cent were positive for bacterial growth and 64 per cent were pure cultures.[47] Organisms cultured most commonly are *E. coli*, *Streptococcus* species, and *Staphylococcus intermedius*, all of which are normal vaginal flora in the bitch (Table 12–2).[47,48]

Complete blood count (CBC) and serum chemistry profile usually are normal in bitches with vaginitis. No specific changes were noted on CBC in 18 of 23 mature bitches with vaginitis.[43] Changes in the CBC, serum chemistry profile, or urinalysis may help pinpoint a primary disease process, such as diabetes mellitus or urinary tract infection. Serology for *B. canis* is strongly recommended for all bitches with persistent vulvar discharge.

Vaginitis must be differentiated from disease of the uterus in intact dogs or uterine stump in spayed female dogs, and from uri-

nary tract diseases; bitches with any of these conditions may present with vulvar discharge (Table 12–1). Vaginitis may occur in animals infected with *B. canis* (see Chapter 11) or canine herpesvirus. While canine herpesvirus is associated with significant reproductive tract disease in female dogs, including infertility, abortion, and stillbirths,[49] the mucosal form of the disease generally is mild and self-limiting.[50] A variable number of papulovesicular lesions form on the vaginal and vestibular mucosa within days of infection, progress to form pock-like firm lesions resembling lymphoid follicles, and regress completely within 14 to 18 days.[49,51,52] Female dogs with mucosal herpesvirus infections rarely show clinical signs.[49,52]

Biopsy of the vaginal wall is rarely diagnostic for cause of vaginitis. The most common histopathologic description is chronic ulcerative vaginitis, a diagnosis that neither leads to a primary disease process nor directs treatment. Occasionally, infiltration of the vaginal wall with lymphocytes, plasma cells, or eosinophils is present.[43] Follicular lesions are nonspecific responses to vaginal irritation and usually are composed histologically of lymphoid aggregates.

Treatment of adult-onset vaginitis requires careful evaluation of the history and physical examination findings and any laboratory work performed to try to determine if a predisposing cause of vaginitis is present. Treatment of the underlying problem usually is curative. In one study, vaginitis resolved in three dogs with congenital vaginal abnormalities after surgical repair of the anomalies.[43] Reported response to systemic glucocorticoid treatment for bitches with presumed inflammatory infiltration of the vaginal wall has been equivocal.[43]

Antibiotics should be used if culture of a specimen collected from the anterior vagina reveals heavy growth of a single organism. Systemic therapy is preferable to topical treatment. Antibiotic choice should be based on culture and sensitivity testing and should be continued for 4 weeks. Vaginal douches with antibiotics or antiseptic agents are ineffective in flushing out significant amounts of vaginal discharge, and may be irritating to the vaginal mucosa, worsening the vaginitis.

Treatment with low doses of oral estrogen (diethylstilbestrol [DES]; 1 mg daily per os for dogs greater than 20 pounds, 0.5 mg daily per os for dogs less than 20 pounds for 7 days, tapering the dose over 2 weeks and maintaining lifelong therapy with the minimal effective dose) may be beneficial in spayed bitches with vaginitis because DES will increase thickness of the vaginal mucosa and promote resistance to invasion of the atrophic vaginal epithelium by normal vaginal flora. As many as a third of dogs presenting with adult-onset vaginitis have no identifiable underlying problem. Administration of glucocorticoids may worsen existing urinary tract infection. Response to estrus or OHE in dogs with adult-onset vaginitis of unknown etiology is variable.[43]

A high percentage of dogs with adult-onset vaginitis recover spontaneously. In one retrospective study, 8 of 11 dogs (73 per cent) with no identifiable primary cause of adult-onset vaginitis recovered spontaneously. Treatment, or lack thereof, did not affect the outcome.[43]

Vestibulitis

Inflammation of the canine vestibule resulting in infiltration with lymphocytes and plasma cells has been observed. No infectious agent was cultured, and the vagina was unaffected. Affected dogs have signs of vestibular/vulvar irritation (scooting, excessive licking), abnormal odor, or vulvar discharge. Secondary clitoral hypertrophy may occur. Vestibulitis can be diagnosed by vaginoscopy in medium- and large-breed dogs. Urine pH should be evaluated, because vestibulitis may be a response to exposure to excessively alkaline urine. Urine pH can be altered by diet or by administration of methionine (200 to 1000 mg per os every 8 hours). Treatment with glucocorticoids may decrease inflammation and prevent self-trauma.

Vaginal/Vulvar Neoplasia

Vaginal and vulvar neoplasia are relatively uncommon in the dog, with reported incidences among all canine tumors of 2.8 per cent (n = 3073)[53] and 3.0 per cent (n = 2361).[54] Among tumors specific to the canine reproductive tract, vaginal tumors account for 41 per cent and vulvar tumors 34.2 per cent (n = 117).[55]

Benign vaginal/vulvar neoplasia comprises 70 per cent (n = 20)[30] to 72 per cent (n = 99) of all vaginal tumors.[56] Benign vaginal/vulvar neoplasms reported include leiomyoma, fibropapilloma (vaginal polyps), fibroma, fibroleiomyoma, lipoma, nerve sheath tumor, fibrous histiocytoma, benign melanoma, myxoma, and myxofibroma.[1,30,50,53,56–62] The

most common vaginal/vulvar neoplasm is the benign leiomyoma; reported incidences among all vaginal and vulvar tumors are 29 per cent ($n = 99$),[56] 30 per cent ($n = 20$),[30] and 78 per cent ($n = 85$).[53]

Incidence of malignant vaginal/vulvar tumors is 27 per cent ($n = 99$)[56] to 30 per cent ($n = 20$) of all vaginal tumors.[30] Malignant vaginal/vulvar neoplasms that have been reported include transmissible venereal tumor (TVT), leiomyosarcoma, squamous cell carcinoma, hemangiosarcoma, adenocarcinoma, fibrosarcoma, mastocytoma, epidermoid carcinoma, anaplastic spindle cell carcinoma, lymphosarcoma, metastatic osteosarcoma, metastatic mammary adenocarcinoma, and locally invasive transitional cell carcinoma.[30,53,56,57,61-69] TVT is the most commonly reported malignant vaginal/vulvar neoplasm, with reported incidences among vaginal/vulvar tumors of 10 per cent ($n = 99$)[56] and 11 per cent ($n = 85$),[53] although incidence varies with climate and with incidence of free-roaming populations of dogs.

Pathogenesis of vaginal/vulvar neoplasia other than TVT is unknown. Neoplastic transformation of vaginal and vulvar smooth muscle cells or surrounding tissues may be dependent on presence of ovarian hormones. In reviews of dogs with vaginal/vulvar neoplasia, 65 per cent,[30] 91 per cent,[56] and 98 per cent[62] of affected females were intact at the time of diagnosis. In one review of 40 dogs with vaginal neoplasia, 13 had been treated previously with estrogen for pregnancy termination or with progestogens for either estrus suppression or false pregnancy.[62]

Mean age at diagnosis of vaginal/vulvar tumors other than TVT is 10.8 to 11.2 years, with a range of 2 to 18 years.[31,53,56,62] Although no breed predisposition has been identified, boxers were over-represented in one study[53] and spaniels in another.[62] A greater tendency for benign vaginal/vulvar neoplasia in small-breed dogs and for malignant vaginal/vulvar neoplasia in large-breed dogs was reported in one retrospective study.[30]

The clinical signs most commonly associated with vaginal/vulvar neoplasia are presence of a mass causing perineal swelling or visibly protruding through the vulvar lips (Fig. 12–8)[30,56,60,64] and vulvar discharge, which may be mucoid, mucopurulent, or sanguineous.[1,56,58,62,68] Other clinical signs reported include dysuria, vulvar licking, anorexia and weight loss, polyuria and polydipsia, and fecal tenesmus.[30,56,59,60,62,65,70] Signs may be associated with concurrent disease; concurrent diseases reported in female dogs with vaginal/vulvar neoplasia include cystic endometrial hyperplasia–pyometra, mammary neoplasia, cystitis, ovarian cystic disease, adrenal tumors, and granulosa cell tumors.[59,62]

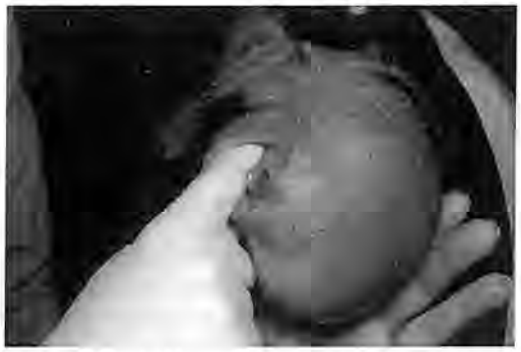

Figure 12–8. Vaginal hemangiosarcoma in an aged Doberman pinscher bitch.

Diagnosis is by physical examination followed by incisional or excisional biopsy. Vaginoscopy or rectal palpation may facilitate localization of masses within the vaginal vault. The primary differential diagnosis is vaginal prolapse, which usually can be differentiated by signalment of the bitch, site of origin of the mass, and changes of the mass with the estrous cycle (Table 12–3). Fine-needle aspiration or imprint of the excoriated tumor mass often is not a good indicator of histologic type for the benign leiomyoma, which does not exfoliate readily.[1] Fine-needle aspiration is, however, recommended with all vaginal masses because many vaginal tumors (TVT, hemangiosarcoma, metastatic tumors) can be diagnosed with this technique; a negative tap does not rule out neoplasia. No correlation has been demonstrated between site of origin of the vaginal/vulvar mass and histologic type.[56] Definitive diagnosis requires histopathologic examination of excised tissue.

Surgical removal is the treatment of choice for vaginal neoplasia in the bitch. Episiotomy may be required for complete visualization of the tumor mass.[56,62] Some masses may be inoperable; in a review of 40 dogs with vaginal neoplasia, 6 (15 per cent) had tumors considered inoperable because of excessive size or number or inaccessible position of tumor masses.[62] Dogs with vulvar involvement may require vulvectomy or vulvovaginectomy with perineal urethrostomy.[56,61] Complete surgical removal of masses yielded mean survival times of 18 months for dogs with benign vagi-

nal/vulvar masses and 11.6 months for dogs with malignant vaginal/vulvar neoplasms in one study.[56] Concurrent OHE may be beneficial; in a review of 74 dogs treated surgically for either benign or malignant vaginal/vulvar neoplasia, none of those undergoing OHE at the time of tumor removal had recurrence of vaginal/vulvar neoplasia.[56]

There is one report of combined CO_2 laser ablation and chemotherapy with doxorubicin HCl (30 mg/m^2 two times, 4 weeks apart) to treat vaginal fibrosarcoma in a 10-year-old miniature poodle. The dog remained disease free for 20 months after therapy.[66]

Transmissible Venereal Tumor

Transmissible venereal tumor (TVT) is also called venereal granuloma, transmissible sarcoma, Sticker's sarcoma, and transmissible venereal sarcoma.[71,72] It has worldwide distribution and is most common in tropical and subtropical urban areas containing large populations of free-roaming dogs.[73,74]

TVT arises from an allogeneic cellular transplant, not neoplastically transformed canine cells.[73] Cells of TVT contain 59 ± 5 chromosomes, and surface antigen characteristics of TVT cells suggest that all TVTs arose from a single, original canine tumor.[74]

Transmission occurs via transplantation of neoplastic cells onto vaginal mucosa during coitus, and onto nasal and oral mucosa by licking of affected genitalia of self or other dogs.[72,73] Transmission to perianal skin and rectal mucosa, and to excoriated skin at other sites, also has been reported.[75] Expression of the tumor is controlled by the immune system, with more rapid growth and metastasis occurring in pediatric or immunosuppressed animals.[72] Icosahedral electron-dense particles have been identified by electron microscopy in degenerating and necrotic TVT cells; significance is unknown.[76]

Local invasion of TVT may occur in as many as 40 per cent of cases.[73] Metastasis is reported to occur in less than 5 per cent[77] and 0 to 17 per cent[74] of cases. Metastasis of TVT to the skin, regional lymph nodes, tonsils, eyes, brain, pituitary, nose, tongue, lips, and thoracic and abdominal viscera has been reported.[73]

Mean age at diagnosis is lower for TVT than for other neoplasms because it is spread by coitus from an outside source. It usually occurs in young, sexually mature animals,[74] with reported mean age at diagnosis of 4.1 to 4.9 years and range of 1.5 to 11 years.[56,78] Female dogs are more susceptible to TVT than male dogs.[77] No breed predisposition has been described. All of the affected dogs in one study (n = 10) were of large-breeds.[56]

Clinical signs of TVT in the bitch are presence of perineal swelling or one or more obvious tumor masses on the vaginal, vestibular, or vulvar mucosa and serosanguineous vulvar discharge.[56,57,74,78] The occasional presence of TVT on nasal or oral mucosa, or presence of metastasis, causes signs referable to those sites. The tumors appear as single or multiple firm, small, grey to red nodules early in the course of disease, progressing to widely pedunculated, cauliflower-like or multilobular hemorrhagic or ulcerated masses that may exceed 10 cm in diameter.[56,72] Signs associated with nasal or ocular TVT include sneezing, epistaxis, epiphora, and lymph node enlargement.[74] Polycythemia has been observed in some animals with a large tumor burden, because TVT may secrete erythropoietin.[72]

Diagnosis is by visual inspection and cytologic examination of exfoliated neoplastic cells. TVT is an easily exfoliated round cell tumor; a diagnostic sample of neoplastic cells can be collected by imprint or fine-needle aspiration.[74] TVT cells are round with a large nucleus:cytoplasmic ratio, prominent chromatin clumping, and large nucleoli. Mitotic figures are commonly seen.

Effective treatment methods described for canine TVT include surgical excision, cryosurgery, radiation therapy, immunotherapy, and chemotherapy. Spontaneous remission has been demonstrated after experimental infection, but has not been reported in natural infections.[73]

Surgical excision is most successful in animals with few, small, circumscribed, accessible lesions with no local invasion or metastases. Although successful treatment with surgery alone has been reported,[56] local recurrence rate has been reported to be as high as 44 per cent.[74] Of 35 female dogs with TVT, 17 per cent of those with genital TVT and 58 per cent of those with extragenital TVT had local recurrence.[79] There is one report of successful treatment of TVT with cryosurgery.[74]

Radiation therapy, with exposure of the neoplastic tissue to 1000 rads at each treatment, induced remission after one to three treatments in most of the cases reported.[72] Immunotherapeutic agents reported include treatment with bacillus Calmette-Guérin, autogenous formalized vaccines, extracts of irradiated tu-

mors, and serum from patients with TVT in remission.[73] Results of immunotherapy are variable, and recurrence is common.[74]

Effective chemotherapeutic regimens reported for TVT include vincristine alone and vincristine in combination with cyclophosphamide and methotrexate. Vincristine, at a dose of 0.025 mg/kg IV weekly, was reported to cause visible tumor regression within 2 weeks, with complete regression after a mean of 3.3 (range 2 to 7) treatments in 39 of 41 dogs.[80] Vincristine at a dose of 0.6 mg/m^2 caused complete remission in 138 of 140 dogs within 2 to 6 weeks.[81] Reported side effects of vincristine treatment are vomiting and transient leukopenia, which occur 7 per cent and 5 per cent of the time, respectively.[80] Animals over 5 years of age were reported to show more gastrointestinal side effects in one study.[81]

Combination therapy with vincristine (0.0125 to 0.025 mg/kg IV weekly), cyclophosphamide (1 mg/kg once daily per os or 50 mg/m^2 every other day per os), and methotrexate (0.03 to 0.05 mg/kg IV weekly or 2.5 mg/m^2 every other day per os) was reported to cause tumor regression in a mean of 10.2 days, with a range of 2 to 40 days.[78] A mean survival time of 524 days, and survivals of greater than 1075 days, have been reported.[56,78] Side effects reported are transient leukopenia and vomiting.[78] There is no apparent advantage of combination chemotherapy over treatment with vincristine alone.

Because several modes of therapy are effective in treating TVT, selection of therapy should be based on the size and location of the tumor, and on availability of treatment modalities. In general, surgical excision followed by chemotherapy is recommended.

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Chapter 13

Disorders of the Mammary Glands of the Bitch

False Pregnancy/Galactorrhea

False pregnancy is also known as pseudopregnancy or pseudocyesis. A related term is *galactorrhea*, defined as spontaneous development of the mammary gland with secretion ranging from clear liquid to true milk.[1] *Galactorrhea* is the more accurate term, because physical signs of the condition commonly referred to as false pregnancy are more indicative of false whelping than false pregnancy. However, because *false pregnancy* is the term most commonly used, it will be used throughout this chapter.

False pregnancy in the bitch is used to describe a syndrome of clinical signs that include mammary development and galactorrhea, nesting behavior, and mothering behavior, to include protection of puppies, kittens, toys, or other inanimate objects.[2,3] Abdominal distention and slight uterine enlargement also are reported.[3] False pregnancy occurs in response to decline in systemic progesterone stimulation; this may occur 2 to 3 months after estrus (at the end of normal diestrus), 3 to 4 days following a diestrual ovariohysterectomy (OHE), or 3 to 4 days following discontinuation of exogenous progestin administration.

False pregnancy is a normal phenomenon in the intact bitch. In one study, 64.3 per cent of intact bitches were reported to exhibit false pregnancy signs regularly, and 7.1 per cent were reported to exhibit signs of false pregnancy intermittently.[4] Eighty-seven per cent of intact dogs are reported to exhibit signs of false pregnancy two or more times in their lives while intact.[5] These numbers should be interpreted with caution, however, because many bitches exhibit mild lactation and galactorrhea with no other noticeable signs.

False pregnancy most commonly occurs at the end of diestrus, after serum progesterone concentrations abruptly decline. Because normal, intact bitches undergo a 2-month luteal phase of diestrus, regardless of breeding and pregnancy status, the canine mammary gland undergoes glandular development (under the influence of progesterone) after every heat cycle. Serum progesterone concentrations rise until 13 to 16 days after the onset of cytologic diestrus, and then decline over the latter portion of diestrus, falling within the last 7 days of diestrus[6–8] (Fig. 13–1). There is no significant difference in this pattern in serum progesterone concentration during diestrus between pregnant and nonpregnant dogs.[8–12] No differences have been reported in serum progesterone concentrations between dogs with covert and overt false pregnancy, or between sterilemated and nonmated bitches.[13] The endocrine events that are necessary for normal whelping and lactation in pregnant dogs also occur secondary to declining serum progesterone concentrations in nonpregnant dogs, that may then develop false pregnancy signs.

Decline in serum progesterone concentration in late diestrus is accompanied by increase in serum estrogen and plasma prolactin concentrations.[1,2,14] Plasma prolactin concentrations are elevated in all intact bitches in late diestrus, with elevations of two (nonpregnant) to five (pregnant) times early diestrus concentrations.[14] Mammary gland development is stimulated by the prolonged exposure to serum progesterone, and production of milk is stimulated by the presence of plasma prolactin. Signs of false pregnancy may occur from 3 to 14 weeks after estrus, with 57.1 per cent of dogs exhibiting overt false pregnancy within 3 to 8 weeks postestrus.[4]

Other conditions causing an abrupt decrease in serum progesterone concentrations—for ex-

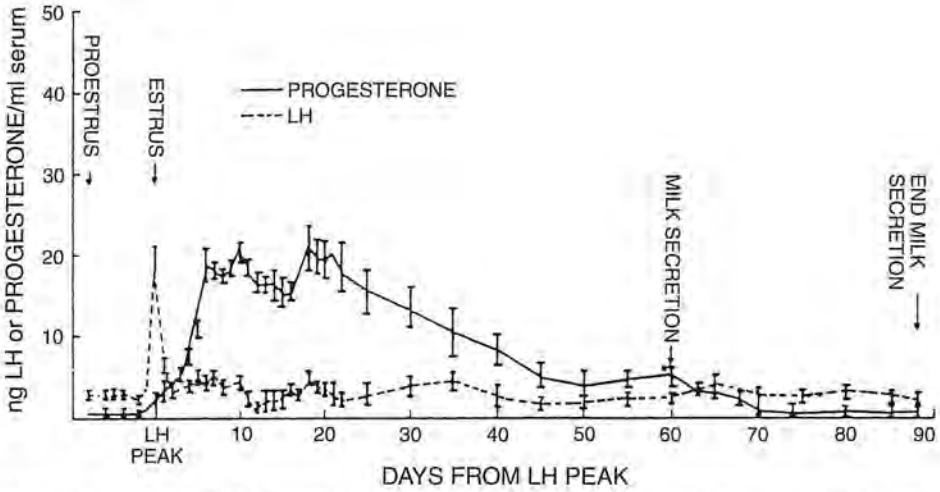

Figure 13–1. Serum concentrations of luteinizing hormone and progesterone during pseudopregnancy and lactation in three females. The vertical bars represent the standard error of the mean. (From Smith MS, McDonald LE: Serum levels of luteinizing hormone and progesterone during the estrous cycle, pseudopregnancy and pregnancy in the dog. Endocrinology 94:404–412, 1974, with permission.)

ample, withdrawal of exogenous progestins or OHE of a diestrual bitch—may precipitate false pregnancy signs. Lactation occurring after OHE of a diestrual bitch is a normal consequence of removal of ovaries containing functional corpora lutea, and should not be considered a negative postoperative complication or indication of improper surgical technique.

False pregnancy does not predispose the bitch to disease of the reproductive tract. No excessive or prolonged luteal function was observed in a study of 12 bitches with false pregnancy.[15] There is no reported correlation with false pregnancy and historical estrus irregularity.[16] In a study correlating history of false pregnancy with pyometra, it was reported that fewer dogs with pyometra had a history of false pregnancy (56.6 per cent) than did reproductively normal age-matched controls (77.4 per cent).[17] Galactorrhea was reported in four bitches with clinical hypothyroidism, possibly resulting from increased release of thyrotropin-releasing hormone, which may stimulate release of prolactin.[18,19] However, normal false pregnancy was not ruled out definitively in these bitches. Galactorrhea resolved with appropriate supplementation with L-thyroxine, but the resolution of signs was not proven to be caused by treatment.

The unique prolonged diestrus of nonpregnant dogs and subsequent predisposition to galactorrhea has been described as an atavism, or vestigial pack behavior.[3] Female dogs housed together tend to cycle together ("dormitory effect") and therefore reach the end of diestrus and either whelp or exhibit galactorrhea at about the same time. Because members of a pack of wild canids usually are related, and because only the dominant animals are likely to successfully breed, it is genetically advantageous for subordinate females in the pack to contribute to raising of the dominant bitch's pups.

The age at onset of false pregnancy varies; 17.9 per cent of bitches exhibited false pregnancy signs after their first estrus, while 7 per cent of bitches did not exhibit false pregnancy signs until more than 4 years of age.[4] Bitches may not exhibit overt false pregnancy after every estrous cycle.[4,5]

Diagnosis is by physical examination and by exclusion of pregnancy. A lateral abdominal radiograph taken more than 54 days after breeding or abdominal ultrasonography performed more than 24 to 28 days after breeding definitively differentiates true from false pregnancy. Although the mammary secretion of false pregnancy does vary from that in a normal lactation, wide variation in protein content of mammary secretions from dogs with overt false pregnancy precludes accurate assessment of abnormal versus normal lactation by analysis of a single mammary fluid sample.[20] Other causes of mammary development are uncommon. Galactorrhea caused by hyperprolactinemia from a functional hypophyseal tumor has not been reported in the dog.

Treatment usually is unnecessary in dogs with mild clinical signs of false pregnancy.[1]

Spontaneous remission occurs as baroreceptors in the engorged mammary glands signal a neuroendocrine reflex causing decreased prolactin secretion and subsequent decreased lactation. Clinical signs of false pregnancy are variable in duration, and may be present for up to 6 weeks. Mild caloric and fluid restriction may hasten remission. Dogs treated with antiprolactin therapies described below may show a faster regression of clinical signs than do untreated bitches.[21] In bitches with discomfort secondary to mammary gland enlargement, alternating cold and warm compresses on the engorged mammae or wrapping the abdomen with Ace bandages may give relief. The owner must be cautioned not to "milk-out" the mammary glands, because that will only stimulate lactogenesis.

Bitches with false pregnancy that exhibit extreme behavioral changes may benefit from mild tranquilization. Short-term (4-day) oral therapy with diazepam, a benzodiazepine tranquilizer, has been described.[22] The use of phenothiazine tranquilizers (e.g., acepromazine) or butyrophenones (e.g., haloperidol) is not recommended, because both of these classes of drugs act as antagonists of dopamine, a prolactin inhibitor, thus resulting in increased prolactin release.[19]

Megestrol acetate (Ovaban; Schering Corporation, Kenilworth, NJ) a progestin, is the only drug currently approved for treatment of overt false pregnancy in female dogs in the United States. Administration of this synthetic progestin after the normal decline of endogenous progesterone late in diestrus reverses the sequence of endocrine events that precipitated the onset of false pregnancy. Megestrol acetate is administered at 2.5 mg/kg/day orally for 8 days.[1] Dogs may show side effects of increased appetite and changes in temperament while receiving the drug,[1] and, unfortunately, often relapse after cessation of therapy when serum progesterone concentrations again decline. Progestins should not be given to bitches with diabetes mellitus or mammary neoplasia, or to pregnant dogs,[1] in which exogenous progestin administration is reported to cause masculinization of female fetuses. Prolonged or repeated use of megestrol acetate may promote development of cystic endometrial hyperplasia, predisposing the bitch to pyometra.[1] Other progestins described in the literature for treatment of false pregnancy include medroxyprogesterone acetate, hydroxyprogesterone acetate, and proligestone.[1,23] All are associated with side effects as described for megestrol acetate and are not recommended for routine clinical use.[1]

Administration of androgens may decrease clinical signs of false pregnancy in bitches. Testosterone, most commonly administered as testosterone proprionate, has variable efficacy.[1] The weaker androgen mibolerone (Cheque; Pharmacia & Upjohn, Peapack, NJ) is more commonly used.[24,25] Doses of 0.6 to 37 μg/kg/day orally for 2 to 10 days have been described.[1,24,25] The optimal dose, defined as that dose at which the majority of bitches with false pregnancy showed a significant decrease in physical and behavioral clinical signs and did not require retreatment, is reported as 16 μg/kg/day orally for 5 days.[24,25] Side effects noted at the dose of 37 μg/kg/day included clitoral hypertrophy, vaginal discharge, mounting behavior, musky body odor, and epiphora.[1] Mibolerone should not be given to pregnant bitches, because it may induce birth defects in puppies exposed in utero.[1] Mibolerone is not approved for treatment of false pregnancy in dogs in the United States.

Use of antiprolactin drugs has been described as effective in treatment of false pregnancy in the bitch. Prolactin secretion is stimulated by serotonin and inhibited by dopamine. Therefore, either serotonin antagonists (e.g., cabergoline[4,26,27] or metergoline[2,21]) or dopamine agonists (e.g., bromocriptine[5,21]) cause a decrease in hypophyseal prolactin release and decreased lactogenesis.

Cabergoline, at doses of 1.5 to 5 μg/kg/day orally for 2 to 8 days, has been reported to cause a clinical response in 3 to 4 days, with clinical signs substantially reduced or absent within 7 days of treatment.[4,26,27] At a dose of 5 μg/kg/day orally for 5 to 7 days, 80 per cent of dogs were reported to show clinical improvement within 7 days, and an overall success rate of 95 per cent was reported.[26,27] No attempt was made to differentiate response to treatment from spontaneous resolution. Side effects include apathy (25.0 per cent), emesis (3.0 to 10.7 per cent), inappetance (5.0 per cent), and ataxia (3.6 per cent).[4,26,27] Approximately 3 per cent of animals are reported to require retreatment because of relapse after 5 to 8 days of therapy.[4] Cabergoline is not available in the United States at this time. Metergoline has been only briefly described; no specific dose was reported.[2] Side effects include whimpering, aggression, emesis, and extreme restlessness.[2,21]

Bromocriptine, at a dose of 30 μg/kg/day orally for 16 days, was reported to cause decreased lactation in 89 per cent and decreased behavioral signs in 90 per cent of dogs treated for false pregnancy.[5] Emesis was reported to occur in 20 to 30 per cent of treated bitches.[5,21] Emesis may be controlled with concurrent treatment with metoclopramide at a dose of 0.5 mg/kg.[5] Metoclopramide has dopamine receptor–blocking properties but has not been shown to decrease efficacy of bromocriptine for treatment of false pregnancy.[5] Treatment with lower doses of bromocriptine and concomitant treatment with mibolerone does not enhance resolution of clinical signs or decrease side effects.[5] Bromocriptine is not approved for use in dogs in the United States.

Estrogens are described as a therapy for false pregnancy but have limited clinical use.[1] Side effects reported include uterine and vaginal hypertrophy and bleeding, blood dyscrasias, prolonged sexual receptivity, and pyometra.[1] Estrogens are not recommended as a treatment for false pregnancy in dogs.

Mastitis

Mastitis occurs in the lactating mammary gland as a sequel to trauma to the nipple from nursing and/or ascent of bacteria on the skin into the milk ducts and gland itself. Mastitis in the bitch with false pregnancy is similar to that in the lactating bitch following parturition (see Chapter 7).

Mammary Dysplasia/Fibrocystic Disease of the Mammary Gland

Mammary dysplasia and hyperplasia have been described as occurring in up to 5 per cent of female dogs presenting with mammary neoplasia.[28–30] One specific mammary dysplastic condition is fibrocystic disease of the mammary gland, also called blue dome cyst or polycystic mastopathy.[31] Middle-aged to older dogs may present with single nodules or multinodular, rubbery masses that are well circumscribed and slow growing. The overlying skin is blue tinged. Serous brown fluid can be aspirated from the mass. The precancerous potential of this and other mammary dysplasias is unknown.[31]

Mammary Neoplasia

Mammary neoplasms are the second most common tumor type reported in female dogs, after skin tumors.[31–34] Incidence of mammary neoplasia as a presenting complaint at one hospital was 3.4 per cent.[35] Incidence of mammary tumors in surveys of neoplastic disease in female dogs averaged 27.1 per cent, with a range of 8.4 to 52.0 per cent.[31–34,36–38]

The World Health Organization has classified mammary tumors into six broad classes (Table 13–1). Mammary neoplasms of dogs may be classified using this scheme, with definition of tumors derived from epithelial cell types (e.g., adenocarcinomas), connective tissue cell types (e.g., sarcomas or lipomas), or a mixture of cell types (e.g., mixed mammary tumors).[28]

Both malignant mammary tumors, defined by local infiltration and tendency to metastasize, and benign mammary tumors occur in the dog. Reported percentages of mammary tumors defined by histology as malignant vary from 39.0 to 91.0, with an average of 55.6; range and average percentages for benign mammary tumors are 9.0 to 61.0 and 44.0, respectively.[31,34,35,39–41]

The three broad categories of malignant mammary tumors described in the dog are carcinomas, malignant mixed tumors, and sarcomas. Adenocarcinomas are reported to be the most common malignant mammary tumor type (37 to 85 per cent), and malignant mixed mammary tumors the second most common (7 per cent).[29,31,38,39,42–44] Malignant mammary carcinomas described include adenocarcinoma, squamous cell carcinoma, basal cell carcinoma, ductular carcinoma, tubular carcinoma, papillary carcinoma, anaplastic carcinoma, and metastatic colonic carcinoma,[31,36,39,42,45] Malignant sarcomas described include osteosarcoma, fibrosarcoma, mastosarcoma, hemangiosarcoma, chondrosarcoma, liposarcoma, and myxosarcoma.[38,39,42,43,46,47] Metastatic malignant melanoma also has been reported in mammary tissue.[47]

Mixed mammary tumors are reported to occur in 50 to 66 per cent of female dogs with mammary neoplasia.[38,48] The majority of these are benign fibroadenomas, the most common type of benign mammary tumor reported.[31,38,41,43] Other benign mammary masses reported in the bitch include myoepithelioma, lipoma, fibroma, ductular papilloma, cystadenoma, mastocytoma, and hemangioma.[38,39,43]

Dogs may experience multiple histologically different mammary tumors concurrently.[31,49,50] Sixty-three per cent of dogs with mammary neoplasia in one study had more than one mammary mass at time of initial diagnosis,[49,50] and 37 per cent had both benign and malignant

Table 13-1. Histologic Classification and Nomenclature of Tumors and Dysplasias of the Mammary Gland

I. Carcinoma
  A. Adenocarcinoma
    1. Tubular
      (a) Simple type*
      (b) Complex type†
    2. Papillary
      (a) Simple type
      (b) Complex type
    3. Papillary cystic
      (a) Simple type
      (b) Complex type
  B. Solid carcinoma
    1. Simple type
    2. Complex type
  C. Spindle cell carcinoma
    1. Simple type
    2. Complex type
  D. Anaplastic carcinoma
  E. Squamous cell carcinoma
  F. Mucinous carcinoma
II. Sarcoma
  A. Osteosarcoma
  B. Fibrosarcoma
  C. Osteochondrosarcoma (fibrolipoosteochondrosarcoma) [combined sarcoma]
  D. Other sarcomas
III. Carcinosarcoma (malignant mixed tumor)
IV. Benign or apparently benign tumors
  A. Adenoma
  B. Papilloma
    1. Duct papilloma
    2. Duct papillomatosis
  C. Fibroadenoma
    1. Pericanalicular
    2. Intracanalicular
      (a) Noncellular type
      (b) Cellular type
    3. Benign mixed tumor
    4. Total fibroadenomatous change
  D. Benign soft-tissue tumor
V. Unclassified tumors
VI. Benign or apparently benign dysplasias‡
  A. Cyst
    1. Nonpapillary
    2. Papillary
  B. Adenosis
  C. Regular typical epithelial proliferation in ducts or lobules
  D. Duct ectasia
  E. Fibrosclerosis
  F. Gynecomastia
  G. Other non-neoplastic proliferative lesions
    1. Noninflammatory lobular hyperplasia
    2. Inflammatory lobular hyperplasia

* The term *simple* is applied to any type of neoplasm or proliferation composed of cells resembling either secretory epithelial cells or myoepithelial cells.
† The term *complex* is applied to any type of neoplasm or proliferation composed of cells resembling both secretory epithelial cells and myoepithelial cells.
‡ The term *dysplasia* is used as defined in the World Health Organization classification of human breast tumors and not in the sense of certain disorderly proliferations together with a certain degree of cytonuclear atypia.
From Hampe JF, Misdorp W: Tumours and dysplasias of the mammary gland. Bull World Health Organ 50:111, 1974, with permission.

masses.[50] Presence of a benign mammary mass increased risk of developing subsequent malignant mammary tumors three-fold in one study.[51] Mammary tumors also may occur concurrently with other neoplasms in the same animal, including vaginal leiomyomas and ovarian thecal cell tumors.[52,53]

Intact female dogs have a four times greater risk of developing both benign and malignant mammary tumors than ovariohysterectomized dogs.[41,49,51,54,55] Dogs ovariohysterectomized before their first estrous cycle have 0.5 per cent the risk, those spayed after one estrous cycle 8.0 per cent the risk, and those spayed after two cycles 26.0 per cent the risk of developing mammary neoplasia of intact females.[54] The sparing effect of OHE is lost after females have cycled more than twice or are more than 2.5 to 4 years of age.[54,55]

Correlation of abnormal reproductive history with incidence of mammary neoplasia is unclear. In one survey of 105 cases of mammary neoplasia, most of the affected bitches were reported to have had few or no pregnancies and multiple false pregnancies.[39] However, several other studies reported no effect of number of previous pregnancies, age at first pregnancy, or history of irregular estrous cycles on incidence of mammary neoplasia in dogs with the above history and in age-matched controls.[16,33,35,49] Several studies also documented that fewer bitches with mammary neoplasia had a history of false pregnancy than age-matched controls.[33,35] In humans, lactation, especially early in life, appears to protect women from eventual mammary neoplasia.[38,56] This effect is undocumented in the bitch. Basal serum prolactin concentrations do not differ between intact dogs with mammary neoplasia and age-matched controls.[57]

Pathogenesis

Mammary cells that undergo neoplastic transformation must be effected by an "initiator" and abnormal growth stimulated by a "promoter." Steroid hormones may act as initiators or promoters, explaining the increased incidence of mammary neoplasia in intact versus neutered female dogs. Progestins stimulate local production of growth hormone, which induces formation of insulin-like growth factor (IGF) and IGF-binding protein.[58] In this highly proliferative environment, risk of malignant transformation may be enhanced.[58] Estrogen may have a similar effect because it is a known mitogen for mammary epithelium.[30,59] In a study of 172 dogs administered human contra-

ceptive steroids containing estrogens and progestins for 5 to 7 years, 66 per cent developed multiple mammary nodules, benign mixed mammary tumors, and malignant mammary tumors; however, no comparison with age-matched intact controls was reported.[60]

Receptors for estrogen, progesterone, epidermal growth factor, and prolactin have been identified in normal mammary tissue of intact and spayed dogs, and in mammary masses.[61–63] Malignant tumors have a significantly smaller number of receptors for the above hormones, with the number of receptors decreasing as masses become less well differentiated.[61–64] This may be due to loss of the gene or gene function encoding for these receptors in very anaplastic mammary tissue[61]; an adenocarcinoma was demonstrated to contain a translocation (t[4;27]) in 25 per cent of metaphases examined, suggesting either stimulation of an oncogene or loss of growth-regulating genes at that site.[65]

Environmental estrogens are hypothesized to be a factor predisposing women to mammary neoplasia. The incidence of human breast cancer has increased significantly since 1940. Some may be an apparent increase in incidence resulting from better detection. Lifestyle factors exposing women to more estrogen in their lifetime—for example, earlier menarche and fewer childbirths—also play a role, as may increased exposure to environmental estrogens, such as xenoestrogens.[56]

Xenoestrogens are foreign substances that activate estrogen receptors or alter estrogen activity in the body.[56] Xenoestrogens are found in soy products, cruciferous vegetables (broccoli and cauliflower), pesticides, drugs, fuels, and plastics.[56] The amount and type of xenoestrogens that may induce neoplastic transformation of mammary epithelium by mimicking estrogen's ability to stimulate epithelial growth, induce release of endogenous estrogen, or promote vascular growth is unknown.[56] The impact of xenoestrogens in the dog is unknown.

Body conformation and diet may contribute to development of mammary neoplasia in the bitch. In one study, bitches that were judged to have been thin at 9 to 12 months of age had a significantly reduced risk of mammary neoplasia in later life, when retrospectively comparing female dogs with mammary neoplasia, age-matched female dogs with non-mammary cancer, and age-matched female dogs without neoplasia.[66] Similarly, bitches judged to have been obese at 1 year of age had a significantly greater risk of developing mammary neoplasia.[67] Female dogs fed homemade (noncommercial) diets, especially diets high in beef or pork, also had an increased risk of developing mammary neoplasia.[67] Women who consume diets high in animal fat or alcohol also may be at increased risk of mammary neoplasia.[56]

Altered immune function may play a role in pathogenesis of mammary neoplasia in the dog. No tumor-specific antigen has been identified that causes an effective humoral or cellular immune response, and immune complexes identified to date in dogs with mammary tumors generally impede rather than enhance antitumor immune response.[68] Fibrin has been demonstrated in canine mammary tumors.[69] In humans, fibrin impedes the immune response by protecting neoplastic cells from cytotoxic cells and decreasing macrophage migration.[69]

Mammary tumors in mice may be induced by viruses. One canine study identified particles associated with a virus in a line of mammary carcinoma cells but did not unequivocally demonstrate viral-induced malignant transformation of mammary cells.[70]

Signalment

Mammary neoplasia is rare in animals less than 5 years of age[49]; in one retrospective study, only 3 per cent of dogs with mammary neoplasia were less than 3 years of age.[29] Mammary dysplasia has been diagnosed in dogs as young as 2 to 3 years of age; the potential of dysplasia as a preneoplastic change is unknown.[71] Mean age at diagnosis of mammary neoplasia in female dogs is 10.1 years, with a range of 8.8 to 11.2 years.[29,32,35,42,72] Reported mean age at diagnosis of benign tumors is slightly lower, at 10.0 years, than is mean age at diagnosis of malignant tumors, at 11.0 years.[41] No correlation has been shown between age at diagnosis and specific tumor type.[43]

Breed predisposition for mammary neoplasia is not well defined in the literature. Studies disagree as to whether purebred dogs are more likely to develop mammary neoplasia than are crossbred dogs.[31,41] Similarly, one study reports no breed predisposition,[39] while others support increased incidence in hunting dogs (German shorthaired pointers, English and Irish setters, English springer and Brittany spaniels, and Labrador retrievers),[43] nonsporting breeds (great Pyrenees, samoyeds, Airedale terriers, miniature and toy poodles, and keeshonds),[73]

and the fox terrier, American cocker spaniel, and Boston terrier.[31] The boxer breed was under-represented in the latter study.[31] Genetics does play a role in human mammary neoplasia but accounts for only 5 per cent of cases.[56] Median inbreeding coefficient does not differ between dogs with malignant mammary neoplasia and normal female dogs.[74]

Clinical Findings

The most common presenting complaint in female dogs with mammary neoplasia is presence of a palpable or visible mass in the mammary gland. More than half of affected dogs have multiple tumors in the same or different glands.[31,49,50] Tumors must be at least 1 cm in diameter to be easily palpable, and may be difficult to feel in glands altered by pseudopregnancy or perineoplastic inflammation.[31] The fourth and fifth glands are most commonly affected, with 52 to 64 per cent of tumors reported to occur there.[29,35,38,39,42,75]

Palpation alone does not allow differentiation of benign from malignant masses. Either may be solitary or multiple.[35] Although malignant tumors generally are larger, great variability in size exists for both types.[35] Benign tumors generally have a slower rate of growth and are freely movable under the skin.[47] Malignant tumors have a more rapid rate of growth; sarcomas can double in volume in 9 days and carcinomas in 16 to 28 days.[76] Malignant masses often are fixed to overlying skin or deep tissues, are locally invasive, and may be associated with ulceration or hemorrhage.[38,47]

Dogs with benign mammary neoplasia often are asymptomatic. Clinical signs of mammary neoplasia may be due to the discomfort caused by the growth of the mass, and include excessive licking at the mass, hesitance to lie down, and resistance to manipulation of the abdominal area.

Metastasis of malignant mammary tumors is common, with a reported incidence of 93 per cent for all malignant mammary tumor types.[42] Fifty-one per cent of malignant mixed mammary tumors, 67 per cent of mammary adenocarcinomas, and 100 per cent of mammary sarcomas are reported to metastasize.[39,46] The most common sites of metastasis for all malignant mammary tumor types are the lungs and regional lymph nodes.[42,46,73] Other reported sites of metastasis include the adrenal glands, kidneys, heart, liver, bone, brain, eyes, nose, spleen, uterus, and skin.[31,42,46,47,73,77,78]

Clinical signs may be referable to the site of metastasis of malignant mammary tumors. Pulmonary metastases may not be clinically evident; inability to auscultate abnormal lung sounds does not rule out pulmonary metastatic disease.[47] Seventy per cent or more of the lung parenchyma must be replaced or obstructed before signs are evident.[31] Dogs may exhibit exercise intolerance, dyspnea, fatigue, rales, and cyanosis.[31,47] Occasionally signs of hypertrophic pulmonary osteoarthropathy, such as lameness, swelling and palpable periosteal new bone formation of the distal extremities, may be present.[31]

Regional lymph node enlargement (axillary or inguinal) may be palpable. Lymphedema may occur, and is more common in the hindlimbs than the forelimbs.[47,78] Metastases to other tissues causes signs specific to those sites, for example, hyphema and iridocyclitis in the eye, lameness or limb swelling with metastasis to bone, and ataxia, convulsions, or circling with brain metastases.[31,77]

Diagnosis and Staging

Presumptive diagnosis of mammary neoplasia is made on physical examination. All mammary glands on both sides should be palpated carefully. A complete physical examination, with auscultation of the chest and palpation of the regional lymph nodes and abdomen, should be performed. Complete blood count, serum chemistry profile, urinalysis, thoracic radiography, and abdominal radiography or ultrasound should be performed.[79] Female dogs with aggressive malignant mammary tumors may have evidence of disseminated intravascular coagulation, with hemorrhage and thrombocytopenia on complete blood count. Serum alkaline phosphatase concentrations may be elevated in female dogs with either benign or malignant mammary neoplasms.[80]

Metastases may not be detected readily on radiographs or by palpation. Geriatric changes in the lungs of aged female dogs may mask small pulmonary metastases. Radionuclide lymphoscintigraphy was demonstrated to accurately identify 100 per cent of lymph nodes with metastatic disease and 82 per cent of lymph nodes free of metastatic disease in dogs in one study.[81]

Collection of neoplastic cells, usually by fine-needle aspiration or excisional biopsy, is required for definitive diagnosis of tumor type and malignancy. Malignant tumor cells are rarely exfoliated into mammary secretions,

and most affected bitches not have mammary secretions that can be expressed. If mammary secretion is present, fluid from affected glands containing benign masses varies from normal milk because of an increased number of red blood cells and the presence of clusters of foam cells and ductal epithelial cells with increased nuclear:cytoplasm ratio.[82]

Fine-needle aspirate of mammary masses often is nondiagnostic. Evaluation of cells collected by fine-needle aspiration or scraping of mammary masses yielded a correct diagnosis in only 34 of 57 cases (60 per cent) in one study.[83] Tumor foci often are interspersed with normal tissue, so collection of a small number of cells may be inadequate for diagnosis of the type of mammary neoplasm present.[31,47] Aggressively growing malignant neoplasms may induce local inflammation that may make accurate evaluation of a cytologic specimen difficult. Interpretation of cytologic specimens is difficult because of the variable morphology of neoplastic mammary cells (Fig. 13–2). Therefore, fine-needle aspiration samples should be considered diagnostic only if definitive evidence of malignancy is present.

Excisional biopsy more readily permits definitive diagnosis of tumor type because a larger sample is collected. Incisional biopsy is not recommended because damage to vessels and lymphatics within the mass may promote dissemination of malignant cells.[47,79] Excisional biopsy is the preferred technique.[79]

Tumors should be staged according to the guidelines of the World Health Organization to allow the best possible recommendation for treatment and assessment of prognosis. A TNM evaluation is performed, assessing characteristics of the tumor itself (T), metastasis to regional lymph nodes (N), and metastasis to distant sites (M) (Tables 13–2 and 13–3). The TNM evaluation may be used to guide treatment.

Treatment

Surgical therapy is always recommended in cases of canine mammary neoplasia because it permits complete removal of localized tumors and debulking of invasive tumors to optimize adjunctive therapies (Table 13–4). Basic principles of oncologic surgery include the following[79]:

1. Avoid local anesthesia; tissue distortion secondary to anesthetic infusion may make assessment of completeness of tumor removal difficult.
2. Minimize manipulation of the tumor mass; exfoliation of malignant cells may lead to local recurrence.
3. Remove a minimum of 1 cm of apparently normal tissue around the tumor.
4. Ligate the blood supply to and venous drainage from the tumor early; this will decrease hematogenous spread of tumor emboli.
5. After removal of the tumor, flush the site with sterile saline to remove exfoliated cells.
6. Always submit excised tissues for histopathology to allow assessment of tumor type and completeness of excision.

Controversy exists as to what extent of surgery provides the best long-term outcome. In one study of 97 dogs with mammary neoplasia, extent of surgery had no effect on cancer-free interval postoperatively.[50] Surgical options include lumpectomy (removal of mass), simple mastectomy (removal of mass and associated gland), radical mastectomy (removal of mass, associated gland, regional lymph nodes, and intervening tissues; Table 13–5), unilateral complete mastectomy (removal of one mammary chain), and bilateral complete mastectomy (removal of both mammary chains). With any kind of surgical therapy, tumor removal is reportedly complete in only about 50 per cent of dogs,[76] and malignant neoplasms often recur, with adenocarcinomas recurring 20 to 44 per cent of the time and malignant mixed mammary tumors recurring 19 per cent of the time.[39,50]

Ovariohysterectomy (OHE) at the time of tumor excision may or may not prolong survival time. In one study of 41 dogs with mammary neoplasia, 23 of which were treated with mastectomy alone and 18 treated with mastectomy and OHE, mean survival times after surgery were 6.1 months and 18.5 months, respectively.[84] However, in all other studies, concurrent OHE at the time of surgical mass removal did not affect survival time or change the biologic behavior of the tumor.[16,49,50] In a study of 108 dogs with mammary neoplasia, tumor growth recurred after surgery in 22 per cent of dogs treated with mass removal alone, and recurred in 43 per cent of dogs treated with mass removal and concurrent OHE.[29] If the two surgeries are performed at the same time, the OHE should be performed first to prevent seeding of mammary tumor cells into the abdominal cavity.

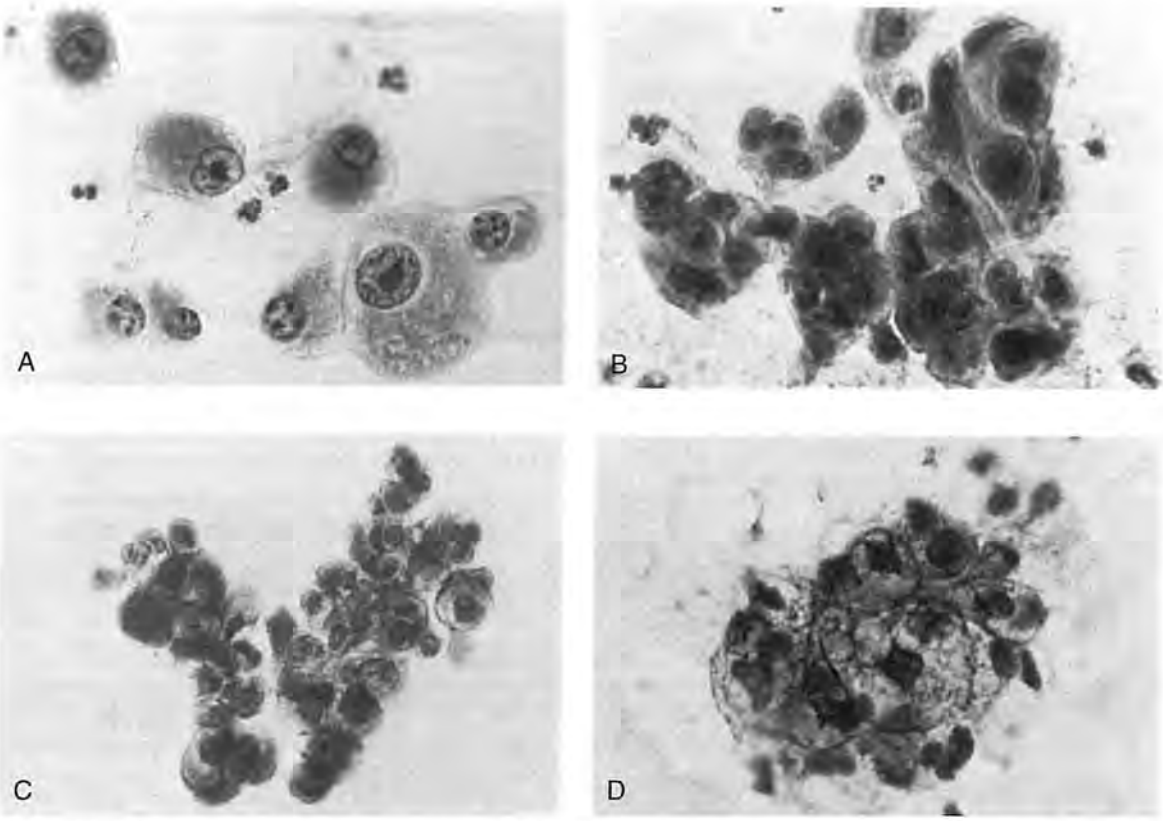

Figure 13–2. Exfoliative cytology in various mammary disorders. Samples were collected by fine-needle aspiration. Note that both benign and malignant cells may be single or in clusters, and that appearance of single neoplastic cells may not be appreciably different from cells seen during normal mammary development and lactation. **A:** Single carcinoma cells in a smear of expressed mammary fluid. The subsequent histologic diagnosis was anaplastic ductal carcinoma. **B:** Loose cluster of carcinoma cells in a scraping from an ulcerated area near the site of a previously removed mass histologically diagnosed as mammary ductal carcinoma. **C:** Papillary cluster of epithelial cells in a smear of canine mammary fluid. The cytologic diagnosis of a benign tumor was supported by the subsequent histologic diagnosis of ductal papilloma. **D:** Cluster of foamy cells in mammary fluid collected from the single enlarged gland of a bitch during pseudocyesis. Following involution this gland appeared normal (Sano's trichrome, 780 X). (From Brodey RS, Goldschmidt MH, Roszel JR: Canine mammary gland neoplasms. J Am Anim Hosp Assoc 19:61–90, 1983, with permission.)

Chemotherapy is most often described as adjunctive therapy after debulking of a primary tumor. Regimens described include doxorubicin (30 mg/m^2 intravenously [IV] every 3 weeks for at least two treatments), and a combination of cyclophosphamide (1 mg/kg orally once daily), vincristine (0.0125 mg/kg IV weekly), and methotrexate (0.3 to 0.5 mg/kg IV weekly).[40,85] Doxorubicin treatment is described as causing partial remission of pulmonary metastatic disease in two female dogs with adenocarcinoma for 12 to 18 months after therapy.[85]

Hormonal agents used to treat mammary neoplasia in the female dog include testosterone and tamoxifen. Testosterone may have a palliative effect; in one study, it increased survival time in dogs also undergoing mass removal and OHE by 10.9 months.[84] Tamoxifen, an estrogen receptor blocker, has been described as a successful treatment for mammary neoplasia at doses of 0.5 to 1.0 mg/kg orally once daily.[86–89] Estrogenic side effects, including vulvar hypertrophy, vulvar discharge, and hair loss, are reported[87–89]; in one study, 56 per cent of owners withdrew treatment because of severity of estrogenic side effects.[86] Side effects may be minimized by using the lower dose or a decreasing dose (1 mg/kg orally once daily, then 1 mg/kg every other day, then 1 mg/kg twice weekly, then 0.5 mg/kg twice weekly, then 0.5 mg/kg weekly).[87] Tamoxifen is not approved for use in dogs in the United States.

Table 13-2. Classification of Primary Canine Tumor (T)

Stage	Size (cm)	Skin	Underlying Tissue
T1	<5	Not involved	Not involved
T2	5-10	Dimpled	Not involved
T3	11-15	Infiltrated or ulcerated	Muscle fixation
T4	>15	Whole gland involved	Chest or abdominal wall

From Brodey RS, Goldschmidt MH, Roszel JR: Canine mammary gland neoplasms. J Am Anim Hosp Assoc 19:72, 1983, with permission.

Radiation therapy is not well described as a treatment for canine mammary neoplasia, and is generally considered an adjunctive therapy for primary tumors after debulking.[47] Radiation therapy is unlikely to affect metastatic disease.[47] There is one report in the literature of regression of multiple intraductal carcinomas after radiation therapy alone in a bitch.[90]

Immunotherapy has been described for treatment of canine mammary neoplasia. Stimulation of the immune system may be effected with levamisole (5 mg/kg three times weekly for 3 months, then weekly).[40] Tumor regression may be promoted with administration of mitomycin- and neuraminidase-treated autologous tumor cells,[68] or infusion of plasma incubated

Table 13-3. World Health Organization TNM Evaluation for Staging of Mammary Tumors*

Circle Appropriate Category		RLN Evaluated: Inguinal	RLN Evaluated: Axillary	Method of RLN Evaluation: Clinical	Method of RLN Evaluation: Radiographic (Lymphangiography)	Method of RLN Evaluation: Histologic
N0	No RLN involved	____	____	____	____	____
N1	Ipsilateral RLN involved	____	____	____	____	____
N2	Bilateral RLN involved	____	____	____	____	____
N3	Distant LN involved	____	____	____	____	____
NR	RLN previously removed	____	____	____	____	____

(check appropriate space)

M (distant metastasis) Categories (Circle appropriate category)

		Method of M Evaluation: Clinical	Radiographic	Histologic
M0	No evidence of lung metastasis	____	____	____
M1	Solitary lung metastasis	____	____	____
M2	Multiple lung metastasis	____	____	____
M3	Other nonlung sites of metastasis	____	____	____
		Specify sites ____		
M4	Solitary or multiple lung metastasis and other nonlung metastatic sites	Specify sites ____		

Summary Data

Mammary glands		Categories: T	Categories: N		Categories: T	Categories: N
TN categories	Left chain gland 1	____	____	Right chain gland 1	____	____
	gland 2	____	____	gland 2	____	____
	gland 3	____	____	gland 3	____	____
	gland 4	____	____	gland 4	____	____
	gland 5	____	____	gland 5	____	____

M category

Final Clinical Stage

	T	N	M
T N M Evaluation	____	____	____

* Characteristics of the tumor (T), metastasis to regional lymph nodes (N), and metastases to distant sites (M) are evaluated. RLN, regional lymph node.

From Brodey RS, Goldschmidt MH, Roszel JR: Canine mammary gland neoplasms. J Am Anim Hosp Assoc 19:73, 1983, with permission.

Table 13–4. Management of Canine Mammary Tumors at the Veterinary Cancer Unit of New York

Extent of Disease	Primary Therapy	Adjuvant Therapy and Follow-up Procedure
I. Local disease		
A. Benign tumor	Surgery*	(a) Recheck semiannually for local recurrence (b) Thoracic radiographs at each recheck
B. Well-circumscribed malignancy ("carcinoma in situ")	Surgery†	(a) Recheck every 2 months (b) Thoracic radiographs at every other recheck
C. Invasive carcinoma	Surgery†	(a) Recheck monthly (b) Thoracic radiographs every 3 months
II. Advanced local disease		
A. Malignant tumor remaining after surgery or regional lymph node metastasis	Surgery†	(a) Recheck monthly (b) Thoracic radiographs every 2 months (c) Repeat excision as needed (d) Immunotherapy‡
III. Disseminated disease		
A. Distant metastasis with or without local recurrence	Surgery†	(a) Recheck monthly (b) Thoracic radiographs every 2 months (c) Immunotherapy (d) Chemotherapy§

* Lumpectomy, removal of affected gland, or radical mastectomy.
† Preferably radical mastectomy (all ipsilateral glands, intervening tissues, and lymphatic drainage).
‡ For the purpose of reducing tumor volume. Includes resection of local recurrence and distant metastasis when possible.
§ Cyclophosphamide (Cytoxan; Bristol-Myers Squibb, Princeton, NJ), 1 mg/kg body weight per day orally; vincristine (Oncovin; Eli Lilly, Indianapolis, IN), 0.0125 mg/kg body weight intravenously weekly; methotrexate (Lederle, Pearle River, NY), 0.3 to 0.5 mg/kg body weight intravenously weekly.
From Harvey HJ, Gilbertson SR: Canine mammary gland tumors. Vet Clin North Am 7:213–219, 1977, with permission.

with protein A–positive *Staphylococcus aureus* Cowan I strain.[91] Neither IV treatment with the bacille Calmette-Guérin strain of *Mycobacterium bovis* nor vaccination with *Corynebacterium parvum* has been shown to be effective in treatment of mammary neoplasia in the dog.[68]

Prognosis of mammary neoplasia varies with tumor type and related criteria. Factors associated with a good prognosis, defined as survival of at least 2 years after diagnosis, include benign tumor type, slow rate of growth, small tumor size (< 5 cm), and lack of local infiltration.[92,93] Factors not associated with prognosis include type of surgical therapy used, gland(s) involved, and duration of signs before diagnosis.[16,49,50,85,92,93] Median survival time after surgical therapy for dogs with malignant mammary neoplasia is 4 to 8 months, with a reported range of 0 to 36 months.[35,42] Fifty-nine per cent of dogs with adenocarcinoma and 39 per cent of dogs with malignant mixed mammary tumors died or were euthanized within 2 years of surgery in one study.[39]

Table 13–5. A Commonly Accepted Protocol for Radical Mastectomy in the Dog

Location of Mammary Tumor	Glands and Lymph Nodes to be Removed
Gland 1	Glands 1, 2, and axillary lymph node (LN)
Gland 2	Glands 1, 2, 3, and axillary LN
Gland 3	Glands 1–5 and inguinal LN
Gland 4	Glands 3, 4, 5, and inguinal LN
Gland 5	Glands 4, 5, and inguinal LN

From Harvey HJ: General principles of veterinary oncologic surgery. J Am Anim Hosp Assoc 12:339, 1976, with permission.

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Chapter 14

Clinical Approach to Infertility in the Bitch

Normal fertility in the bitch requires ovulation of normal ova into a patent, healthy reproductive tract, insemination with normal semen near the time of ovulation, and maintenance of pregnancy for approximately 2 months.[1] Infertility is a clinical sign or presenting complaint that requires confirmation, characterization, and localization before an etiologic diagnosis can be established. Diagnosis is confounded by the inaccessibility of much of the female canine reproductive tract, the infrequent cycling of the normal bitch (the average interestrous interval is 5 to 8 months), and the relatively advanced age of show bitches before breeding is attempted.[2] Confirmation of the complaint of infertility usually must be made by interpreting historical events. An effort should be made to gather as much of the following information as possible.

1. *What is the signalment of the bitch?*
   Small-breed dogs may cycle earlier in life and more frequently than large- or giant-breed dogs. Average age at onset of pubertal estrus ranges from 9.6 to 13.9 months, and may be as late as 23 months in some normal bitches.[3]
2. *What is the environment in which the bitch is housed?*
   Bitches housed with other intact females may be induced to cycle in synchrony with those bitches, the "dormitory effect." If the bitch is housed with or exposed to an experienced male dog, that may help the owner pinpoint subtle signs of estrus. Vaccination and worming protocols and hygiene in the kennel should be determined. If the kennel is a closed colony, the isolation protocol employed for incoming animals should be reviewed.
3. *What is the general health of the bitch?*
   A complete medical history should be taken, to include medications she has received in the past? Previous endocrine therapy and endocrine disease should be noted.
4. *What is the reproductive history of the bitch?*
   Nature and timing of the estrous cycle may be similar within a family of bitches.[3] The following should be collected for the bitch in question. An abbreviated reproductive history should be taken for the bitch's dam and female siblings.
   - Dates of onset of proestrual bleeding
   - Dates of onset of first receptivity
   - Day of the estrous cycles on which ovulation occurred, if known (see Chapter 4)
   - Breeding dates and whether the bitch was bred by natural service and, if so, what were the nature and duration of the copulatory locks (ties). Although the duration of the copulatory lock is not correlated with fertility, it does suggest that ejaculation occurred.[3] If bred by artificial insemination, the type of fresh semen (fresh, chilled, frozen) and route of insemination (vaginal, intrauterine) should be noted (see Chapter 4).
   - Dates of first refusal of mating
   - Length of interestrous interval
   - Male fertility and whether the males used sired litters or have undergone semen evaluation within the 6 months prior to breeding the bitch. The ages of the males used and previous semen culture information, if any, should be recorded.
   - *Brucella canis* antibody status of the bitch and male(s) used, and dates of blood sample collection
   - Pregnancy diagnosis history, including the method used (palpation vs. ultrasound / radiographs).

- Previous whelpings and whether labor was normal. Occurance of dystocia, and cause(s), if known, should be recorded. Litter size, stillbirths, stunted puppies and puppies that died within the first several weeks of life should be noted.
- False pregnancy—False pregnancy is not indicative of nor does it predispose bitches to reproductive tract disease. Signs of false pregnancy are indicative of maintenance of a normal luteal phase with subsequent normal decline in serum progesterone concentration (see Chapter 13).
- Whether prior reproductive tract disease been diagnosed, such as ovarian cyst, or pyometra
- Whether the bitch has been treated with hormones for estrus suppression, estrus induction, pregnancy termination, false pregnancy.

Information acquired in the history can be used to determine whether the cycles were normal, whether breeding management was appropriate, and to generate a well-defined problem. Etiologic diagnoses can be considered based on whether the bitch is cycling, whether normal copulation occurred, and whether breedings that occurred were performed at the optimum time of the bitch's estrous cycle.

Routine laboratory assessment of the infertile bitch includes *Brucella canis* serology, canine herpesvirus serology, a complete blood count, serum chemistry profile, urinalysis, and thyroid hormone profile to include measurement of free thyroxine (T4) and canine thyroid-stimulating hormone (cTSH). Clinicians should discuss with the owner the possibility that successful treatment of infertility and subsequent conception may generate offspring with similar reproductive abnormalities.[2]

Persistent Anestrus

Primary Anestrus

Primary anestrus in the bitch is lack of estrous cycling by 24 months of age.[4] The clinician should ascertain whether the bitch is housed with cycling bitches, who may be able to induce the anestrous bitch to cycle (the dormitory effect). The bitch should be on an appropriate plane of nutrition for her activity level. If the bitch is being shown or worked heavily, she may be more likely to cycle if retired from constant stress and activity. Causes of primary anestrus in the bitch include the following differential diagnoses.

PREVIOUS OVARIOHYSTERECTOMY

Because some puppies are now neutered as early as 7 weeks of age, an owner may be unaware of reproductive status of animals purchased. Prior ovariohysterectomy (OHE) may be diagnosed by history, palpation or visualization of an OHE scar at the ventral abdominal midline, or via exploratory laparotomy. Serum concentrations of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are elevated in bitches that have undergone OHE and in bitches with ovarian dysgenesis or premature ovarian failure.[5] Lack of negative feedback from the nonfunctional or absent ovary to the anterior pituitary causes elevation of serum LH concentration to greater than 200 ng/ml and serum FSH concentration to greater than 290 ng/ml.[6] Serum gonadotropin concentrations can be assayed by radioimmunoassay at the Rothgerber Endocrinology Laboratory at Colorado State University (303-491-5620, Fort Collins, CO) or with an in-house enzyme-linked immunosorbent assay (ELISA) test marketed for breeding management (Status-LH; International Canine Genetics, Synbiotics Corporation, San Diego, CA). Challenge testing of serum estrogen also has been described. The gonadotropin releasing hormone analogue, buserelin, is administered intravenously (IV) at a dose of 0.02 to 0.03 μg/kg, and blood drawn 60 to 90 minutes later. Intact bitches, at any stage of the reproductive cycle, will show an elevation in serum estrogen concentration to greater than 15 to 20 pg/ml.[7] However, estrogen is very difficult to measure consistently in the bitch due to the low concentrations present and normal variability between and within dogs, and practitioners are advised to contact the laboratory used for information regarding accuracy of their specific estrogen assay for assessment of reproductive status in dogs.[8]

SILENT HEAT

Silent heat is defined as ovarian activity with no concomitant vulvar swelling, exudation of serosanguineous vulvar discharge, or attraction of male dogs.[4] In one survey of 108 bitches presented for reproductive dysfunction, 18 (17 per cent) were classified as having had a silent heat.[9] Silent heat can be diagnosed by monthly assay of serum progesterone concentrations,

with a value of greater than 2 ng/ml indicating presence of functional luteal tissue, or by weekly assessment of vaginal cytology, with increasing percentages of cornified vaginal epithelial cells indicative of rising serum estrogen concentrations.[4] There is one report of treatment of silent heat by estrus induction with FSH; 18 of 18 (100 per cent) bitches diagnosed with silent heat were successfully induced to cycle normally by administration of 25 to 50 IU FSH subcutaneously (SC) or intramuscularly (IM) for 6 to 8 days.[9]

ABNORMALITIES OF SEXUAL DIFFERENTIATION

Dogs that appear female that have abnormal chromosome complements may have a 78,XO, 79,XXX, 79, XXY, or 78,XX/78,XY karyotype.[4,37] Male pseudohermaphrodites, that have male gonads and female external genitalia, also may present for primary anestrus. A 4-year-old, intact female Airedale terrier presenting with primary anestrus was diagnosed with ovarian dysgenesis secondary to abnormal chromosome complement; she had a 79,XXX karyotype, and significantly elevated serum gonadotropin concentrations (LH, 670 ng/ml [normal is < 200 ng/ml], FSH, 11,210 ng/ml [normal is < 290 ng/ml]).[6] Diagnosis of abnormality of sexual differentiation is made by visual inspection of abnormal external genitalia (Fig. 14–1), histopathology of excised gonadal tissue, measurement of serum gonadotropin concentrations, and/or assessment of the karyotype (see Chapter 10).

DRUG-INDUCED ANESTRUS

Bitches receiving androgens or progestogens usually will not cycle. A thorough inquiry should be made regarding use of performance-enhancing drugs in show, field trial, working, and racing dogs. Similarly, the owner should be questioned regarding medications the bitch received for health or behavioral problems. Dogs receiving exogenous glucocorticoids have been shown to have decreased serum concentrations of LH and, presumably, decreased fertility.[10]

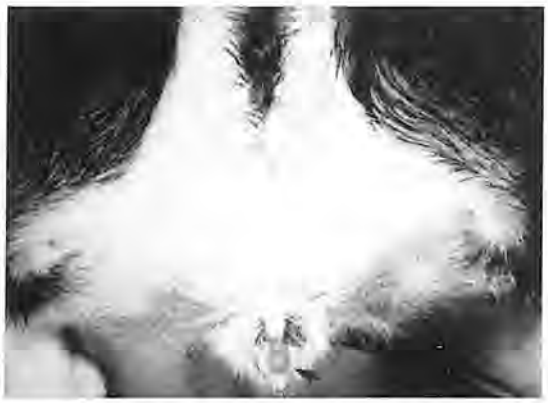

Figure 14–1. Enlarged clitoris in a canine true hermaphrodite. (From Fitzgerald AL, Murphy DA: Bilateral ovotestes in an intersex, mixed breed dog. Lab Anim Sci 40:647–650, 1990, with permission.)

HYPOTHYROIDISM

Hypothyroidism is the most common endocrine disorder of dogs, with reported prevalence of 0.2 per cent.[11] Prevalence may be increased in populations of infertile dogs; in one survey of 24 infertile bitches, 9 (37.5 per cent) were hypothyroid.[12]

Hypothyroidism may be classified as primary, in which the underlying abnormality is localized at the thyroid gland; secondary, localized at the pituitary; or tertiary, localized at the hypothalamus.[8] Primary hypothyroidism is the most common form in dogs.[13] Idiopathic thyroid atrophy, with replacement of normal glandular epithelial cells with adipose tissue, is the most common form of the disease.[13,14] Immune-mediated thyroiditis, with infiltration of the thyroid follicles with lymphocytes, also is described, and may be a preliminary step in progression to thyroid atrophy.[12] Immune complexes may lodge in the basement membrane of thyroid follicles, leading to damage by complement or natural killer (NK) cells.[14] Thyroid neoplasia is uncommon in the dog.[13]

Hypothyroidism may cause infertility by interfering with gamete maturation.[15] Thyroid hormones support granulosa cell function in developing ovarian follicles and are required for normal placental trophoblast function once conception has occurred.[16] However, dogs may have reproductive dysfunction and concurrent, noncausative hypothyroidism.[17] Hyperthyroidism has been shown to cause infertility in women; veterinary clients should be questioned as to whether an infertile bitch has been supplemented with thyroid hormone, and whether serum thyroid hormone concentrations have been measured in the supplemented animal.[18]

Although mode of inheritance has not been rigorously defined, hypothyroidism is considered a familial disorder.[14,19] Breeds predisposed to hypothyroidism include the golden retriever, Doberman pinscher, dachshund, Shetland sheepdog, Irish setter, Pomeranian,

miniature schnauzer, American cocker spaniel, Airedale terrier, bulldog, basenji, Great Dane, boxer, beagle, poodle, and borzoi.[11,13,14] Sexually intact female dogs are reported to be at relatively lower risk of developing hypothyroidism than females that have undergone OHE.[11]

Clinical reproductive signs have been reported in 5 of 53 (9 per cent) hypothyroid bitches. These include primary anestrus; prolonged or irregular interestrous intervals; prolonged proestrus; decreased intensity or duration of estrous cycles; galactorrhea; and increased incidence of spontaneous abortion, stillbirths, mummified fetuses, and puppies with low birth weight.[13,16,18-21] Extrareproductive signs of hypothyroidism in the dog include lethargy and mental dullness, obesity, heat seeking, bilaterally symmetrical truncal alopecia, slow regrowth of hair, seborrhea, change in character of pelage, hyperpigmentation of skin, bradycardia, weakness, and facial paralysis.[11,13]

Tentative diagnosis of hypothyroidism is based on history and clinical signs. Changes in a complete blood count, serum chemistry profile, and electrocardiogram that support a diagnosis of hypothyroidism include nonregenerative anemia, hypercholesterolemia, hypertriglyceridemia, and low-voltage R waves with a bradycardic trace on the electrocardiogram.[11,13,19] Diagnosis of canine hypothyroidism by measurement of serum triiodothyronine (T3) and T4 concentrations is confounded because many nonthyroidal factors influence serum concentrations of these hormones. Serum T3 and T4 concentrations are artifactually increased or decreased by nonthyroidal illness such as hyperadrenocorticism, and by therapy with glucocorticoids and other drugs.[8,13,22] Other factors that artifactually increase or decrease serum T3 and T4 concentrations include age, obesity, and stage of the estrous cycle, with serum T3 concentration increased during diestrus and serum T4 concentration increased during diestrus and pregnancy (Table 14–1).[23,24]

Circulating T3 and T4 are highly protein bound. Only the free form of T4 is physiologically active, suggesting that measurement of total hormone concentrations may not be as valuable as measurement of free hormone concentrations. Although one study reported that concentrations of free T4, measured by a dialysis technique (fT4D), did not differ from concentrations of total T4,[25] measurement of fT4D is considered the "gold standard," because it measures the fraction of hormone that diffuses through the dialysis membrane, mimicking movement of hormone through the cell wall.[22] Dialysis techniques for measurement of free T4 have been demonstrated to have a sensitivity of 0.98 and a specificity of 0.92, compared to a nondialysis technique that had a sensitivity and specificity of 0.9 and 0.83, respectively.[26]

Canine TSH (cTSH) is elevated in animals with hypothyroidism, due to lack of negative

Table 14–1. The Effect of Physiologic Conditions, Nonthyroidal Illness, and Drugs on Thyroid Function in Dogs

	TT4	fT4D	TT3
Condition present			
Neonate	↑		
Aging	↓	↓ then ↑	↓ then ↑
Pregnant	↑		
Fasting	N to ↓		↓
Obesity	↑		↑
Hyperadrenocorticism	N or ↓	N or ↓	N or ↓
Diabetes mellitus	N or ↓		↑ or ↓
Hypoadrenocorticism	N or ↓		
Renal disease	↓	N or ↓	N or ↓
Hepatic disease	N or ↓		N
Drug Therapy Used			
Glucocorticoid therapy	↓		↓
Anticonvulsant therapy	↓		N
Phenylbutazone	↓		

* Arrows indicate increase or decrease from normal. N indicates that results are generally within the normal range.
TT4, total thyroxine; fT4D, free thyroxine; TT3, total triiodothyronine.
Adapted from Figures 1, 3, and 4 from Ferguson DC: The effect of nonthyroidal factors on thyroid function tests in dogs. Compend Contin Educ Pract Vet 10:1365–1377, 1988, with permission.

feedback to the pituitary.[27] Canine TSH assays are commercially available but are neither sensitive (0.75) nor specific (0.90) enough to allow diagnosis of hypothyroidism based on cTSH values alone.[26,27] Twelve per cent of euthyroid dogs had increased serum cTSH concentrations and 38 per cent of hypothyroid dogs had normal serum cTSH concentrations in one study.[28]

Assay of autoantibodies to T3 (T3AA), T4 (T4AA), and thyroglobulin (TgAA) may be valuable in diagnosis of canine hypothyroidism. Presence of autoantibodies may artifactually alter results of radioimmunoassays for T3 and T4, and may exacerbate disease by binding free hormone.[29] Although dogs with decreased serum concentrations of T3, T4, and cTSH have been reported to be more likely to have significant concentrations of circulating thyroid hormone autoantibodies,[30] 13 per cent of euthryoid dogs have been reported to have elevated concentrations of TgAA and 40 per cent of euthyroid dogs have been reported to have elevated concentrations of T3AA.[31,32] Elevated concentrations of TgAA may or may not be indicative of increased T3AA and T4AA concentrations.[22,29] Presence of TgAA does not alter measurement of free serum T4 by dialysis.[26]

The current recommendation for definitive diagnosis of canine hypothyroidism is assessment of fT4D and cTSH.[33] Concurrent assessment increases sensitivity to 0.98, yielding only 2 per cent false-positive results (i.e., euthryoid dogs falsely diagnosed as hypothyroid).[26]

Canine hypothyroidism is treated by supplementation with 0.01 to 0.02 mg/kg L-thyroxine twice daily per os.[1,13] Absorption, metabolism, and excretion of the drug vary widely between animals and between products; serum concentrations relative to dose administered were shown to be superior for the veterinary product (Soloxine; Daniels Pharmaceuticals, St. Petersburg, FL) compared to a human product (Synthroid; Boots Pharmaceuticals, Lincolnshire, IL) and a generic product.[34,35] Recheck of serum T4 concentrations should be undertaken 4 to 6 weeks after treatment is instituted.[13] Postpill samples should be drawn 4 to 8 hours after the pill is regularly administered.[13,35] Do not recheck by assay of cTSH alone, because currently available assays are not sensitive enough to allow accurate monitoring of function of the pituitary / thyroid axis.[34] Thyroid hormone concentrations should be rechecked every 6 months while the animal is being treated. Certification of euthyroidism by the Orthopedic Foundation for Animals (OFA) requires yearly measurement of fT4D, cTSH, and TgAA until the animal is 5 years of age.[26]

Normal cycling should resume within 4 to 6 months of adequate replacement therapy, if no other reproductive tract dysfunction exists.[1] An infertile, hypothyroid, 5-year-old, intact female boxer whelped a litter of four pups 11 months after institution of thyroid replacement therapy.[36]

SYSTEMIC DISEASE

Bitches with systemic disease, such as renal failure or cancer cachexia, may be less likely to cycle than normal bitches. A complete blood count, serum chemistry profile, and urinalysis should be performed on all bitches with primary anestrus.

Hyperadrenocorticism has been reported to cause persistent anestrus with or without clitoral hypertrophy in greater than 75 per cent of affected bitches.[8] Elevated serum cortisol concentrations cause a decrease in LH synthesis or release from the pituitary with subsequent decrease in fertility.[10] Hyperadrenocorticism is best diagnosed with challenge testing and plasma adrenocorticotropic hormone (ACTH) concentrations, since baseline cortisol concentrations are affected by stress and medical therapy. Treatment for hyperadrenocorticism is dependent on whether the primary defect is in the pituitary or adrenal gland.

PROGESTERONE-SECRETING OVARIAN CYST

Functional ovarian luteal cysts may produce serum progesterone concentrations greater than 2 ng/ml. Negative feedback to the pituitary decreases gonadotropin release and inhibits cycling. Diagnosis requires demonstration of persistently elevated serum progesterone concentrations (see Chapter 10).

OVARIAN APLASIA

Ovarian aplasia is a rare congenital anomaly in dogs, in which there is defective prenatal germ cell migration.[15] Serum gonadotropin concentrations are elevated in affected dogs.[4]

IMMUNE-MEDIATED OOPHORITIS

Autoimmune destruction of the ovary has been described in the dog (see Chapter 10).

Secondary Anestrus

Secondary anestrus, or prolongation of the interestrous interval, is failure to cycle by 10 to 18 months of the previous cycle.[1,38] In a survey of 108 bitches presenting with reproductive dysfunction, 19 (18 per cent) presented with secondary anestrus.[9] The basenji, dingo, and wolf-dog hybrid typically cycle at 1-year intervals, so that prolonged interestrous intervals in these breeds is longer than those described for other dogs.[2]

Apparent prolongation of the interestrous interval occurs in dogs with silent heat, defined as ovarian activity in the absence of overt physical and behavioral changes characteristic of canine estrus.[4] Diagnosis requires monthly assay of serum progesterone concentrations, with a value of greater than 2 ng/ml indicative of functional luteal tissue, or weekly collection of vaginal epithelial cells, with increasing cornification indicative of elevated serum estrogen concentrations. A 30-month-old, intact female Chesapeake Bay retriever with secondary anestrus was determined to have had a silent heat, as evidenced by signs of false pregnancy secondary to the normal postdiestrual decline in serum progesterone concentrations.[39]

Prolongation of the interestrous interval may be associated with hypothyroidism and hyperadrenocorticism in the dog. Similarly, animals receiving exogenous glucocorticoids may show secondary anestrus.[10] Functional luteal cysts, that secrete progesterone, also may suppress normal cyclicity in bitches. Diagnosis requires demonstration of persistently elevated serum progesterone concentrations. There is one report of a 3.5-year-old, intact female collie with secondary anestrus due to lateral hermaphroditism, in which one of her gonads was a testis and the other an intermittently functional ovary.[40] Finally, estrus may be delayed by 1 to 2 months after pregnancy in some bitches; cause of this delay in cycling is unknown.[2,41]

INDUCTION OF ESTRUS

Estrus induction may be considered in a bitch either as a means of treating primary or secondary anestrus for which no underlying cause has been found, or to induce cycling, pregnancy, and whelping at a more convenient time than that permitted by her natural cycles. In normal dogs, the transition from anestrus to proestrus occurs in the presence of elevated serum FSH concentrations after increased pulsatile secretion of gonadotropin-releasing hormone (GnRH) and LH for several days, resulting in a mean increase in serum LH concentrations.[42] Serum concentrations of progesterone and prolactin are low at this time.[42] The three categories of drug protocols described for estrus induction are those that stimulate the ovarian follicle directly—pregnant mare serum gonadotropin (PMSG = equine chorionic gonadotropin [eCG]), FSH, LH, estrogen—those that stimulate release of pituitary gonadotropins (GnRH and its analogues), and those that shorten anestrus by inhibiting synthesis or release of prolactin (dopamine agonists) (Table 14–2).[43]

Availability and potency of hormone preparations for estrus induction in the bitch are, however, extremely variable. In addition, failure to achieve pregnancy is common. Side effects reported include thrombocytopenia due to hyperestrogenism in 29 per cent of dogs treated with PMSG (20 IU/kg SC once daily for 10 days),[44] and transient vomiting in 16.2 per cent of dogs treated with a dopamine agonist, cabergoline (5 μg/kg once daily per os [PO]).[45] Failure of protocols to induce estrus in dogs with normal ovaries is most likely caused by inadequate gonadotropin stimulation of developing ovarian follicles. Failure to achieve or maintain pregnancy is most likely caused by an insufficient luteal phase.[46] No safety studies have been reported that indicate whether these hormonal therapies alter future reproductive cycles.

Persistent Estrus

Persistent estrus is defined as combined proestrus and estrus of greater than 6 weeks.[7] Bitches with persistent estrus should be evaluated for evidence of bone marrow suppression secondary to estrogen toxicity, including nonregenerative anemia, leukopenia, and thrombocytopenia. Treatment of bone marrow suppression due to hyperestrogenism requires aggressive administration of blood products, supportive care, and treatment to remove the underlying cause of hyperestrogenism.

Persistent estrus is suspected in bitches with other conditions causing exudation of sanguineous vaginal discharge and attraction of male dogs, such as vaginitis.[2,66] True persistent estrus is most commonly caused by presence of functional ovarian follicular cysts or functional ovarian granulosa cell tumors (see

Table 14–2. Success of Reported Estrus Induction Protocols in the Dog

Protocol	Proestrus	Ovulation	Pregnancy	Reference
Gonadotropins				
PMSG, 150 or 300 IU, 3 times on alternate days	0%	—	—	Van Haften et al.[47]
PMSG, 20 IU/kg sid × 5 days + hCG, 500 IU IM	—	—	50% (n = 6)	Arnold et al.[44]
PMSG, 20 IU/kg sid × 10 days + hCG, 500 IU IM	—	—	35% (n = 17)	Arnold et al.[44]
PMSG, 20 IU/kg sid IM × 5 days + hCG, 500 IU IM	—	—	56% (n = 9)	Levya-Ocariz[48]
PMSG, 20–500 IU/kg SC × 10 days + hCG, 500 IU SC	—	56% (n = 25)	—	Thun et al.[49]
PMSG, 44 IU/kg sid IM × 9 days + hCG, 500 IU IM	64% (n = 11)	—	18% (n = 11)	Nakao et al.[46]
PMSG, 44 IU/kg sid IM × 9 days + hCG, 500 IU IM	100% (n = 5)	80% (n = 5)	—	Archbald et al.[50]
PMSG, 44 IU/kg sid SC × 9 days + hCG, 500 IU IM	60% (n = 5)	80% (n = 5)	—	Archbald et al.[50]
PMSG, 44 IU/kg IM × 5–9 days + hCG, 500 IU IM	—	—	9% (n = 11)	Tsuda et al.[51]
PSMG, 250 IU/kg SC × 20 days or until proestrus induced + hCG, 500 IU SC	62.5% (n = 8)	40% (n = 5)	—	Wright[52]
PMSG, 500 IU/kg IM × 10 days + hCG, 500 IU IM	100% (n = 15)	—	20% (n = 15)	Chaffaux et al.[53]
PMSG, 500 IU/kg IM × 10 days + GnRH, 50 μg	100% (n = 5)		0% (n = 5)	Chaffaux et al.[53]
hMG, 75 IU sid IM × 9 days	90% (n = 10)	67% (n = 9)	44% (n = 9)	Wanke et al.[54]
LH, 0.1 IU/kg tid × 7 days	100% (n = 16)	—	31% (n = 16)	Verstegen et al.[55]
FSH, 25–50 IU SC or IM + estrogen, 0.1–0.5 mg SC or IM	94% (n = 67)	—	—	Arbeiter and Dreier[9]
FSH, 10 mg single injection	40% (n = 5)	—	—	Shille et al.[56]
FSH, 1, 2, 4, 8, and 16 mg for 2 days each	50% (n = 4)	—	—	Shille et al.[56]
FSH + LH, 1:1, 1:1, 2:1, 4:1, 8:2.5, 16:5 on days 1, 3, 5, 7, 9, and 11	0% (n = 4)	—	—	Shille et al.[56]
Estrogen				
DES, 5 mg sid PO × 6–9 days, until proestrus induced	100% (n = 5)	—	100% (n = 5)	Bouchard et al.[57]
DES, 5 mg sid PO × 7 days or until proestrus induced + LH, 5 mg IM on day 5 of induced proestrus and FSH, 5 mg IM days 9 and 11 of induced proestrus	100%	—	—	Moses et al.[58]
DES, 5 mg sid PO × 5–14 days or until proestrus induced + hCG, 1000 IU IM on day 5 of induced proestrus and FSH, 10 mg IM days 9 and 11 of induced proestrus	100% (n = 5) (40% estrus)	—	—	Shille et al.[59]
GnRH and GnRH Analogues				
GnRH, 0.04–0.43 μg/kg SC every 90 minutes × 6–12 days	83% (n = 6)	80% (n = 5)	60% (n = 6)	Vanderlip et al.[60]
GnRH, 1.25 μg SC every 90 minutes × 11–13 days	—	—	86% (n = 7)	Cain et al.[61]
GnRH, 280–500 ng/kg SC every 90 minutes × 7–9 days	100% (n = 12)	80% (n = 12)	33% (n = 12)	Concannon et al.[62]
GnRH, 85–270 ng/kg SC every 90 minutes × 7–9 days	84% (n = 12)	42% (n = 12)	8% (n = 12)	Concannon et al.[62]
GnRH, 15–85 ng/kg SC every 90 minutes × 7–9 days	58% (n = 12)	33% (n = 12)	8% (n = 12)	Concannon et al.[62]
Lutrelin, constant SC infusion × 14 days	—	75% (n = 24)	37.5% (n = 24)	Concannon[63]
Dopamine Agonists				
Bromocriptine, 20 μg/kg bid PO × 21 days	100% (n = 6)	—	83% (n = 6)	Van Haaften et al.[47]
Bromocriptine, 20 μg/kg bid PO × 21 days + PMSG, 300 IU 3 times on alternate days	71% (n = 7)	57% (n = 7)	—	Van Haaften et al.[47]
Bromocriptine, 100 μg/kg sid or bid PO until proestrus induced	100%	—	—	Concannon and Verstegen[42]
Bromocriptine, 250 μg/kg bid PO until proestrus induced	100% (n = 4)	—	—	Okkens et al.[64]
Cabergoline, 5 μg/kg sid PO × 7–10 days	100% (n = 28)	—	93.3% (n = 28)	Jöchle et al.[45]
Cabergoline, 5 μg/kg sid PO until proestrus induced	93% (n = 15)	—	86% (n = 14)	Verstegen et al.[65]

PMSG, pregnant mare serum gonadotropin; sid, once daily; hCG, human chorionic gonadotropin; SC, subcutaneously; IM, intramuscularly; GnRH, gonadotropin-releasing hormone; hMG, human menopausal gonadotropin; LH, luteinizing hormone; tid, three times a day; FSH, follicle-stimulating hormone; DES, diethylstilbestrol; PO, per os (orally); bid, twice daily.

Chapter 10). Bitches with hepatic portosystemic shunts may show persistent estrus due to delayed metabolism of circulating hormones.[2] A 3.5-year-old Rhodesian Ridgeback bitch with persistent estrus was reported to have idiopathic lymphocytic oophoritis.[67]

Irregular Estrus

The normal canine uterus requires 130 to 150 days after an estrous cycle for endometrial involution and repair.[2] Interestrous intervals of less than 4 months usually are infertile, possibly due to incomplete endometrial repair after the previous cycle.[2,38]

Length of the interestrous interval is influenced by breed, with a heritability of 35 per cent.[68] Breeds with significantly shorter interestrous intervals than the average of 5 to 8 months include German shepherds, rottweilers, basset hounds, cocker spaniels, and Labrador retrievers.[2,69,70] There is no influence on interestrous interval of breed size or season of the year (day length).[68]

Irregular interestrous intervals may be seen in bitches with uterine disease.[2] Prostaglandins released from the hyperplastic endometrium may cause premature luteolysis in affected diestrual bitches, causing an early return to estrus. Tentative diagnosis of cystic endometrial hyperplasia may be possible with visualization of the thickened uterine endothelium by ultrasound; definitive diagnosis requires uterine biopsy.

Treatment of bitches with short interestrous intervals due to uterine disease is induction of anestrus with mibolerone (Cheque drops; Upjohn, Kalamazoo, MI; 0.3 ml (30 μg) per 25 lb body weight once daily PO, not to exceed 1.8 ml, except in German shepherd purebreds or crosses, which should receive 1.8 ml daily regardless of weight) or testosterone (testosterone cypionate, 0.5 mg/kg IM every 5 days).[38] Bitches treated may fail to show normal cyclicity and have decreased fertility, especially at the first estrus after cessation of androgen treatment.[38]

Apparent irregular interestrous intervals may be misinterpreted in bitches with split heats. Split heat is appearance of physical and behavioral changes characteristic of proestrus, such as vulvar swelling, serosanguineous discharge from the vulva, and attraction of male dogs, with no progression to estrus, then a short anestrus period of several weeks, followed by a normal estrous cycle. In one survey of 1152 bitches presented for breeding management, 3 (0.3 per cent) underwent a split heat, with an average anestrous period of 4 weeks between initial signs and normal, progressive estrus.[71] Split heat may be caused by insufficient gonadotropin release or breakthrough bleeding at onset of folliculogenesis.[66] The second appearance of estrual signs and behavior usually is a fertile estrus.

An irregular interestrous interval may be seen after an anovulatory cycle in the bitch. In a survey of 1152 dogs, 11 (1 per cent) were documented to have had an anovulatory cycle, in which serum progesterone concentrations never rose above 3.5 ng/ml.[71] Five of these 11 bitches (45 per cent) had a normal, ovulatory estrus at their next season.[71] Dogs with persistent anovulation may be treated by ovulation induction with GnRH or human chorionic gonadotropin (hCG).[2]

Nonreceptive Behavior

Poor Timing of Breeding Attempts

Bitches show a gradual increase in receptivity toward the male during proestrus and will, by definition, stand for breeding attempts by the male when in estrus.[72] Receptivity declines in early diestrus.[72] Assessment of vaginal cytology specimens and serum progesterone concentrations can be used to determine if a nonreceptive bitch is in proestrus, estrus or diestrus (see Chapter 3).

Physical Abnormality of the Vulva, Vestibule, or Vagina

Congenital anomalies or changes in the size or shape of the vulva, vestibule, or vagina may prevent normal copulation in the dog. Male dogs may experience difficulty achieving intromission of the penis in young bitches with an infantile vulva.[2] Bitches with abnormalities of sexual differentiation may have abnormal external genitalia. A 9-month-old intact, female, nonreceptive Doberman pinscher from which an enlarged clitoris containing an os clitoris was surgically removed was later successfully bred by natural service.[73]

The most common vaginal/vestibular physical abnormalities preventing natural service are septae and circumferential strictures. The hymen forms at the junction of the vagina and vestibule, just cranial to the urethral papilla, where the anastomosing paired paramesone-

phric ducts fuse with the urogenital sinus, from which the vestibule and vulva are formed. In a survey of 13 bitches with anatomic abnormalities of the caudal reproductive tract, 2 (15 per cent) had circumferential vestibulovulvar strictures, 4 (30 per cent) had circumferential vaginovestibular strictures, and 7 (55 per cent) had vaginal septa or retained hymenal remnants (Fig. 14–2).[74] The most common presenting complaint in bitches with these congenital anomalies is inability to copulate naturally; other complaints include chronic vaginitis, chronic urinary tract infections, and urinary incontinence.[74–76] Specifics of diagnosis and treatment are described elsewhere (see Chapter 12).

Vaginal prolapse, also called vaginal hypertrophy or vaginal hyperplasia, is protrusion of edematous vaginal tissue into the vaginal lumen and possibly through the vulvar lips of sexually intact female dogs under the influence of estrogen.[77] The condition is most commonly seen in young, large- or giant-breed dogs during proestrus or estrus, but may arise at the end of gestation as well.[77] Affected bitches may refuse mating, but can be bred by artificial insemination and may require cesarean section for delivery. The condition usually recurs at subsequent cycles; in one survey of 13 bitches with vaginal prolapse, 100 per cent had recurrence of the condition at the two cycles after initial diagnosis.[78] Heritability of the condition is unknown (see Chapter 12).

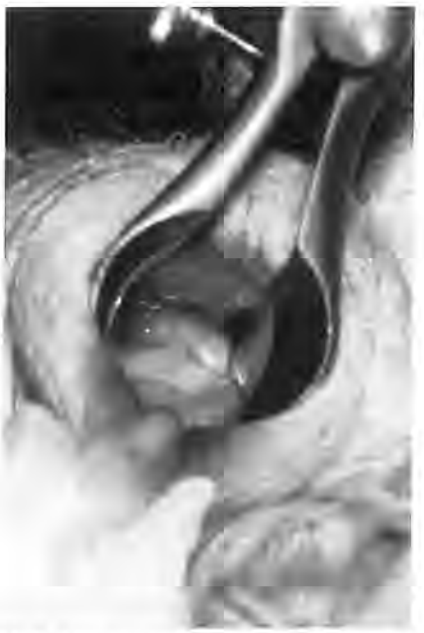

Figure 14–2. Canine vaginal septum viewed through a speculum. (From Olson PN, Behrendt MD, Weiss DE: Reproductive problems in the bitch: Formulating your diagnostic plan. Vet Med 82:482–496, 1987, with permission.)

Abnormal Sexual Behavior

Sexual behavior in female dogs is influenced by inherited factors, learning and early experience, and specific differentiation in the brain due to gonadal hormone exposure prenatally and in the early postnatal period.[79] Bitch puppies supplemented with testosterone postnatally until 42 to 89 days of age did not show sexually receptive behavior toward male dogs at puberty.[80] Gonadal hormones released at puberty activate responsiveness to ovarian hormones by the brain mechanisms that mediate sexual behavior.[79,80] Bitches that undergo pubertal estrus very young are less likely to show normal sexual receptivity, as are bitches with low serum estrogen concentrations during proestrus and low or late rising serum progesterone concentrations in estrus.[81]

Normal bitches show increased sexual receptivity during proestrus, with restlessness, roaming, and an increased tendency to urinate, especially in the presence of the male.[72,79] Copulatory behavior occurs during estrus, and includes flagging (lateral deviation of the tail and muscular elevation of the vulva), and standing rigidly during intromission.[72,79] Sexual receptivity declines in early diestrus.[72]

Psychological factors may cause decreased sexual receptivity in bitches. Hierarchical incompatibilities may prevent dominant bitches from allowing subordinate males to mount and copulate.[82] Inexperienced females may not show a normal progression of receptive behaviors through estrus, while submissive bitches may show standing behavior when not in estrus.

Behavioral causes for lack of normal copulation are best treated by artificial insemination. Tranquilization of uncooperative bitches is not recommended.

Conception Failure Caused by Mistimed Breedings

Conception failure caused by mistimed breeding is the most common cause of infertility in the bitch, with reported incidences of 40 per cent to greater than 50 per cent of infertile bitches.[1,83] Bitches may ovulate as early as 3 to 4 days or as late as 25 to 26 days after proestrus onset.[1] The average bitch ovulates about 12

days after first signs of vulvar swelling and sanguineous vulvar discharge.[1] Historically, many bitches have been bred on days 9 to 13 after proestrus onset. It has been demonstrated that breeding by day of the cycle alone yields pregnancy rates as low as 78 per cent.[84] Pregnancy rate can be greatly enhanced if breeding management is performed using determination of ovulation day. Methods for assessment of ovulation and optimal breeding time include breeding history, use of physical indicators of estrous progression, including evaluation of vaginal cytology specimens, and measurement of serum progesterone concentrations.

Breeding history is a poor indicator of current events in a given bitch's estrous cycle. Sixteen of 36 bitches (44 per cent) were demonstrated to have significant variation in optimal breeding time between estrous cycles.[85]

Physical indicators of estrus progression include changes in the size and turgidity of the vulva (Fig. 14–3), change in color of vulvar discharge, changes in color and character of the vaginal mucosal rugae,[86] and ferning of vaginal fluid on a glass slide.[84] The population of vaginal epithelial cells collected on a vaginal swab changes as bitches progress from proestrus through estrus and into diestrus, with a gradual increase in percentage cornified (keratinized) cells through proestrus and an abrupt return to predominant noncornification on the first day of diestrus.[87,88] Ovulation cannot be predicted prospectively from vaginal cytology alone but can be determined retrospectively by counting back 6 days from the cytologic onset of diestrus[87,89,90] (see Chapter 3).

Ovulation can be predicted prospectively by measurement of serum progesterone concentrations (see Chapter 4). Serum progesterone concentration ranges from 4 to 10 ng/ml on ovulation day.[90,91]

Canine spermatozoa can live in a normal female reproductive tract for at least 5 days, forming a reservoir at the lower isthmus of the uterine tube, where they adhere to mucosal cells.[92] The bitch ovulates a primary oocyte that requires 24 to 48 hours to mature to a fertilizable secondary oocyte, which remains viable for about 24 hours.[92] Conception rate is best in bitches bred from 3 days before to 4 days after ovulation, with maximal litter size if bred 2 days after ovulation[88] (see Chapter 4).

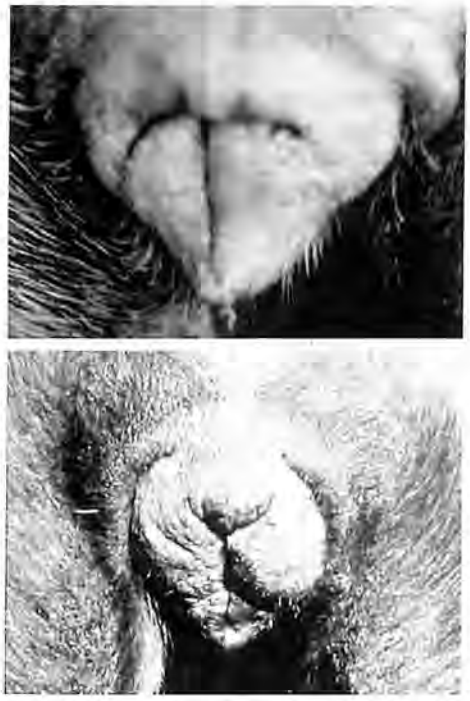

Figure 14–3. Comparative vulvar morphology of the bitch in heat. The proestrus vulva at the top is swollen and turgid under the fluid retentive effects of estrogen. The vulva below is still enlarged and swollen but flaccid. (From Thomas PGA, Perkins NR: History-taking and diagnostic assessment of the subfertile bitch. Aust Vet Pract 23:198–206, 1993, with permission.)

Conception Failure with Good Breeding Management

Male Infertility

After mistimed breeding, male infertility is the next most common cause of conception failure in bitches presenting with infertility.[1] A complete breeding soundness examination, including physical examination with rectal palpation of the prostate, *Brucella canis* serology, and semen collection and evaluation, including semen culture, is the best proof of male fertility.[82]

Uterine Infection

Subclinical uterine infection is a reported cause of infertility in the bitch.[1,93–97] Infection may cause conception failure by creating a hostile environment for the sperm and eggs, or by causing early embryonic death.[2]

Because the uterus of the bitch is relatively inaccessible to the veterinarian unless laparotomy and hysterotomy are performed, culture samples drawn from the vagina have traditionally been used to infer presence of infection in the uterus. However, the normal canine vagina is not sterile. In 5 normal anestrous bitches

and 59 normal bitches from which samples were collected at monthly intervals for 18 months, aerobic bacteria were cultured from 100 per cent and 94.8 per cent of specimens, respectively.[98,99] Accuracy may be enhanced by requesting quantitative culture results, with the assumption that organisms with light or moderate growth are probably normal flora; by collecting specimens from the anterior vagina, where fewer organisms are present in the normal birth[100]; and by culturing during proestrus or estrus when the cervix is open so that uterine infection, if present, may be detected. Frequency of detecting positive uterine cultures is greatly increased during proestrus and estrus, when the presence of sanguinous discharge originating in the uterus may contain representative uterine flora (Fig. 14–4).[100–103] In one study, aerobic bacteria isolated from the uterus of a given bitch were always isolated from the cervix and vagina, while in only 7 of 13 bitches (54 per cent) were bacteria isolated from the cervix and vagina also found in the uterus.[103]

Specific microbial organisms causing infertility in the bitch include aerobic bacteria that are normal residents of the vagina (enterobacteriaceae, gram-positive cocci), *Brucella canis*, canine herpesvirus, and mycoplasma. Canine brucellosis and herpesvirus have been described (see Chapters 11 and 5, respectively).

The role of mycoplasma as a cause of infertility in the bitch is not well defined, but it is probably similar to that of other vaginal flora. Percentage of bitches from which *M. canis* was

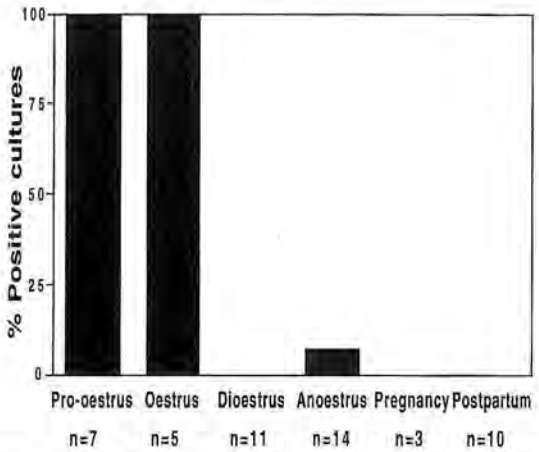

Figure 14–4. Frequency of positive uterine cultures from normal bitches throughout the reproductive cycle. (From Watts JR, Wright PJ, Whithear KC: Uterine, cervical and vaginal microflora of the normal bitch throughout the reproductive cycle. J Small Anim Pract 37:54–60, 1996, with permission.)

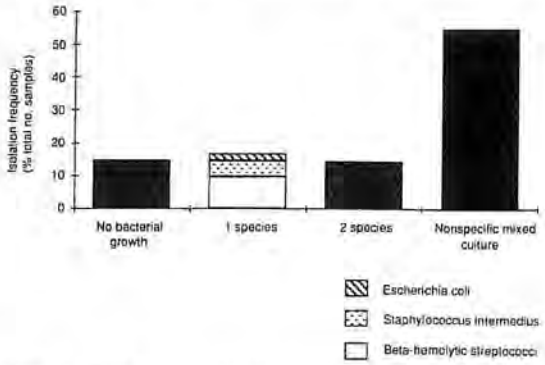

Figure 14–5. Frequency distribution of the number of bacterial species per sample in vaginal cultures of 42 infertile bitches. (From Bjurström L: Aerobic bacteria occurring in the vagina of bitches with reproductive disorders. Acta Vet Scand 34:29–34, 1993, with permission.)

cultured from vaginal swabs in one report did not differ between reproductively normal bitches ($n = 10$) and infertile bitches ($n = 27$).[104] Mycoplasmas are a part of the normal vaginal flora; in one survey of 59 normal bitches from which vaginal swabs were collected weekly for 18 months, *M. canis* was isolated from 8.8 per cent of the specimens.[99] All of those bitches whelped at least one normal litter during the testing period.[99] Mycoplasma are fastidious organisms, making quantitative culture techniques less sensitive than for the more common aerobic bacteria. Growth of mycoplasmas can be inhibited by tetracycline and the fluoroquinolones.

In a survey of normal ($n = 10$) and infertile ($n = 14$) bitches, similar populations of aerobic and anaerobic bacteria were isolated from the vaginas of the two groups.[105] A survey of 42 infertile bitches revealed no growth of aerobic bacteria in 14.3 per cent of vaginal samples and presence of a nonspecific mixed culture in the majority of bitches (Fig. 14–5).[106] Organisms cultured from vaginal swab specimens of normal dogs are the same as those cultured from infertile dogs (Table 14–3). It is, therefore, impossible to implicate any aerobic organism except *Brucella canis* as a cause of infertility in the bitch based solely on its presence in the vagina.

Infertile dogs from which aerobic bacteria have been retrieved at vaginal culture have been reported to conceive and whelp normal litters successfully after antibiotic therapy.[93,94] However, aerobic organisms have been shown to recolonize the vagina within 4 days of cessation of antibiotic therapy in normal dogs, and isolation of *M. canis* was more common in

Table 14–3. Aerobic Organisms Commonly Cultured from the Anterior Vagina of Normal and Infertile Bitches

	Normal Bitches		Infertile Bitches	
Organisms cultured from the anterior vagina	*Pasteurella multocida* β-hemolytic *Streptococcus* sp. *E. coli* *Staphylococcus intermedius* *Mycoplasma* sp.	*E. coli* β-hemolytic *Streptococcus* sp. α-hemolytic *Streptococcus* sp. coagulase + *Staphylococcus* sp. coagulese − *Staphylococcus* sp. *Pasteurella multocida*	β-hemolytic *Streptococcus* sp. *Pasteurella multocida* *E. coli* *Staphylococcus intermedius* *Pasteurella multocida* *Proteus mirabilis*	*E. coli* *Streptococcus* sp. *Staphylococcus* sp. *Pseudomonas* sp. *Proteus* sp. *Klebsiella* sp. *Bacillus* sp.
References	Bjurström et al.[99] (n = 59)	Olson and Mather[100] (n = 81)	Bjurström[106] (n = 42)	Gandotra et al.[107] (N = 43)

dogs treated with ampicillin or trimethroprim-sulfamethoxazole.[98] Unwarranted use of antibiotics in breeding bitches should be avoided. Hysterotomy for uterine culture should be considered in some cases.

Uterine Pathology

Bitches with cystic endometrial hyperplasia (CEH) may be infertile due to implantation failure after conception.[2] The thickened endometrium may be visible ultrasonographically grossly or histologically. Definitive diagnosis requires hysterotomy for uterine biopsy. Biopsy sites should be chosen so as to preserve maximal uterine luminal diameter after healing. The uterus may be incised and a wedge of endometrial tissue removed, or a 2- to 4-mm skin biopsy punch may be used to gather a full-thickness uterine biopsy sample (Fig. 14–6).[108] No grading system, as is used for assessment of equine uterine biopsy specimens, has been reported for the dog.

CEH is irreversible in bitches, although there is one report of a possible positive effect in bitches with CEH treated with mibolerone (Cheque drops; Upjohn, Kalamazoo, MI; 0.3 ml (30 μg) per 25 lb body weight once daily PO, not to exceed 1.8 ml, except in German shepherd purebreds or crosses, which should receive 1.8 ml daily regardless of weight) for 6 months, and bred at the next cycle.[2] Pregnancy in bitches treated medically for pyometra also suggests that some healing of the uterine mucosa may occur following pregnancy in bitches with CEH.

Hypoluteoidism

In the dog, all progesterone for pregnancy maintenance is secreted from luteal tissue on the ovaries.[109,110] Bitches with an insufficient luteal phase are unable to maintain pregnancy. Luteal dysfunction is generally considered to be a primary ovarian problem, but secondary luteal insufficiency has been described in a Great Dane with a pituitary defect.[111] Diagnosis requires documentation of low serum progesterone concentrations in diestrus. Blood samples should be drawn at no less than weekly intervals when assessing bitches for luteal function with serum progesterone concentrations. If serum progesterone concentration falls to less than 2 ng/ml for greater than 48 hours, pregnancy may be terminated. In one report, a 2-year-old intact female silky terrier with a history of late-term abortion was documented to have a serum progesterone concentration of 2 to 5 ng/ml at 50 days of gestation.[112]

Bitches in which progesterone supplementation appears necessary should be placed on empiric antibiotic therapy to ensure intrauterine infection is not present. Progestogens reported for successful pregnancy maintenance in bitches with spontaneously low diestrual serum progesterone concentration,[112] or bitches that have been experimentally ovariectomized,[113] include progesterone in oil and ally-trenbolone (Regumate; Hoechst-Roussel Agrivet, Summerville, NJ). Progesterone in oil is administered parenterally at a dose of 2 mg/kg every 3 days, to no later than 58 days from ovulation or 52 days from the first diestrous vaginal smear.[112] Ally-trenbolone is administered at a dose of 0.088 mg/kg once daily orally, to no later than 61 days from ovulation or 55 days from the first diestrous vaginal smear.[113] It is imperative that the normal decline in progesterone be mimicked in the last 2 to 3 days of gestation; prolongation of gestation with exogenous progestogens for 2 days beyond the due date greatly increases incidence of stillbirths.[110] Bitches receiving the oral progestogen, ally-trenbolone, may have poor milk production in the early postpartum period.[113]

Figure 14–6. Use of a 2- to 4-mm skin biopsy punch for retrieval of a full-thickness uterine biopsy specimen in the bitch. (From Downs M, Miller-Liebl D, Fayrer-Hosken R, et al: Obtaining a useful uterine biopsy specimen in dogs. Vet Med 89:1055–1059, 1994, with permission.)

Advanced Age

Dogs do not undergo cessation of reproductive cycling with age, but continue to cycle throughout their lives.[82] Age-related changes in fertility include increased interestrous interval,[82] decreased conception rate (with greater than 50 per cent of beagle bitches 5 years of age or older failing to conceive in one study[21]), and decreased litter size after age 7.[114]

Drugs

Dogs that receive medications causing early fetal death and subsequent reabsorption may appear to be infertile (see Chapter 5).[115]

Impatent Female Tubular Reproductive Tract

Occlusion of the vaginal, uterine, or uterine tubal lumen may occur as a result of congenital segmental aplasia or may occur secondary to infection or trauma after whelping or cesarean section.[116] Bitches have been described with segmental aplasia of the cranial and caudal vagina.[117,118] Both bitches cycled, although the bitch with caudal vaginal agenesis did not show sanguineous vulvar discharge in proestrus/estrus, as fluid from each estrous cycle pooled in her cranial vagina (hematocolpos).[117] The bitch with cranial vaginal agenesis underwent surgical removal of the obstructed portion and vaginal anastomosis, and later conceived and whelped a normal litter.[118]

Diagnosis of an impatent tubular reproductive tract usually requires exploratory laparatomy. A distended portion of the vagina cranial to an occlusion may be visible on radiographs. The radiographic contrast technique, hysterosalpingography, in which radiopaque contrast medium is infused through the vagina and cervix so as to distend the uterine body and horns, often shows equivocal results.[119,120] Similarly, the normal tight uterotubal junction of bitches makes patency of the uterine tubes difficult or impossible to assess by attempting to flush fluid into the uterine tubes from the uterus.[121]

Immunologic Infertility

Although antisperm antibodies have been identified and artificially induced in the dog, there are no documented reports of spontaneous immunologic infertility in the bitch.[82]

Systemic Disease

Hypoadrenocorticism was identified in 1 of 24 infertile bitches.[12] Mean diestrual serum progesterone concentration was decreased in bitches with insulin-dependent diabetes mellitus ($n = 7$) and hyperadrenocorticism ($n = 6$).[48] Bitches with hyperadrenocorticism may have decreased secretion of luteal progesterone, because elevated serum cortisol concentrations cause decrease in the synthesis and release of luteotropic LH.

Anovulatory Cycles

Anovulatory cycles, in which serum progesterone concentrations never rose above 3.5 ng/ml, were reported in 11 of 1152 (1 per cent) bitches.[71] Five of these 11 bitches (45 per cent) had a normal, ovulatory estrus at their next season.[71] Dogs with persistent anovulation may be treated by ovulation induction with GnRH or hCG.[2]

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Section II

THE DOG

Chapter 15

Sexual Differentiation and Normal Anatomy of the Dog

Sexual Differentiation

Normal sexual differentiation in the male begins with fusion of a gamete bearing one X chromosome with a gamete bearing a Y chromosome (chromosomal sex), followed by development of testes (gonadal sex) and male internal and external genitalia (phenotypic sex).[1] Vertebrate embryos are inherently female. The Y chromosome is necessary for differentiation of the testes and development of male internal and external genitalia. A portion of the short arm of the Y chromosome, sometimes called the testis determining factor, induces the indifferent gonad to undergo testicular development.[1,2]

Early in sexual development, an indifferent gonad with sexual bipotentiality forms.[5] In the indifferent gonad, the epithelium of the genital ridge proliferates to form sex cords that surround germ cells.[6] Differentiation of the indifferent gonad into a testis involves differentiation of Sertoli cells from the sex cords followed by fusion of the sex cords to form a network of medullary sex cords and the rete testis.[6,7] The sex cords eventually lose contact with the surface epithelium and are separated from it by the thick tunica albuginea.[6] Interstitial mesenchymal cells differentiate to form the Leydig (interstitial) cells,[6] and hormone production and secretion begins.

The two major hormones produced by the embryonic testis facilitate regression of the female ductal system and promote differentiation of the male internal and external genitalia (Fig. 15–1).[8] Müllerian inhibiting substance (MIS), also called antimüllerian hormone and müllerian regression factor,[9] is a glycoprotein secreted by the Sertoli cells that promotes regression of the müllerian, or paramesonephric, ducts between 36 and 46 days of gestation in the dog by inducing migration or death of mesenchymal cells in these structures.[1,6,10,11] The other hormone secreted by the embryonic testis is testosterone (T), a steroid secreted by Leydig cells of the testis. Testosterone and its metabolite, 5α-dihydrotestosterone (DHT), induce formation of the internal and external genitalia.[1] Under the influence of T, the wolffian, or mesonephric, ducts form the epididymes and ductuli deferentes. Under the influence of DHT, the urogenital sinus forms the urethra and prostate, the genital tubercle lengthens to form the penis, the genital folds enclose the penis and form the prepuce, and the genital swellings form the scrotum (Fig. 15–1).[1,8,10,12]

The embryonic testes develop caudal to the kidneys. The gubernaculum testis, a mesenchymal cord containing fibroblasts, collagen fibers, and mucopolysaccharides, attaches to

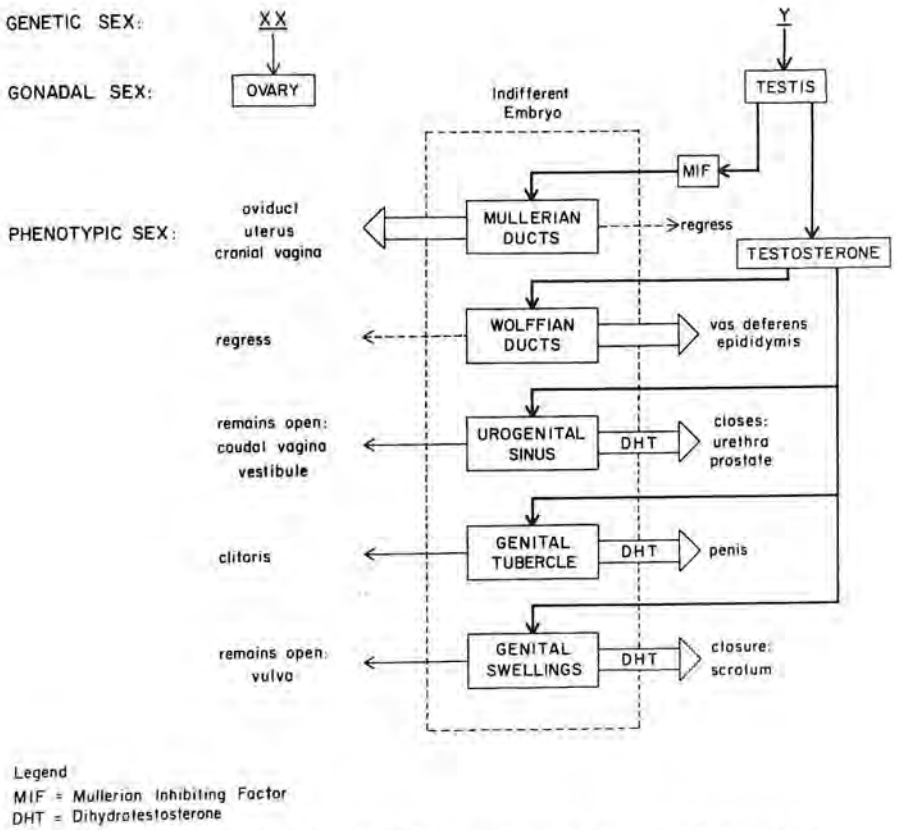

Figure 15–1. Normal sexual development in the dog. (From Meyers-Wallen VN, Patterson DF: Disorders of sexual development in the dog. *In* Morrow DA [ed]: Current Therapy in Theriogenology: Treatment, Diagnosis, and Prevention of Reproductive Diseases in Small and Large Animals, 2nd ed. Philadelphia, WB Saunders, 1986, pp 567–574, with permission.)

the caudal pole of the testis, runs through the inguinal canal, and attaches distally in the scrotum.[13,14] Normal descent of the testis into the scrotum occurs passively, and primarily is due to changes in the gubernaculum. In the first phase, which lasts until about 5 days after birth, outgrowth of the gubernaculum occurs with an enormous increase in length and volume, expansion of the inguinal canal, and distal movement of the testis and epididymis.[13,15] The testes pass through the inguinal canal 3 to 4 days after birth.[15] In the second phase, regression of the gubernaculum occurs, which draws the testes to their final position in the scrotum. Mucopolysaccharides disappear, and the gubernaculum decreases in size as it becomes more fibrous.[13,14] The canine testes generally reach their final position in the scrotum by 35 days of life.[15]

Normal testicular descent is controlled by hereditary, hormonal, and mechanical factors.[16–19] If the testes are removed from fetal dogs, the epididymes will not descend. Supplementation of T to the above dogs results in delayed epididymal descent. Removal of the testis at birth, without T supplementation, also causes delayed descent. This suggests that T is not the sole agent responsible for gubernacular outgrowth, and that either mechanical factors or production of a nonandrogenic substance is required.[16–18] Structures besides the gubernaculum also may impact testicular descent; bilateral cryptorchidism due to persistence of cranial gonadal suspensory ligaments has been described.[20]

On average, the inguinal canal remains open in dogs until they reach 6 months of age, allowing free movement of testes proximal to the scrotum until closure of the inguinal canals is complete. Dogs should not be diagnosed as cryptorchid until after 6 months of age.

The age at which puberty (first ejaculation) is reached varies by breed; in general, small-breed dogs achieve physical maturity and puberty earlier than large-breed dogs, some of which reach puberty after 12 months of age.

Serum T concentrations increase after 24 weeks of age, reaching adult concentrations by 32 weeks of age.[21,22] The embryonic sex cords surrounding the germ cells hollow out to form seminiferous tubules at about 16 weeks of age in the dog.[6] Diameter of the seminiferous tubules and epididymal ducts increases dramatically from 20 to 28 weeks of age (Fig. 15–2).[6,23] Testicular weight increases markedly at 24 to 32 weeks of age.[24] Spermatogenesis begins at about 20 weeks of age, and large numbers of spermatozoa are present in the epididymis after 32 weeks of age.[23] Spermatozoa first appear in the ejaculate at about 7 to 9 months of age.[22,25] Puberty in the dog refers to first appearance of spermatozoa in the ejaculate, and the sexual desire and the ability to copulate. Average age of puberty onset in the dog has been described as 7 to 10 months, with a range of 5 to 12 months, but many normal male dogs will not ejaculate until after a year of age.[26]

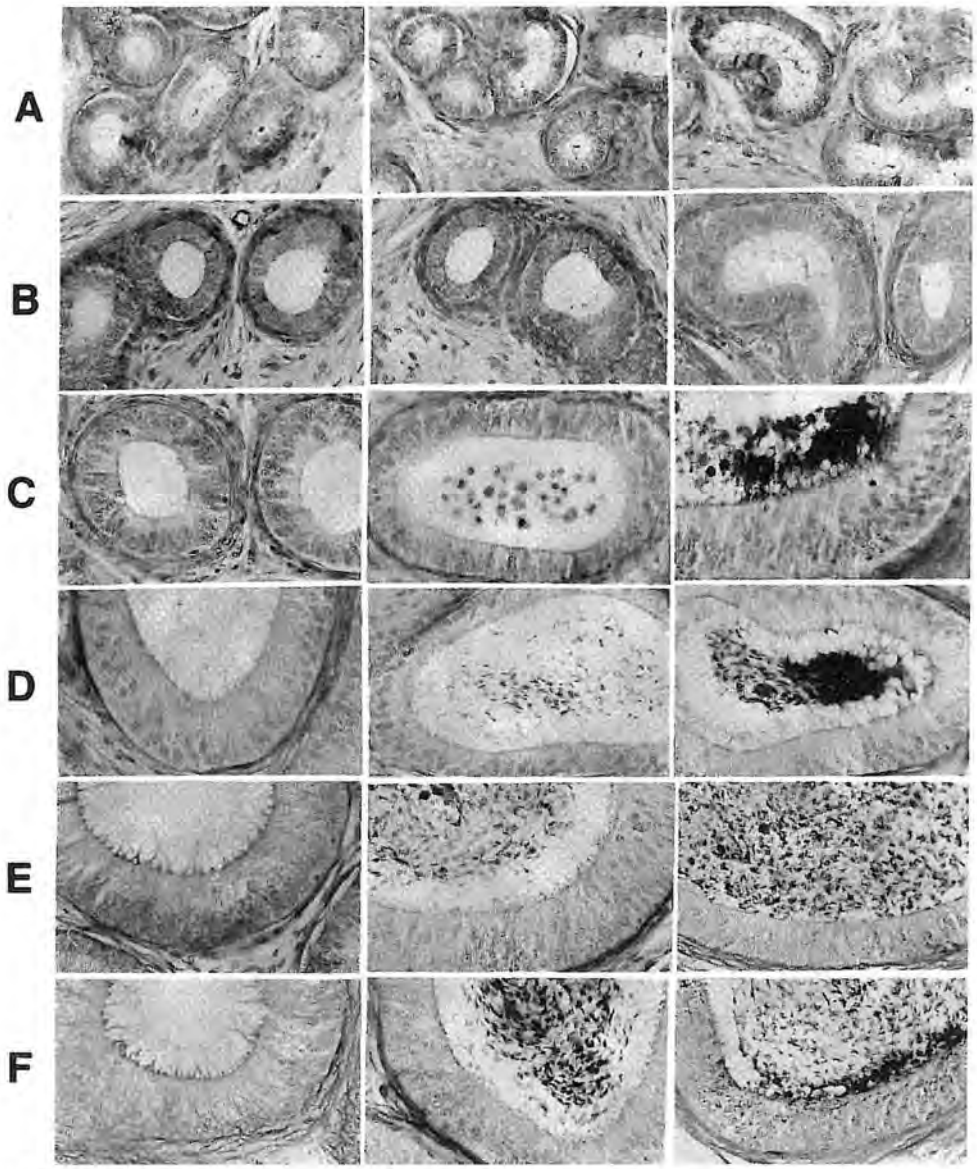

Figure 15–2. Histological changes of the head (caput, left), body (corpus, middle), and tail (cauda, right) of the epididymis in dogs. PAS–hematoxylin stain; 100×. **A:** 0 weeks of age. **B:** 16 weeks of age. **C:** 24 weeks of age. Germ cells are present in the lumen of the body and tail of the epididymis. **D:** 28 weeks of age. Spermatozoa are present in the lumen of the body and tail of the epididymis. **E:** 36 weeks of age. **F:** 48 weeks of age. (From Kawakami E, Tsutsui T, Ogasa A: Histological observations of the reproductive organs of the male dog from birth to sexual maturity. J Vet Med Sci 53:241–248, with permission.)

Anatomy of the Male Reproductive Organs of the Dog

The anatomy of the scrotum, testes and epididymes, ductus deferens and spermatic cord, prostate, and penis and prepuce of the dog is depicted in Figure 15–3.

Scrotum

The scrotum is a pouch of thin, lightly haired, pigmented skin that contains the two testes. Beneath the integument lie well-developed sebaceous glands and a poorly defined layer of smooth muscle commingled with collagenous and elastic fibers, the dartos. Within the scrotum, the testes are surrounded by a double-walled vaginal tunic formed by an evagination of the peritoneum covered by spermatic fascia of the abdominal wall, called the vaginal process. The two layers of the vaginal tunic are the parietal, also called the superficial or common layer, and the visceral, also called the deep or proper layer. The potential space between them is the cavity of the vaginal process. The cremaster muscle arises from the free border of the internal abdominal oblique muscle and inserts on the parietal vaginal tunic. Contraction and relaxation of the cremaster and dartos allow movement of the scrotum and testes in relation to the body. This ability of the scrotum to move, the presence of well-developed sebaceous glands, and the thin skin with minimal hair coat permit the scrotum to act as a thermoregulator for the testes and epididymes.[13] Major vessels supplying the scrotum are the external pudendal artery and vein, and innervation occurs via the superficial perineal nerve.[13,27]

TESTES/EPIDIDYMES

Testicular size varies with body weight in the dog. Body weight is positively correlated with testicular weight, testicular volume, total epididymal mass, and total scrotal width in normal male dogs.[28–31] Measurement of body

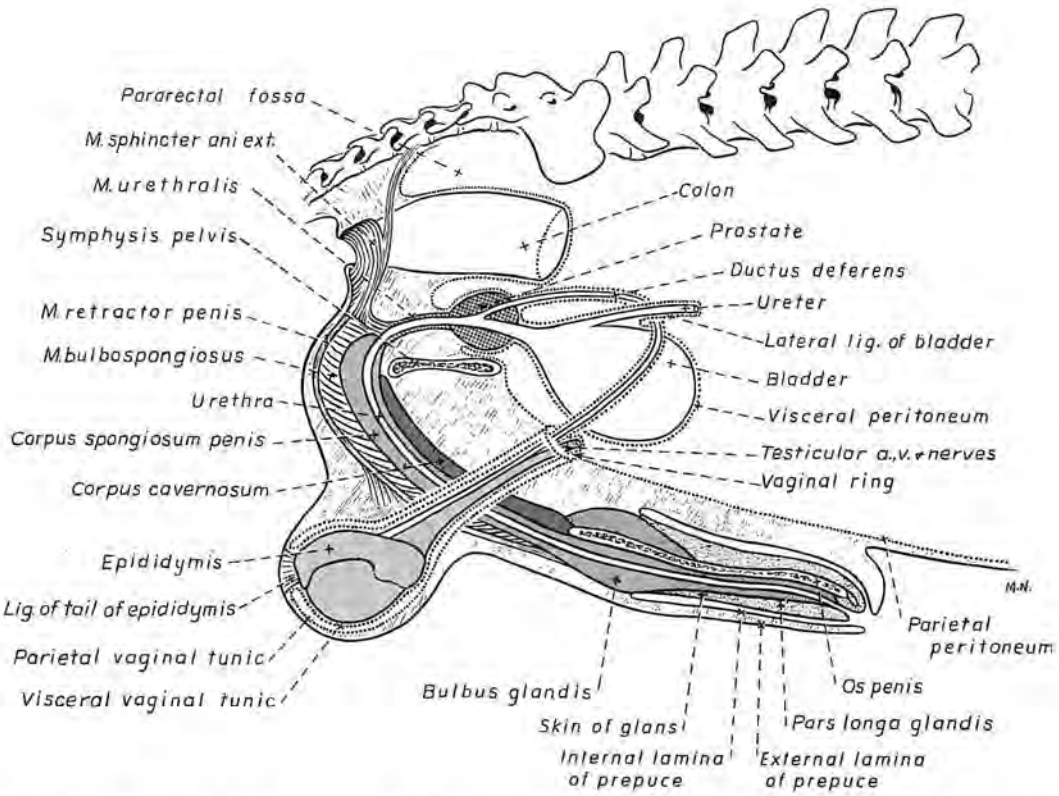

Figure 15–3. Diagram of male internal and external genitalia. (From Evans HE, Christensen GC: The urogenital system. *In* Evans HE [ed]: Miller's Anatomy of the Dog. Philadelphia, WB Saunders, 1993, pp 494–558, with permission.)

weight and total scrotal width permits evaluation of dogs for normal testicular size (see Chapter 23 and Fig. 23–4).

The testes are positioned obliquely within the scrotum. The long axis is oriented dorsocaudally. The head (caput) of the epididymis is at the cranial pole of the testis, the body (corpus) runs over the dorsolateral surface, and the tail (cauda) is attached to the caudal end of the testis by the proper ligament of the testis; the spermatic cord exits the tail of the epididymis at the caudomedial aspect of the testis, and extends, medial to the testis, up through the inguinal canal to the inguinal ring. The ligament of the tail of the epididymis attaches the testis and epididymis to the vaginal tunic (Fig. 15–4).[13] The major blood vessels supplying the testes are the testicular (internal spermatic) arteries and the testicular veins, which form the pampiniform plexus, a countercurrent exchange that cools arterial blood before it enters the testis.[13,32] Innervation is via the testicular (internal spermatic) plexus of the sympathetic nervous system.[13]

The testis is covered by the visceral vaginal tunic and the tunica albuginea, a dense white fibrous capsule. At the site of attachment of the head of the epididymis, the tunica albuginea joins the mediastinum testis, a 0.2-cm-wide cord of connective tissue running centrally through the long axis of the testis.[13,33] The testicular parenchyma is split into lobules by connective tissue septae. The lobules contain the seminiferous tubules; the seminiferous tubules within each lobule connect to straight tubules that enter the rete testis, which is continuous with the epididymis.[13]

The testis consists of three functional compartments: (1) the interstitial compartment, which contains blood vessels, Leydig (interstitial) cells, and supportive tissue, which functions to supply the seminiferous tubules with hormones and nutrients; (2) the basal compartment, which contains spermatogonia and Sertoli cells; and (3) the adluminal compartment, which contains developing spermatozoa and is separated from the basal compartment by the blood-testis barrier (Fig. 15–5).[34,35] The basal and adluminal compartments are within the seminiferous tubules, which, in man, comprise 66 per cent of testicular volume.[34] Sertoli cells rest on the basement membrane of the seminiferous tubules and have cytoplasmic extensions that surround the germ cells and extend into the adluminal compartment. The Sertoli cells are connected by junctional complexes through which developing spermatozoa must pass to gain entry into the adluminal compartment. These junctional complexes are a major component of the blood-testis barrier. Developing spermatozoa are protected from noxious influences, since they are only exposed to molecules that pass through the Sertoli cells. Similarly, as the developing spermatozoa within the adluminal compartment express unique surface antigens, they are protected from detection by the dog's immune system.[34]

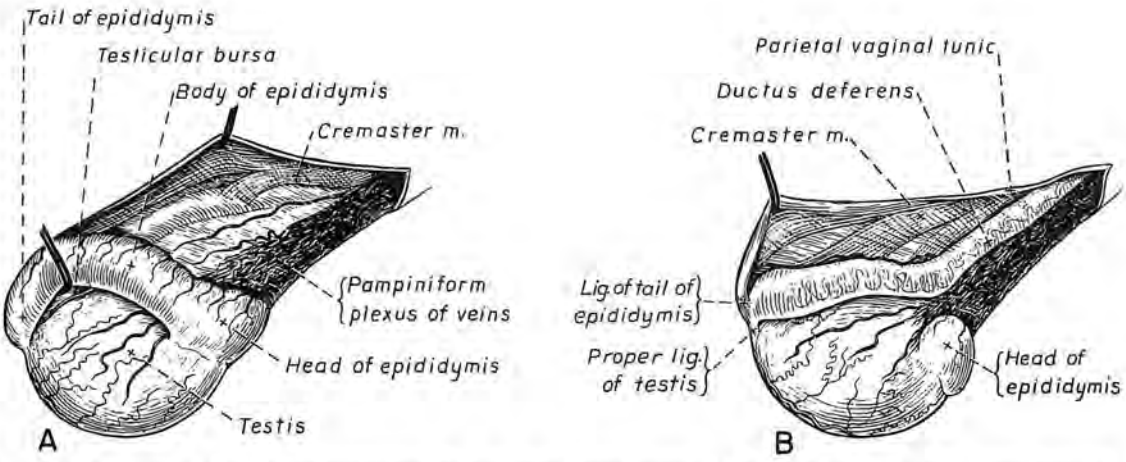

Figure 15–4. Diagram of testes and epididymes. **A:** Right testis, lateral aspect. **B:** Left testis, medial aspect. (From Evans HE, Christensen GC: The urogenital system. *In* Evans HE [ed]: Miller's Anatomy of the Dog. Philadelphia, WB Saunders, 1993, pp 494-558, with permission.)

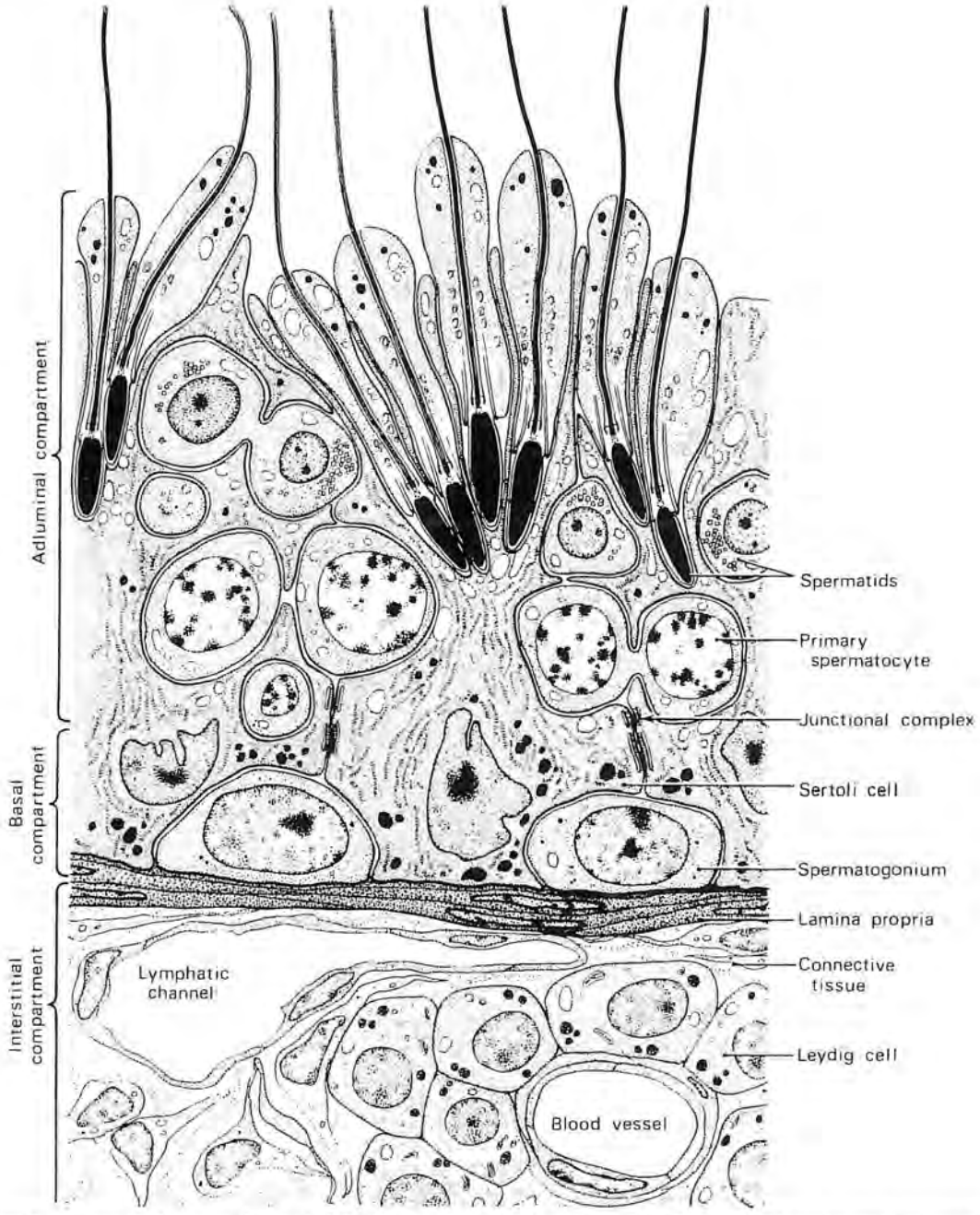

Figure 15–5. Structure of the seminiferous tubule and spermatogenesis. (From Amann RP: Reproductive physiology and endocrinology of the dog. *In* Morrow DA [ed]: Current Therapy in Theriogenology: Treatment, Diagnosis, and Prevention of Reproductive Diseases in Small and Large Animals, 2nd ed. Philadelphia, WB Saunders, 1986, pp 532–538, with permission.)

The testis performs two major functions, spermatogenesis and production and secretion of hormones. The Leydig (interstitial) cells secrete T in response to luteinizing hormone (LH) stimulation. Activin and inhibin, protein hormones secreted by the Sertoli cells, and T exert feedback on the pituitary, controlling secretion of the gonadotropins LH and follicle-stimulating hormone (FSH). Formation of an androgen-binding protein in Sertoli cells is

stimulated by FSH. Binding of T to this protein maintains intratesticular T concentrations necessary for spermatogenesis.

Spermatogenesis

Spermatogenesis consists of spermatocytogenesis, formation of spermatids from spermatogonia, and spermiogenesis, differentiation of spermatids into spermatozoa. Spermatogenesis takes place within the germinal epithelium of the seminiferous tubules.

The least differentiated germ cell in the testis is the spermatogonium. Two populations of spermatogonia exist: the reserve population, which is resistant to radiation and toxic injury, and the proliferating population. Proliferating spermatogonia divide by mitosis, changing from type A to type B spermatogonia. Cytokinesis is incomplete, allowing development of cells as a cohort via maintenance of intercellular bridges.[6,34] The type B spermatogonia divide to form primary spermatocytes, which undergo meiosis to form short-lived secondary spermatocytes and eventually spermatids. The haploid spermatids turn with what will be the head of the spermatozoon facing the basement membrane, and complete development by undergoing elongation of the nucleus, formation of the head with the overlying acrosome, development of the flagellum with encircling mitochondria at the midpiece, and jettisoning of the droplet of excessive cytoplasm (Fig. 15–5).[6] The immature spermatozoa are released from the seminiferous tubule into the straight tubules, then through the rete testis and into the epididymis.[32] Spermatozoa gain motility and fertilizing ability during passage through the epididymis, and are stored in the tail of the epididymis.[34]

The proliferating spermatozoa are stimulated to divide at a fixed time interval which, in dogs, is every 13.6 ± 0.7 days.[36,37] Life span of the various developing spermatozoal cell types in the dog are 20.9 days for primary spermatocytes, 0.5 days for secondary spermatocytes, and 21.1 days for spermatids.[36] Total duration of spermatogenesis is approximately 4.5 times the length of one spermatogenic cycle, or 62 days, in the dog.[34] Within a given seminiferous tubule, four to five cohorts or generations of developing cells are present. Characteristic groupings of cells, called cellular associations, can be defined. The same cellular associations recur in a seminiferous tubule every 13.6 ± 0.7 days.[34] Eight cellular associations have been defined in the dog (Table 15–1).[36,37] Spermatogenesis continues constantly, regardless of frequency of ejaculation.[34]

Ductus Deferens/Spermatic Cord

The paired ductuli deferentes (vasa deferentia) are a continuation of the epididymal ducts. On each side, the ductus deferens runs along the dorsomedial surface of the testis, ascends into the abdominal cavity through the inguinal canal, crosses ventral to the ureter, and penetrates the dorsal median surface of the prostate to open into the prostatic urethra lateral to the urethral crest, a longitudinal fold on the dorsal aspect of the urethral lumen.[13,32] Although a dilation of the ductus deferens is present in which some spermatozoa may be stored, no distinct ampulla of the ductus deferens is described in dogs.[13] Major blood vessels supplying the ductus deferens are the artery and vein of the ductus deferens and the middle rectal artery.[13,38]

The spermatic cord on each side is made up of the ductus deferens, testicular vessels and nerves, and artery and vein of the ductus deferens, enwrapped in the mesoductus deferens and mesorchium, parietal tunic, and the spermatic fascia, which form the vaginal process (Fig. 15–6). The vaginal ring is formed where the spermatic cord and associated vaginal process enter the deep inguinal ring. The inguinal canal is an opening through the abdominal musculature connecting the deep and superficial inguinal rings, bounded by the rectus abdominus muscle medially, the internal abdominal oblique muscle cranially, and the aponeurosis of the external abdominal oblique muscle laterally and caudally.[13]

Prostate

The prostate is the only accessory sex gland of the male dog. It is a retroperitoneal organ that encircles the urethra at the neck of the urinary bladder; it is bounded dorsally by the rectum and ventrally by the symphysis pubis or ventral abdominal wall.[13] The craniocaudal position is dependent on size of the organ; as prostatic size increases with age in the sexually intact male dog, the prostate pushes the freely movable urinary bladder from the pelvis into the abdomen. The prostate is an androgen-dependent organ. Castration causes significant atrophy of the prostate.[39] Persistent androgen

Table 15–1. The Eight Cellular Associations of Spermatogenesis in the Dog

Cell Types Present*	Cellular Association							
	1	2	3	4	5	6	7	8
Elongated spermatids				x	x	x	x	x
			x				x	x
		x				x		
Round spermatids	x			x	x			
Secondary spermatocytes				x	x			
					x	x	x	
Mature primary spermatocytes	x	x	x	x				x
Immature primary spermatocytes	x	x	x	x				x
Type B spermatogonia						x	x	
				x	x			x
Type A spermatogonia	x	x	x			x	x	x

* Increasing height in table indicates change in nuclear size with maturation. For example, elongated spermatids that are least mature appear in cellular associations 2 and 6; those of greatest maturity appear in association 8.
Data from Foote RH, Swierstra EE, Hunt WL: Spermatogenesis in the dog. Anat Rec 173:341–352, 1972 and Ibach B, Weissbach L, Hilscher B: Stages of the cycle of the seminiferous epithelium in the dog. Andrologia 8:297–307, 1976.

secretion over the life of the dog causes gradual enlargement of the gland due to proliferation of glandular and stromal components, and increase in size and number of prostatic epithelial cells.[40] Prostatic weight, height, length, width, and volume all are positively correlated with age until 11 years, after which time senile involution occurs.[39–43] The bilobed prostate is covered by a thick capsule containing smooth muscle fibers. Major blood vessels supplying the prostate are the prostatic artery, which arises from the internal pudendal artery, and the prostatic and urethral veins.[13,38] Parasympathetic innervation via the pelvic nerve from the prostatic plexus induces secretion of prostatic fluid. Sympathetic innervation from the hypogastric nerve stimulates smooth muscle contraction and subsequent expulsion of prostatic fluid into the prostatic ducts and urethra.[13,44]

The prostate is split into two major lobes by a prominent fibrous medial septum. The prostatic urethra passes through the center of the gland dorsally, and is V-shaped on cross section due to presence of a dorsal longitudinal fold, the urethral crest.[13,32] The two main lobes are separated into lobules by connective tissue septae. The lobules are made up of compound tubuloalveolar glands lined by columnar epithelium. The numerous prostatic ducts into which prostatic secretion empties do not coalesce but instead empty into the prostatic urethra around the openings of the ductuli deferentes, forming the colliculus seminalis.[13]

Penis/Prepuce

The canine penis is composed of three parts: the root, the body, and the glans (Fig. 15–7). The proximal root, or crus penis, contains the corpus cavernosum and is covered by a thick tunica albuginea and the ischiocavernosus muscle. The penile root is adhered to the ischial arch between the ischial tuberosities.[13] The body, or corpus penis, begins where the two crura join. It is made up of two separate erectile bodies separated by a median connective tissue septum. The urethra lies ventrally within the body of the penis, enwrapped by the corpus spongiosum. The two sides of the penile body fuse at the base of the os penis.[13] The third part of the penis, the glans, consists of two parts, the bulbus glandis and the pars longa glandis. The bulbus glandis is a barrel-shaped expansion of the corpus spongiosum. The os penis, or baculum, runs through the bulbus glandis, to which it is tightly adhered, and the pars longa glandis. The penile urethra runs ventrally along the base and grooved body of the os penis.[13,45] Major vessels supplying the penis are the internal pudendal and perineal arteries, and the internal and external pudendal veins and dorsal vein of the penis. Parasympathetic innervation is via the pelvic nerve and sympathetic innervation is via the hypogastric nerve.

Erection of the penis is a result of parasympathetic stimulation.[46] Pressure increases in the

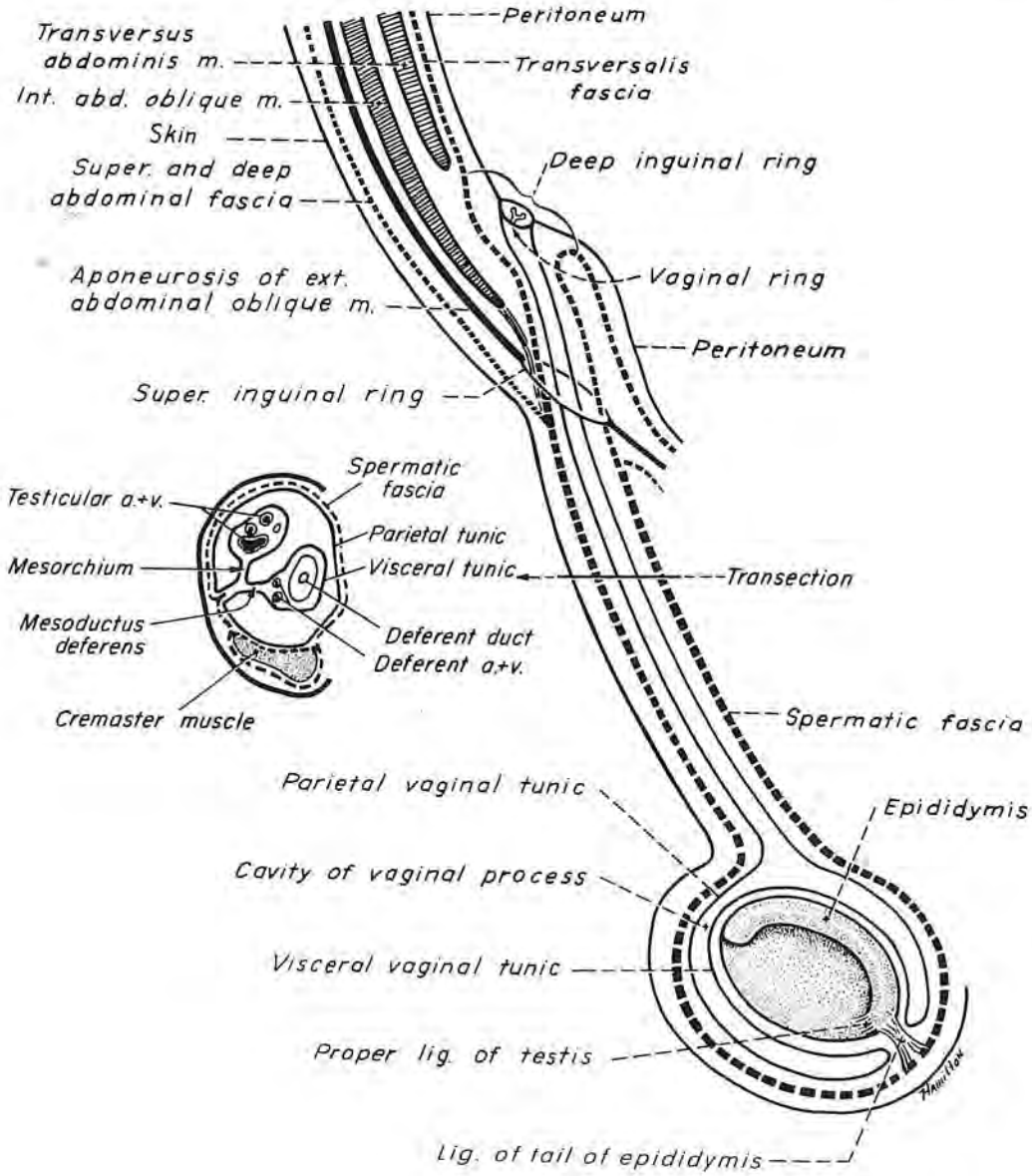

Figure 15–6. Diagram of the spermatic cord of the dog. (From Evans HE, Christensen GC: The urogenital system. *In* Evans HE [ed]: Miller's Anatomy of the Dog. Philadelphia, WB Saunders, 1993, pp 494–558, with permission.)

corpus spongiosum first. Engorgement of the corpus cavernosum is delayed, but eventually reaches three times the pressure within the corpus spongiosum.[47] Relaxation of smooth muscle fibers in trabeculae of the corpus cavernosum decreases intracavernosal resistance with subsequent increased arterial flow, compression of the root of the penis and bulbus glandis by the ischiocavernosus and bulbospongiosus muscles, respectively, and occlusion of venous outflow by obstruction of the venous lumen at the tunica albuginea and compression by the ischiourethralis muscle.[46–48] Venous occlusion is not complete; some blood flow has been demonstrated even when corpus cavernosal pressure is maximal.[46] Erection primarily affects the glans penis. A ligamentous attachment from distal cartilage of the os penis to connective tissue of the glans above the urethra and free movement of the pars longa glandis over the os penis cause deformation of the distal glans to form a corona glandis, which permits ejaculation of semen through the now dorsally directed urethral ori-

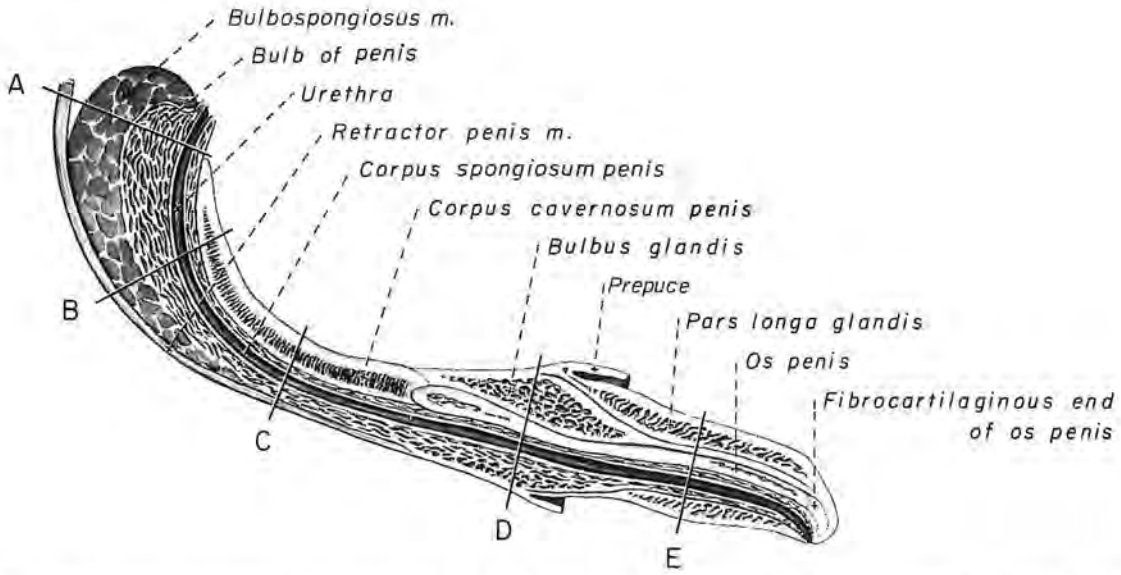

Figure 15–7. Internal morphology of the canine penis. (From Evans HE, Christensen GC: The urogenital system. *In* Evans HE [ed]: Miller's Anatomy of the Dog. Philadelphia, WB Saunders, 1993, pp 494–558, with permission.)

fice toward the cervix during the copulatory lock.[13,49] The penis of male dogs twists 180 degrees in a lateral plane during the copulatory lock (Fig. 15–8). This twist may assist with venous occlusion and maintenance of erection despite high intravaginal pressure during the copulatory lock, which is unique to dogs among domestic animals.[49]

Detumescence of the penis occurs as smooth muscle contracts in sinusoidal walls of the cavernosal tissue, permitting widening of venous lumina and restoring venous outflow.[47] Sym-

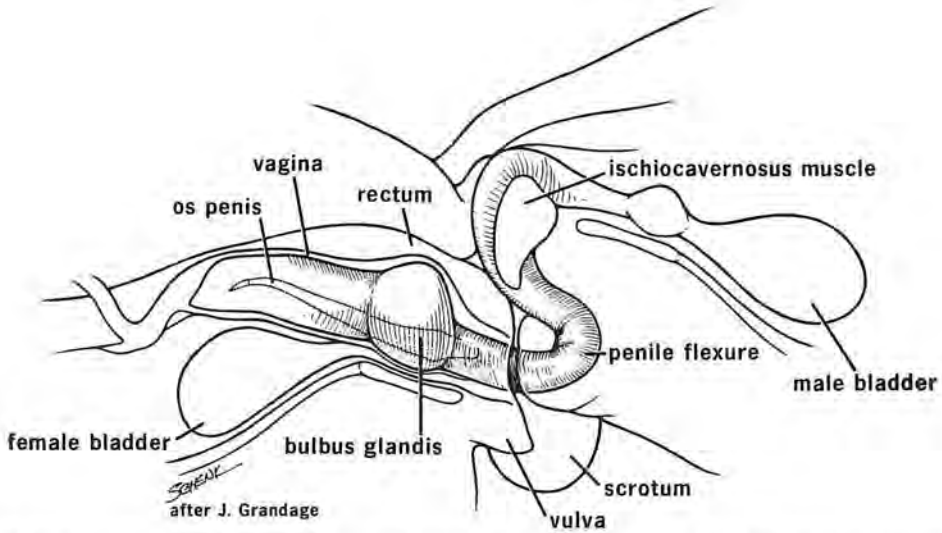

Figure 15–8. Anatomy of the male and female reproductive tracts during copulation in the dog. (Modified from Grandage J: The erect dog penis: A paradox of flexible rigidity. Vet Rec 91:141–147, 1972, with permission.)

pathetic innervation via the hypogastric nerve increases arterial resistance, causing a decrease in corpus cavernosal pressure.[46]

The prepuce is a fold of haired skin covering the glans of the penis. The preputial mucosa becomes continuous with the penile mucosa at the fornix. The preputial muscle is a small portion of the cutaneous trunci muscle, which prevents the prepuce from hanging loosely when the penis is flaccid and pulls the prepuce back over the glans penis during detumescence.[13]

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Chapter 16

Semen Collection, Evaluation, and Preservation

Canine semen collection may be indicated for evaluation of semen as part of a complete breeding soundness examination, for assessment of suspect subfertile or infertile dogs, for collection of specimens for seminal fluid cytology and microbial culture, for immediate artificial insemination, or for preservation for insemination in the near (chilled extended semen) or distant (frozen semen) future. The American Kennel Club (AKC) requires that semen evaluation and demonstration of breeding soundness be performed by a veterinarian before registration of a litter for all sires less than 7 months and more than 12 years of age.

Semen Collection

Manual ejaculation is the most common method of semen collection used in dogs. Electroejaculation has been reported, but usually is not necessary, and may be associated with urine contamination of the ejaculate.

Canine semen collection should be performed in a comfortable, quiet environment with nonslip flooring. Although most male dogs will ejaculate during manual stimulation of the penis in the absence of a teaser bitch, use of a teaser is recommended whenever possible. Occasionally, experienced stud dogs will not ejaculate unless an estrous bitch is present and, if quality of semen obtained in the absence of a teaser is not normal, the veterinarian should attempt recollection after recruiting an estrous bitch. Males with poor libido may be less distracted and ejaculate more readily in the presence of a teaser bitch,[1] and the presence of a teaser bitch, especially if she is in estrus, will improve quality of the ejaculate collected.[1,2] The teaser bitch need not be of the same breed as the stud but should approximate him in size.

If an estrous teaser bitch is not available, some investigators report success when the pheromone methyl *p*-hydroxybenzoate (Aldrich Chemical, Milwaukee, WI) is applied to the vulva of a nonestrous bitch; however, some dogs do not respond to this compound.[3] Alternatively, swabs of vaginal discharge collected from a disease-free estrual bitch can be stored frozen, then thawed and used to tease male dogs before semen collection.[4] Gonadotropin-releasing hormone (GnRH), 3.3 μg/kg intramuscularly (IM), can be administered to the male dog once daily during a bitch's standing heat or 60 minutes before breeding or semen collection to stimulate libido by causing release of endogenous testosterone.[5] Caution must be used with this technique in dogs with androgen-dependent disease, such as benign prostatic hypertrophy or perianal gland adenoma. Administration of exogenous testosterone to stimulate libido should be avoided, as this hormone exerts negative feedback on the pituitary, causing a decline in endogenous testosterone release, decreased spermatogenesis with a decline in total number of spermatozoa per ejaculate, and eventually causing testicular degeneration.[6,7]

If possible, canine semen should be collected using a latex artificial vagina (Nasco, Fort Atkinson, WI) attached to a sterile, graduated, plastic centrifuge tube (Fig. 16–1), because this best simulates natural service and the copulatory lock, and ensures that no part of the ejaculate is lost during collection. Alternatively, the semen can be collected into any clean vessel. Other receptacles described include stainless steel or styrofoam cups, plastic sandwich bags, and glass tubes. Thin, disposable polypropyl-

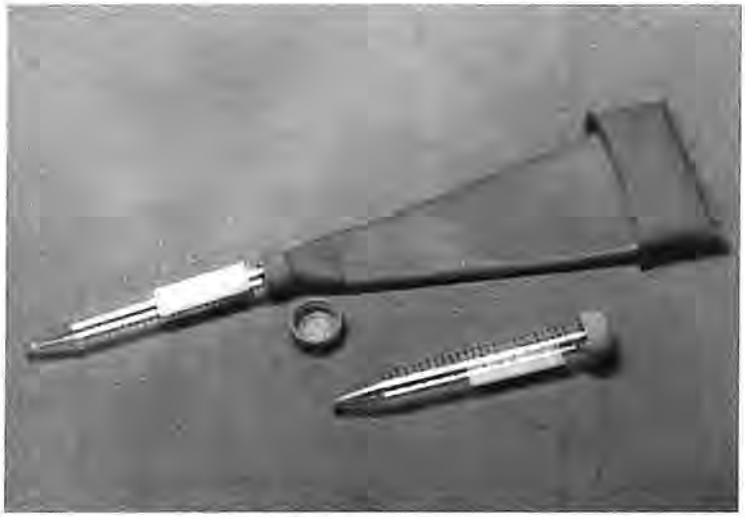

Figure 16–1. Latex collecting cone and centrifuge tube for collection of canine semen. (From Purswell BJ, Althouse GC, Root MV: Guidelines for using the canine breeding soundness evaluation form. Nashville, Society for Theriogenology, 1992, with permission.)

ene collecting cones are marketed for the dog. Caution should be exercised when using all collecting cones near the erect penis, since any sharp edge formed where the collecting cone is joined during manufacture may cut the penis with even minimal contact. Although exposure to latex has been shown to decrease motility of spermaotozoa,[8] if proper technique is used, there is minimal exposure of the semen to the latex during ejaculation. The narrow end of the collecting cone is pulled over a sterile centrifuge tube. It is not possible to collect a sterile semen sample, since there are normal bacterial flora on the penile mucosa and in the distal urethra that contaminate the sample. The centrifuge tube and collecting cone should be rinsed thoroughly with distilled water and allowed to air-dry, ensuring that any spermicidal residues from manufacture are removed, and it is recommended that collection tubes be sterile so as not to contaminate any sample that is collected.[9] The top of the latex collecting cone is folded over to form a final length such that the tip of the erect penis will be just above the centrifuge tube, minimizing contact of the ejaculate with the latex cone but not permitting the tip of the penis to be traumatized by the collecting tube. A small amount of water-soluble lubricant is placed around the top fold of the collecting cone, which facilitates later removal. Lubricant should not be allowed to come in contact with the ejaculate, since many commonly used lubricants decrease spermatozoal motility.[10,11]

Two handlers should be present, one for the teaser bitch and one for the male. The bitch should be muzzled to prevent her biting the stud dog or handler during mounting. The handler of the bitch should kneel in front of her and keep her standing. The male dog is allowed to sniff at the bitch's hindquarters, and he may be allowed to mount her. Some normal males do not mount the bitch during this procedure. Brisk and enthusiastic massage of the bulbus glandis through the prepuce of the male will elicit erection. As soon as erection begins, the hand manipulating the prepuce can be used to move the prepuce proximal to the bulbus glandis while the other hand introduces the collecting cone with attached centrifuge tube over the engorging penis. Full erection of the bulbus glandis within the prepuce may be uncomfortable to some dogs, cause incomplete ejaculation, and prevent turning the penis to simulate the copulatory lock, but some dogs ejaculate normally despite presence of the taut prepuce around the erect penis.[4] Ideally, the collecting cone is passed to just proximal to the bulbus glandis and a tight grip maintained at that area. Application of circumferential pressure proximal to the bulbus glandis simulates the pressure of the lips of the vulva during the copulatory lock, and the pressure of the latex cone around the erect penis simulates intravaginal pressure. The dog usually will thrust vigorously for several minutes, ejaculating the pre-sperm and sperm-rich fractions of semen, on average, 20.7 seconds after manual stimulation is begun.[12] The male will then rest briefly before ejaculating the prostatic fluid fraction of the ejaculate. The dog may try to step over the operator's arm

Figure 16–2. Caudal repositioning of the erect canine penis, within the latex collecting cone, during ejaculation of the third fraction of semen. (From Purswell BJ, Althouse GC, Root MV: Guidelines for using the canine breeding soundness evaluation form. Nashville, Society for Theriogenology, 1992, with permission.)

during this rest to simulate the copulatory lock, at which time the operator should lift the dog's rear leg over his or her arm and redirect the penis 180 degrees in a horizontal plane until it is directed caudally, as it would be during the copulatory lock (Fig. 16–2).

Semen is ejaculated in three fractions (Table 16–1). The first, or pre-sperm fraction, probably originates in the prostate.[13] It usually is small in volume, although occasional dogs may ejaculate as much as 5 ml or more; the pre-sperm fraction is clear and acellular. This fraction usually is ejaculated during rapid thrusting by the male. The second, or sperm-rich fraction, originates in the tail of the epididymis where spermatozoa are stored. It is variable in volume (typically 1 to 4 ml) and opalescent in color. The sperm-rich fraction may be ejaculated either during vigorous thrusting or immediately thereafter. The third, or prostatic, fraction of the semen usually is a large volume of clear fluid in the normal dog. During ejaculation of prostatic fluid, the operator should feel rhythmic pulsations in the penile urethra concurrent with visualization of anal contractions. Unlike the first two fractions, which are secreted without visible force, the third fraction appears to be ejaculated with propulsion such that surges of prostatic fluid spurt into the collection tube.

Once semen collection is complete, manual pressure proximal to the bulbus glandis is released, and the collecting cone is gently peeled off of the engorged penis. The male often will continue to ejaculate pulses of prostatic fluid for several minutes after removal of the collecting cone. Detumescence of the penis can be hastened by allowing the male to lick at the penis, or exercising the dog away from the environment in which semen was collected. The male should not be kenneled away from observers until penile detumescence is complete; occasionally the prepuce rolls in on itself during detumescence, leaving the tip of the penis exposed to drying and trauma. This can be resolved by lubrication and manipulation of the tip of the penis into the prepuce.

Semen collection can be attempted again within 1 hour in the dog, if necessary, although some males will not ejaculate more than once in a day. In a study in which semen was collected from dogs twice within 45 to 75 minutes (mean = 63 minutes), total number of spermatozoa was significantly lower in the sec-

Table 16–1. Normal Semen Quality in the Dog

	Fraction 1	Fraction 2	Fraction 3	Total Ejaculate
Volume (ml)	0.5–5.0	1.0–4.0	1.0–80.0	2.5–>80.0
Color	Clear	Opalescent	Clear	Opalescent
Concentration (10^6/ml)	–	4–400	–	4–400
Total sperm per ejaculate (10^9/ml)	–	300–2000	–	300–2000
Percentage progressively motile spermatozoa	–	>70%	–	>70%
Percentage morphologically normal spermatozoa	–	>80%	–	>80%
pH	–	–	6.3–6.7	6.3–6.7
White blood cells/hpf*	0–3	0–3	–	≤6

* Centrifuged sample.

ond ejaculate.[14] The two collections, pooled, contained on average 70 per cent more spermatozoa than the single first ejaculate, suggesting that this technique may be useful when collecting semen for artificial insemination or semen preservation.

Semen quality is best if collected no more frequently than every 2 to 5 days.[1,2,15,16] Dogs that ejaculate infrequently may have excessive debris or decreased percentages of progressively motile and morphologically normal spermatozoa in the first ejaculate after a sexual rest, presumably due to presence of large numbers of aged spermatozoa from the epididymes in that ejaculate. Daily semen collection removes spermatozoa from storage in the epididymes. In one study, daily semen collection caused a gradual decline in total number of spermatozoa per ejaculate with stabilization at the amount the testes produced daily, termed the daily sperm output (DSO), over a period of 6 days.[2] In another study, daily semen collection for 5 days did not alter semen quality.[17] Short-term daily semen collection did not affect libido, but libido did decrease in five dogs collected daily for 12 weeks.[18] More frequent collection (e.g., two or three times daily) caused a dramatic decrease in total number of spermatozoa per ejaculate and libido.[17] Less frequent collection, such as twice weekly, did not cause a decline in libido or semen quality even after 6 months.[19] Some investigators believe that daily ejaculation until DSO is reached is the best measure of spermatogenic function in dogs.

Semen Evaluation

Evaluation of canine semen includes determination of volume, color, pH of the third (prostatic fluid) fraction, percentage of progressively motile spermatozoa, concentration and total number of spermatozoa in the ejaculate, percentage of morphologically normal spermatozoa, and assessment of seminal fluid cytology and microbial culture. Completion of a standard semen evaluation form (Fig. 16–3) ensures collection of all necessary history and physical examination findings, and is useful for accurate record-keeping and monitoring changes in semen quality over time.[9]

Canine semen quality may vary due to change in the environment in which semen is collected, presence of disease of the male reproductive tract, systemic disease, age and breed of the animal, and season of the year. Very young dogs and very old dogs have poor semen quality. Beagles have been demonstrated to have a gradual increase in semen quality from their first ejaculation of spermatozoa, occurring on average at 235 days of age, until 1 year of age.[20] In studies of dalmatians and rottweilers, those less than 6 years of age had significantly better semen quality, with higher total number of spermatozoa per ejaculate and higher percentage progressively motile spermatozoa, than those more than 6 years of age.[21,22] Differences in semen quality due to breed generally are differences in total number of spermatozoa per ejaculate, which is dependent on grams of testicular tissue present and is, therefore, higher in large-breed dogs with large testes. Mongrels (n = 4) have been reported to have better quality semen than purebred dogs (n = 8).[23] Season of the year may have some effect on semen quality; studies have demonstrated the peak of semen quality in spring and the nadir in summer or fall in the Northern hemisphere, but, in these studies, total number of spermatozoa per ejaculate never fell below normal limits.[23,24] Abnormal semen quality is discussed elsewhere (see Chapter 23). Reported values for parameters in normal canine semen are listed in Table 16–2.

Volume

Normal semen volume in the dog ranges from 1.0 to 80.0 ml.[9,12] Volume is not indicative of semen quality, since it is dependent on the amount of prostatic fluid collected by the operator. Dogs have been reported to ejaculate prostatic fluid for up to 20 minutes during semen collection.[12] The volume of semen should be recorded before removing any aliquots, since this volume is needed for calculation of total number of spermatozoa in the ejaculate.

Color

Normal canine semen is cloudy white to opalescent. Cloudy samples should be examined microscopically for presence of spermatozoa, because, occasionally, ejaculates containing large numbers of fat droplets or bacteria and inflammatory cells mimic the normal appearance of those containing spermatozoa. Yellow color indicates contamination with urine or inflammatory exudate, green may indicate presence of purulent exudate, red indicates blood, brown indicates old blood, usually originating in the prostate, and a clear sample indicates azoospermia is present.

CANINE BREEDING SOUNDNESS EVALUATION

Guidelines Established by Society for Theriogenology
P.O. Box 2118, Hastings, NE 68902-2118
Phone(402)463-0392 FAX(402)461-4103

Case # _____ Date: _____
Client: _____ Address: _____
Dog's Name: _____ Breed & Color: _____
Date of Birth: _____ Registration Number: _____ Tattoo: _____

HISTORY

Reason for evaluation: _____ Date of last breeding: _____
Date of last litter: _____ Brucellosis tested: _____ Type of test: _____
Pedigree available: _____ Infertile relatives: _____

PHYSICAL EXAMINATION

Physical condition: _____ Weight: _____
Pertinent other health problems: _____
Penis/Prepuce: _____ Spermatic cord: _____
Scrotum: _____ Prostate: _____
Epididymides: (R) _____ (L) _____
Testes: Width (R) _____ (L) _____ Total: _____
Consistency (hard/normal/soft): (R) _____ (L) _____
Masses/fluid/pain/other: _____

SEMEN COLLECTION

Date last collected: _____ Libido/ease of collection: _____
Teaser bitch present: _____ Stage of cycle: _____ Pheromone used: _____
Equipment used (AV/other): _____

SEMEN EVALUATION

	Color	Volume	pH	Conc (sperm/ml)	Total Sperm Number (sperm/ejac)
Fraction 1	_____	_____	XXX*	XXXXXXXXXXXXXXX*	XXXXXXXXXXXXXXXXX*
Fraction 2	_____	_____	XXX*	_____	_____
Fraction 3	_____	XXXXXXXX*	_____	XXXXXXXXXXXXXXX*	XXXXXXXXXXXXXXXXX*

*Not applicable and/or necessary

MOTILITY:
Total Motility (%): _____ Progressive Motility (%): _____
Diluent (if used): _____ Speed: slow moderate fast

MORPHOLOGY:
Method (Stain): _____ Phase Contrast ( )
% Normal: _____ Total normal (% normal X sperm/ejac): _____
Head abnormalities: _____
Midpiece abnormalities: _____
Tail abnormalities: _____

CYTOLOGY (0-4+) (RBC, WBC, Epith., Bact., Other):
Fraction 1: _____ Fraction 2: _____ Fraction 3: _____

CONCLUSIONS:

Signed: _____ Clinic Name: _____
Member-Society for Theriogenology

Figure 16–3. Canine breeding soundness examination form. (From Purswell BJ, Althouse GC, Root MV: Guidelines for using the canine breeding soundness evaluation form. Nashville, Society for Theriogenology, 1992, with permission.)

Table 16–2. Reported Mean Values for Percentage Progressively Motile Spermatozoa, Total Number of Spermatozoa in the Ejaculate, and Percentage Morphologically Normal Spermatozoa in Canine Semen

Breed (Sample Size)	Mean Progressively Motile Spermatozoa (%)	Mean Total Number of Spermatozoa in the Ejaculate ($\times 10^6$)	Mean Morphologically Normal Spermatozoa (%)	References
Variable (n = 34)	89.5	332.8	88.4	England and Allen[33]
Mongrel (n = 4), beagle (n = 8)	81.2	508.6	92.4	Takeishi et al.[23]
Spitz (n = 10)	89.3	383.5	93.9	Kuroda and Hiroe[24]
Dalmatian (n = 28)	81	563	79	Schubert and Seager[21]
Rottweiler (n = 23)	77	795	72	Seager and Schubert[22]
Variable (n = 45)	75.6	—	79.3	Oettle[34]
Mongrel (n = 3)	—	528	85	Barlett[35]
Variable (n = 150)	75	365	78	Stockner and Bardwick[35]
Irish wolfhound (n = 25)	63.1	—	65.4	Dahlbom et al.[36]
Variable (n = 44)	87.2	769	78.6	Dahlbom et al.[36]
Irish wolfhound (n = 25)	79.9	—	68.5	Dahlbom et al.[37]
Variable (n = 44)	87.3	744	78.7	Dahlbom et al.[37]
Variable (n = 41)	–	844.6	–	Root Kustritz et al.[38]
Variable (n = 245, n = 167)	—	860 *	69.1†	Morton and Bruce[39]
Average	79.8	585.8	83.1	

Progressive Motility

Percentage of progressively motile spermatozoa in the canine semen sample is assessed by placing a drop of undiluted semen on a prewashed glass slide and examining it microscopically at 100× magnification. Normal percentage progressively motile spermatozoa is 70 per cent or greater (Table 16–2).[9] Canine spermatozoa are resistant to cold shock, so the slide need not be warmed. Percentage progressive spermatozoal motility has been demonstrated to decline less quickly in samples held at room temperature than in those held at body temperature.[25] A cover slip may be applied. Highly concentrated samples can be diluted with autologous prostatic fluid, phosphate-buffered saline, 2.9 per cent sodium citrate solution, or a semen extender.[4] Autologous prostatic fluid has been reported to have neither a beneficial nor a detrimental effect on percentage progressive motility of spermatozoa.[26] In one study that reported a detrimental effect of prostatic fluid on percentage progressive motility of spermatozoa, pH of the prostatic fluid was abnormally high, at 7.0.[27] Variable pH of saline preparations may decrease percentage progressive spermatozoal motility, and many extenders contain viscous substances, such as egg yolk, which decrease the speed of motile spermatozoa.

The drop of semen should be evaluated under 100× magnification (10× objective plus 10× oculars) and a subjective assessment made of percentage of spermatozoa that are moving forward. Assessment also can be made of speed of progression of the spermatozoa, rating speed as slow, moderate, or fast.[4] A subjective evaluation for agglutination of spermatozoa also should be made at this time. Examination should be done immediately, as motility declines as the light from the microscope heats the drop. Motility is artifactually increased near air bubbles, and decreased near the edge of the slide.[28]

Percentage of progressively motile sperm is increased in samples collected after 5 days of sexual rest, compared to those collected after 1 day of sexual rest.[26] Percentage of progressively motile sperm also may be increased by addition of an extender (see Semen Preservation, below). Increased percentage of progressively motile sperm was demonstrated in a second sample, collected 12 hours after first ejaculation; a concurrent decrease in morphologic abnormalities of spermatozoa also was present.[29] Percentage morphologically normal spermatozoa and percentage progressive motility of canine spermatozoa usually are positively correlated.[19,30]

Decline in percentage of progressively motile spermatozoa often is an early, nonspecific indicator of reproductive tract infection or testicular insult.[9] Artifactual decreases in percentage of progressively motile sperm may occur due to exposure of the spermatozoa to toxic residues of manufacture of the plastic components used for semen collection or evaluation, or to excessive exposure to latex or lubricants. Many lubricants are spermicidal, although petroleum jelly is reported not to affect motility of canine spermatozoa.[10,11] Both incubation of canine spermatozoa with latex for 15 minutes, and running the semen sample over a latex-gloved hand have been shown to decrease progressive motility of canine spermatozoa.[8]

No correlation between speed of progression of spermatozoa, percentage progressively motile spermatozoa, and fertilizing capability of canine spermatozoa has been reported. However, in dogs, percentage progressively motile spermatozoa is a more sensitive indicator of semen quality than is viability of spermatozoa measured by filter penetration tests[32] and, in humans, assessment of speed and linearity of progression of spermatozoa has been reported to be the best predictor in the spermiogram of the couple's future fertility.[31]

pH

The pH of the third (prostatic) fraction averages 6.5 with a range of 6.3 to 6.7.[9,36,41] Knowledge of pH of prostatic fluid may be used to guide antibiotic therapy in dogs with prostatitis (see Chapter 20).

Concentration/Total Number of Spermatozoa

Concentration is not an indicator of semen quality in the dog, except in those cases in which no spermatozoa are present in the ejaculate. In ejaculates containing spermatozoa, concentration is dependent on the amount of prostatic fluid collected, and may range from 4 to 400 million per milliliter. Concentration should be determined to allow calculation of total number of spermatozoa in the ejaculate, which is the number of interest. Concentration may be measured using the white blood cell (WBC) Unopette system (Becton-Dickinson, Rutherford, NJ) (Fig. 16–4). Semen is drawn into the 20-μl pipette, and dispensed into the

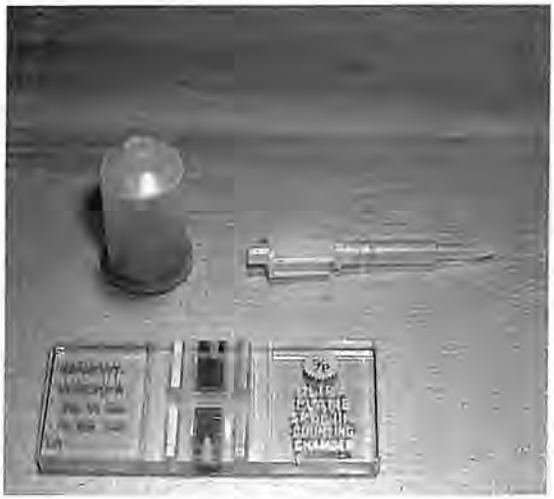

Figure 16–4. White blood cell Unopette system and Neubauer hemacytometer for measurement of concentration of canine spermatozoa in the ejaculate.

reservoir chamber, which contains 2 ml of diluent. Diluted semen is then dispensed into both chambers of a Neubauer hemacytometer and the central square of the grid, made up of 25 small squares in 1-mm square, is counted on each side of the hemacytometer, and averaged (Fig. 16–5). This central 1-mm square fills the field of the light microscope using the 10× objective. The number of spermatozoa in the central square is the concentration, in million spermatozoa per milliliter of semen.

A technique has been described for determining sperm concentration in semen from other species in which samples placed in a 10-μm counting chamber are compared to standard photomicrographs of spermatozoal concentrations ranging from 100 to 1500 million per milliliter.[41] This technique is not suitable for samples with spermatozoal concentrations less than 100 million per milliliter, which often is the case in the dog.[41]

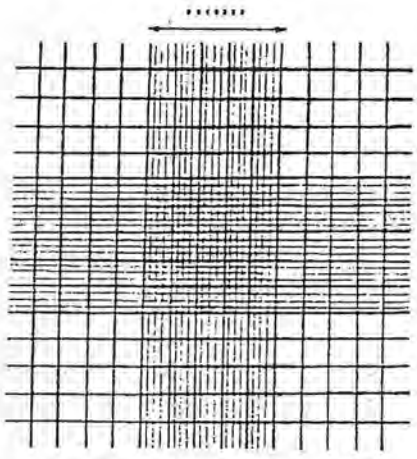

Figure 16–5. Grid of the Neubauer hemacytometer. The number of spermatozoa counted within the large central square, of the 9 large squares shown, is the number of million spermatozoa per milliliter in that ejaculate.

Normal total number of spermatozoa in the canine ejaculate is 300 million to 2 billion.[9] Multiplication of concentration in millions per milliliter times the volume collected in milliliters yields the total number of spermatozoa in millions per ejaculate. The wide range of total sperm in the canine ejaculate reflects that fact that sperm production is dependent on grams of testicular tissue; small-breed dogs do not produce as many spermatozoa as large-breed dogs with larger testes (Table 16–3).

Total number of spermatozoa in the ejaculate may be decreased in very young and very old dogs, and in inbred dogs.[42] Male dogs should not be labeled as subfertile or infertile after a single semen collection, since apprehension, absence of a teaser bitch, and pain in the prostate, spine, or rear limbs may decrease number of spermatozoa ejaculated. Seminal fluid from samples lacking spermatozoa should be evaluated for alkaline phosphatase concentration, which comes from the epididymis, to ensure that a complete ejaculate was obtained (see Chapter 23).[43]

Morphology

Normal percentage of morphologically normal spermatozoa (%MNS) in normal canine semen should be greater than or equal to 80 per cent (Table 16–2).[9] Examination by phase contrast microscopy of spermatozoa in undiluted semen or semen diluted with phosphate-buffered saline allows visualization of morphologic abnormalities. Examination of spermatozoa by light microscopy requires staining of the cells. Stains described for use with dog spermatozoa include eosin-nigrosin stain (SFT stain; Lane Manufacturing, Denver, CO) and modified Giemsa stain (DiffQuik; Baxter Healthcare, Miami, FL). To stain spermatozoa with eosin-nigrosin, a drop of semen and a drop of stain are placed on one end of a glass slide. Another slide is used to gently mix the two solutions by rocking, and to draw the mixture out to form a thick film, which is allowed to air-dry. Staining spermatozoa with DiffQuik can be done in two ways. With either method, a drop of undiluted semen is placed on one end of a glass slide, drawn out as for a blood smear, and allowed to air-dry. In the first stain-

Table 16-3. Influence of Body Weight on the Reproductive Capacity of Adult Dogs*

Characteristic	Body Weight (lb)		
	10–34	35–39	60–84
Total scrotal testes width (mm)	36 ± 2§	50 ± 1¶	56 ± 1**
Paired testes weight (g)	16 ± 1§	31 ± 1¶	44 ± 2**
DSP/g parenchyma (10^6)	20 ± 2	17 ± 1	20 ± 3
DSP/dog (10^6)	287 ± 33	472 ± 32	750 ± 111
Extragonadal spermatozoal reserves (10^9) at sexual rest			
Caput epididymidis	0.07 ± 0.01	0.23 ± 0.04	0.23 ± 0.05
Corpus epididymidis	1.10 ± 0.18	1.85 ± 0.16	2.27 ± 0.24
Cauda epididymidis	2.06 ± 0.31	3.30 ± 0.36	4.68 ± 0.39
Ductus deferens†	0.06 ± 0.02	0.21 ± 0.03	0.23 ± 0.04
Semen ejaculated after sexual rest			
Volume (ml)‡	2.4 ± 0.3	3.9 ± 0.5	5.4 ± 1.3
Concentration (10^6/ml)‡	209 ± 42	359 ± 72	228 ± 58
Total sperm (10^9)	0.4 ± 0.11	1.12 ± 0.13	1.43 ± 0.46

* Mean (± SEM) for 30, 53, and 32 dogs in the 10–34, 35–59, and 60–84 lb groups, but data on extragonadal spermatozoal reserves are for 17, 32, and 14 dogs and data for characteristics of a single ejaculate collected after ≥7 days of sexual rest are for 12, 14, and 11 dogs, respectively. DSP = daily spermatozoal production. For a characteristic, means without a superscript symbol (§, ¶, **) or with the same superscript symbol are not different ($p > 0.05$).

† Not all of the ductus deferens was available for dogs castrated rather than euthanized.

‡ The presperm and sperm-rich fractions were collected together, but ejaculation was terminated when ejaculation of the postsperm prostatic fluid started.

From Amann RP: Reproductive physiology and endocrinology of the dog. *In* Morrow DA (ed): Current Therapy in Theriogenology. Philadelphia, WB Saunders, 1986, p 536, with permission.

ing method, the slide is placed in each of the three DiffQuik solutions for 5 minutes each, then rinsed and allowed to dry. In the second, the slide is dipped in the first and second solutions 7 to 10 times, and in the final solution 10 to 15 times. The slide is not rinsed, and the back of the slide is wiped dry. The slide is allowed to dry. Staining artifacts may be minimized by blowing on the slide or placing the slide on a plate heated to 37°C to hasten drying.[44] At least 100 spermatozoa are evaluated with light microscopy under the oil immersion (100×) objective.

Any staining technique causes some artifactual changes in spermatozoa. The defects vary with the stain used.[45–48] Detached heads, coiled and bent tails, and reflex midpieces are the most common preparation artifacts described.[49,50] In bulls, it has been shown that artifacts of spermatozoal morphology due to staining vary by bull; consistency of technique is required for accuracy in serial evaluations.[50] Assessment of percentage morphologically normal spermatozoa (%MNS) is the only consistently repeatable measure between or within investigators evaluating canine or human spermatozoa.[46,51]

Abnormalities of spermatozoal morphology may be classified by area within which they occur (Figs. 16–6 and 16–7), as primary defects (those occurring during spermatogenesis) or secondary defects (those occurring during epididymal storage or preparation of the sample) (Fig. 16–8), or as minor defects (unassociated with fertility) or major defects (those negatively correlated with fertility).[52] Morphologic abnormalities associated with infertility in the dog all are abnormalities of the midpiece or its attachment.[53–55] Samples containing large percentages of spermatozoa with proximal cytoplasmic droplets, bent or coiled tails, or reflex midpieces may be suitable for immediate use but will not withstand cryopreservation.[39] Causes of abnormal spermatozoal morphology in the dog include infection of the reproductive tract, fever, and testicular trauma.[56,57] The spermatogenic cycle of the dog is approximately 62 days, so abnormal morphology may be present well after the initial insult. Samples collected after a prolonged sexual rest that contain aged spermatozoa from the epididymis may have a higher percentage of morphologically abnormal spermatozoa.[29,58]

Normal canine spermatozoa from all breeds have a total length of 6.8 μm, with a head capped by an acrosome of uniform thickness, a midpiece 1.1 μm in length, and a tail made up of the principal piece and end piece, 5.0 μm in length (Fig. 16–6).[25,59] Both straight and gently curved spermatozoa with no overt abnormalities of the head, midpiece, or tail are designated as normal. The minimum acceptable value for %MNS defined in one study was 60 per cent; male dogs with greater than or

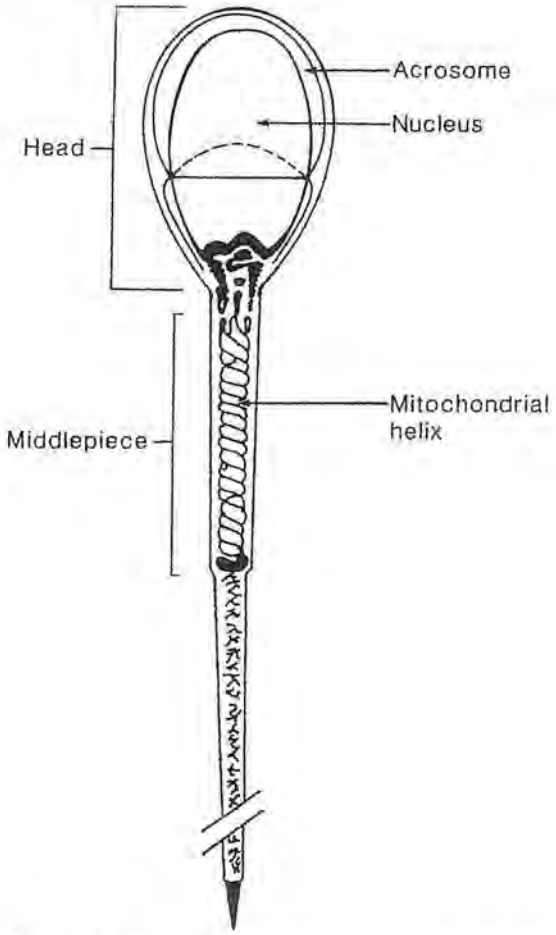

Figure 16–6. Drawing of a spermatozoon as seen with the light microscope. (From Smith FO: Cryopreservation of canine semen: technique and performance. PhD thesis, University of Minnesota, 1984.)

equal to 60 per cent MNS had a conception rate of 61 per cent (n = 23), while male dogs with less than 60 per cent MNS had a conception rate of 13 per cent (n = 15).[34]

Cytology/Culture

Cytologic evaluation of seminal fluid is best performed on the pellet remaining after 0.3 to 0.5 milliliters of the fluid has been centrifuged at 120g for 7 minutes. Normal findings include presence of mature spermatozoa, occasional WBCs and bacteria, and epithelial cells. Fertile male dogs may have as many as 2000 WBCs/μl, or 2 to 4 WBCs per high-power field (hpf) in the first and second fractions of the ejaculate.[35,60] Number of WBCs/hpf in a smear of unconcentrated semen may be graded on a scale from 0 to 4+, with 3+ and 4+ indicative of pathologic inflammation (Table 16–4).[4] Epithelial cells may be present in amounts as great as 10 to 20 per high-power field in normal dogs, especially after sexual rest.[4,35]

Semen is not sterile, because of presence of normal bacterial flora on the penile and preputial mucosa and in the distal urethra. Aerobic culture of semen is suggestive of reproductive tract infection only if more than 10,000 colony forming units (CFUs) per milliliter are present in the semen. With the exception of *Brucella canis*, organisms commonly isolated from semen are the same ones present in normal urethral flora, and include *Staphylococcus* sp., *Streptococcus* sp., *E. coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Haemophilus* sp., *Pasteurella multocida*, *Corynebacterium* sp., *Moraxella* sp., *Pseudomonas* sp., and mycoplasma.[9,38] It is recommended that culture for aerobic, anaerobic, and mycoplasma organisms be performed on all samples of semen. Significance of positive aerobic, anaerobic, and mycoplasma culture results are described elsewhere (see Chapter 23).

Assessment of cytology of seminal fluid for presence of inflammatory cells should not preclude microbial culture. In a survey of male dogs examined for breeding soundness or reproductive tract disease, only 12 of 95 (12.6 per cent) had negative microbial cultures and noninflammatory cytology (true-negative). Five of 95 (5.3 per cent) had negative microbial cultures with inflammatory cytology (false-positive). Forty-five of 95 (45.3 per cent) had noninflammatory cytology despite significant growth of aerobic or anaerobic organisms from seminal fluid (false-negative).[38]

Physical and chemical analyses, including trace mineral content, of prostatic fluid from normal dogs have been reported (Table 16–5).[61] Although the concentration of zinc in prostatic fluid varies from normal in human males with prostatic disease, intraprostatic zinc concentrations in experimentally infected dogs have not been demonstrated to vary significantly from zinc concentrations in prostatic tissue or fluid from normal dogs.[61,62] Antibacterial activity has been demonstrated in canine semen.[63] Source of the antibacterial activity is undefined; concentrations of zinc, a known natural antibacterial factor, decrease after castration, but the atrophic prostate in castrated male dogs is not more readily colonized by bacteria than is the normal prostate of intact male dogs.[63,64]

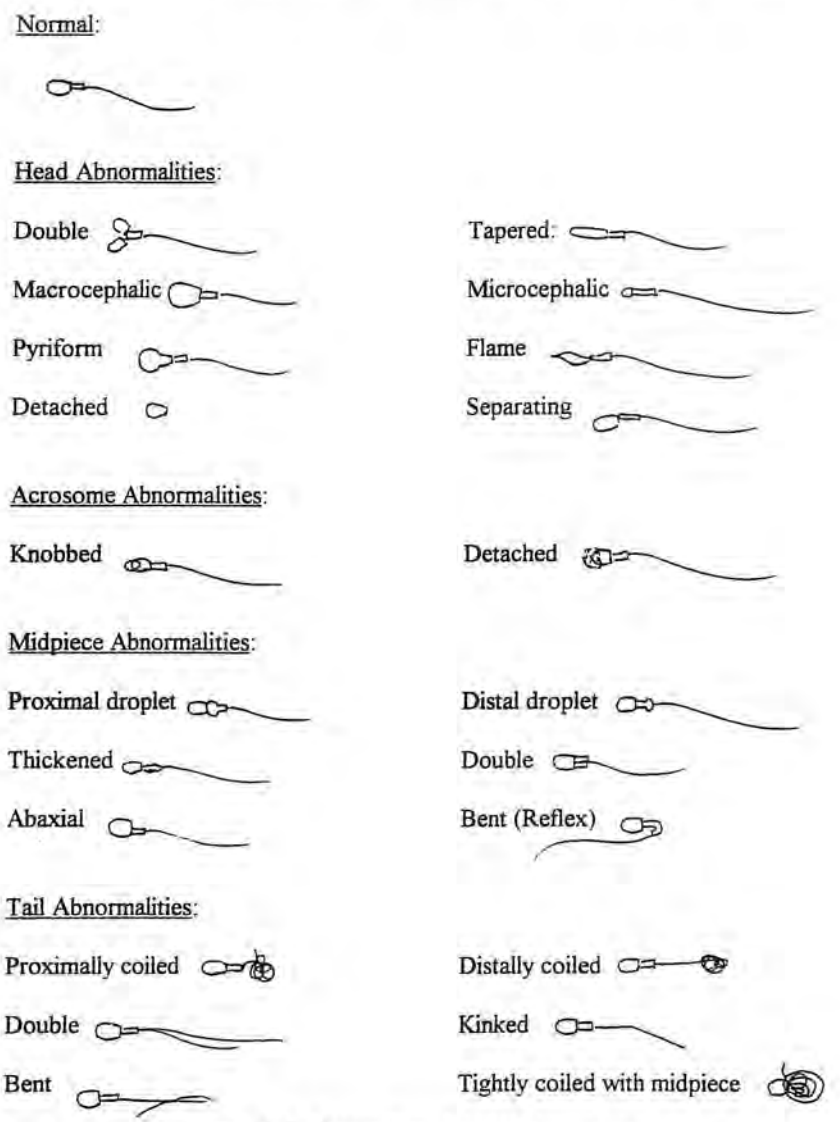

Figure 16–7. Abnormalities of canine spermatozoa.

Interpretation of Semen Evaluation Results

Azoospermic canine semen samples should be submitted for laboratory determination of alkaline phosphatase concentration, as seminal fluid alkaline phosphatase is an epididymal marker (see Chapter 23). Repeat collection of semen under optimal conditions with a teaser bitch present is recommended for dogs exhibiting azoospermia. Dogs with normal semen quality are classified as fertile, although tests of spermatozoal fertilizing ability are not routinely available. Dog spermatozoa do not penetrate zona-free hamster oocytes, but do penetrate zona-free canine oocytes.

Subfertile dogs, those with one or more parameters below the normal range, are more difficult to classify. Dogs with percentage progressively motile spermatozoa as low as 10 per cent and %MNS as low as 9 per cent, and total number of spermatozoa in the ejaculate as low as 36 million have been demonstrated fertile by breeding.[33,65] A pregnancy rate of 81.5 per cent can be achieved by introduction of at least 250 million normal spermatozoa into the bitch over her fertile period, from 3 days before to 4 days after ovulation.[65] Therefore, as an example, a dog with only 45 per cent MNS and total number of 200 million sperm in the ejaculate, or 90 million morphologically normal spermatozoa in the ejaculate, would be able to achieve

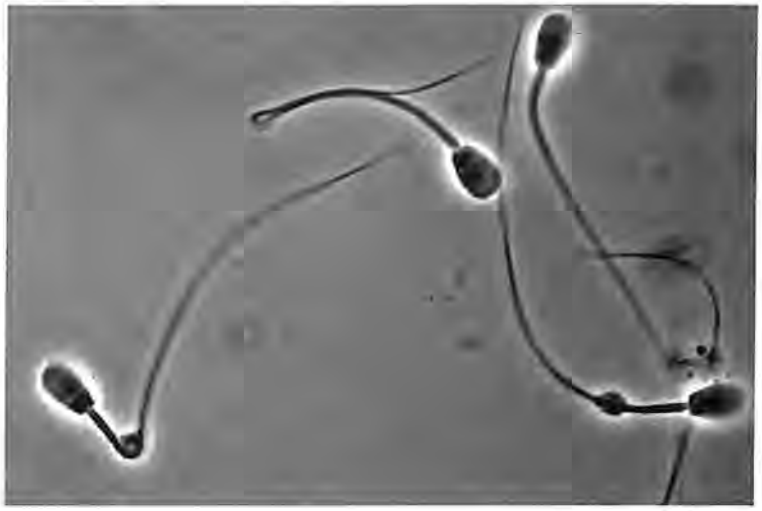

Figure 16–8. Morphologic abnormalities of canine spermatozoa, unstained, examined with phase-contrast microscopy, showing bent tails and distal droplets.

a pregnancy rate of 81.5 per cent with three breedings over a given bitch's fertile period. Individual inseminates may be improved by pooling two ejaculates collected approximately 1 hour apart. Although the second sample usually is of significantly poorer quality than the first, the pooled sample contains, on average, 70 per cent more spermatozoa than the first ejaculate alone.[14]

Semen Preservation

Canine semen can be chilled to refrigerator temperature (about 4°C), buffered, and used within several days, or frozen and maintained in liquid nitrogen indefinitely. With either technique, the spermatozoa are mixed with an extender to provide nutrients, prevent growth of bacteria, and buffer the semen. Chilled semen is useful when a chosen male is geographically distant from the bitch to be bred or has a work or show schedule requiring frequent travel, or when dogs cannot be shipped because of extreme environmental temperatures. Disease transmission may be decreased by lack of physical contact between the mating pair. Frozen semen has the same advantages and, in addition, allows breeding with semen from male dogs that have died and/or breeding of female dogs in other countries, which generally have fewer import restrictions for properly prepared frozen semen than for live animals. Conception rate with preserved semen is lower than that with artificial insemination with fresh semen, or with natural service.

Table 16–4. Grading Scale for Number of White Blood Cells (WBCs) per High-Power Field (hpf) with Light Microscopic Evaluation of a smear of Canine Seminal Plasma

Score	WBC/hpf
0	<1
1	1–3
2	4–6
3	7–10
4	>10

From Purswell BJ et al: Guidelines for using the canine breeding soundness evaluation form. Nashville Society for Theriogenology, 1992, with permission.

Extenders

Extenders are added to semen to buffer changes in pH that might otherwise occur with time; to provide an energy source for the spermatozoa; to prevent growth of bacteria; and to prevent injury to spermatozoa during cooling, freezing, and thawing.[66,67] Some extenders may be used for both chilled and frozen semen preparation; addition of a cryoprotectant such as glycerol is required only in extenders used for semen freezing (Table 16–6). After preparation, extender can be frozen in aliquots, with a shelf life of about 1 year.[67] In addition, proprietary canine extenders, of unreported composition, are commercially available (Synbiotics, San Diego, CA; Camelot Farms, College Station, TX; International Canine Semen Bank [ICSB], Sandy, OR). Equine semen extenders, such as the Kenney-type skim milk semen extender, are suitable for use in dogs.[77] Studies comparing extenders for preservation of canine semen do not include the commonly

Table 16–5. Selected Physical and Chemical Analyses of Prostatic Fluid from Normal Dogs

	Mean ± SD (range)	Sample Size
pH	6.2 ± 0.3 (5.5–7.1)	$n = 43$
Specific gravity	1.018 ± 0.005 (1.008–1.028)	$n = 40$
Cholesterol (mg/dl)	27.0 ± 17.0 (8.0–73.0)	$n = 29$
Zinc (μg/ml)	62.3 ± 35.3 (10.3–120.6)	$n = 20$
Copper (μg/ml)	7.1 ± 4.8 (1.3–19.5)	$n = 20$
Iron (μg/ml)	0.7 ± 0.5 (0–1.6)	$n = 20$
Calcium (μg/ml)	13.3 ± 20.2 (0.3–97.0)	$n = 20$
Magnesium (μg/ml)	16.4 ± 9.5 (3.4–40.0)	$n = 20$

Data from Branam et al.[61]

Table 16–6. Reported Composition of Extenders Used for Preservation of Canine Semen by Chilling or Freezing

References	Extenders for Chilled Semen: Components (dissolved in 1000 ml distilled water; exceptions marked⊕)*
Held[67] = Extender I†	14.5 g Na citrate, 12.5 g dextrose, 250 ml egg yolk, 1000 U/ml K penicillin, 1000 μg/ml streptomycin
Olar et al.[69]	800 g Na citrate, 200 g egg yolk
Province et al.[72] and Gill et al.[73]	0.7 g Na citrate monohydrate, 11.6 g Na citrate dihydrate, 1.7 g Na bicarbonate, 0.3 g KCl, 7.5 g glycine, 2.4 g glucose, 200 ml egg yolk
Gill et al.[73]	15.6 g Na citrate, 7.8 g glycine, 2.3 g glucose, 10 ml 25% *N*-caproic acid, 10 ml 45 mg% catalase, 200 ml egg yolk
Seager[74]	24 g TRIS, 13 g Na citrate, 10 g fructose, 38 ml glycerol, 200 ml egg yolk
Gill et al.[73]	Skim milk heated at 95°C for 10 minutes, then cooled⊕
Olar et al.[69]	Sterilized homogenized milk with 2% fat⊕
Linde-Forsberg[78]	800 g cream (12% fat), 200 g egg yolk, 1000 U ml benzyl penicillin, 1 mg/ml dihydrosterptomycin
References	**Extenders for Frozen Semen: Components (dissolved in 1000 ml distilled water)**
Held[67] = Extender II†	14.5 g Na citrate, 12.5 g dextrose, 250 ml egg yolk, 1000 U/ml K penicillin, 1000 μg/ml streptomycin, 80 ml glycerol
Foote[71]	24 g TRIS, 14 g Na citrate, 10 g fructose, 200 ml egg yolk, 90 ml glycerol
Foote[71]	9 g TRIS, 40 g TES, 1.6 g fructose, 150 ml egg yolk, 60 ml glycerol
Foote[71]	15 g PIPES, 0.9 g K hydroxide, 6 g Na citrate, 9.8 g glucose, 200 ml egg yolk, 90 ml glycerol
Province et al.[72]	30.3 g TRIS, 16.9 g Na citrate, 12.5 g glucose, 200 ml egg yolk, 1 million U K penicillin, 1 g streptomycin, 100 ml glycerol
Gill et al.[73]	24.4 g TRIS, 13.6 g Na citrate, 8.2 g glucose, 200 ml egg yolk, 500,000 U K penicillin, 1 g streptomycin, 30 ml glycerol
Seager[74]	30 g TRIS, 17 g Na citrate, 12.5 g fructose, 150 ml egg yolk, 1.6 g gentamicin, 80 ml glycerol
Paccamonti[77]	29 g TRIS, 13.2 g sodium citrate, 12.5 g fructose, 200 ml egg yolk, 80 ml glycerol
Foote[71]	110 g lactose, 200 ml egg yolk, 40 ml glycerol
Olar[75] and Paccamonti[77]	110 g lactose, 200 ml egg yolk, 1 million U K penicillin, 1 g streptomycin, 40 ml glycerol
Yubi et al.[76]	71 g lactose, 200 ml egg yolk, 500,000 U K penicillin, 1 g streptomycin, 40 ml glycerol
Smith[79]	0.3 m solution of PIPES (50 ml) titrated to pH 7.0 by adding sequential 0.1 ml aliquots of 0.3 m KOH (~4 ml); osmolality adjusted to 300 ± 10 m Osm with double distilled deionized water if necessary.⊕

* All volumes represent replacement of buffer volume for volume (v/v).
† See How to Extend and Chill or Freeze Canine Semen.
TRIS, tris(hydroxymethyl)aminomethane; TES, *N*-tris(hydroxymethyl)methyl-2-aminomethane-sulfonic acid; PIPES, piperazine-*N*-*N*-bis(2-ethane sulfonic acid).

used commercial preparations, and contain too many variables, such as dilution rate, cooling rate, and freezing and thawing techniques, to allow meaningful comparison between studies.

Buffers are used to maintain ionic balance and pH in the extender solution. Optimal pH of extended semen is 6.75 to 7.50; optimal osmolality is 300 to 325 mOsm.[79] Use of zwitterionic buffers, such as tris(hydroxymethyl) aminomethane (TRIS), and potassium buffers, such as potassium hydroxide, has been reported in canine semen extenders.[79] Sodium citrate binds heavy metals in seminal plasma.

Glucose, dextrose, and lactose are described as energy sources in canine semen extenders. Canine seminal fluid has a much lower concentration of fructose than does seminal fluid from other species, perhaps because male dogs do not have seminal vesicles. However, canine spermatozoa can use fructose as an energy source.[80]

Egg yolk and glycerol are the most commonly used compounds in canine semen extenders for protection of spermatozoa from cold shock and disruption during freezing and thawing. Egg yolk contains the lipids lecithin and cephalen, high-molecular-weight compounds that do not enter the sperm cell but instead are hygroscopic and change the extracellular space, creating room for fluid to freeze without damaging the spermatozoa.[79] Percentage of progressively motile spermatozoa after freezing and thawing is highest with extenders containing 20 per cent egg yolk by volume, compared to 5 or 10 per cent.[81] Glycerol has a low molecular weight, and so can enter the sperm cell and bind intracellular water, decreasing intracellular ice formation and helping to dehydrate the cell slowly.[79] Percentage progressive motility of newly extended or frozen-thawed spermatozoa decreases with increasing concentration of glycerol by volume.[66,82–84] High concentrations of glycerol are associated with a decreased percentage of frozen-thawed spermatozoa with intact acrosomes.[84] Glycerol concentration by volume that is associated with highest post-thaw percentage progressively motile spermatozoa is about 4 per cent.[75,83]

Dilution of canine spermatozoa with extender can be done either by diluting at a particular volume ratio, or by determining concentration of spermatozoa in the initial solution and diluting with extender to a preferred concentration of spermatozoa.[66] The former method provides a more consistent environment for the spermatozoa, since the amount of extender added is not arbitrary and is dependent on the fluid volume to which it is added, permitting exact calculation of percentage concentrations of buffer and cryoprotectants.[83] Very high dilution rates, such as 1 part semen to 16 or 32 parts extender, cause a significant percentage decrease in progressive spermatozoal motility.[27,83] Dilution rate associated with highest postextension percentage progressive spermatozoal motility in dogs in one study was 1 part semen to 4 parts extender.[83]

Extended Chilled Semen

Prior to extending canine semen, the sample should be evaluated and cultured for aerobes, anaerobes, and mycoplasma, because poor-quality semen does not warrant extension and shipment. Only the second (sperm-rich) fraction need be extended, chilled, and shipped; either the semen can be fractionated during collection or centrifuged after collection with extension of the resultant pellet. Beginning January 1, 2000, the AKC required DNA identification of all male dogs from which semen is collected for extension, chilling, and shipment. The DNA sample is retrieved from a cheek swab collected by the owner (Fig. 16–9).[85]

Extenders for chilled extended canine semen often contain egg yolk, to help protect the spermatozoa from cold shock. Percentage progressively motile spermatozoa in extended samples chilled to 4° to 5°C declines gradually with time.[73,86–88] The decline in motility is significantly less severe in extenders containing 20 per cent egg yolk than with skim milk–based extenders.[72,88–90] If skim milk is to be used, it must be heated to 92° to 95°C for 10 minutes to denature enzymes in the milk.[80] Percentage progressively motile spermatozoa in warmed samples of extended semen declines to a level of about 50 per cent by 4.9 ± 1 days after extension and chilling.[86] Although no change in percentage of spermatozoa with damaged cellular membranes has been demonstrated with extension and chilling, spermatozoal function, assessed by transmigration rate, declines with time.[91,92] To optimize conception rate, extended chilled canine semen is best used within 48 hours of collection.[86] Canine semen usually is extended at a rate of 1 part semen to 2 to 3 parts extender.[67]

Fertile male dogs may have semen that does not tolerate extension and chilling. Well before semen is collected and shipped for breeding,

When a sample is collected, swabs are taken from their protective wrappers and grasped only by the plastic handle.

The dog's head is held firmly and the swab is placed against the inside surface of the dog's cheek.

The swab is firmly rotated for 10 to 20 seconds and immediately reinserted in its wrapper, which is then sealed and placed in an envelope for processing.

Figure 16–9. Collection of cheek swab samples for DNA identification of male dogs with semen frozen. (From Edwards J, Mandeville J, Slay B: DNA and the AKC. AKC Gazette 114:55–57, 1997, with permission.)

semen should be collected, extended, and placed in the refrigerator for 24 hours, and the motility reassessed after rewarming. One drop of semen can be warmed with the heat generated by the light microscope; if no motility is observed after several minutes, likelihood of impregnating a bitch is reduced.

Chilled extended canine semen can be shipped either in a commercial canine or equine system or in any container that will maintain refrigerator temperature (4°C) (see How to Extend and Chill or Freeze Canine Semen below).[77]

Frozen Semen

Freezing of canine semen and insemination of bitches with frozen-thawed semen is not as commonly used as in the bovine and equine species. Male dogs used must have good to excellent semen quality, and bitches bred with the semen should be of breeding age with no history of infertility or reproductive tract disease. Many variables affect success with frozen semen in dogs (Table 16–7).

The AKC has accepted registrations for litters created with frozen semen since 1981. Beginning January 1, 1999, the AKC required DNA identification of all male dogs from which semen is frozen. The DNA sample is retrieved from a cheek swab collected by the owner.[85] Facilities that freeze canine semen must be approved by the AKC; this approval is not an indication of quality but instead verifies accurate record-keeping regarding semen collection and storage.[77]

For freezing of canine semen, semen is collected by manual ejaculation and evaluated, as described previously. Percentage of progressively motile spermatozoa declines significantly with freezing and thawing, so only good to excellent samples, with total number of spermatozoa adequate for the dog, based on body weight or total scrotal width (Table 16–3) and percentage progressively spermatozoal motility greater than 70 per cent, should be considered for cryopreservation. Semen may be collected on several occasions to allow freezing and storage of a number of insemination doses.

After collection, canine semen is centrifuged and the sperm-rich fraction extended. The extended spermatozoa are cooled prior to freezing (equilibration) to reduce damage due to cold shock.[66,93] Optimal equilibration time has not been defined. Cooling to refrigerator temperature (4° to 5°C) for 1, 2, or 3 hours does not result in different semen quality after thawing.[75]

Semen can be frozen in pellets, glass ampoules or straws. Pellets are formed when cooled extended semen is deposited in 50- to 100-μl aliquots into depressions on solid dry ice, and then stored in perforated nylon vials in liquid nitrogen.[66] Polyvinylchloride (PVC) straws, 0.25 or 0.50 ml in volume, can be filled with cooled extended semen and an air bubble,

Table 16–7. Variables Affecting Success of Artificial Insemination with Frozen Semen in Dogs

Male:	Fertility of male dog; ability of semen from individual males to be frozen
Semen collection:	Contamination with presperm, fraction I; prostatic fluid, fraction III; urine or blood; lubricants or detergents. Composition and surface of collection container; contamination with preputial debris; temperature of collecting vessel
Semen quality:	Sperm number and concentration; sperm motility and progressive motility; percentage of live sperm; percentage of morphologically normal sperm; sperm-agglutination problems
Semen dilution:	Avoidance of cold shock; composition of extender; time between collection and dilution; temperature of initial dilution; amount of semen dilution (volume); extent of sperm dilution (sperm concentration); number of dilution steps and solutions; force of any centrifugation; amount of light exposure
Equilibration:	Rate of cooling to 5°C; duration of storage at 5°C before freezing
Freezing:	Freezing form (pellets, ampules, or straws); freezing unit size; freezing method (dry ice; liquid nitrogen vapors); freezing rate(s) from liquid to solid (–40°C); freezing rate(s) to final temperature
Thawing:	Thawing medium (original vs. new diluent); thawing rate (i.e., at 23° vs. 37° vs. 45° vs. 75°C); post-thaw storage temperature and time; post-thaw temperature damage; post-thaw motility and vitiality; post-thaw morphology and acrosome integrity; post-thaw thermolability and longevity
Insemination:	Time relative to ovulation; site of insemination (vaginal vs. uterine); volume of insemination; number of live sperm per insemination; genital manipulations

From Concannon PW, Battista M: Canine semen freezing and artificial insemination. *In* Kirk RW (ed): Current Veterinary Therapy X. Philadelphia, WB Saunders, 1989, p 1249, with permission.

plugged with cotton on one end and powdered PVC pyrrolidone on the other, and then frozen while suspended in liquid nitrogen vapor before plunging the straw into the liquid nitrogen.[66,67,79] Quality of canine semen samples after thawing may vary between those frozen as pellets and those frozen in straws.[70,81] Variables such as extender used also impact post-thaw canine semen quality, so method of freezing should be evaluated for each technique used.

Optimal canine semen freezing rate is dependent on extender and cryoprotectant used and volume of the sample.[66] If the sample freezes too quickly, intracellular ice forms, rupturing the spermatozoa. If the sample freezes too slowly, an osmotic gradient forms with subsequent dehydration and shrinking of the cells.[66] Reported values for freezing rate vary from 1.89° to 5°C per minute until the sample reaches –15°C, then 10° to 20°C per minute until the sample reaches –100°C, after which the sample is plunged into liquid nitrogen.[75,79,94,95]

The thawing rate is as important to survival of canine spermatozoa as is the freezing rate.[96] Comparison of short-term immersion of either straws or pellets in thaw medium in a 75°C water bath for 6 seconds with immersion in a cooler water bath (30 seconds [0.25-ml straws] or 60 seconds [0.50-ml straws or pellets in thaw medium] at 37°C) yields equivocal results for highest post-thaw percentage progressive spermatozoal motility.[75,76] The latter is the more commonly used thawing technique for frozen canine sperm.[97]

Prior to long-term storage of the frozen semen, a sample of pellets or a straw containing frozen semen that has been immersed in liquid nitrogen for at least 5 minutes should be thawed and evaluated to allow recording of post-thaw semen quality.[67] Progressive motility is the parameter most commonly assessed for evaluation of quality of frozen-thawed canine spermatozoa. Percentage of progressively motile spermatozoa is decreased post-thaw in the dog, with reported values of about 50 to 60 per cent.[67,98] Investigators differ as to whether percentage progressive spermatozoal motility may or may not be associated with damage to the acrosome or plasma membrane of the canine spermatozoa.[99,100] Post-thaw percentage of progressive motility of canine spermatozoa is a poor indicator of semen quality, as motile spermatozoa with damaged acrosomes may be incapable of fertilizing oocytes.[101] In one study, despite normal post-thaw motility and min-

imal membrane damage, few spermatozoa bound to canine oocytes in an oocyte penetration test of spermatozoal function after freezing and thawing.[102] In a survey of seven pooled ejaculates frozen with four different extenders and techniques, the sample with the lowest post-thaw motility yielded the highest conception rate in breeding trials.[103] Addition of a methylxanthine, pentoxifylline, to the thaw medium increased percentage progressive spermatozoal motility of frozen-thawed canine spermatozoa,[104] but effect on fertilizing ability of treated spermatozoa is unknown.

The spermatozoa of individual fertile male dogs respond differently to semen freezing.[81,105] Even male dogs without morphologic defects associated with poor post-thaw survival of spermatozoa, such as proximal cytoplasmic droplets and bent and coiled tails,[39,98] may have spermatozoa that do not survive freezing and thawing, perhaps due to individual variance in their seminal fluid chemistry.

Frozen-thawed spermatozoa have a much shortened life span than that of newly ejaculated spermatozoa.[75] An insemination dose of 100 to 150 million live spermatozoa has been associated with a conception rate of 75 per cent or higher, depending on health of the bitch, timing of insemination, and placement of semen within the bitch's reproductive tract.[79,97,98]

Many methods for canine semen freezing have been reported, including techniques using the extenders and materials described,[39,67,69,70,73,99,100,102,105–108] one in which extended semen is dropped directly into liquid nitrogen,[109] and a technique using methanol as the freezing medium.[110,111] Several commonly used techniques belong to private companies with proprietary methods. The authors are unaware of any large-scale study comparing these various techniques for semen freezing.

How to Extend and Chill or Freeze Canine Semen[68]

Chilled Semen

- Evaluate the male for breeding soundness with a complete physical examination and semen evaluation, including semen culture and *Brucella canis* serology, and tests for genetic defects specific to the breed.
- Collect semen for extension and shipment by manual ejaculation. Isolate second (sperm-rich) fraction by changing tubes during collection or by centrifuging the sample at 300 to 500*g* for 10 minutes.*
- Dilute the sperm-rich fraction with Extender I (Table 16–6) at room temperature, at a ratio of 1 part semen to 2 parts extender.
- Secure the extended sample in a centrifuge tube with a tight cap and no vent hole. Pack in a small styrofoam box or wrap tightly in several layers of newspaper. Place in a larger styrofoam box with two frozen ice packs, both wrapped in newspaper. Ship to reach the bitch as soon as possible, ideally within 24 hours (see Chapter 4).

Frozen Semen

- Evaluate the male for breeding soundness with a complete physical examination and semen evaluation, including semen culture and *Brucella canis* serology, and tests for genetic defects specific to the breed.
- Collect semen for extension and shipment by manual ejaculation. Isolate second (sperm-rich) fraction by changing tubes during collection or by centrifuging the sample at 300 to 500*g* for 10 minutes.*
- Aspirate the pellet and add Extender I (Table 16–6), at room temperature, at a 1:1 ratio. Place at refrigerator temperature for 1 hour. Label 0.5 ml PVC straws with facility identification number, the animal's AKC number and breed, and the date.
- Add 2 parts Extender II (Table 16–6), at refrigerator temperature, in four 0.5-ml aliquots over a 45-minute period for a final glycerol concentration of 4 per cent. Fill the 0.5-ml straws, introduce an air bubble to prevent expulsion of the seal during freezing, seal the straw, and place in refrigerator temperature for at least 1.5 hours. Fill the bottom of a styrofoam box with 10 cm of liquid nitrogen while you are waiting, and leave it in the cold room or refrigerator.
- Place the straws on a rack 5 cm above the surface of the liquid nitrogen for 6 minutes, then drop the straws into the liquid nitrogen. After at least 5 minutes, transfer to a permanent tank and thaw one straw (60 seconds in a 37°C water bath) to assess post-thaw semen quality.
- Ship semen samples in a canister containing liquid nitrogen or liquid nitrogen vapor in a

* Centrifuge *g* force = 11.18×10^{-6} times RN^2, with R = radius from centrifuge spindle to media in tube (cm) and N = speed of spindle in revolutions per minute.

"dry shipper." The latter will hold its charge for 1 to 3 weeks; either recharge the canister on arrival at its destination or weigh it daily to assess loss of liquid nitrogen and possible thawing of the straws it contains. Information that should be sent with the semen includes thawing instructions, the number of spermatozoa in each straw, and percentage post-thaw progressive motility.

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Chapter 17

Prevention of Fertility in the Male Dog

A single male dog may sire many more puppies than a single female is capable of producing. Prevention of fertility in male dogs is requested by owners not only to avoid the siring of unintended litters but also to decrease incidence of undesirable male behaviors, such as mounting, roaming, aggression to other male dogs, and undesirable urination behavior.[1] The ideal contraceptive is effective, safe, readily available, and acceptable to owners both in its use and possible side effects.[2]

Surgical Sterilization

Orchiectomy

Castration and neuter are defined as removal of one or both gonads. The correct terms for removal of one or both testes are orchidectomy and orchiectomy; the two terms are synonymous.[3]

Bilateral orchiectomy is the most common form of sterilization in male dogs in the United States, and is the treatment of choice for sterilization of the male dog of this species. Open and closed techniques have been described that refer to incision of the tunics investing the spermatic cord, and the latter is more common. General anesthesia is induced and the inguinal and prescrotal areas are clipped and prepared for sterile surgery. A prescrotal incision is made on the midline and a testis manipulated to the incision. The subcutaneous tissue, spermatic fascia, and scrotal ligaments are severed or broken manually, and the tunics are stripped free of fat so that the testis may be exteriorized. The spermatic cord is exposed, still encased within the testicular tunics, clamped, ligated, and severed. The process is repeated with the other testis.[4] Dogs undergoing closed orchiectomy with a prescrotal approach have been demonstrated to have less hemorrhage from the pedicle, less scrotal swelling postoperatively, and less seromas at the incision site than dogs undergoing open orchiectomy, in which the testicular tunics were incised to expose the spermatic cord.[4] In a survey describing 24 male dogs in which closed orchiectomy with a prescrotal approach was compared to closed orchiectomy with a postscrotal approach, open orchiectomy, and scrotal ablation, dogs undergoing closed orchiectomy with a prescrotal approach had the shortest surgery time, best postoperative cosmetic appearance, fewest postoperative complications, and fastest healing time.[5]

Bilateral orchiectomy is a completely effective contraceptive method. Other benefits of bilateral orchiectomy include prevention of testicular and epididymal disorders such as neoplasia, torsion of the spermatic cord, and orchitis/epididymitis; and suppression or eradication of clinical signs associated with androgen-dependent diseases such as benign prostatic hypertrophy, chronic prostatitis, perianal adenomas, perineal hernias, and castration-responsive alopecia.[1,3,6–9]

Objectionable male behaviors decline in most dogs after bilateral orchiectomy, with greatest effect seen on sexually dimorphic reproductive behaviors, such as mounting and roaming.[10] Most male dogs undergoing bilateral orchiectomy before puberty do not develop some male behaviors at all.[1,11,12] Previous sexual experience of male dogs is not a good predictor of likelihood of reduction in sexual behaviors after orchiectomy.[10] In a survey describing behavior of 42 male dogs after orchiectomy, 70 per cent showed a decrease in mounting behavior, 60 per cent showed a decline in intermale aggression, and greater than 90 per

cent showed a decrease in roaming behavior after bilateral orchiectomy.[13] Mounting and thrusting behavior may take months to years to diminish, suggesting nonhormonal, or learned, components to the behavior.[12,14] Orchiectomized dogs with persistent mounting behavior rarely achieve intromission of the penis and if intromission does occur, the copulatory lock is very short or does not occur at all.[12]

Postoperative complications after bilateral orchiectomy have been reported in 4.5 per cent of male dogs with bilaterally descended testes (n = 240) and in 6.5 per cent of dogs with one or both testes retained (n = 46).[15] Reported short-term postoperative complications of orchiectomy in the dog include hemorrhage from the pedicle of the spermatic cord, scrotal bruising or swelling, infection at the incision site, and hemiprostatic urethral avulsion after exploration for a retained testicle with an ovariohysterectomy hook.[16,17] An arteriovenous fistula involving the testicular artery and vein, artery of the ductus deferens, and cremasteric artery developed in the inguinal region of a male dog 4.5 years after bilateral orchiectomy.[18]

Complications reported to occur after bilateral orchiectomy include development of scirrhous (spermatic) cords, obesity,[1,19–21] decrease in secondary sex characteristics,[22] urinary incontinence due to urethral sphincter mechanism incompetence,[3,22] delayed closure of physes of long bones,[22,24] possible predisposition to pancreatitis[25] and prostatic neoplasia,[26] and endocrine alopecia.[3,27] Dogs castrated with active orchitis, epididymitis, or prostatitis are at risk of developing scirrhous (infected) spermatic cords. These patients should be treated with antibiotics prior to surgery. Bilateral orchiectomy is a risk factor for obesity, along with owner, diet, breed, age, and activity level factors.[19,20] Orchiectomized male dogs were more likely to be overweight than intact males in a retrospective survey.[21] However, in two prospective studies, body weight, food intake, and depth of back fat were not increased in male dogs orchiectomized at 7 months of age compared to age-matched intact male dogs.[28,29] Development of urinary incontinence due to urethral sphincter mechanism incompetence has been demonstrated more frequently in orchiectomized than intact male dogs and in those with an intrapelvic bladder neck, perhaps related to decreased prostatic weight after orchiectomy.[23]

Early spay-neuter, or prepuberal gonadectomy, refers to surgical sterilization of sexually immature animals. Male dogs undergoing bilateral orchiectomy at 7 to 14 weeks of age have been reported to have delayed closure of physes of long bones and decreased development of secondary sex characteristics compared to male dogs gonadectomized at 7 months of age or left intact.[29] Prepuberally gonadectomized male dogs have not been reported to be more obese than those gonadectomized at 7 months of age or left intact.[28,29] Surgery time is decreased in animals undergoing gonadectomy prepuberally, and no significant surgical, anesthetic, or postoperative complications have been described following this surgery in pediatric patients.[30,31] For a complete discussion of early spay-neuter in dogs, see Chapter 9.

Vasectomy

Vasectomy, bilateral removal or occlusion of a segment of the ductus deferens, prevents fertility both by obstructing ejaculation of spermatozoa and by causing secondary testicular atrophy.[32] Androgen-dependent diseases and undesirable male sexual behaviors may still occur, since steroidogenesis is not affected.

After induction of general anesthesia, the inguinal and prescrotal areas are clipped and prepared for sterile surgery. The spermatic cord is identified either by direct observation and exteriorization (prescrotal) or by laparoscopy (abdominal). The tunic is incised over the spermatic cord and the ductus deferens exposed. A segment is either occluded with electrocautery, or surgically removed and the severed ends ligated.[33–35] Azoospermia has been reported to develop from 2 to 21 days after bilateral vasectomy.[34–36]

Epididymal/Testicular Sclerosing Agents

Sclerosing agents prevent fertility by inducing fibrous occlusion when injected into the epididymes (Fig. 17–1). Agents injected into the testicular parenchyma cause testicular atrophy and decrease spermatogenesis. It may be better to target the epididymis than the testicle, as breeching of the blood-testis barrier in the latter may be more likely to cause a systemic immune response and local inflammation with release of testicular autoantigens. General anesthesia is indicated both with epididymal and testicular injection.

Agents that have been demonstrated to induce infertility after injection into both cauda epididymes include zinc arginine, zinc tan-

Figure 17–1. Injecting a sclerosing agent into the cauda epididymis of a beagle dog. (From Olson PN, Nett TM, Bowen RA, et al: A need for sterilization, contraceptives, and abortifacients: Abandoned and unwanted pets. Part I. Current methods of sterilizing pets. Compend Contin Educ Pract Vet 8:87–92, 1986, with permission.)

nate, methylcyanoacrylate, chlorhexidine gluconate, chlorhexidine in ethylcellulose, ethylcellulose in dimethyl sulfoxide (DMSO) and formalin, and acrylic hydrogel in DMSO.[37–40] Zinc arginine injection in the cauda epididymes of 10 dogs was described to induce azoospermia by 90 days after injection.[37] Histology of the epididymes and testes 1 year after injection showed atrophy of the epithelium of the corpus and caput epididymis with no granuloma formation, variable atrophy of the seminiferous tubules, and atrophy of the rete testes.[37,38] Increased intratesticular zinc concentrations were hypothesized to inhibit replication of germ cells.[38] Zinc arginine is nonmutagenic, nonteratogenic, and noncarcinogenic.[37] Injection of 0.5 ml of 4.5% aqueous chlorhexidine gluconate solution into the cauda epididymes of seven dogs caused azoospermia in all dogs by 35 days after treatment.[40] The azoospermia persisted for 4 months. Transient testicular swelling occurred, but the dogs were able to work and hunt within 1 day of treatment.[40]

Intratesticular injection of pure lactic acid prevented development of both testes in immature dogs; the testes were not palpable in the scrotum by 7 weeks after injection. Injection of pure lactic acid into the testes of adult dogs significantly decreased plasma testosterone concentrations and libido immediately after injection. Degenerative changes of seminiferous tubules and germ cell atrophy were present in testicular biopsy samples 4 months after injection.[41] Treated dogs showed no discomfort after intratesticular injection with lactic acid.[41] Intratesticular injection of zinc tannate decreased testosterone production and caused azoospermia, with the effect on spermatogenesis positively correlated with the dose administered.[42]

Medical Suppression of Spermatogenesis

Medical therapies for contraception that cause either a significant decline in semen quality or complete azoospermia have been demonstrated in male dogs. Medical therapy for induction of infertility in dogs is less invasive than are surgical techniques or administration of epididymal/testicular sclerosing agents, and generally are reversible. The two classes of drugs best described for contraception in male dogs are progestins and gonadotropin-releasing hormone (GnRH) agonists.

Progestins described as contraceptive agents for male dogs are megestrol acetate and medroxyprogesterone acetate. Megestrol acetate is not an effective contraceptive agent; even at the highest reported dose (4 mg/kg once daily per os [PO] for 7 days), semen quality was not appreciably diminished, although a small percentage of spermatozoa with secondary morphologic defects was noted.[43] Megestrol acetate has been demonstrated to suppress the pituitary-adrenal axis.[44] Medroxyprogesterone acetate (MPA) is an effective contraceptive agent at high doses (20 mg/kg subcutaneously [SC]).[45] Treatment with MPA induces a rapid decrease in total number of spermatozoa ejaculated, percentage morphologically normal spermatozoa, and percentage progressively motile spermatozoa.[43] Serum luteinizing hormone (LH) concentrations are not suppressed with MPA treatment, the effect is rapid, and the morphologic defects induced in spermatozoa are those that occur during maturation, suggesting an effect of MPA directly at the level of the epididymis.[43] Concurrent treatment with MPA and testosterone esters causes a more rapid decrease in semen quality than

does treatment with MPA alone, but does not cause azoospermia.[43] The authors do not recommend use of progestins for contraception in male dogs because of the side effects (diabetes mellitus, mammary nodules) these drugs may cause.

Androgens suppress release of LH from the pituitary with secondary declines in serum and intratesticular testosterone concentrations and an eventual decrease in spermatogenesis. Daily administration of 50 mg of methyltestosterone to male dogs for 90 days decreased spermatogenesis and mean testicular length.[46] Treatment with androgens for prevention of fertility is not recommended, since predisposition to androgen-dependent diseases will be increased.[7]

GnRH agonists, including nafarelin acetate (0.5 or 2.0 μg/kg SC once daily for 44 days) and leuprolide acetate (0.1 or 1.0 mg/kg SC), have been demonstrated to cause transient increases in serum LH and testosterone concentrations followed by declines to nondetectable concentrations by 7 to 14 days.[47–50] Reported changes in semen quality varied from low total number of spermatozoa ejaculated to azoospermia 3 to 8 weeks after the drug was administered. Testicular histopathology revealed cessation of spermatogenesis in most seminiferous tubules.[47–50] Return to normal semen quality and normal testicular histology was evident by 140 to 252 days after treatment.[49,50] Other drugs reported to inhibit spermatogenesis in male canids include oral bisdiamine[51] and α-chlorohydrin, an alkylating agent.[52]

Immunosterilization

Ten male dogs immunized with an androgen-binding protein isolated from the canine cauda epididymis were demonstrated to have disruption of the epithelium of the cauda epididymes and marked testicular damage.[53] Semen quality was not reported. Immunization of 1-year-old male beagles with 30 to 50 μg of a GnRH analogue conjugated to tuberculin protein had a variable effect, with extent of degeneration of spermatogonia and lack of spermatogenesis, and subsequent decrease in total number of spermatozoa ejaculated correlated with titer.[54] Immunization with human chorionic gonadotropin (hCG) elicited formation of antibodies, but did not cause infertility, since the antibodies were not cross-reactive with canine gonadotropins.[55] Immunization of dogs with a single 10-mg dose of ovine gonadotropin induced antibody formation and ejaculatory failure, but results were inconsistent; some dogs maintained spermatogenesis at normal levels despite presence of high antibody titers.[55]

Irradiation

Male dogs irradiated with a total of 300 rad over 60 days had a decrease in number of spermatogonia.[55] Permanent cessation of spermatogenesis required exposure of at least 3 rad per day, localized at the testis, for 475 consecutive days.[56] Irradiation is not considered a feasible or humane contraceptive method in the dog because of the duration of treatment necessary to be effective.[56,57]

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Chapter 18

Disorders of the Canine Testes and Epididymes

Congenital Abnormalities

Development of testes in the embryonic male dog is dependent on presence of a gene on the Y chromosome that signals production of testis-determining factor. Subsequent development of male internal and external genitalia is dependent on müllerian-inhibiting substance (MIS), a glycoprotein hormone secreted by Sertoli cells in the embryonic testis, and testosterone (T) secreted by embryonic testicular interstitial (Leydig) cells. Müllerian-inhibiting substance induces regression of the müllerian duct system, destined in animals without testes to persist as the female uterine tubes, uterus, and cranial vagina. Testosterone stimulates differentiation of the epididymes and vasa deferentia from the wolffian ducts. Testosterone also is converted to dihydrotestosterone (DHT), which effects differentiation of the prostate, urethra, penis, and scrotum. Abnormalities of sexual differentiation in the male dog include abnormalities of chromosomal sex, of gonadal sex, and of phenotypic sex.[1]

Abnormalities of chromosomal sex refer to abnormal complement of sex chromosomes; those that have been reported in the phenotypic male dog include the 79,XXY, 78,XX/78,XY, and 78,XX/79,XXY karyotypes (see Chapter 10) (Fig. 18–1).[1–3] The 79,XXY males have scrotal testes that are small and soft, and are composed histologically of interstitial cells and Sertoli cells lining the seminiferous tubules, but no spermatogenesis.[2] The 78,XX/78,XY and 78,XX/79,XXY chimeras may have testes or ovotestes; the gonads are retained in the abdomen.[3]

Abnormalities of gonadal sex are defined as those with presence of gonads opposite that expected with the chromosomal complement. The only abnormality of gonadal sex reported in dogs is XX sex reversal, in which phenotypic male dogs with testes and male external genitalia have a 78,XX karyotype. XX sex reversal is inherited as an autosomal recessive trait in American cocker spaniels.[1] It also has been reported in beagles, English cocker spaniels, Weimeraners, German shorthaired pointers, Chinese pugs and Kerry blue terriers.[1,4–6] The hypothesized cause is translocation of a portion of the Y chromosome to an autosome.[1] Male dogs with XX sex reversal have bilateral testes and epididymes, which often are retained in the abdomen, and histologically show no spermatogenesis.[1,6] Ambiguous external genitalia including penile hypoplasia and hypospadias may be present.[1,6]

Abnormalities of phenotypic sex occur in animals in which the external genitalia or the internal tubular reproductive organs are of a different sex than the gonads. These are pseudohermaphrodites. Female pseudohermaphrodites appear male externally in the presence of ovaries; these are uncommon, except in cases where the individual has been masculinized by exogenous androgen administration to or androgen secretion by the mother during her pregnancy. Male pseudohermaphrodites (male gonads with female phenotype), which are more common, and are due to failure of secretion or action of MIS or T.[2,7,8] Male pseudohermaphroditism may be due to inadequate binding of T or DHT in target tissues, with subsequent lack of development of normal male external genitalia.[9]

Dogs that appear phenotypically male and that have a normal 78,XY karyotype and normal testes may have a uterus masculinus, which is a type of male pseudohermaphroditism. In the miniature schnauzer, an inherited

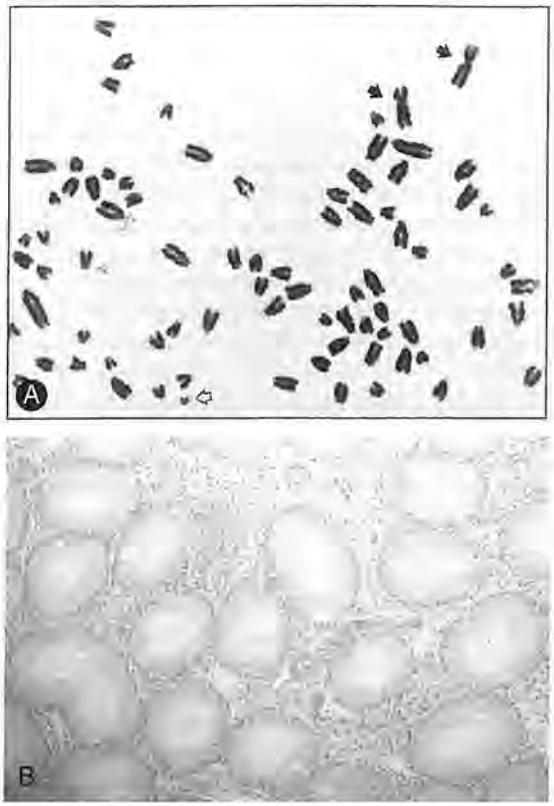

Figure 18–1. 79,XXY chromosome complement (**A**) and testicular histology (**B**) of a 21-month-old male Norwich terrier that was azoospermic. Sex chromosomes are indicated (open arrow, Y chromosome; dark arrows, X chromosomes). Seminiferous tubules contain only Sertoli cells, not spermatogenic elements. (From Nie GJ, Johnston SD, Hayden DW, et al: Theriogenology question of the month: Male dog with 79,XXY chromosome complement. J Am Vet Med Assoc 212:1545–1547, 1998, with permission.)

form of male pseudohermaphroditism associated with unilateral or bilateral cryptorchid testes, attached to a complete uterus, and normal male external genitalia, has been reported.[10–12] These dogs commonly present with Sertoli cell tumor of the cryptorchid testis and cystic endometrial hyperplasia/pyometra of the uterus masculinus.

Monorchidism, aplasia of one testis, has not been reported in the dog. If only one scrotal testis is evident, careful ultrasonographic or surgical exploration of the contralateral inguinal canal and abdomen, and tracing of the vas deferens retrograde from its insertion on the dorsal aspect of the prostate may be necessary to find the retained testis. Presence of retained testes in the abdomen may be misdiagnosed as testicular aplasia, and can be diagnosed by measurement of serum testosterone following challenge testing. Protocols for challenge testing include (1) gonadotropin-releasing hormone (GnRH), 2 μg/kg intramuscularly (IM), with blood sample drawn 1 hour later for measurement of serum testosterone; or (2) human chorionic gonadotropin (hCG), 20 IU/kg IM, with blood sample drawn 4 hours later for measurement of serum testosterone. Serum testosterone concentrations exceeding 1 ng/ml following challenge testing support the diagnosis of abdominal testes; concentrations often exceed 5 ng/ml.

Both unilateral and bilateral aplasia of a portion of the epididymis have been reported.[13,14] A Siberian husky with unilateral epididymal aplasia had moderate degeneration of the ipsilateral testis, but was a fertile male.[14]

Cryptorchidism

Cryptorchidism is a developmental defect in male dogs in which descent of one or both testes into the scrotum does not occur by 6 months of age. Cryptorchidism ("hidden testis") is the preferred term; monorchidism ("single testis") implies unilateral testicular aplasia, a condition that has not been reported in male dogs.

In normal male dogs, the testes usually have descended into the scrotum by 10 days of age.[15,16] In one study of 105 male puppies, 97 per cent had bilateral testicular descent by 42 days of life.[17] Neonatal testes are small, soft, and mobile, and may be difficult to palpate.[18] In puppies, the testes can move freely between the scrotum and inguinal canal.[19] Contraction of the cremaster muscle can pull the testis from the scrotum into the inguinal canal if the pup is cold, frightened, or stressed.[18]

The inguinal rings of most dogs are closed by 6 months of age, precluding movement of testes from the abdomen to the inguinal canal if that has not already occurred. In a review of 1494 male dogs with undescended testes, 30 of the 122 that were under 6 months of age at initial diagnosis showed spontaneous descent of at least one testis by 6 months of age, with 19 dogs previously diagnosed as unilateral cryptorchids and 5 dogs previously diagnosed as bilaterally cryptorchid achieving normal scrotal placement of both testes.[20] In that study, no dog showed testicular descent after 6 months of age.[20] Lack of testicular descent after 6 months of age is most likely due to functional closure of the inguinal rings and increasing testicular size, and may, therefore, occur earlier in small-breed dogs and later in

large- and giant-breed dogs that mature later in life. In general, cryptorchidism should not be definitively diagnosed in dogs less than 6 months of age.[19]

Reported incidences of canine cryptorchidism are 1.2 per cent (n = 2365,[21] n = 1.8 million[22]), 2.5 per cent (n = 1679),[23] and 5.0 per cent (n = 198).[24] In boxers, the reported incidence in one study was 10.1 per cent (n = 3431).[25] Age of the male dogs at time of diagnosis was not reported in the majority of these studies, leaving the true incidence of cryptorchidism in question.

Among cryptorchid dogs, unilateral cryptorchidism is more common than bilateral cryptorchidism, with overall reported incidences of 79.8 and 20.2 per cent, respectively.[21,24–27] In unilaterally cryptorchid dogs, the right testis is more often retained than the left, with respective incidences of 65.7 and 34.3 per cent.[21,27,28] Testes may be retained in the prescrotal subcutaneous tissue, the inguinal area, or the abdomen. Inguinal retention is most common[24]; in one survey of 29 unilaterally cryptorchid dogs, 21 (72 per cent) had inguinal testicular retention.[21]

Normal testicular descent occurs in three phases.[18] In phase I, intra-abdominal migration, the embryonic testes, which develop caudal to the kidney, are pulled caudally by outgrowth of the distal portion of the gubernaculum, a gelatinous tissue of mesenchymal origin connecting the caudal pole of the testis to the external opening of the inguinal canal. Phase II is intrainguinal migration of the testis. Phase III, extrainguinal migration of the testis into the scrotum, is due to regression of the gubernaculum. Gubernacular outgrowth and regression are regulated by testosterone and nonandrogenic factors released from the fetal testes.[29,30] In a series of elegant experiments in which fetal and neonatal puppies underwent orchidectomy with or without concurrent supplementation with testosterone, gubernacular outgrowth and regression and subsequent descent of the remaining epididymis were demonstrated to require physical presence of the testis.[29] Gubernacular outgrowth was minimally affected by testosterone supplementation, while a more profound effect of testosterone on gubernacular regression was demonstrated, suggesting that testosterone plays a larger role in Phase III of testicular descent.[30]

Canine cryptorchidism is heritable. It is a sex-limited autosomal recessive trait. Mode of inheritance, including number and penetrance of genes involved, is undefined. Because the gene(s) responsible for testicular descent are autosomal, the cryptorchidism trait can be carried by both males and females.[15] Concentration of the defect within lines of inbred cocker spaniels and miniature schnauzers has been demonstrated.[27,31] Other congenital defects that have been reported with increased frequency in cryptorchid male dogs include inguinal hernia, umbilical hernia, hip dysplasia, patellar luxation, and penile/preputial defects.[22,32]

Breeds at increased and decreased risk for cryptorchidism have been identified (Table 18–1).[15,22,25,28,32,33] In general, small-breed dogs (<9.1 kg) are at increased risk compared to medium (9.1 to 18.1 kg) and large-breed dogs (>18.1 kg).[34] The risk in the smaller counterpart within a breed is higher; toy poodles are at greater risk of cryptorchidism than are standard poodles.[22]

Pathogenesis of abnormal testicular descent in the dog is not well defined. There is one report of bilaterally retained testes in a dog with extremely strong cranial suspensory ligaments joining the cranial end of the testes to the area craniolateral to the ipsilateral kidney and preventing outgrowth of the gubernaculum and subsequent testicular migration, suggesting that breakdown of the cranial suspensory ligament may be an essential component

Table 18–1. Risk for Canine Cryptorchidism by Breed

Breeds at increased risk (in decreasing order)
Toy poodle
Pomeranian
Yorkshire terrier
Miniature dachshund
Cairn terrier
Chihuahua
Maltese
Boxer
Pekingese
English bulldog
Old English sheepdog
Miniature poodle
Miniature schnauzer
Shetland sheepdog
Siberian husky
Standard poodle
Breeds at decreased risk
Mongrel
Beagle
Labrador retriever
Golden retriever
Saint Bernard
Great Dane
English setter

Data from Hoskins and Taboada,[15] Hayes et al.,[22] Turba and Willer,[25] Reif and Brodey,[28] Pendergrass and Hayes,[32] and Lüerssen.[33]

of normal testicular descent.[35] No significant difference has been demonstrated in serum estrogen concentrations between unilaterally cryptorchid and normal male dogs.[36,37] Serum testosterone and luteinizing hormone (LH) concentrations may or may not be decreased in cryptorchid dogs.[36–39] Inadequate secretion of GnRH, LH, or T has been hypothesized as causative of cryptorchidism due to the occasional descent of testes in human males at puberty, and occasional reports of successful treatment of canine cryptorchidism with gonadotropic agents.[18] Cryptorchid dogs have been reported to have lower serum T concentrations after administration of a GnRH analogue than normal dogs.[39] However, lack of consistent success with gonadotropic agents as a treatment for canine cryptorchidism argues against insufficiency of the hypothalamic-pituitary-testicular axis as an inciting cause of cryptorchidism in this species. Finally, the increased risk of cryptorchidism in the smaller counterpart within a breed suggests that abnormal testicular descent may be related to physical size or rate of growth of the testes, epididymes, and/or gubernaculum.[22]

Nonhereditary causes of canine cryptorchidism have been hypothesized. Any process that alters intra-abdominal pressure, such as umbilical infection with peritonitis, or causes inflammation or adhesions in the inguinal canal or scrotum, such as trauma to the inguinal region, may prevent normal testicular descent.[18] In humans, excessive weight of the mother is associated with a decline in concentration of sex-hormone–binding globulin, which increases free serum estrogen concentrations and causes fetal testicular hypoplasia[40]; a similar sequence in dogs has not been reported.

Retained testes are smaller than scrotal testes, with size positively correlated with degree of retention. Abdominally retained testes weigh less than inguinally retained testes.[36] Compensatory enlargement of the scrotal testis in unilaterally cryptorchid dogs has not been reported.[36] Histologically, diameter of seminiferous tubules in retained testes is reduced by up to 60 per cent, compared to scrotal testes.[41] The seminiferous tubules are lined by Sertoli cells, which may be atrophic. Spermatogonia may be present, but there is no spermatogenesis.[24,36,41] Interstitial cells are present and may be either atrophic[41] or hypertrophic.[42] Hypertrophy of interstitial cells was identified in 5 of 28 retained testes in one study.[42] Scrotal testes showed histologic abnormalities in 40 per cent of 34 unilaterally cryptorchid dogs in one study.[42]

Retained testes are capable of steroidogenesis[36–39] but are not capable of normal spermatogenesis. Twenty-seven of 28 retained testes showed histologic abnormalities in one study, with no evidence of spermatogenesis in 22 of the 27.[42]

Although three of four bilaterally cryptorchid dogs achieved erection in one study, only two of the four ejaculated and neither sample contained live spermatozoa.[42] Bilaterally cryptorchid dogs may be azoospermic.[38] Human beings with bilateral testicular maldescent are twice as likely to be azoospermic as men with unilateral testicular maldescent.[43]

Dogs with unilateral cryptorchidism show variable semen quality. In a review of 34 unilaterally cryptorchid dogs, 91 per cent achieved erection.[42] Forty-seven ($n = 34$)[42] to 58 per cent ($n = 19$)[21] of unilaterally cryptorchid dogs were demonstrated to ejaculate, and as many as a quarter of the samples were azoospermic.[42] Oligozoospermia is common; in a survey of ejaculates from 16 unilaterally cryptorchid dogs, only one had normal total number of spermatozoa in the ejaculate (>300 million), normal progressive motility (>70 per cent), and normal per centage morphologically normal spermatozoa (≥80 per cent).[42] In a review of semen quality from 11 unilaterally cryptorchid dogs, total number of spermatozoa did not vary between unilaterally cryptorchid dogs with inguinally retained testes ($n = 7$) and those with abdominally retained testes ($n = 4$), and was abnormally low in both, at 50 to 200 million and 20 to 240 million, respectively.[21] Percentage morphologically normal spermatozoa did vary between the groups. Percentage morphologically normal spermatozoa was abnormal in unilaterally cryptorchid dogs with an inguinally retained testis, averaging 72.5 per cent, but was normal in unilaterally cryptorchid dogs with an abdominally retained testis, averaging 87.2 per cent.[21] Although abnormal semen quality usually is present in unilaterally cryptorchid dogs, these dogs should be considered fertile if allowed to mate with a female.

Retained testes are predisposed to neoplasia. The increased risk of developing neoplasia in a retained versus a descended testis has been reported as 9.2 times ($n = 2912$),[22] 10.9 times ($n = 1266$),[32] and 13.6 times ($n = 410$).[44] In a survey of dogs with testicular neoplasia, 54.7 per cent of 108 dogs with Sertoli cell tumors and 33.8 per cent of dogs with seminomas were

cryptorchid.[28] Matched pair analysis in that study showed a significant degree of association between cryptorchidism and testicular neoplasia (see Testicular Neoplasia below).[28]

The spermatic cord attached to a retained testis is predisposed to torsion. There are many published reports of torsion of a spermatic cord attached to an enlarged, sometimes neoplastic, retained testis; in one review of 13 cases of torsion of the spermatic cord, 12 of the testes involved were retained (see Torsion of the Spermatic Cord below).[26,45–48]

Diagnosis of cryptorchidism is by visual inspection and careful palpation of the scrotum and inguinal region in dogs older than 6 months of age. Inguinal lymph nodes and fat may feel like small inguinal testes.[49] The small, freely movable testes may be palpable beneath or lateral to the proximal portion of the prepuce. Abdominally retained testes are not palpable, unless they are enlarged due to neoplasia or torsion of the spermatic cord. Ultrasonography of the inguinal canals and abdomen may be attempted, but the decreased size and large number of possible locations of the retained testis decrease likelihood of identifying it by this method. Differentiation of bilaterally cryptorchid dogs from castrated male dogs may be done by rectal palpation of the prostate (enlarged in intact dogs)[50] or hormone assay. Serum testosterone following challenge testing is elevated above baseline concentrations in dogs with testes. Since testosterone is secreted pulsatiley, challenge testing is recommended (GnRH; 2 μg/kg or 50 μg/dog IM, draw blood sample 60 minutes following injection).[51] Serum LH and follicle-stimulating hormone (FSH) are elevated in castrated animals due to lack of negative feedback to the pituitary from the gonads.[50] Work is ongoing to determine the value of commercially available in-house test kits (Status-LH, Synbiotics Corp, Inc, San Diego, CA) for diagnosis of bilateral cryptorchidism in dogs (R. Lofstedt, personal communication).

The treatment of choice for cryptorchidism is bilateral castration. Although bilaterally cryptorchid dogs are sterile, predisposition of the retained testes to neoplasia and torsion of the spermatic cord makes surgical removal of both of the testes the best choice for the health of the animal. Unilaterally cryptorchid dogs should be castrated to decrease possible transmission of this hereditary defect, and to decrease the predisposition of the retained testis to neoplasia and torsion of the spermatic cord.

Surgical approach for removal of the retained testis depends on its suspected location. Abdominally retained testes may be anywhere on the path from the caudal pole of the kidney to the inguinal canal.[49] Bilaterally retained testes often are located caudal to the kidneys, while a unilaterally retained testis is more commonly found in the caudal abdomen, lateral to the bladder.[27,49] To find a testis in the caudal abdomen, retroflex the urinary bladder and identify the ductus deferens as it leaves the prostate or courses around the ureters on the lateral aspect of the urinary bladder.[49] Removal of retained testes is similar to that for scrotal testes. Complications reported in dogs after surgical removal of retained testes include avulsion of the prostatic urethra after trauma by an ovariohysterectomy hook used for retrieval of the retained testis,[52] and inadvertent prostatectomy.[49] Laparoscopic removal of retained testes has been described.[53]

Surgical placement of the retained testis in the scrotal sac (orchiopexy) has been described, but is not recommended.[37,54,55] Testes repositioned in the scrotum do regain some spermatogenic function; increased diameter of the seminiferous tubules was identified in both the repositioned testis and scrotal testis in one study.[37] Unilaterally cryptorchid dogs from which the scrotal testis was removed and the retained testis surgically replaced in the scrotum showed gradual improvement in semen quality by 1 year after surgery. Inseminates from these dogs successfully impregnated 3 of 11 bitches bred.[54,55] Orchiopexy and subsequent improvement in semen quality is not a preferred consequence of veterinary intervention in unilaterally cryptorchid dogs, which should not be bred. Correction of cryptorchidism by orchiopexy also may increase the dog's value fraudulently; cryptorchid dogs cannot be shown in American Kennel Club (AKC)-sanctioned conformation competitions. Finally, the repositioned testis of human beings treated for cryptorchidism with orchiopexy is considered to retain its predisposition to neoplasia, regardless of age of the boy at time of surgery.[28] The authors discourage orchiopexy as a treatment for unilateral cryptorchidism in dogs.

Medical treatments for canine cryptorchidism with GnRH, and drugs with LH activity, including hCG and equine chorionic gonadotropin (eCG), have been described (Table 18–2).[17,33,56–59] Most reports did not include a control group in the study; 20 per cent of dogs with one or both testes retained underwent

Table 18–2. Medical Therapies Described for Treatment of Canine Cryptorchidism

Dose Regimen Employed	Age When Treated	Recovery Rate	Reference
Gonadotropin-releasing hormone (GnRH)			
50–750 μg 1–6 times	2–4 mo	26.6% (n = 301)	Humke[57]
Human chorionic gonadotropin (hCG)			
100–1000 IU IM 4 times in a 2-wk period	< 16 wk	84.0% (n = 25) [0% in 28 controls]	Feldman and Nelson[58]
300–1000 IU 3–4 times	—	75%	Ravaszova et al.[17]

spontaneous testicular descent by 6 months of age in one study.[20] Medical treatment for cryptorchidism may increase fertility in a dog that should not be bred, and may increase value of a dog with a heritable defect. The predisposition to neoplasia remains whether the retained testis descends or not. The authors strongly discourage medical treatment for canine cryptorchidism.

Anecdotal reports exist of success in pulling inguinally retained testes into the scrotum by gentle downward traction of the testis several times daily.[59,60] The authors are unaware of controlled studies evaluating this practice.

Canine cryptorchidism is best controlled by removing cryptorchid dogs and, ideally, their dam and sire, from the breeding program. Presence of the carrier state in females is difficult to define without breeding trials. Production of at least 40 male pups surviving to 6 months of age is required from a given bitch to exonerate her as a carrier for cryptorchidism.[61]

Orchitis/Epididymitis

Orchitis is inflammation of the testis. Epididymitis is inflammation of the epididymis. The two conditions may occur separately, jointly (orchiepididymitis), or with extension to the vaginal tunic and adhesions to the scrotum (periorchiepididymitis).[62]

Orchitis/epididymitis usually occurs in young dogs. Mean and median ages at time of diagnosis are 3.7 and 2.0 years, respectively (n = 10), with a range from 11 months to 10 years.[63–67] There is no breed predisposition reported for canine orchitis/epididymitis.

Dogs may present with acute or chronic disease. Acute illness is characterized by sudden onset of painful swelling of the scrotal contents. Possible accompanying signs of acute orchitis/epididymitis include pyrexia, lethargy, hindlimb lameness, scrotal edema, and purulent preputial discharge.[62,63,68] Dogs with chronic orchitis/epididymitis present with nonpainful enlargement of scrotal contents, sometimes accompanied by atrophy of the unaffected testis.[62,67]

Pathogenesis of orchitis/epididymitis in the dog involves inflammation of the scrotal contents due to infection or autoimmune destruction of testicular and epididymal tissue.[62] Possible routes of entry of infectious agents include retrograde movement of organisms from the prostate or lower urinary tract, hematogenous spread, and direct entry via a penetrating wound or insect bite.[62,63,69] Initial infection and inflammation cause testicular and epididymal swelling with formation of numerous tiny intraluminal abscesses. Chronic inflammation results in testicular degeneration and fibrosis or atrophy. The epididymis frequently becomes fibrotic.[62] Organisms that were isolated from cases of orchitis, epididymitis, or orchitis/epididymitis include *Brucella canis* (see Brucellosis in the Stud Dog, below), *Escherichia coli* (isolated from 5 of 10 cases reported), *Proteus vulgaris*, *Staphylococcus* sp., *Streptococcus* sp., blastomycosis and other fungi, *Mycoplasma canis*, and canine distemper virus.[62,63,65–68,70] A noninfectious cause of inflammatory testicular/epididymal disease is injection of sterile urine via the vas deferens, as may occur when a dog with a full urinary bladder undergoes a full body trauma, such as being hit by a car.[62]

Autoimmune destruction of the testes occurs when trauma or inflammation exposes immunologically privileged tissue to the immune system. In the normal male dog, Sertoli cell tight junctions form the blood-testis barrier, which isolates maturing germ cells from the immune system.[71] Damage to the blood-testis barrier, either directly or from inflammation within the testis, may allow leakage of antigens unique to mature spermatozoa, infiltration of lymphocytes into the testes, and sensitization of T cells.[72] Both cell-mediated and humoral factors may be involved, with destruction of spermatogenic tissue and formation of antisperm antibodies.[72]

Autoimmune orchitis may occur secondary to a known infection, may be a component of a widespread autoimmune disorder, or may be idiopathic. Lymphocytic orchitis was diagnosed concurrently with lymphocytic thyroiditis in 32 per cent of 69 beagles over 1 year of age in one colony.[73] Infiltration of the testes with lymphocytes, variable degrees of damage to the seminiferous tubules, and grossly evident testicular atrophy with oligozoospermia or azoospermia were present. A hereditary component was identified.[73] Idiopathic autoimmune orchitis was diagnosed in two related Labrador retrievers with focal inflammatory lesions in 20 to 40 per cent of the seminiferous tubules, and azoospermia.[74,75]

Diagnosis of orchitis/epididymitis is by visual inspection and palpation of the enlarged testis. Differential diagnoses include scrotal hernia, torsion of the spermatic cord, testicular neoplasia, hydrocele, and sperm granuloma.[62] Dogs with acute orchitis/epididymitis may resist handling of the scrotal contents due to pain. If the testes are palpable, they are enlarged and firm. The epididymes are enlarged and doughy. In cases of chronic orchitis/epididymitis, the testes may be soft (atrophic) or very firm and irregular (fibrotic). The epididymes are frequently "woody" on palpation due to fibrosis. Formation of adhesions within the scrotum may preclude free movement of the testes within the scrotum.[62]

Fine-needle aspiration (FNA) of the enlarged testes/epididymes yields samples for cytology, and aerobic, anaerobic, and mycoplasma cultures. Numerous polymorphonuclear leukocytes are present in cases of suppurative orchitis/epididymitis (Fig. 18–2). Minimal or no exfoliative inflammatory response is present in cases of granulomatous orchitis/epididymitis.[68]

Dogs with orchitis/epididymitis may be too painful to achieve erection and permit collection of an ejaculate. If the dog is capable of ejaculating, semen samples may be submitted for culture; quantitative culture should be performed.[76] Growth of more than 10,000 bacteria per milliliter of a single organism is significant, but does not localize infection to the testes, epididymes, or prostate. The authors are unaware of reports correlating presence of orchitis/epididymitis with semen cultures.

All dogs presenting with scrotal enlargement should be tested for canine brucellosis, regardless of age or status as a breeding animal (see Brucellosis in the Stud Dog below). No significant changes have been reported on complete blood counts, serum chemistry profiles, or urinalyses of dogs with orchitis/epididymitis.[63]

Ultrasonography of the enlarged scrotum may allow differentiation of structures that are not palpable due to pain or scrotal edema. Testes with inflammatory changes have a patchy hypoechoic pattern.[67] In epididymitis, the epididymes are irregular in contour, and may contain either hypoechoic areas and flocculent material (suppurative disease), or both hypo- and hyperechoic areas, with or without mineralization (granulomatous disease).[63]

Treatment of orchitis/epididymitis requires removal of the affected testes. Antibiotic therapy alone is rarely curative[62]; in one case, antibiotic therapy resolved the acute clinical signs,

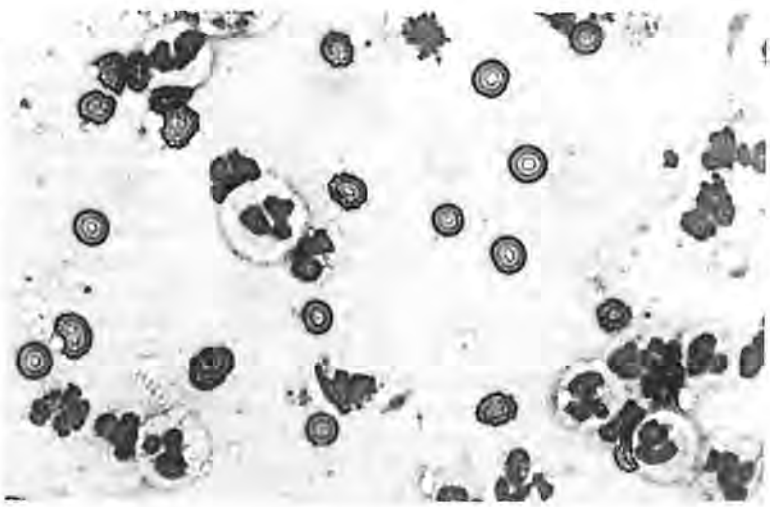

Figure 18–2. Testicular smear from a case of orchitis/epididymitis showing few spermatogenic cells and a large number of polymorphonuclear cells. A bacteriologically pure culture of *Escherichia coli* was detected. Hemacolor; 400×. (From Dahlbom M, Mäkinen A, Suominen J: Testicular fine needle aspiration cytology as a diagnostic tool in dog infertility. J Small Anim Pract 38:506–512, 1997, with permission.)

but the testis atrophied to 90 per cent of its initial volume over the next year,[69] perhaps due to ongoing inflammation. Human beings with a history of orchitis/epididymis have smaller testes and increased prevalence of oligozoospermia or azoospermia compared to men without that history.[43] Glucocorticoid therapy, in either infectious or autoimmune orchitis, has not been described in the dog. Glucocorticoids may decrease spermatogenesis in male dogs.

Dogs with bilateral orchitis/epididymitis should be castrated. Presurgical treatment with antibiotics helps prevent formation of a scirrhous cord, a painful swelling of the remaining vasa deferens, as a postsurgical complication.

Unilateral orchitis/epididymitis in dogs that are not intended for breeding should be treated with bilateral castration. In valuable stud dogs, unilateral orchiectomy may be performed. Both the testis and epididymis on the affected side should be removed. Unilateral orchiectomy should be performed as soon as possible after diagnosis to prevent atrophy of the contralateral testis due to inflammation and increased intrascrotal temperature. Appropriate antibiotic therapy should be instituted prior to surgery; empiric treatment with a highly soluble, broad-spectrum antibiotic, such as enrofloxacin, is recommended.

Semen quality may return to normal more than 62 days after therapy, or after a complete spermatogenic cycle in the dog.[77] Sperm numbers decline by 50 per cent immediately after unilateral orchiectomy.[78] Compensatory hypertrophy of the remaining testis, characterized by increased diameter of seminiferous tubules, may be evident as early as 3 months after surgery.[78,79] Return to normal semen quality by 2.5 to 6 months after unilateral orchiectomy was reported in two of three dogs.[63,64] Prognosis is better if neither atrophy nor fibrosis is palpable in the remaining testis.

Brucellosis in the Stud Dog

Brucella canis is a nonmotile, gram-negative coccobacillus with a host range limited to domestic and wild canids. Reported incidence in the United States and Canada varies from 0.2 to 9.0 per cent, with an overall mean of 3.5 per cent ($n = 5963$).[80–86] Incidence is higher in stray dogs than in pets.[84–87]

Infected animals shed organisms in urine and semen and, to a lesser extent, in saliva and nasal secretions.[88–90] Transmission occurs primarily by ingestion or inhalation.[88] Disease transmission has been identified in dogs housed together but without sexual contact; 1 of 13 susceptible dogs housed with an infected dog seroconverted in one study.[90,91] Venereal transmission also may occur.[88] A slight but real zoonotic potential exists.

The organism is phagocytized by macrophages and initially transported to the lymph nodes. Transient lymphadenopathy is followed by a prolonged bacteremia of 6 to 64 months' duration.[88,92] The organism replicates within lymphocytes and lymphoreticular tissues for an indefinite period of time. Antibody titers rise after initial infection and fall as bacteremia abates; decreasing serum antibody titers are not indicative of clearing of infection from the body.[88,92,93] The organs most commonly infected in male dogs are the testes and epididymes, causing signs of orchitis/epididymitis, scrotal dermatitis, oligozoospermia, and infertility.[88,95] The organism can be recovered from the testes and epididymes within 5 weeks of experimental infection.[89] Epididymitis alone occurs more commonly than orchitis or orchitis/epididymitis (Fig. 18–3).[88,96] Testicular atrophy associated with teratozoospermia, sperm agglutination due to formation of antisperm antibodies, and eventual spermatogenic arrest may occur in chronic infections.[88,96] *Brucella canis* infection also has been reported as a cause of uveitis, meningitis, diskospondylitis, glomerulonephritis, osteomyelitis, cystitis, and pyogranulomatous dermatitis in the dog.[88,91,97]

Definitive diagnosis requires culture of *Brucella canis* from blood, lymph node aspirates, bone marrow, or infected tissues or discharges.[92] However, *Brucella canis* is a fastidious organism; one negative culture does not rule out the disease. Blood culture is the most accurate test available in the first 8 weeks following infection.[88,92,98]

Serologic testing is readily available and diagnostic once significant antibody titers develop, at least 8 weeks following infection.[88] Agglutination tests identify presence of antibodies to cell wall antigens of *Brucella canis*. A rapid card agglutination test (RCAT; card test) and tube agglutination test (TAT; tube test) are commercially available. The RCAT gives a positive/negative result while the TAT yields a titer. A titer of greater than 1:200 is considered a positive result.[99,100] Agglutination tests are sensitive (accurate when negative) but not specific (accurate when positive). Negative test results are 95 to 99.7 per cent accurate.[87,92,99]

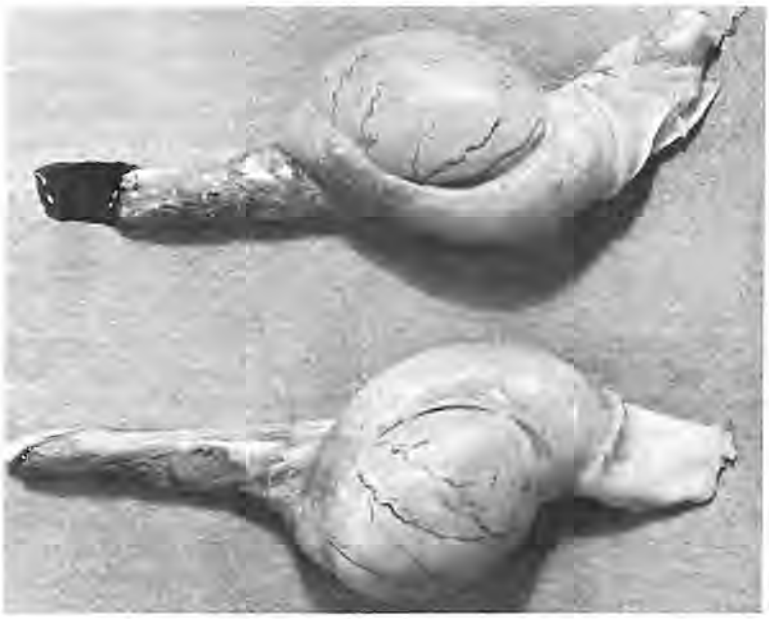

Figure 18–3. Enlarged epididymes from a dog naturally infected with *Brucella canis*. (From Carmichael LE, Kenney RM: Canine abortion caused by Brucella canis. J Am Vet Med Assoc 152:605–616, 1968, with permission.)

Positive test results may be inaccurate, as many common organisms share cell wall antigens with *Brucella canis;* false-positive results of 20 to 50 per cent have been reported.[92] Specificity of the test may be enhanced with addition of 2-mercaptoethanol to the test serum. Common cross-reacting organisms include *Pseudomonas aeruginosa, Staphylococcus* sp., and *Bordetella bronchiseptica.*[88] Agglutination tests remain positive in infected animals from 8 to 12 weeks following infection to 3 months after the animal becomes abacteremic.[88] These are excellent screening tests, but positive results should always be verified with another testing method before diagnosis of *Brucella canis* infection is considered definitive.

The most accurate serologic test currently available is the agar gel immunodiffusion test (AGID) available through Cornell University. This test identifies antibodies to cytoplasmic antigens of *Brucella canis*, which are very specific to the *Brucella* sp. Since dogs are rarely infected with other species of *Brucella* (e.g., *B. abortus, B. melitensis*) a positive result is considered definitive for a diagnosis of canine brucellosis. AGID tests are positive in infected animals from 12 weeks following infection to 36 months after the animal becomes abacteremic.[88,92]

An enzyme-linked immunosorbent assay (ELISA) for canine brucellosis has been described, but is not yet commercially available.[92,101] It has been reported to be quite accurate, yielding a false-positive rate of only 2 per cent in one study.[101]

Treatment recommendations for canine brucellosis vary with housing and breeding status of the animal. Treatment with antibiotics has not been shown to effect a long-term cure.[88,93,94] It must be remembered that decreasing titers occur in natural infection, and that negligible titers are not indicative of complete clearing of infection from the body.[88,94]

Pet animals that are housed singly may be neutered and treated with antibiotics to decrease bacteremia and subsequent shedding of the organism.[88] Antibiotic regimens that have been described include minocycline (25 mg/kg per os [PO] once daily for 14 days) with dihydrostreptomycin (5 mg/kg IM twice daily for 7 days) and tetracycline (30 mg/kg PO twice daily for 21 days) with streptomycin (20 mg/kg IM once daily for 14 days).[88,93] The zoonotic potential of the disease must be stressed, especially in households with young children or immunosuppressed occupants.

Control of *Brucella canis* in a kennel includes the following steps: (1) confirm disease, (2) quarantine kennel (no animals allowed in or out), (3) determine source of infection (the index case is not necessarily the source of infection), (4) eliminate mode of transmission within kennel, (5) identify and cull infected animals, and (6) initiate practices to prevent further outbreaks.[94] Kennels should be consid-

ered clear of disease only when all animals have tested negative for 3 consecutive months.[88] In closed kennels, complete eradication of the disease may require 5 to 7 months of testing and culling. In open kennels, the disease may never be eradicated. Canine brucellosis is a reportable disease in some states.

Spermatocele/Sperm Granuloma

Spermatoceles are localized areas of spermiostasis within the epididymis. Sperm granulomas are palpable as discrete, firm, nonpainful swellings in the epididymis. On gross examination, they are most commonly found in the caudae epididymes, are tan in color, and contain milky brown fluid.[102] The histologic description of sperm granulomas in one report was scattered normal tubules with lumens packed with spermatozoa and abnormal tubules with flattened, nonciliated epithelial cells, surrounded by inflammatory cells and fibrous tissue,[102] suggesting that spermatoceles may precede sperm granulomas. Hypothesized causes include trauma, infection, and congenital anomalies.[24,102] Trauma may cause sperm granulomas by allowing exposure of spermatozoa, which are normally immunologically privileged, to the immunocompenent cells within the epididymis.[24,71,102] Definitive diagnosis requires biopsy or surgical excision of tissue. Dogs with a unilateral sperm granuloma may be fertile. Dogs with bilateral sperm granulomas are infertile. No treatment for sperm granulomas in dogs has been described.

Testicular Degeneration/Atrophy

Testicular degeneration is evidenced by decrease in size and softening in consistency of the testis. Testicular size and weight in normal dogs are positively correlated with body weight,[103,104] and are best estimated by measurement of total scrotal width (see Chapter 23).[104,105] Normal testicular consistency is similar to that of a peeled, hard-boiled hen's egg.

Causes of testicular degeneration include noninfectious inflammatory testicular disorders, such as autoimmune orchitis, chronic infection of the testis, such as canine brucellosis (see Orchitis/Epididymitis, Brucellosis in the Stud Dog above), testicular neoplasia (see Testicular Neoplasia, below), retention of the testis in the inguinal canal or abdomen (see Cryptorchidism above), pyrexia, and any process causing inflammation of the scrotum, such as a penetrating scrotal wound.[67,68,88,106] Testicular degeneration has been induced experimentally by bilateral vasectomy and ligation of the caudae epididymes, suggesting that it may occur in patients with epididymal occlusion.[107,108] Degeneration of the contralateral testis may occur due to increased intrascrotal temperature secondary to inflammation,[106] or hormone production by the affected testis, as may occur in dogs with a unilateral Sertoli cell tumor producing estrogen.[109–112]

Fine-needle aspiration of atrophic testes yields low cell density.[68] Spermatogenic cells may be present. In a report of three dogs with testicular degeneration in a testis contralateral to one containing a functional Sertoli cell tumor, none of the atrophic testes had changed in volume or consistency when re-evaluated 6 to 10 months after surgical removal of the affected testis, but all three dogs did sire litters.[110]

How to Perform Fine-Needle Aspiration, Core Biopsy, and Incisional Biopsy of the Canine Testis

Fine-Needle Aspiration of the Testis

Fine-needle aspiration of the canine testis is a relatively noninvasive technique that permits retrieval of testicular tissue specimens that can be examined cytologically or submitted for culture. The dog is placed under general anesthesia. A 20-gauge needle attached to a 10-ml syringe is introduced at the testicular midline, and suction is applied. The needle is redirected three to four times, with suction applied after each redirection. The needle is withdrawn and digital pressure applied to control bleeding, if necessary. The sample is expelled onto a glass slide, smeared out, and allowed to air-dry. After fixation with methanol, the sample is stained as for a blood smear and examined under a light microscope under the oil immersion (100×) objective.[68]

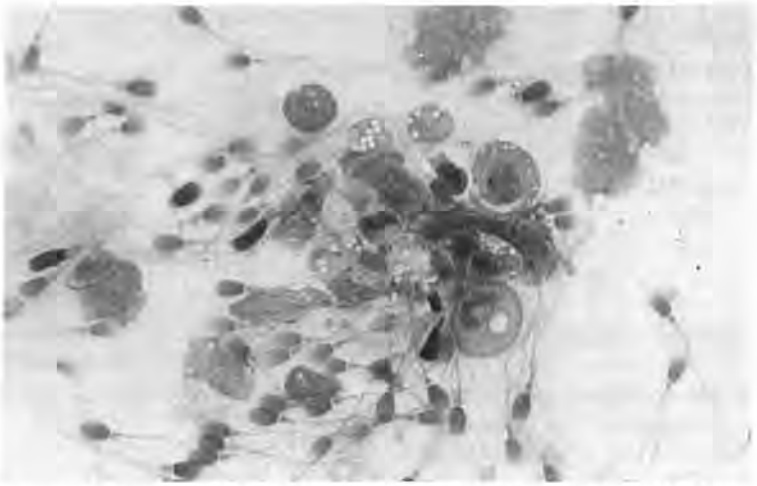

Figure 18–4. Active spermatogenesis in the testicular aspirate of a dog. (From Dahlbom M, Mäkinen A, Suominen J: Testicular fine needle aspiration cytology as a diagnostic tool in dog infertility. J Small Anim Pract 38:506–512, 1997, with permission.)

The sample collected by FNA does not allow evaluation of testicular architecture or dynamics of spermatogenesis, but does allow the investigator to ascertain whether spermatogenesis is occurring (Fig. 18–4). Interstitial (Leydig) cells are rarely identified. Sertoli cells, and immature and mature spermatozoa are readily identified. Tails of mature spermatozoa collected by this technique often are absent.[68]

In a survey of four normal dogs and one unilateral cryptorchid dog undergoing FNA of the testis, no changes in libido or semen quality were noted after the procedure, and follow-up histology of the testis was normal.[68] However, hemorrhage within the testis at the site of aspiration may occur (Fig. 18–5).

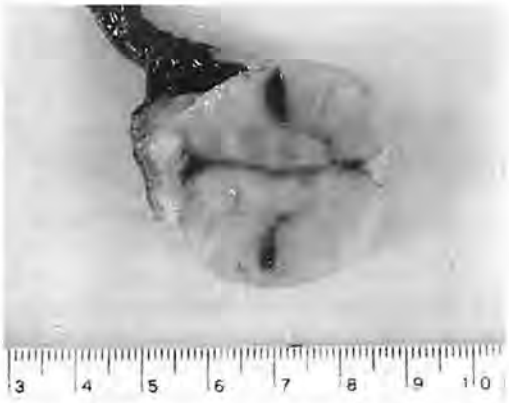

Figure 18–5. Hemorrhage in a testis after an aspiration biopsy with a 20-gauge needle. (From Freshman JL, Amann RP, Bowen RA, et al: Clinical evaluation of infertility in dogs. Compend Contin Educ Pract Vet 10:443–461, 1988, with permission.)

Because FNA as well as core and incisional biopsy of the testis disrupts the blood-testis barrier and induces potential for immune-mediated orchitis, the benefit of these diagnostic techniques must be weighed against their risks. In many dogs, information gained from semen evaluation with measurement of seminal plasma alkaline phosphatase and testicular ultrasound may preclude need for biopsy.

Core Biopsy of the Testis

Core biopsy of the testis permits removal of a large enough piece of tissue for complete evaluation of dynamics of spermatogenesis. The dog is placed under general anesthesia and the prescrotal area shaved and prepared for sterile surgery. A prescrotal incision is made, the testis is advanced to the incision and a small incision is made through the testicular tunics. A sliding biopsy instrument (Trucut biopsy needle; Travenol Laboratories, Inc, Deerfield, IL) is introduced, and the instrument is closed, trapping a piece of testicular tissue within it. The needle is withdrawn, and the tunica albuginea, subcutaneous tissue, and skin are closed routinely. The sample is fixed, embedded in paraffin, and stained.[113] Recommended fixatives include Zenker's and modified Bouin's; formalin fixation may cause artifactual changes in testicular histology. It is recommended that the sample be evaluated by someone familiar with canine testicular morphology.[114] Percutaneous biopsy with a needle 1.0 mm

Figure 18–6. The method of obtaining a biopsy from the testis. Small incisions have been made in the skin cranial to the scrotum, the tunica vaginalis, and the tunica albuginea. (From Freshman JL, Amann RP, Bowen RA, et al: Clinical evaluation of infertility in dogs. Compend Contin Educ Pract Vet 10:443–461, 1988, with permission.)

in diameter and 40.0 mm in length attached to a 5-ml syringe also has been described.[115]

Gross and microscopic testicular lesions have been described in dogs undergoing core needle biopsy.[113,115] Of 36 dogs undergoing core needle biopsy of the testis, 33 per cent had hemorrhage at the biopsy site, and 3 per cent had adhesions between the tunica vaginalis and tunica albuginea by 36 days after biopsy.[113] Microscopic lesions described included tubular degeneration, interstitial fibrosis, coagulation necrosis, and maturation arrest at the biopsy site; the microscopic lesions were considered mild in 65 per cent of the dogs, moderate in 30 per cent, and severe in 5 per cent.[113] Similar changes were described in eight dogs in which percutaneous core needle biopsy was performed monthly for 7 months.[115] Neither semen quality nor serum testosterone concentrations varied in those eight dogs over the study. Testicular volume decreased in one of eight.[115]

Incisional Biopsy of the Testis

Incisional biopsy is the most invasive of the biopsy techniques described, but it yields the largest specimen. Because a relatively large sample of testicular tissue is obtained, testicular architecture and dynamics of spermatogenesis may be evaluated. The dog is anesthetized and the prescrotal area shaved and prepared for sterile surgery. A prescrotal incision is made and the testis is advanced to the incision. The tunica vaginalis is incised and the testis exposed. A scalpel is used to pierce the tunica albuginea and the tissue that bulges out of the capsule is shaved off (Fig. 18–6). The tunica albuginea, subcutaneous tissue, and skin are closed routinely. The tissue is fixed, embedded in paraffin, and stained.[113,114] As with core biopsy, fixation with modified Bouin's or Zenker's solution is preferred to formalin fixation (Fig. 18–7).

Gross and histologic changes are more numerous and more severe in dogs undergoing incisional testicular biopsy than in those undergoing core needle biopsy.[113] Hemorrhage at the biopsy site was present in 28 per cent (n = 39) and adhesions of the tunica albuginea to the tunica vaginalis in 44 per cent of dogs undergoing incisional testicular biopsy. Microscopic lesions were present in 90 per cent of the dogs, and were considered mild in 13 per cent, moderate in 36 per cent, and severe in 51 per cent, with 26 per cent exhibiting maturation arrest.[113] Semen quality was not reported in these dogs after incisional testicular biopsy.

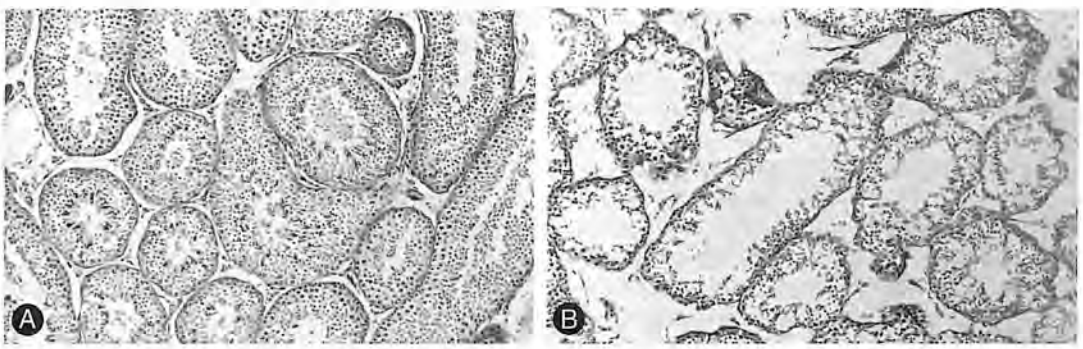

Figure 18–7. A: Photomicrograph of canine testicular tissue fixed in Bouin's fixative and stained with hematoxylin and eosin and periodic acid–Schiff stains; 100×. **B:** Photomicrograph of testicular tissue from the patient, this time fixed in buffered formaldehyde. Note the severe artifact. 100×. (From Freshman JL, Amann RP, Bowen RA, et al: Clinical evaluation of infertility in dogs. Compend Contin Educ Pract Vet 10:443–461, 1988, with permission.)

Testicular Neoplasia

Testicular neoplasia is the second most common tumor type in male dogs, after skin tumors.[44] Reported incidence is 0.91 per cent.[116] Testicular tumors comprised 91 per cent (n = 77) of tumors of the male genital system in one review.[117]

Mean age at diagnosis of affected dogs is reported as 9.0 years (n = 70),[117] 10.2 years (n = 198),[118] 10.8 years (n = 12),[67] and 11.0 years (n = 205),[119] with a range of 2 to 19 years. Mean age at diagnosis is decreased for boxers, at 7.2 years.[118] The boxer is reported to be at increased risk of developing testicular neoplasia.[118,120] Breeds reported to be at decreased risk of testicular neoplasia include the dachshund, beagle, Labrador retriever, and mongrel.[44] Testicular neoplasia occurs more commonly in retained than in descended testes, with reported relative increased risks of 9.2 times,[22] 10.9 times,[32] and 13.6 times (see Cryptorchidism above).[44]

Testicular tumors may be unilateral or bilateral, and multiple cell types may be present. Bilateral neoplasia is reported to occur in 45 per cent (n = 177) of cases.[121] Presence of two or more tumor types concurrently in one or both testes has been reported to occur in 11.2 per cent (n = 410),[117] 23.0 per cent (n = 165),[118] and 35.0 per cent (n = 177) of cases.[121]

The three most common types of testicular tumor reported in the dog are Sertoli cell tumor (SCT), seminoma (SEM), and interstitial (Leydig) cell tumor (ICT). Review of the literature yields overall incidences of 44 per cent for SCT, 31 per cent for SEM, and 25 per cent for ICT.[24,32,67,117–119,122–124] Incidence varies between retained and scrotal testes for the various cell types. SCT and SEM are significantly more common in retained testes than is ICT.[118,122] Multiple tumor types in one or both testes that have been described include SCT/ICT, SCT/SEM, SEM/ICT, and SCT/SEM/ICT.[8,24,44,118,125,126]

Less common testicular neoplasms reported in dogs include benign epidermoid cysts, fibrosarcoma, hemangioma, anaplastic carcinoma, gonadoblastoma, embryonal carcinoma, sarcoma, lymphoma, and granulosa cell tumor.[44,117,121,123,127–131] Sarcoma of the spermatic cord and leiomyoma of the tunica vaginalis also have been described in dogs.[117,132]

Dogs with testicular neoplasia most often present with testicular enlargement as the only clinical sign, and age at diagnosis with absence of pain provides presumptive diagnosis of neoplasia instead of orchitis. Clinical signs specific to a paraneoplastic syndrome may be present with some tumors (see Sertoli Cell Tumor below). Clinical signs specific to metastasis may be seen in dogs with malignant tumors accompanied by distant metastases.

Presumptive diagnosis also is provided by palpation or ultrasound of the scrotum. The neoplastic testis may or may not be significantly enlarged. Atrophy of the contralateral testis may be present due to hormone production by the neoplastic tumor, or increased intrascrotal temperature. Fine-needle aspiration of testicular masses may not yield a definitive diagnosis of tumor type.[68] Nonpalpable tumors may be visualized by ultrasound. Tumors disrupt the normal internal architecture of the testis, and often are visible as hyper- or hypoechoic foci, that may or may not be surrounded by a well-defined hyperechoic capsule.[67,110] Tumor type cannot be differentiated by ultrasound.[67,133]

Treatment of choice is orchiectomy. Because of the relatively high incidence of bilateral neoplasia, and atrophy of the unaffected testis, bilateral orchiectomy is recommended. Unilateral orchiectomy may be considered in valuable breeding dogs. Sperm numbers decline by 50 per cent immediately after unilateral orchiectomy.[78] Compensatory hypertrophy of the remaining testis, characterized by increased diameter of seminiferous tubules, may be evident as early as 3 months after surgery.[78,79]

Sertoli Cell Tumor

Sertoli cell tumor is a neoplasm of the Sertoli (nurse) cells of the testis. Mean age at time of diagnosis has been reported as 7.0 years (n = 8),[134] 7.4 years (n = 5),[135] 9.2 years (n = 46),[118] and 10.0 years (n = 33),[121] with a range of 2.5 to 16 years. Boxers and Weimeraners are reported to be at increased risk for SCT.[28,44] A syndrome has been reported in miniature schnauzers with cryptorchidism, SCT of the retained testis or testes, and cystic endometrial hyperplasia/pyometra of a uterus masculinus.[10–12]

SCTs are the most common type of testicular neoplasm in retained testes. Of 239 SCTs described in the literature, 115 (48 per cent) were in retained testes and 124 (52 per cent) were in scrotal testes.[24,28,117,118,121,122] Of 53 SCTs in retained testes described in the literature, 58 per cent were abdominal and 42 per cent were

inguinal.[28,135,136] Dogs with cryptorchid testes are reported to have a 23 times greater risk of developing SCT than dogs with two scrotal testes.[44]

Tumor size varies from 1 to 12 cm in diameter, with most (15 of 33) tumors reported to be less than 1 cm in diameter in one study.[121] SCT is a discrête, white to pale yellow, firm mass.[121,136] Malignancy is low, reported as 2 per cent ($n = 46$)[118] and 6 per cent ($n = 33$).[121] Metastases to the regional lymph nodes (iliac, sublumbar, and inguinal lymph nodes), paraaortic and mesenteric lymph nodes, lungs, and spleen have been reported.[118,121,137,138]

The testis containing a SCT is enlarged and firm. Atrophy of the contralateral testis may be present.[109–112,138] Small SCTs in scrotal testes may not be palpable within the testicular parenchyma; three dogs presenting with infertility that were diagnosed with nonpalpable scrotal SCT have been described.[110] SCT is visible within the testicular parenchyma by ultrasound as a hypoechoic to anechoic area that may or may not be surrounded by a hyperechoic capsule (Fig. 18–8).[110,139]

SCTs commonly are associated with a feminizing paraneoplastic syndrome caused by estrogen secretion, which may also lead to estrogen-induced pancytopenia.[109,140,141] Hyperprogesteronemia also has been described in dogs with SCT and feminizing signs.[111] Five dogs with feminizing signs were reported to have normal serum estrogen concentrations, but increased inhibin and decreased serum luteinizing hormone and follicle-stimulating hormone concentrations, suggesting that secretory products from the SCT may suppress gonadotropin secretion.[135]

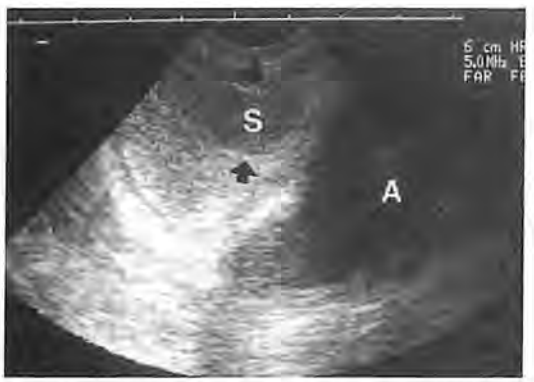

Figure 18–8. Ultrasound image of Sertoli cell tumor (S) within the testicular parenchyma of a dog. The capsule is well defined (arrows). An air artifact (A) is present adjacent to the caudal surface of the scrotum. Scale in centimeters. (From England GCW: Ultrasonographic diagnosis of non-palpable Sertoli cell tumours in infertile dogs. J Small Anim Pract 36:476–480, 1995, with permission.)

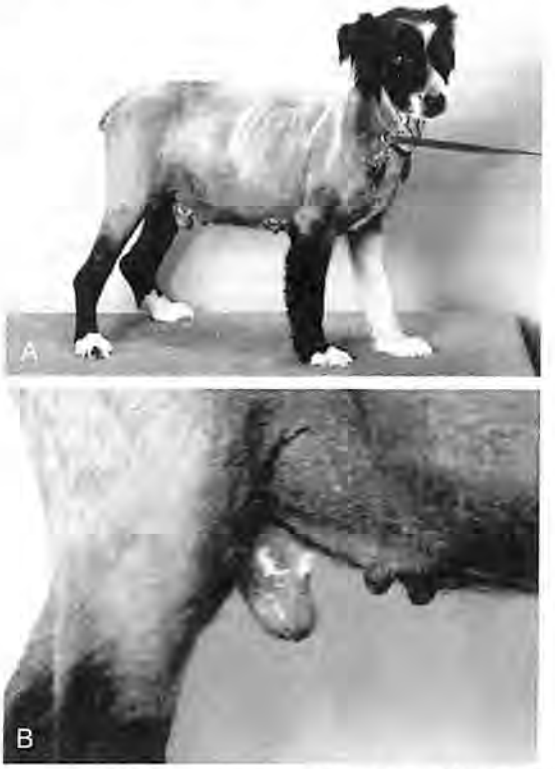

Figure 18–9. A 10-year-old male border collie with (**A**) bilateral alopecia and (**B**) pendulous prepuce and gynecomastia secondary to an estrogen-producing testicular tumor. (From Chastain CB: Compendium challenge: Feminizing testicular tumor. Compend Contin Educ Pract Vet 15:197–201, 1993, with permission.)

Feminizing signs are reported to occur in 24 per cent ([$n = 46$],[118] [$n = 33$][121]) and 39 per cent ($n = 95$)[28] of dogs with SCTs. Retained testes with SCTs are more likely to produce estrogen than are descended testes with SCTs; in one study, 10 of 11 dogs with feminizing signs had retained testes.[118] Feminizing signs were reported present in 16.7 per cent of dogs with scrotal testes containing SCTs ($n = 48$), in 50.0 per cent of dogs with inguinally retained testes containing SCTs ($n = 20$), and 70.4 per cent of dogs with abdominally retained testes containing SCTs ($n = 27$).[28]

Clinical signs of the paraneoplastic syndrome accompanying SCT include bilaterally symmetrical alopecia of the trunk and flanks with hyperpigmentation of inguinal skin and a dry, easily epilated coat, gynecomastia, a pendulous preputial sheath, squamous metaplasia of the prostate that may appear clinically as prostatitis, and attraction of male dogs (Fig. 18–9).[109,111,112,118,136,140–143] Keratinization of the

preputial mucosa may be present. Bone marrow hypoplasia with nonregenerative anemia, pale mucous membranes and lethargy, leukopenia, thrombocytopenia, petechiae of mucous membranes, epistaxis, hematemesis, melena, and hematuria also have been described in dogs with functional SCTs.[112,134,140,144] Prothrombin time, partial thromboplastin time, and fibrin degradation products are normal in these dogs.[134]

Feminizing signs are reported to resolve within 21 days of surgical removal of the SCT.[109] Prognosis for dogs with bone marrow hypoplasia secondary to SCT is guarded. Seven of eight dogs with SCT and bone marrow hypoplasia characterized by a mean packed cell volume (PCV) of 19 per cent, mean total white blood cell (WBC) number of 2080 cells/mm^3, and mean platelet count of 24,000 cells/mm^3 were treated with castration and supportive care including antibiotics, fluid therapy, hematinics, androgenic steroids, and transfusion of fresh whole blood or platelet-rich plasma.[134] Only two of the seven (29 per cent) recovered, and clinical improvement was not evident until 2 to 3 weeks after castration.[134]

Treatment of choice for SCT, with or without feminizing signs, is bilateral castration. Hemicastration may be considered for valuable stud dogs. Three male dogs presented for infertility, diagnosed with nonpalpable SCT of scrotal testes by ultrasound, and treated with castration of the affected testis only, went on to sire litters, although all had persistently low total numbers of sperm in the ejaculate.[110] Chemotherapy with cisplatin (60 mg/m^2 with a 6-hour saline diuresis, every 3 weeks for 2 to 5 cycles) and combination chemotherapy with vinblastine, cyclophosphamide, and methotrexate have been described for treatment of metastatic SCT.[131,145]

Exfoliative cytology of the preputial mucosa may be useful in confirming presence or recurrence of hyperestrogenism in dogs with SCT. Preputial cytology of the normal male dog yields noncornified epithelial cells, whereas cytology of dogs exposed to estrogen yields cornified epithelial cells with similar morphology to vaginal epithelial cells of estrous bitches.

Seminoma

Seminomas are tumors of the germ cells within the testes. Mean age at time of diagnosis of affected dogs is reported as 10.0 years (n = 47)[118] and 11.0 years (n = 22).[121] German shepherds reportedly are predisposed to SEM.[44] An increased incidence of SEM was reported in German shepherd dogs that had performed military service in Okinawa, Japan, and Vietnam, compared to age-matched German shepherds that had performed comparable military service in the United States.[146]

Of 116 SEM in dogs described in the literature, 26 (22 per cent) were in retained testes and 90 (78 per cent) were in scrotal testes.[117,118,121,122] SEM are more common in abdominally retained testes than in inguinally retained testes, with relative incidences of 73 per cent and 27 per cent, respectively.[118]

The tumor varies in size from 1 to 10 cm in diameter, and is a homogeneous or lobulated mass that is cream to pink-gray to khaki color on cut surface.[121] Although malignancy of SEM is considered low, metastases to regional lymph nodes, abdominal and thoracic viscera, brain, eye, and lung have been described.[118,124,137,147–149] Local invasion was reported to occur in 15 per cent of 20 cases.[150] Distant metastases have been reported to occur in 6 per cent (n = 47)[118] and 10 per cent (n = 20)[150] of cases.

Paraneoplastic syndromes that have been reported in dogs with SEM include progressive, nonpruritic alopecia and hyperpigmentation of the trunk, prostate disease, and non–insulin-dependent diabetes mellitus.[151,152] Relationship of these clinical signs to the tumor are unexplained, but all resolved after castration.

Treatment of metastatic SEM with chemotherapy (cisplatin, 60 mg/m^2 with a 6-hour saline diuresis, every 3 weeks for 3 cycles),[145] or radiotherapy have been described. Four dogs with metastatic SEM were castrated, and then treated with cesium-137 so as to receive a minimum total tumor dose of 17 to 40 Gy, divided into 8 to 10 fractions and given 3 fractions per week. The metastatic SEM resolved in all four dogs; all died or were euthanized for reasons other than SEM 6 to 57 months after therapy.[148]

Interstitial (Leydig) Cell Tumor

Interstitial cell tumors (ICTs) arise from the endocrine cells of the testis, and also are called interstitial cell adenomas. Mean reported age at time of diagnosis with ICT in dogs is 10.0 years (n = 88).[121] There is no breed predisposition for ICT.

Interstitial cell tumor is a tumor of scrotal testes. Of 187 ICTs reported in dogs in the

literature, 186 (99 per cent) were in scrotal testes.[117,118,121,122,153,154] Dogs with cryptorchidism are not at increased risk of developing ICT compared to normal intact dogs.

Grossly, ICTs are small, usually less than 1 cm in diameter, and vary in color from yellow to brown on cut surface.[12,124,155] They often are not detected clinically but are incidental findings at necropsy.[155] The contralateral testis may be atrophic.[153,154] Interstitial cell tumors are considered to be of low malignancy.[118,121,124,137]

Paraneoplastic syndromes that have been reported with ICT are due either to hyperestrogenism or hypertestosteronism. Clinical signs reported in dogs with ICT associated with elevated serum estrogen concentrations (>30 pg/ml) include bone marrow hypoplasia with aplastic anemia, pale mucous membranes and petechiation, prolonged bleeding time, and pyrexia, and bilaterally symmetrical, nonpruritic alopecia of the trunk and flanks.[153,154] Clinical signs reported in dogs with ICT associated with elevated serum testosterone concentrations include prostate disease, perianal adenoma, perianal and tail gland hyperplasia, and perineal hernia.[118,141]

How to Perform Testicular Ultrasound

The dog is placed in either dorsal or lateral recumbency. Sedation is rarely necessary. B-mode (brightness mode) ultrasonography is most often used. Either a 5.0- or 7.5-MHz transducer can be used; a gel standoff may be used to ensure that the testes are in the focal zone of the transducer used.[139,156] Both the transverse and sagittal planes of both testes should be scanned, and testicular architecture, echogenicity and size, and size and distribution of mass lesions evaluated.[156]

In a normal male dog, the testes are the same size and have a coarse, homogeneous appearance. The fibrous mediastinum testis may be visible as a hyperechoic central band (Fig. 18–10). The tunica albuginea is normally hyperechoic. The epididymes lie dorsoterally to the testes and have a hypoechoic to anechoic appearance relative to the testicular parenchyma.[139]

Abnormalities that may be identified with B-mode ultrasonography of the testes/epididymes include testicular neoplasia, epididymal mass lesions such as sperm granulomas, and torsion of the spermatic cord. Testes retained in the inguinal area or abdomen of cryptorchid dogs are very difficult to identify ultrasonographically unless they are neoplastic and enlarged. Specifics of diagnosis of testicular/epididymal abnormalities by ultrasound are described in the respective sections of the text.

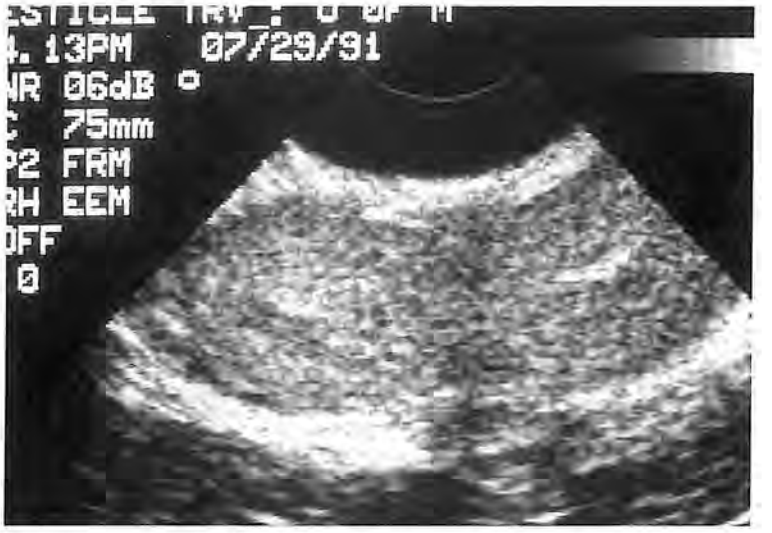

Figure 18–10. Sonogram of normal canine testes.

Torsion of the Spermatic Cord

Torsion of the spermatic cord historically has been referred to as testicular torsion. Torsion of the spermatic cord is the preferred term. Torsion varies from a loose 360-degree torsion to several tight revolutions.[26]

Incidence of torsion of the spermatic cord is unknown, but the condition is not commonly reported in reviews of testicular disease in dogs. A review of 28 cases reported in the literature yields mean and median ages at time of diagnosis of 5.9 and 8.0 years, respectively, with a range of 5 months to 10 years.[26,45–48,157] No breed predisposition has been described; in one review of nine cases of torsion of the spermatic cord, 4 of the dogs were boxers, but in a review of 13 cases, 4 of the dogs were Pekingese and only 1 was a boxer.[26]

Dogs with torsion of the spermatic cord usually have signs of an acute abdomen, including acute onset of abdominal pain, vomiting, abdominal distention, lethargy, anorexia, stiff gait, dysuria, hematuria, and pyrexia.[26,47,48,157] Swelling of the scrotum or inguinal area may or may not be painful.[26,157] Other signs reported in dogs with torsion of the spermatic cord may be related to concurrent testicular neoplasia, and include symmetrical alopecia and pendulous prepuce.[26] There are two reports of apparently asymptomatic torsion of the spermatic cord in the dog.[46,52]

Torsion of the spermatic cord is more common with retained than with scrotal testes. In one review of 13 cases of torsion of the spermatic cord, 11 involved abdominally retained testes and 1 involved an inguinally retained testis.[26] It has been hypothesized that intra-abdominal testes are more prone to torsion of the spermatic cord than are testes retained in the inguinal area or normally descended into the scrotum because of greater mobility of the testis within the abdominal cavity. Testes attached to torsed spermatic cords often are enlarged at the time of diagnosis. This enlargement may occur before the torsion; neoplastic testes are heavier and more pendulous, and may predispose to torsion of the spermatic cord. Of 28 cases reviewed in the literature, 10 (36 per cent) involved neoplastic testes.[26,45–48,157] Sertoli cell tumors, seminonas, and interstitial cell tumors have been described in affected testes.[26,46,48] Non-neoplastic testes are enlarged after torsion of the spermatic cord due to venous occlusion, edema, and inflammation.[48] Histologic descriptions of affected non-neoplastic testes describe ischemic necrosis, intratesticular hemorrhage, and epididymal edema.[26,45,47]

Presumptive diagnosis is based on clinical signs and presence of concurrent cryptorchidism in the dog. The enlarged testis may be palpable in the abdomen.[26,48,157] On ultrasound, there is uniform decrease in echogenicity in testes with a torsed spermatic cord.[139] Color flow Doppler ultrasonography may be used to demonstrate absence of blood flow to and from the affected testis. Definitive diagnosis requires exploratory surgery.

Treatment is surgical removal of the affected testis and spermatic cord. Because the condition is common in dogs with cryptorchidism, a heritable defect, bilateral orchidectomy is recommended. In a review of 13 cases of torsion of the spermatic cord, 2 animals died before surgery could be performed due to uremia in one case and circulatory failure and shock in the other; 1 died several hours after surgery due to severe anemia, and 10 (77 per cent) recovered.[26]

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Chapter 19

Disorders of the Canine Scrotum

Scrotal Hernia

The scrotum in the normal, intact, adult male dog is a pouch of skin containing two testes which are separated by a median partition of connective tissue. The layers of the scrotum, from external to internal, are skin, smooth muscle (dartos m.), external and internal spermatic fascia, the parietal vaginal tunic, vaginal cavity, and the visceral vaginal tunic, which closely apposes the tunica albuginea of the testis. The vaginal process, composed of the parietal and visceral vaginal tunics with central vaginal cavity, is an outpouching of the peritoneum, formed during testicular descent, which, in the normal male dog, occurs by 7 to 8 weeks of age. The vaginal cavity communicates with the peritoneal cavity at the deep inguinal ring in the dog.

Scrotal hernias are a variant of inguinal hernias in which abdominal contents pass through the inguinal canal and into the scrotum. Indirect scrotal hernias, in which abdominal contents pass into the vaginal process, are much more common than direct scrotal hernias, in which abdominal contents invested in a separate peritoneal outpocketing come to lie adjacent to the vaginal process.[1-3] Unilateral herniation is most common, but bilateral herniation has been reported.[1-3] Hypothesized predisposing causes of scrotal hernias include trauma and genetic factors.[1]

Scrotal hernias are uncommon, with reported incidence of 0.02 per cent.[4] Differential diagnoses for scrotal hernia include orchitis, testicular torsion, testicular or scrotal neoplasia, sperm granulomas, and trauma.[1,5]

Scrotal hernias occur in young male dogs; reported age at time of diagnosis ranges from 5 months to 4 years, and averages 1.73 years.[1-3,5] Scrotal hernias have been reported in both intact and castrated male dogs, and in purebred and crossbred dogs.[1-6]

The most prominent clinical sign of scrotal hernia in the dog is fluctuant to firm swelling of the scrotum, sometimes associated with ipsilateral inguinal swelling. The swelling may be persistent or intermittent, and may vary in size over weeks or months.[1,2,5,6] It may or may not be reducible through the inguinal canal,[3] and may or may not be painful. In scrotal hernias uncomplicated by entrapment and ischemia of herniated tissues, the dog may show no other abnormal signs.[2,3,5] If ischemia and necrosis of entrapped tissue are present, leukocytosis with a left shift, vomiting, anorexia, and scrotal pain may be present.[1]

Definitive diagnosis is made by careful palpation of the scrotum and inguinal areas. Abdominal contents may be palpated emerging from the superficial inguinal ring.[2,3] The testis and epididymis on the affected side may not be palpable due to excessive inflammation or presence of intrascrotal tenacious, serosanguineous fluid.[2,5] Contents of the scrotal sac may be identified by radiography or ultrasonography.[1,5] Nuclear scintigraphy has been reported as a technique to rule out testicular torsion by identifying normal blood flow through the spermatic cord.[5]

Treatment is surgical removal or replacement of herniated tissues.[1,2,6] An incision is made carefully over the hernial sac. Viable tissues are replaced within the abdomen if possible. Irreducible or nonviable tissues are resected if possible; if nonviable intestinal loops are resected, an abdominal incision may be necessary for anastomosis of viable portions of the intestine. The hernial ring is closed with mattress sutures. Redundant vaginal tunic is

trimmed and ligated close to the spermatic cord, and dead space obliterated by suturing the subcutaneous tissue to the fascia of the external oblique muscle with continuous sutures. Closure is routine.[1,2,6]

Concurrent castration may or may not be beneficial. Advantages of concurrent castration include removal of possibly damaged testes, prevention of fertility in a dog with a potentially heritable condition, and more complete closure of the inguinal ring after ligation and removal of the distal spermatic cord.[3] Fertility of dogs left intact is best evaluated more than 2 months after hernia repair to allow completion of one spermatogenic cycle in testes after intrascrotal temperature is returned to normal. Palpably abnormal testes (enlarged or small and soft) are unlikely to regain normal function.

Hydrocele

Hydrocele is a circumscribed collection of fluid in the vaginal process and along the spermatic cord. Composition of the fluid varies with underlying cause of the condition. In humans, hydrocele most commonly occurs secondary to orchitis, testicular neoplasia, or trauma.[7] In bulls, ascites and venous or lymphatic congestion are hypothesized causes.[8,9] Reported primary conditions in the dog include compromised lymphatic drainage due to testicular lymphosarcoma (Fig. 19–1), inguinal hernia, orchitis due to infection with *Blastomyces dermatitidis*, trauma, and testicular torsion.[10,11] Hydrocele in the dog also may be idiopathic.[10]

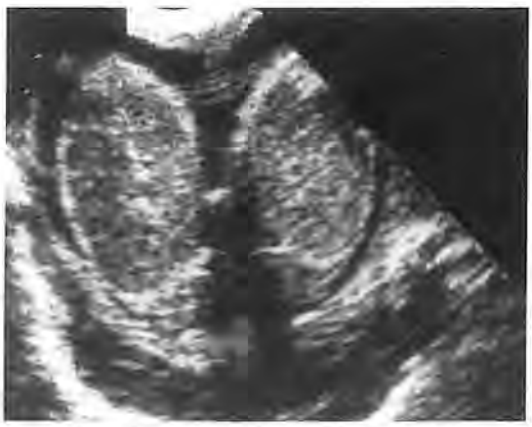

Figure 19–1. Dorsal plane scrotal sonogram of a 7-year-old German shepherd dog with a pendulous swollen scrotum, lymphadenopathy, and subsequent diagnosis of lymphosarcoma. Ultrasound showed a grossly thickened (edematous) scrotal wall and extratesticular fluid. (From Pugh CR, Konde LJ: Sonographic evaluation of canine testicular and scrotal abnormalities: A review of 26 case histories. Vet Radiol 32:243–250, 1991, with permission.)

Age and intact status of dogs presenting with hydrocele vary with the primary condition, as do concurrent clinical signs. Consistent clinical signs are turgid, non painful distention of the scrotum, and thickening of the scrotal wall due to edema.[10,11]

Palpation of the scrotum is not diagnostic for hydrocele due to the turgidity of the grossly distended scrotum. Scrotal contents should be imaged with ultrasonography.[10] Percutaneous drainage of the fluid may be performed blindly[11] or with ultrasound guidance, and may facilitate palpation or visualization of intrascrotal structures.

Treatment depends on the primary condition. There is one report of hydrocele secondary to inguinal hernia in a young dog that was successfully treated with surgical repair of the hernia and percutaneous drainage of intrascrotal fluid postoperatively.[11] A total of 135 ml of aseptic, serosanguineous fluid was drained percutaneously over 3 days before resolution.[11] In bulls, spontaneous resolution is reported to occur in 85 per cent of animals by 120 days after diagnosis, with return to normal semen quality and breeding function in 77 per cent of animals by 120 days.[8]

Scrotal Dermatitis

Scrotal dermatitis with varying degrees of pruritis and scrotal self-mutilation may occur as a component of infectious diseases, autoimmune disorders, mycotoxin ingestion, sperm granulomas, as a drug reaction, or as a direct or allergic reaction to environmental irritants. Inflammation of the scrotum may cause increased intrascrotal temperature and subsequent decreased fertility.

Infectious diseases reported to cause scrotal dermatitis in the dog include canine brucellosis and Rocky Mountain spotted fever.[12,13] Infection of male dogs with *Brucella canis* causes transient lymphadenopathy, followed by epididymitis sometimes associated with self-inflicted scrotal mutilation, and an increase in abnormal morphology of ejaculated spermatozoa by 5 weeks following infection.[12] Diagnosis and treatment of canine brucellosis are discussed in detail in Chapter 18. Rocky Mountain spotted fever is caused by transmission of *Rickettsia rickettsii* from infected ticks to susceptible dogs.[13] Clinical signs include depres-

sion, anorexia, fever, multifocal neurologic signs, petechiation due to profound thrombocytopenia, and scrotal dermatitis.[13] The condition is diagnosed serologically, with a single high titer or rise in titer in paired sera, using an indirect fluorescent antibody (IFA) test. Tetracycline therapy may be attempted, but prognosis is poor; in one study, three of five affected dogs died of systemic necrotizing vasculitis despite tetracycline therapy.[13]

Autoimmune disorders associated with scrotal dermatitis in the dog include pemphigus erythematosus and Vogt-Koyanagi-Harada–like syndrome.[14,15] Dermatitis and crusting are noted at mucocutaneous junctions and on the scrotum. Treatment with topical and systemic glucocorticoids and possibly azathioprine may be required to control pruritis and excessive inflammatory damage.[14,15] These medications may suppress spermatogenesis.

Miscellaneous reported causes of canine scrotal dermatitis include necrolytic dermatitis with associated hepatic changes due to mycotoxin ingestion,[16] self-mutilation and scrotal ulceration associated with presence of bilateral sperm granulomas,[17] and localized erythematous drug eruptions as an idiosyncratic reaction to prophylactic heartworm therapy with diethylcarbamazine.[18] Scrotal dermatitis resolved in all these dogs with treatment of the primary condition or removal of the offending feed or medication.

Two types of scrotal contact dermatitis are described in the dog. Allergic contact dermatitis is a cell-mediated (Type IV) hypersensitivity reaction. Variably pruritic, maculopapular dermatitis occurs after a sensitization period of 6 months to 2 years. Reported allergens include plant pollens and resins, topical insecticides including flea control products, shampoos containing tars and creosols, wool and nylon fibers, cleansers, polishes, and detergents.[19] Irritant contact dermatitis occurs when an offending substance causes cutaneous irritation without requiring an allergic response. Absolute primary irritants are corrosives that injure skin on first contact, and include petroleum distillates and disinfectants. Relative primary irritants require repeated contact to cause dermatitis and include soaps, detergents, solvents, lawn fertilizers, and road salt. Irritant contact dermatitis is characterized by intense pruritis and self-trauma, with erythema, crusts, and excoriation.[20]

Diagnosis of contact dermatitis requires identification of the offending substance. The cutaneous changes of contact dermatitis seen in skin biopsy specimens are not pathognomonic. Treatment involves removal of the offending substance and soothing baths. Intensely pruritic animals may benefit from treatment with topical or systemic glucocorticoids.[19,20]

Scrotal Neoplasia

Neoplasia of the canine scrotum occurs in the overlying dermis. The three most common types are squamous cell carcinoma, melanoma, and mast cell tumor.[21–23]

Squamous cell carcinoma appears as proliferative or ulcerative firm nodules on the legs, head, lips, and/or scrotum. This tumor is locally invasive but slow to metastasize. Primary therapy is wide surgical excision. Adjunctive therapy includes cryotherapy and radiotherapy.[21]

Melanomas appear as slow growing, small brown-black macules or rapidly growing, large (>2 cm diameter) black ulcerative masses in the oral cavity, or on the digits or scrotum. Lesions usually are solitary. Twenty-five to 50 per cent of scrotal melanomas are malignant and locally invasive. Wide surgical excision is the treatment of choice. Postoperative chemotherapy may be beneficial.[21]

Mast cell tumors appear as well circumscribed, raised, firm masses less than 3 cm in diameter that may be erythematous or ulcerated.[22] Mast cell tumors may arise virtually anywhere on cutaneous surfaces of the body; in one study, 22 per cent of male dogs with mast cell tumor had involvement of the scrotum.[23] Secondary systemic effects may be seen due to release of histamine, serotonin, heparin, and other bioactive products from metachromatic granules within tumor cells. Mast cell tumors are often locally invasive and may metastasize to regional lymph nodes or the lungs.[22,23] Wide surgical excision is the treatment of choice. Animals with mast cell tumors may benefit from presurgical treatment with antihistamines. Successful adjunctive therapies include cryosurgery, chemotherapy, and radiation therapy. Experimental immunotherapy also is described.[22]

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Chapter 20

Disorders of the Canine Prostate

Benign Prostatic Hypertrophy/Hyperplasia

The normal prostate in the intact male dog increases in weight, due to normal growth and glandular hyperplasia, for the first 1 to 5 years of the dog's life, with a peak in secretory function, as assessed by ejaculate volume and total ejaculated protein content, at 4 years of age.[1-3] As many as 16 per cent of dogs have been reported to have histologic evidence of benign hyperplasia (increase in cell number) and hypertrophy (increase in cell size) of the prostate by 2 years of age.[4-6] Further cystic hyperplasia and hypertrophy develop as the animal ages, with 50 per cent of dogs exhibiting histologic evidence of benign prostatic hypertrophy (BPH) by 5 years of age.[4] Senile involution of the prostate occurs in animals aged 11 years or more.[1]

Prostatic growth and secretion are modulated by 5α-dihydrotestosterone (DHT), a metabolite of testosterone (T) formed from the action of the (inhibitable) enzyme 5α-reductase.[7] DHT is the active androgen at the intracellular level, because it has a twofold greater binding affinity for the intracellular androgen receptor and a five times lower dissociation rate than T.[8] Although intraprostatic concentrations of DHT do not vary throughout the gland, epithelial cell morphology and response to DHT do vary with location in the gland; the epithelial cells proximal to the prostatic urethra are squamous to low cuboidal and are undergoing cell death; a deeper zone consisting of tall columnar cells is mitotically quiescent; and a distal zone is made up of tall columnar cells, which are undergoing active cell division.[9] Significance of this variation in cellular activity is unknown. Unidentified humoral and tissue factors act as modulators of epithelial cell growth.[10]

In aged dogs with BPH, there is an increase in the intraprostatic estrogen : androgen ratio. Some studies suggest that this is caused by declining concentrations of androgen in the presence of stable estrogen levels,[1] while others have demonstrated higher serum and intraprostatic concentrations of estrogen in older dogs with hyperplastic prostates compared to young dogs with normal prostate glands.[11] Prostatic weight is positively correlated with intraprostatic DHT concentrations, but increased intraprostatic DHT concentration is not demonstrated consistently in animals with BPH.[12] Other reports document increased ability of the BPH prostate to take up and metabolize steroids, with net formation of DHT and subsequent increased intraprostatic DHT concentrations.[13,14] Secretory function of the Leydig cells may be altered in dogs with BPH; decreased endoplasmic reticulum has been demonstrated in testicular androgen-producing cells of dogs with BPH, suggesting decreased androgen production.[6]

Experimental induction of BPH requires both estrogens and androgens.[15-18] Treatment with 17β-estradiol alone induces stromal and glandular hyperplasia, squamous metaplasia with subsequent decreased secretory function of metaplastic epithelial cells, and increase in number of prostatic intranuclear estrogen receptors.[16,17] Dogs treated only with an androgen, 3α-androstanediol, show mild glandular proliferation and histologic evidence of mild BPH.[18] Only dogs treated with both 17β-estradiol and 3α-androstanediol develop overt BPH, with squamous metaplasia and "florid" hypertrophy and hyperplasia of prostatic epithelial cells.[15,18] Hypothesized reasons for this

requirement for both hormones for induction of BPH include androgen-stimulated growth of prostatic epithelial cells damaged by metabolites of estrogen with free radical activity,[19] and enhanced sensitivity of the gland to estrogen-mediated changes due to a permissive rather than inductive role of elevated intraprostatic DHT concentrations.[12]

BPH is the most commonly diagnosed prostatic disease in the dog.[20,21] In the only retrospective study in which BPH was not the most commonly diagnosed prostatic disease, the authors required histologic confirmation of BPH by biopsy and disregarded a large number of dogs with apparent BPH from which no biopsy specimens were collected.[22] Incidence of BPH has been reported as 0.78 per cent.[21] There is no known breed predisposition.[21] This is a disease of older, intact male dogs, with a reported mean age at onset of clinical signs of 8.0 years of age.[21] Due to the hormone dependence of this condition, BPH occurs spontaneously only in intact dogs or in castrated male dogs treated with androgens.

Dogs with BPH may be asymptomatic.[22] Intraparenchymal cysts that communicate with the prostatic urethra may develop within the hypertrophied prostate.[1,23] Light yellow to sanguineous fluid from these cysts or frank blood from a hyperplastic gland with increased vascularity may appear as urethral discharge.[23] This serous to serosanguineous urethral discharge, unassociated with urination, is the primary presenting clinical sign in BPH; in one study, 20 of 28 dogs (71.5 per cent) with BPH presented with sanguineous urethral discharge as the sole clinical sign of disease.[21] Hematuria may be seen grossly, or noted on urinalysis.[21,22] Other signs are referable to increased size of the gland, and include rectal tenesmus, dysuria, caudal abdominal pain, and infertility.[22] Urinary tract signs occur in 27 per cent, and gastrointestinal signs in 9.1 per cent of dogs with BPH.[22] Systemic signs of disease are rarely reported.[22] In men, increased stromal proliferation around the prostatic urethra causes a primary clinical complaint of dysuria with pollakiuria.[24] This is rarely seen in dogs.

Diagnosis of BPH requires demonstration of prostatic enlargement and exclusion of other prostatic disorders associated with prostatomegaly, such as prostatitis or neoplasia. Complete blood count usually is normal.[21] Aerobic and anaerobic cultures of seminal fluid and urine are negative unless infection is superimposed on BPH.[21] In one report semen was collected by manual ejaculation in 69 per cent of dogs with BPH; hematospermia often was present.[25] If a sample cannot be collected by manual ejaculation, a prostatic wash may be necessary for collection of prostatic fluid. Prostatic epithelial cells also may be collected with a urethral brush technique, which has been shown to diagnose BPH cytologically in 64 per cent of dogs.[25] In this technique, a sterile microbiologic specimen brush within a double-sheathed catheter is passed into the urethra to the level of the prostate. The prostate is vigorously massaged per rectum for 1 minute. The specimen brush is advanced and retracted five to six times and withdrawn. The brush and any fluid collected are placed in sterile saline and centrifuged, and the pellet is examined cytologically.[25]

The canine prostate is palpable per rectum unless prostatomegaly is severe enough to cause cranial movement of the prostate into the abdomen. If this has occurred, simultaneously palpate the rectum and place upward pressure on the caudal abdomen or "wheelbarrow" the animal up to stand on his hind legs so as to push the prostate back within reach of the gloved finger within the rectum. The prostate usually is symmetrical and smooth, with a distinct dorsal median raphe.[21] The dog does not feel pain when pressure is placed on the normal or hypertrophied prostate.

On survey radiographs of the abdomen, the prostate with BPH may not be visible as a distinct entity, although cranial displacement of the bladder and dorsal displacement of the colon may be seen (Fig. 20–1). Retrograde cystourethrography may be used to demonstrate prostatomegaly and a normal urethra.[26] Reflux of contrast medium into the prostatic parenchyma usually is minimal in the normal prostrate; if reflux is present, it extends less than the measurement of the prostatic urethral diameter away from the seminal colliculus.[27] Contrast medium reflux may exceed the measurement of maximal urethral diameter in the dog with BPH. Prostatic ultrasound reveals prostatomegaly, with a homogeneous parenchyma with or without cavitating cystic lesion[28] (Fig. 20–2).

Presumptive diagnosis of BPH is based on signalment, history, physical examination, examination of prostatic fluid, and prostatic imaging. Definitive diagnosis of BPH may be made with biopsy of the gland, either trans- or perirectally, or transabdominally. Dilated acini surrounded by smooth muscle and fibrous connective tissue, and no inflammatory cells or organisms, are seen.[29] Prostatitis should be ruled out by quantitative culture of pros-

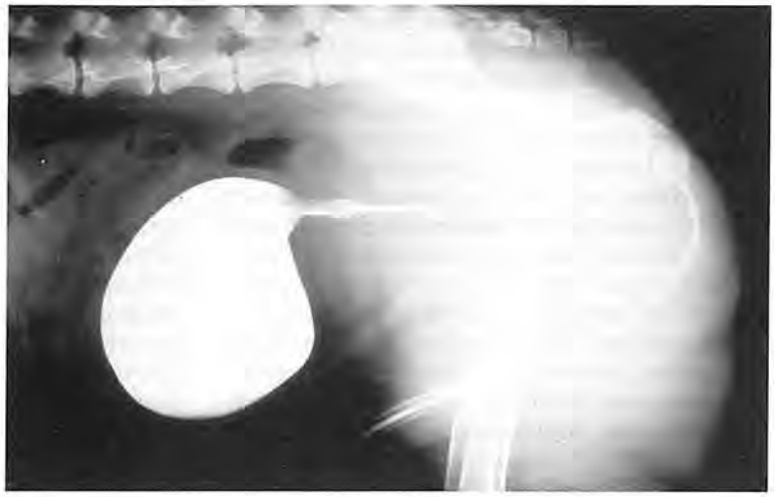

Figure 20–1. Retrograde cystourethrogram of canine prostate with benign prostatic hypertrophy (BPH). Note cranial displacement and flattening of the caudal edge of the urinary bladder.

tatic fluid collected by manual ejaculation or prostatic wash before the gland is biopsied. Biopsy rarely is required, because BPH is most often diagnosed by exclusion of other disorders.

Secretory proteins, measured in man as evidence of prostate disease, have not been used extensively in veterinary medicine. Human prostate-specific antigen (PSA) has not been consistently demonstrated in either serum or seminal plasma of normal dogs or dogs with prostatic disease.[30] Acid phosphatase measured in serum does not differ between normal dogs and dogs with prostatic disease.[30] Canine prostatic secretory esterase (CPSE), also called arginine esterase, is the primary secretory protein of the canine prostate, and is expressed 1000 times more from the prostate than from liver, muscle, kidney, or pancreas.[30,31] Concentrations of CPSE are higher in dogs with BPH than in normal dogs, but do not differ significantly between dogs with BPH, prostatitis, and prostatic neoplasia, perhaps because many dogs with the latter two disorders also have BPH.[30] These serologic tests are not used routinely for diagnosis of prostate disease in the dog at this time.

Dogs with BPH need be treated only if symptomatic.[32] One dog with clinical signs of BPH that was left untreated successfully impregnated bitches for 7 years after diagnosis.[21] However, the hypertrophied gland may be susceptible to ascending infection by normal urethral organisms (see Acute and Chronic

Figure 20–2. Sonogram of canine prostate with benign prostatic hypertrophy (BPH). The prostate is symmetrically enlarged and the prostatic parenchyma is homogeneous in echogenicity.

Prostatitis below), suggesting that treatment of BPH in valuable breeding animals may prevent progression of prostatic disease and subsequent subfertility.

Castration is the treatment of choice for BPH. No drug therapy has been demonstrated to be as effective as castration in decreasing prostatic size and subsequently decreasing clinical signs of BPH.[7] After castration, prostatic involution begins within days and is palpably obvious by 1 week.[23,32] The prostate decreases in volume by 50 per cent within 3 weeks, and by 70 to 75 per cent within 9 weeks of surgery.[23,33] Serum DHT concentrations fall by more than 98 per cent postsurgically.[33] In one study, eight of nine dogs with urethral bleeding secondary to BPH showed complete resolution of signs within 4 weeks of castration.[21]

Estrogenic compounds may cause a decrease in prostatic size by inhibiting gonadotropin secretion from the pituitary, and so may reduce clinical signs of BPH.[34] Although cellular mass will decrease within the prostate in estrogen treated dogs, cystic changes may not resolve.[34] Diethylstilbestrol (DES) at a dose of 0.2 to 1 mg/d per os (PO) for 5 days has been demonstrated to decrease prostatic secretory function for up to 2 months.[32,33] Use of injectable estradiol cypionate (ECP) at a dose of 0.1 mg/kg to a total dose of 2.0 mg also has been described.[32] An antiestrogen compound, tamoxifen, which has weak estrogenic activity, has been demonstrated to decrease glandular but not stromal proliferation in dogs with BPH.[17] Estrogen has been demonstrated to cause squamous metaplasia of the prostate gland and subsequent secretory stasis (see Squamous Metaplasia of the Prostate below), which may predispose the gland to ascending infection.[32] Thrombocytopenia, leukopenia, and fatal aplastic anemia may occur due to bone marrow suppression after repeated doses of estrogen, high doses of estrogen, or as an idiosyncratic reaction after a single dose.[34,35] Estrogens are not approved for use in dogs in the United States and are not recommended by the authors as a treatment for BPH.

Synthetic progestins also may exert negative feedback on the pituitary, decreasing gonadotropin release and serum T concentration.[36,37] Synthetic progestins include oral megestrol acetate, injectable medroxyprogesterone acetate, and implants of chlormadinone acetate.[34,36,38–41] Megestrol acetate, administered at a dose of 0.5 mg/kg day PO for 4 to 8 weeks, decreased prostatic size in one study.[34] One dog continued to receive 0.5 mg/kg weekly and showed no adverse effects.[34] Use of medroxyprogesterone acetate for canine BPH has been reported at doses of 3 to 4 mg/kg subcutaneously (SC), with subsequent doses administered at no less than 10-week intervals.[35,36] Resolution of clinical signs occurred in 84 per cent of dogs, and decrease in prostatic size occurred in 53 per cent of dogs by 4 to 6 weeks after therapy.[35,36] Side effects of progestin treatment include increased appetite in the early weeks after treatment in 31 per cent of dogs treated, and hypothyroidism or diabetes mellitus in 5 per cent of dogs treated.[36] Chlormadinone acetate, implanted subcutaneously at doses of 0.5 to 20 mg/kg for 1 to 26 weeks, has been shown to cause a dose-dependent decrease in prostatic size, with doses as low as 5 mg/kg causing a decrease of 52 to 61 per cent in prostatic size after 26 weeks of treatment.[38–40] Prostatic size returned to 74 to 85 per cent of pretreatment values by 22 weeks after the drug was withdrawn.[38] At high doses, chlormadinone acetate causes a decrease in total sperm number, progressive spermatozoal motility, and per cent morphologically normal spermatozoa.[38] Progestins are not approved for use in male dogs in the United States and are not recommended by the authors for treatment of BPH in the dog.

Antiandrogens have been described for treatment of canine BPH. Delmadinone acetate is an antigonadotropic and direct antiandrogenic compound that has been demonstrated to have little clinical efficacy in treating BPH, resulting in good or excellent results in only two of nine dogs treated.[21] TZP-4238 is an experimental steroidal antiandrogen that has been demonstrated to decrease prostatic size, intraprostatic concentrations of testosterone and DHT, and 5α-reductase activity, and to cause down-regulation of intracellular androgen receptors.[40] WIN-49596 is a steroidal androgen receptor antagonist. At doses of 0.625 to 40 mg/kg/d for 16 weeks, it has been shown to cause a dose-dependent decrease in prostatic size, increased incidence and severity of prostatic atrophy, and decreased prostatic secretory function as evidenced by decreased levels and activity of CPSE within the prostate.[41,42] WIN-49596 does not affect testicular weight or semen quality, although mild Leydig cell hyperplasia may occur at high doses.[42] Flutamide and hydroxyflutamide at doses of 5 mg/kg/d per os for 7 weeks cause decrease in prostatic size identifiable by ultrasound within 10 to 14 days of treatment, with statistically significant decrease in prostatic size by

47 days.[23,43] Flutamide also causes decrease in CPSE secretion and activity within the prostate.[41] Treatment with flutamide has not been demonstrated to cause changes in libido or sperm production.[23] Antiandrogen compounds are not approved for use in male dogs in the United States.

Azasteroids, compounds that inhibit activity of the enzyme 5α-reductase and therefore prohibit conversion of T to DHT, have been demonstrated to decrease prostatic size in humans and dogs with BPH.[7,44–51] The azasteroid best described is finasteride (Proscar; Merck, Rahway, NJ). Doses of 0.1 to 1.0 mg/kg/d per os, or one 5 mg tablet per os per day for dogs weighing 5 to 50 kg for 8 to 53 weeks have been described as effective.[7,44–51] Doses of 0.1 to 1 mg/kg/d (one 5-mg tablet PO daily for dogs weighing 5 to 50 kg) cause decline in serum DHT to baseline concentrations, and this is the authors' recommended dose.[48] Finasteride has been reported to cause a decrease in prostatic size of 48 to 70 per cent by 8 to 12 weeks of treatment, with microscopic evidence of atrophy of both the glandular and stromal compartments of the gland.[7,45,47,50] Optimum length of treatment is unknown. Prostate size has been shown to return to near pretreatment values by 6 to 8 weeks after the drug is withdrawn.[47]

Intraprostatic DHT concentration decreases by 70 to 85 per cent with anasteride treatment in dogs.[7,44,49] Serum concentrations of DHT were reported to decrease in dogs treated with low doses of finasteride (0.1, 0.25, or 0.5 mg/kg PO for 7 days).[48] Secretory function of the gland also may be decreased, as evidenced by a 50 per cent decline in PSA in men, and decreased intraprostatic CPSE concentrations and decreased semen volume in dogs treated with finasteride.[46,47,51].

No side effects have been reported with administration of this drug in the dog.[47] No changes have been demonstrated in testicular weight or histomorphology, daily sperm production, or fertility.[47,51] Although men are recommended to avoid unprotected intercourse with a woman pregnant with a male child while on this drug, due to concerns about absorption of the drug from seminal fluid and induction of birth defects in male fetuses, no puppies with visible abnormalities are reported to have been sired by dogs on this drug.[47] Finasteride is not approved for use in dogs in the United States.

Other modalities of treatment for canine BPH have been described in the literature but are not routinely used or recommended. Herbal extracts of various plants containing active phytosterols have been shown to mediate prostatic contractility in vitro, and so may be more useful in humans than in dogs.[52] Techniques involving destruction of the gland itself via transurethral high-intensity focused ultrasound,[53,54] phototherapy after sensitization with etiopurpurin dichloride,[55] electrovaporization,[56] ablation and coagulation with a rotating electrode,[57] and enzymatic digestion with injection of a collagenase/hyaluronidase solution have been reported.[58] Gossypol acetate at 20 mg/kg every other day for 1 month has been shown to prevent development of BPH in dogs treated with 17β-estradiol and 3α-androstanediol, and has been hypothesized to act as an antiandrogen.[59]

Prostatic Cysts

Diffuse cystic change associated with androgen-dependent BPH may occur in the dog. Diagnosis and treatment are as described for BPH.

True (retention) prostatic cysts and paraprostatic cysts are defined as cavitating lesions with a distinct wall, containing clear to turbid fluid, either within (retention) or outside (paraprostatic) the prostatic parenchyma. These are the cystic prostatic disorders described below.

The pathogenesis of cystic prostatic disorders is unknown. A hypothesized cause of retention cysts is dilation of prostatic acini secondary to squamous metaplasia induced by endogenous or exogenous estrogen compounds. Prostatic retention cysts occurring concurrently with an estrogen-secreting Sertoli cell tumor have been described.[60] Paraprostatic cysts usually arise either craniolateral to the prostate, displacing the bladder cranially and ventrally,[61,62] or caudal to the gland, within the pelvis,[61] and are hypothesized to be dilated embryonal remnants of wolffian ducts.[61,62]

Cystic prostatic disorders occur most commonly in older, large-breed dogs.[63,64] Reported mean age at time of diagnosis of paraprostatic cysts is 8.0 years.[64] In affected dogs that are symptomatic, presenting signs include lethargy and anorexia, abdominal distention, rectal tenesmus and straining to defecate, dysuria and intermittent bloody urethral discharge; in dogs with concurrent Sertoli cell tumor, signs of estrogen toxicity such as feminization and anemia may occur.[22,60–66] In one study, signs of urinary tract disease were reported in 26 per cent, gastrointestinal signs in 37 per cent, and

systemic signs of disease in 48 per cent of dogs with cystic prostatic disease.[22]

Dogs with cystic prostatic disease may be asymptomatic; in one study of large-breed dogs (mean weight = 31 kg) with average age of 7.9 years and presenting with signs other than those of prostatic disease, 14 per cent had ultrasonographically identifiable prostatic cysts, and 42 per cent of those cysts contained aerobic bacteria.[67]

On physical examination, the enlarged prostate may be palpable per abdomen.[62] The enlarged and frequently asymmetrical prostate may not be palpable per rectum if the increased weight of the prostate at the urinary bladder neck has pulled the bladder into the abdomen. There is one report of cystic prostatic disease contributing to a perineal hernia.[65] Hematuria and slight proteinuria may be present on urinalysis.[62]

Definitive diagnosis is possible with radiography and ultrasonography. Prostatomegaly is visible on survey abdominal radiographs and on retrograde cystourethrography, with prostatomegaly defined as prostate diameter greater than 70 per cent of the distance from the cranial aspect of the pubic bone to the sacral promontory.[26] Although degree of prostatomegaly is not well correlated with specific type of prostatic disease, extreme prostatomegaly is more common in cystic prostate disease and prostatic neoplasia, and asymmetry is most common with focal disease, such as cystic prostatic disease, neoplasia, and prostatic abscessation.[26] Intramural mineralization often is present and may be visible on radiographs, especially with paraprostatic cysts.[61–63,68] Ultrasonographically, cystic prostatic disorders in the dog are visible as discrete hypoechoic or anechoic lesions that may demonstrate distant enhancement.[28,63,64] Echogenic sediment may be visible within the cyst.[63] Internal septa may also be visualized.[64] Distention of the urinary bladder with saline may be required to differentiate a large cyst from the normal fluid-filled bladder[63] (Fig. 20–3).

Medical therapy for prostatic cysts has not been described. Types of surgical intervention include cyst drainage, cyst resection with or without drain placement, marsupialization, and partial prostatectomy.[60,66,69–72]

Simple drainage of the cystic structure has been described[60] but usually is not recommended because the continuing presence of the well-defined capsule allows the cyst to recur. Large solitary cysts may be resected.[71,72] The difficulty of cyst resection surgery is dependent on size of the cyst and its adherence to surrounding structures.[71] Packing of the residual cyst cavity with omentum may help prevent recurrence,[72] as may placement of drains.[69] The prostate is isolated with laparotomy sponges and the cystic area opened and drained. Penrose drains are placed within the cyst cavity and extended so as to exit through the ventrolateral abdominal wall.[69] Urinary incontinence has been reported to occur in 9 to 28 per cent of dogs immediately after cyst resection,[71,72] although many of these animals go on to recover spontaneously or are well controlled with phenylpropanolamine therapy.[72] Other reported complications include edema of the inguinal area and/or hindlimbs, hypoproteinemia, hypoglycemia, hypokalemia, anemia, and urethral fistulization.[69]

Marsupialization of large solitary cysts allows drainage and subsequent collapse of

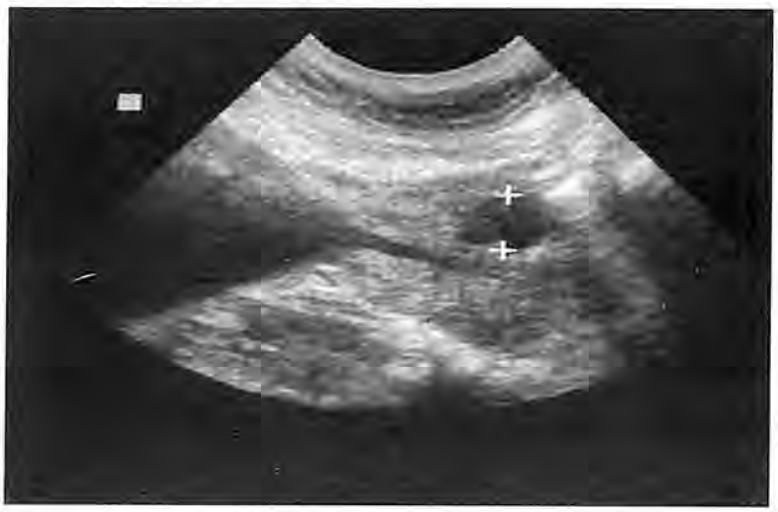

Figure 20–3. Sonogram of canine prostate with true (retention) cyst. Overt prostatomegaly is not present. The cyst is visible as a discrete, hypoechoic lesion within the parenchyma.

the capsule. The overlying skin and musculature are resected, the cyst capsule opened, and the edges of the capsule sutured to the skin.[69] The stoma remains patent, and is allowed to drain, for 1 to 2 months.[69] Disease may recur if the capsule persists.[69]

Partial prostatectomy may be required for resolution of large retention cysts. Ventral and lateral aspects of the prostate gland are resected as necessary, taking care to avoid the prostatic urethra.[69] Complications are those involving the urethra, including urine leakage at the urethra and urinary incontinence.[69] A technique using an ultrasonic aspirator to resect cystic prostatic tissue has been described.[70] In 20 dogs treated with this technique, no return of clinical signs of cystic prostatic disease or cystic prostatomegaly were noted within 12 months of surgery, and only five dogs showed the persistent complication of urinary incontinence, with that being intermittent in all five animals.[70]

Castration or finasteride therapy should be performed before or concurrently with these other procedures. Effect of castration on resolution and recurrence of cystic prostatic disease is not known.

Squamous Metaplasia of the Prostate

Squamous metaplasia of the canine prostate is both a morphologic and physiologic change which is a component of other progressive prostatic disorders. The normal low cuboidal to tall columnar prostatic epithelial cells are altered to form concentric circles of flattened cells toward the center of the prostatic acini.[29] The acini often contain eosinophilic debris and polymorphonuclear cells.[29] These metaplastic cells are not metabolically active, leading to secretory stasis within the gland.

Squamous metaplasia occurs secondary to exogenous or endogenous estrogen exposure. In one survey, 67 per cent of the cases identified were due to administration of estradiol cyclopentylpropionate.[29] Squamous metaplasia also may occur in the presence of functional Sertoli cell tumors that secrete estrogen.[18,29,73,74]

Dogs with squamous metaplasia may be asymptomatic, but, as the altered gland is predisposed to ascending infection, presenting signs may be those of prostatitis (see Acute and Chronic Prostatitis below).

Definitive diagnosis requires prostatic biopsy. Caution must be employed in collection of biopsy specimens from a potentially infected gland. Collection of exfoliated metaplastic cells with the urethral brush technique described previously has not been shown to be uniformly successful.[25]

Acute and Chronic Prostatitis

Prostatitis is inflammation of the prostate gland. Both septic and nonseptic inflammatory prostate disease are described in humans, but in the dog bacterial prostatitis is the most common type seen.[75] Prostatic infection may progress to development of intraparenchymal abscesses.

Dogs may be predisposed to infection of the prostate by increased number of bacterial organisms in the periprostatic urethra, compromise of host local immunity, disease of the urinary tract, or altered prostatic tissue or fluid.[76] Examples of prostatic defense mechanisms include micturition, urethral pressure, and local production of IgA and antibacterial factor, a zinc-associated protein.[38] Examples of alterations in prostatic tissue include BPH, cystic disorders, squamous metaplasia, and prostatic neoplasia.[75]

A diagnosis of lymphoplasmacytic prostatitis was made histologically after experimental induction of bacterial prostatitis with *Escherichia coli*.[77] Prostatic abscesses form as purulent material accumulates in pockets within the chronically inflamed prostatic parenchyma.[38]

Infection develops most commonly due to ascension of normal urethral flora, although hematogenous spread or extension from the testes, epididymes, or peritoneal cavity may occur.[76] Seventy per cent of cases reported have been due to infection with a single organism.[76] Aerobic organisms predominate as pathogens in this condition, with *E. coli* the most common organism reported.[6,76,78] Other aerobic organisms reported include *Staphylococcus* sp., *Streptococcus* sp., *Proteus mirabilis*, *Klebsiella* sp., *Enterobacter* sp., *Haemophilus* sp., *Pseudomonas* sp., and *Pasteurella* sp.[22,76,78] Prostatitis due to infection with *Brucella canis* has been reported.[76] Anaerobic infection is rare but does occur.[75] *Mycoplasma* sp. are frequently cultured from dogs with prostatitis.[76,78] *Blastomyces dermatitidis* is reported as a fungal cause of prostatitis in dogs.[22,75,79]

Prostatitis may occur secondary to diseases of the prostate of intact dogs, such as BPH, or secondary to diseases of intact or neutered

dogs, such as prostatic neoplasia. Therefore, it is identified in both castrated and intact dogs but is much more common in intact dogs. Age at onset of disease varies with underlying cause of the infection.

Clinical signs of prostatitis vary with progression of the disease. Dogs with acute prostatitis may be febrile, anorexic, and lethargic, and have urethral discharge.[76] The dog may feel pain when the prostate is palpated per rectum.[76] Dogs with chronic prostatitis may be asymptomatic, or may have hematuria, lethargy, anorexia, straining to defecate, poor semen quality, or urethral discharge varying in character from clear to bloody to purulent.[22,76] In one study, 26 per cent of dogs diagnosed with prostatitis showed signs of urinary tract disease, 37 per cent showed gastrointestinal signs, and 48 per cent showed signs of systemic disease.[22] Dogs with chronic prostatitis usually do not feel pain on rectal examination of the prostate.[76] Dogs with prostatic abscessation may have fever and caudal abdominal pain,[80] or signs of peritonitis and septic shock if an abscess ruptures into the peritoneal cavity. One dog developed signs of a perineal hernia subsequent to rupture and fibrous adhesion of a prostatic abscess into the perineal area.[81] Prostatomegaly may or may not be present in dogs with prostatitis, depending on the underlying prostatic disease present and the presence or absence of abscesses.

A neutrophilia with left shift usually is present in dogs with acute prostatitis.[76] The hemogram may be normal in dogs with chronic prostatitis.[76] Dogs with experimentally induced prostatitis did not show a change in blood leukocyte number with development of prostatic disease.[82] Seventy-five per cent of dogs with prostatic abscesses are reported to exhibit neutrophilia in a complete blood count.[80]

Serum chemistry profile results do not vary predictably in dogs with prostatitis.[76] In one study, 35 per cent of dogs with prostatic abscessation showed abnormalities on a serum chemistry profile, with elevation of alkaline phosphatase the most common abnormality noted; significance of this finding is unknown.[80]

Urinalysis should be performed on a sample collected by antepubic cystocentesis from dogs with prostatitis. Since prostatic fluid is secreted constantly and normally drains into the urinary bladder, hematuria, pyuria, and bacteriuria are present in dogs with either acute or chronic prostatitis.[76] Urinalysis of dogs with well-walled-off prostatic abscesses may be normal. Urine cultures are not diagnostic for prostatitis, but may be used to localize inflammatory disease, as described below.

Survey abdominal radiographs and retrograde cystourethrography may reveal prostatomegaly, mineralization, and reflux of contrast medium into the prostatic parenchyma in dogs with prostatitis.[26,27,83] Prostatomegaly is more severe in dogs with prostatic abscessation, as is asymmetry of the gland.[26] Prostatic (mineralization) is more common in dogs with chronic prostatitis than in those with acute prostatitis, and also may be seen in dogs with prostatic neoplasia.[26] Irregular reflux of contrast medium into the prostatic parenchyma farther than one width of the prostatic urethra is more commonly seen in dogs with prostate infection than in dogs with BPH, but no good correlation exists between extent of reflux and type or extent of disease.[83] Ultrasound of the infected prostate reveals focal or diffuse hypoechoic changes, giving the parenchyma a mottled or "moth-eaten" appearance[28]; occasionally, with chronic prostatitis, diffuse slightly hyperechoic appearance of the prostate may be observed on ultrasound, which can be distinguished from the ultrasonographic appearance of a neoplastic prostate, which has mottled hyperechoic regions. Ultrasonographic changes have not been shown to be well correlated with results of culture of prostatic tissue.[84] Abscesses are visible as discrete hypo- or anechoic lesions with or without distant enhancement[12] (Fig. 20–4). Abscesses cannot be differentiated from prostatic cysts by ultrasound alone.

Changes in semen quality may occur in dogs with prostatitis, depending on the primary disease present and duration of infection. Five weeks after induction of prostatitis with *E. coli*, no changes were noted in progressive motility, sperm concentration, or percentage morphologically normal spermatozoa of canine semen samples.[85] The testes of these dogs were histologically normal.[85] Prostatic fluid pH, specific gravity, and cholesterol and zinc concentrations did not differ between normal dogs and dogs with experimentally induced prostatic infection.[82,86] However, most dogs with spontaneous chronic prostatitis show decreased progressive motility and increased secondary morphologic abnormalities of spermatozoa, which return to normal after specific antibiotic therapy. This suggests that deposition of infected prostatic fluid into the semen

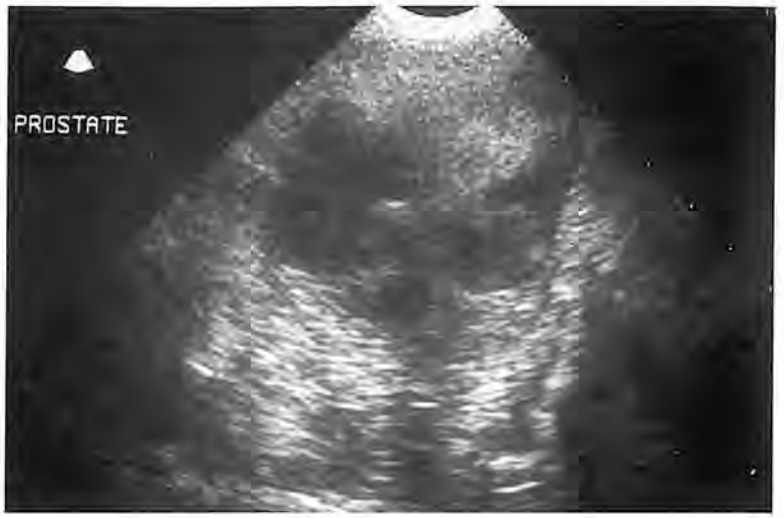

Figure 20–4. Sonogram of canine prostate with abscessation. Note prostatomegaly and presence of multiple discrete hypoechoic lesions.

at the time of ejaculation is detrimental to fertility.

Definitive diagnosis of prostatic infection includes culture of the causative organisms from prostatic fluid or tissue. As retrieval of tissue by fine-needle aspirate for biopsy is contraindicated in the presence of active infection, because of the potential of creating a septic needle tract, other samples must be evaluated by cytologic examination and quantitative aerobic culture to infer presence of prostatic infection. Prostatic fluid has been reported to be successfully collected by manual ejaculation in 29 to 33 per cent of dogs with prostatitis, and this percentage is higher with chronic prostatitis.[25] Fractionation of the ejaculate to allow evaluation of prostatic fluid alone may be performed; however, prostatic fluid is ejaculated into all three fractions of the semen, so the entire semen sample may be evaluated if fractionation is difficult. In dogs too painful to ejaculate or in castrated dogs, samples should be collected by prostatic wash, or with the urethral brush technique previously described. Caution should be taken when manipulating a gland that may contain a prostatic abscess, as too vigorous manipulation may cause abscess rupture.

Collection of prostatic fluid by any technique is not a sterile procedure, due to the presence of normal aerobic bacterial flora in the urethra of dogs.[87] Significant aerobic bacterial growth in dog semen has been defined as more than 10,000 bacteria per milliliter of a single organism[88] or greater than 2 $\log_{10}$ organisms in prostatic fluid compared to concurrent urethral culture or culture of urine collected by antepubic cystocentesis.[78,84] With the latter technique, 26 per cent of dogs so defined were asymptomatic for prostate infection in one study.[78] If it is difficult to differentiate urinary tract disease from prostatic infection, pretreatment with ampicillin, an antibiotic that is excreted in urine but penetrates the prostate poorly, for 24 hours before sampling, may permit collection of a prostatic fluid sample for quantitative culture that contains fewer artifactual organisms.[82] This technique may not work in dogs with acute prostatitis, because ampicillin may more readily diffuse into the acutely inflamed prostate than into the normal prostate. Quantitative culture of prostatic fluid collected by ejaculate or prostatic massage has been demonstrated to have an 80 to 100 per cent correlation with culture of prostatic tissue.[77,82,84]

Cytologic evaluation of prostatic fluid in dogs with prostatitis has been shown to have a greater than 80 per cent correlation with histologic evidence of inflammation.[82] Prostatic fluid from dogs with prostatitis contains many polymorphonuclear cells.[88] Cytologic evaluation of prostatic fluid is poorly correlated with culture of prostatic fluid,[82] however, and so should not be used as a sole diagnostic test for prostatitis in the dog. Samples collected with the urethral brush technique successfully identified prostatitis in 66 per cent of the affected dogs sampled[25]; the technique had a sensitivity of 69 per cent and specificity of 92 per cent.[89]

The danger of collection of canine prostatic tissue by fine-needle aspirate or biopsy lies in the risk of inducing an infected needle tract if the tissue is septic. Quantitative culture and cytologic examination of prostatic fluid, and treatment with an appropriate antibiotic, if

needed, should precede these procedures. Inflammatory cells are present within the prostatic parenchyma of infected dogs.[29] Successful diagnosis of prostatitis by fine-needle aspirate has been reported in 70 per cent of infected dogs.[90]

Treatment of prostatic infection includes appropriate use of antibiotics, chosen by culture and sensitivity of prostatic fluid, that achieve intraprostatic concentrations greater than the minimum inhibitory concentration (MIC) of most pathogens.[76] In acute prostatitis, the blood-prostate barrier is disrupted and virtually any antibiotic will penetrate the prostatic tissue.[76] Treatment should be instituted with an antibiotic chosen by culture and sensitivity, and continued for 4 to 6 weeks. Prostatic fluid should be rechecked by quantitative aerobic culture after 7 to 10 days and again 30 days after antibiotic therapy is concluded to ensure clearance of infection.[76]

In chronic prostatitis, the prostatic capsule is intact, and antibiotics may penetrate the prostatic parenchyma less readily.[76] Characteristics of an antibiotic that allow good penetration of the prostrate include high lipid solubility, pK_a allowing diffusion of the nonionized form of the drug across the lipid membrane, and low protein binding.[76]

Examples of drugs with poor lipid solubility include ampicillin, cephalosporins, oxytetracycline, and the aminoglycosides.[76] Antibiotics that are highly lipid soluble include chloramphenicol, erythromycin, trimethroprim, and the fluoroquinolones, ciprofloxacin and enrofloxacin (Table 20–1).[76,91]

In general, the tissue of the prostate gland is more acidic than blood and prostatic interstitium.[76] Weakly alkaline antibiotics, with a high pK_a, diffuse readily across the prostatic capsule in the nonionized form, ionize within the acidic prostatic tissue, and remain trapped within the capsule.[76] Drugs with high pK_a include trimethoprim, which is effective against gram-negative organisms, and clindamycin and erythromycin, which are effective against gram-positive organisms.[76] The fluoroquinolones are zwitterions, having multiple pK_as, which allow them to diffuse into the prostate regardless of intra- and periprostatic pH.[76,91] Enrofloxacin, at a dose of 5 mg/kg per os twice daily, has been shown to concentrate in prostatic tissue at concentrations well above the MIC of most prostatic pathogens.[91] Ciprofloxacin, at a dose of 10 mg/kg per os twice daily, has been shown to effect 100 per cent recovery in animals with prostatic infection, with no side effects noted[92] (Table 20–1).

Dogs with systemic signs of disease secondary to acute prostatitis or prostatic abscessation may require empiric antibiotic therapy while culture results are pending. The authors recommend enrofloxacin (5 mg/kg twice daily PO) to control infection with gram-negative organisms and *Mycoplasma* sp. plus ampicillin (20 mg/kg three times daily PO) to control infection with gram-positive organisms and anaerobic organisms. Antibiotic therapy should be altered as necessary when culture and sensitivity results become available.

Animals with prostatitis should be treated with appropriate antibiotics for 4 to 6 weeks.[76] Prostatic fluid should be rechecked with quantitative aerobic culture 7 to 10 days and again 30 days after antibiotics are withdrawn. If the infection is resistant to appropriate antibiotic therapy, low-dose chronic antibiotic therapy may be considered.

Owners must be cautioned of the side effects of long-term antibiotic therapy. Resistant organisms may develop if broad-spectrum antibiotics are used indiscriminately. Long-term treatment with trimethoprim-sulfadiazine has been associated with keratoconjunctivitis sicca, reversible decline in thyroid function, sulfadiazine urolithiasis, immune arthropa-

Table 20–1. Characteristics of Antibiotics for Treatment of Canine Prostatitis

Antibiotic	Lipid Solubility	Diffusion into Prostate	Effective Against
Ampicillin	Poor	Poor	Anaerobes
Cephalosporins	Poor	Poor	N/A
Oxytetracycline	Poor	Poor	N/A
Aminoglycosides	Poor	Poor	N/A
Erythromycin	Good	Good	Gram-positive
Chloramphenicol	Good	Good at high dose	Gram-positive and -negative
Trimethoprim	Good	Good	Gram-negative
Ciprofloxacin	Good	Good	Gram-positive and -negative
Enrofloxacin	Good	Good	Gram-positive and -negative

Adapted from Barsanti JA, Finco DR: Canine bacterial prostatitis. Vet Clin North Am 9:670–700, 1979, with permission.

thy, liver disease, and anemia due to folate deficiency.[93-96] Anemia may be ameliorated with supplementation of folic acid, 5 mg/d PO.[93]

Concurrent treatment for prostatic disorders also may help resolve infection. Castration of dogs with experimentally induced bacterial prostatitis shortened mean duration of infection by 5.3 weeks, compared to sham-operated controls.[77] Veterinarians are encouraged to pretreat dogs with infected prostrates with antibiotics for several days prior to castration in order to prevent development of scirrhous cords. Medical treatment with finasteride to decrease prostatic size may be beneficial in valuable breeding animals.

Successful treatment for prostatitis caused by *Blastomyces dermatitidis* with ketoconazole (20 mg/kg once daily for 60 days) and amphotericin B (0.5 mg/kg intravenously [IV] for 3 days) has been reported.[79] Eradication of fungal infection from the prostate can be achieved with these or newer antifungal pharmaceuticals.

Prostatic abscesses cannot be treated with antibiotic therapy alone, as drugs diffuse poorly through the abscess capsule; if the capsule is not resected it may serve as a pocket for recurring fluid accumulation. Culture of purulent fluid from within the abscess should be performed at the time of drainage. Antibiotics generally effective against gram-positive organisms include erythromycin, clindamycin, trimethoprim, and chloramphenicol.[23] Antibiotics generally effective against gram-negative organisms include enrofloxacin, trimethroprim, and chloramphenicol.[23] Chloramphenicol is highly protein bound and so must be administered at the high end of the dose range.[34] Treatment of choice for prostatic abscessation is induction of prostatic involution by castration or finasteride treatment with concurrent antibiotic therapy and subsequent surgical treatment if necessary.

Surgical correction of prostatic abscessation has been reported, but is associated with a high percentage of adverse sequelae in treated dogs. Techniques used include marsupialization, placement of multiple Penrose drains, drainage with omentalization of the cavities remaining, and partial prostatectomy.[69,70,80,98,99] Marsupialization involves resection of the skin and musculature overlying the prostate, lancing and draining of the abscess, and suturing of the edges of the prostatic capsule to the skin to create a stoma through which drainage may continue for weeks to months.[69] Complications include inadequate drainage and recurrence of abscessation.[69]

Placement of Penrose drains (two to eight) into the drained abscess cavity and periprostatically, with extension of the drains through the ventrolateral abdominal wall, has been associated with many short- and long-term complications, including urinary incontinence; recurrent abscessation; hypoproteinemia; edema of the scrotum, prepuce, and hindlimbs; and anemia.[98] Twenty-three of 92 animals treated with this technique died during surgery or in the immediate postoperative period, with most succumbing to sepsis and shock.[98] A modified technique, in which no drains are placed that penetrate the dorsal or dorsolateral capsule of the prostate, was associated with greater success, with only 3 of 17 dogs showing short-term complications and 100 per cent of the owners reporting good or excellent results at 1 year following treatment.[99]

Drainage with subsequent packing of the abscess cavity with omentum also has been described as a successful surgical technique.[80] In a study involving 20 dogs, 19 of 20 showed complete resolution of disease after treatment, with the remaining dog developing recurrent abscessation.[80] Mortality in that study was zero per cent.[80]

Partial prostatectomy with an ultrasonic aspirator, when used concurrently with castration and appropriate antibiotic therapy, is reported as a successful therapy for prostatic abscessation in the dog.[70] Of 20 dogs treated, only 5 showed any side effects, that being minor, intermittent urinary incontinence.[70]

Nonsurgical drainage of prostatic abscesses with ultrasound guidance, while attractive as a less invasive mode of therapy, is not recommended. Persistence of the capsule may more readily allow recurrence of disease, completeness of drainage is difficult to assess, and seeding of the infectious material may occur along the needle tract.

Prostatic Neoplasia

The most common prostatic neoplasm reported in the dog is malignant adenocarcinoma.[23] Extension of transitional cell carcinoma from the urinary tract, and metastasis of lymphosarcoma, hemangiosarcoma, and squamous cell carcinoma to the prostate also have been reported.[23] One case of benign prostatic adenoma has been reported, but there was

some debate as to whether this was truly a neoplasm or a nodular hyperplasia.[100]

In humans, a histologic change called prostatic intraepithelial neoplasia is a precursor to prostatic carcinoma.[101] Prostatic intraepithelial neoplasia has been identified in 66 per cent of dogs with prostatic adenocarcinoma, and in 55 per cent of intact dogs 7 years of age or greater.[102] Significance of this histologic change as a precursor to neoplasia in dogs is unknown.

Prostatic carcinoma is the most common prostatic disease in castrated dogs.[6] In dogs, prostatic neoplasia is considered to be hormonally independent, or to be primarily influenced by nontesticular steroids.[103] Administration of estrogen and androgen to normal dogs induces changes in glycoprotein moieties on prostatic epithelial cells that are the same changes seen in cells that have undergone spontaneous neoplastic transformation.[104] However, several studies have demonstrated an interval of 3 or more years between castration and diagnosis of prostatic carcinoma.[103,105] Studies report either no association between intact status and occurrence of neoplasia,[6] no "sparing effect" of castration on risk of developing prostatic neoplasia,[103] or a 2.38-fold risk of developing prostatic carcinoma in castrated over intact male dogs.[104] Prostatic carcinoma has a reported incidence of 0.29 to 0.60 per cent.[106]

Other prostatic diseases in the dog do not predispose the prostate to neoplastic change. There is one report in the literature of a dog with infected prostatic cysts and abscesses that subsequently developed prostatic adenocarcinoma, but no cause-and-effect relationship was demonstrated.[107] No breed predisposition to prostatic adenocarcinoma has been reported, although there is increased prevalence among medium- to large-sized dogs in general.[106]

In humans, environmental chemicals that have hormonal activity or act as endocrine disrupters are theorized to cause preneoplastic or overtly neoplastic changes in many systems, including the reproductive tract. Significance of environmental chemicals in pathogenesis of spontaneous prostatic neoplasia in the dog is unknown.

Signs of prostatic carcinoma are referable both to the increased size of the gland and subsequent pressure on periprostatic structures, and to metastases. Presenting signs of disease include tenesmus, constipation and dyschezia, stranguria and hematuria, weight loss, neck pain, and ataxia.[22,105,108] Urinary tract signs have been reported in 61 per cent of dogs with prostatic carcinoma, gastrointestinal signs in 31 to 35 per cent, specific rectal/colonic signs in 45 per cent, systemic signs in 23 per cent, and hindlimb weakness in 7.7 per cent.[22,105] Forty-five per cent of dogs with prostate cancer have been reported to have palpable prostatomegaly; 32 per cent of dogs have significant asymmetry of the enlarged prostate.[105] The affected dog may exhibit pain on either rectal or abdominal palpation of the prostate and surrounding structures.[105,108]

Leukocytosis and neutrophilia were reported in 14 of 27 dogs with prostatic adenocarcinoma, with 11 of those dogs showing a left shift.[105] It was not reported whether these dogs had evidence of prostatitis secondary to the neoplasia. The most common change noted on serum chemistry profiles of affected dogs is elevated alkaline phosphotase, reported in 70 per cent.[105] Pyuria (62 per cent) and hematuria (66 per cent) also are observed.[105] Recognition of atypical cells in the urine or semen sediment occurred in only 4 of 24 dogs.[105] Because many of the affected dogs are castrated well before onset of disease, successful collection of an ejaculate for evaluation of prostatic fluid may not be successful.[25]

Prostatomegaly may be identified on survey abdominal radiographs and by retrograde cystourethrography, but, as with other prostatic diseases, degree of prostatomegaly is not well correlated with type of prostatic disease.[26,105] Mineralization of the neoplastic prostatic parenchyma may be visible.[26,105] Irregular or extensive reflux of contrast medium into the prostatic parenchyma may be noted.[83,105] If the urethra is involved in the neoplastic process, mural discontinuity or compression of the prostatic urethra may be seen[105] (Fig. 20–5).

Ultrasonographically, the neoplastic prostatic parenchyma contains focal to diffuse hyperechoic areas, suggestive of mineralization[28,105] (Fig. 20–6). This change has been reported in 67 per cent of dogs with prostatic carcinoma.[105] Fifty-eight per cent of dogs with prostatic carcinoma have an irregular and/or discontinuous prostatic contour[105] (Fig. 20–5).

Definitive diagnosis requires identification of neoplastic prostatic cells. Collection of prostatic fluid by manual ejaculation is desirable, but usually is not successful.[25] Prostatic fluid may be collected by prostatic wash; however, absence of exfoliated neoplastic cells on cytologic examination of prostatic fluid does not rule out prostatic carcinoma. Prostatic adeno-

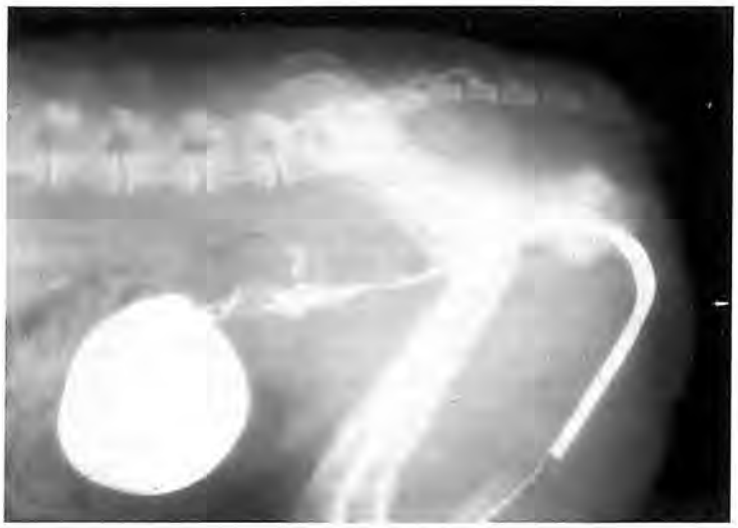

Figure 20–5. Retrograde cystourethrogram of canine prostate with adenocarcinoma. Note cranial displacement and flattening of the caudal edge of the urinary bladder, reflux of contrast medium into the prostatic parenchyma, and mural discontinuity of the urethra.

carcinoma was diagnosed correctly in 75 per cent of dogs from which prostatic cells were collected with a urethral brush technique.[25]

Collection of prostatic cells by fine-needle aspiration has been shown to correctly diagnose prostatic carcinoma in 79 to 80 per cent of dogs.[90,105] Inadequate sample size and inability to aspirate cells from a gland with focal disease contribute to this technique's inaccuracy.[105] Fine-needle aspiration accompanied by prostatic biopsy has been shown to correctly diagnose 89 per cent of dogs with prostatic carcinoma.[90] Neoplastic prostatic epithelial cells are polygonal in shape, with large vesicular nuclei and prominent nucleoli.[29]

Metastasis usually has occurred by the time of diagnosis of this highly malignant neoplasm. Reported sites of metastasis, in order from most to least common, are the lungs, regional lymph nodes, liver, urethra, spleen, colon and rectum, urinary bladder, bone, heart, kidney, distant lymph nodes, and adrenal glands.[23,105,108,109] Radiography is not a definitive test for identification of metastatic disease. One necropsy study reported metastases in the lungs of 40 per cent of dogs that had had no visible metastases on thoracic radiographs.[105] Small lung metastases may be difficult to differentiate from normal aging changes in the lungs of older dogs.[105] In another study, bony metastases were not identified on radiographs but were noted in multiple sites, including the axial skeleton, ribs, humerus, and femur, by scintigraphy.[108]

In humans, the concentration of the secretory protein PSA is used to screen for prostatic

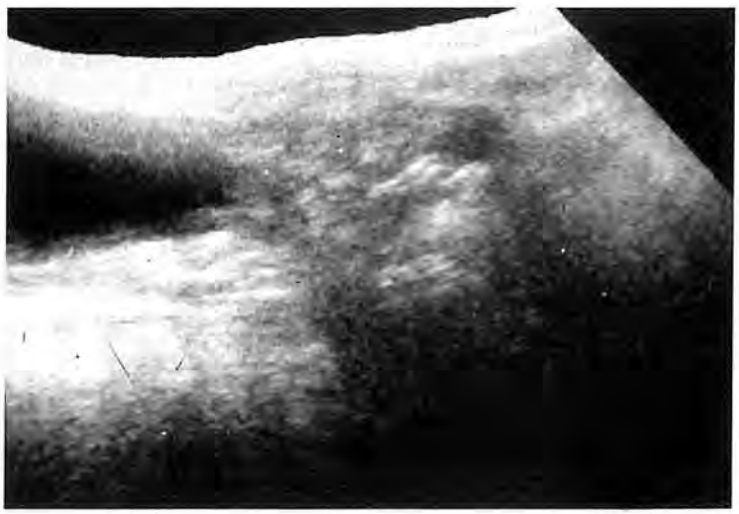

Figure 20–6. Sonogram of canine prostate with adenocarcinoma. Diffuse hyperechoic changes are present within the prostatic parenchyma.

How to Perform a Prostatic Wash

1. The dog is allowed to urinate.
2. Mild sedation occasionally is necessary if the dog is painful on rectal palpation of the prostate.
3. The bladder is catheterized with aseptic technique using a red rubber catheter and the bladder emptied of any remaining urine. The bladder is flushed with 1 to 5 ml sterile saline and again emptied. This fluid is reserved for cytologic examination and quantitative aerobic culture (sample 1, optional).
4. A gloved finger is inserted into the rectum and the prostate identified. The urinary catheter is withdrawn until the tip is palpated (per rectum) just distal to the prostate, and the prostate vigorously massaged per rectum for 1 minute.
5. After digital occlusion of the urethral orifice, 5 ml sterile saline is slowly injected and the urinary catheter advanced while aspirating. This sample also is evaluated by cytology examination and quantitative aerobic culture (sample 2).
6. Compare the two samples. If sample 2 shows greater evidence of inflammatory disease and significant bacterial growth than sample 1, disease can be localized to the prostate. If interpretation is difficult due to severe urinary tract disease, pretreatment with ampicillin for 24 hours before prostatic wash may enhance differences between urinary and prostatic samples. Sample 2 alone may be evaluated for culture and cytology in the presence of other evidence of prostatic disease, such as ultrasonographic evidence.

carcinoma, but has limited usefulness in defining extent of disease.[110] None of the prostate secretory proteins described in the dog have proven useful in differentiating normal dogs from dogs with prostatic carcinoma.[30]

Treatment of prostatic adenocarcinoma in dogs is palliative and not curative. Castration after diagnosis of prostatic adenocarcinoma causes atrophy of the non-neoplastic portions of the gland, but does not affect morphology of neoplastic cells or progression of disease.[23,105,112] Orthovoltage radiation treatment has limited success.[23] In one survey of 21 dogs treated medically with either estrogen or ketoconazole, 16 died or were euthanized within 10 days of diagnosis, regardless of treatment used, and only 1 had a survival time of greater than 4 months.[105]

The surgical treatment most often described is total prostatectomy, a surgery associated with many postoperative complications. Urinary incontinence, seen in 33 to 93 per cent of treated dogs, is caused by decreased urethral pressure, detrussor instability, shortened urethral length, and pudendal nerve damage.[69,113,114] Other reported complications include stranguria and hematuria, tenesmus, and hindlimb edema.[114]

Use of an ultrasonic aspirator to resect up to 80 per cent of prostatic tissue from within the prostatic capsule has been described.[115] Vessels and nerves are spared, and the pros-

How to Collect a Fine-Needle Aspiration Sample or Biopsy from the Prostate

1. Ensure that prostatic infection is not present, by quantitative aerobic culture and cytologic examination of prostatic fluid collected by manual ejaculation or prostatic wash.
2. Transrectal sample collection:
   A. Sedation occasionally is necessary if the dog is painful on rectal palpation of the prostate.
   B. A transrectal needle guide (Franzen transrectal needle guide; Precision Dynamics Corporation, Burbank, CA) is placed over a gloved hand and another glove is placed over the instrument, which provides a path through which a spinal needle will pass exactly beyond the center of the tip of the index finger. The double-

gloved hand is introduced into the rectum, the prostate is palpated, and the area intended for biopsy identified. A spinal needle is passed through the guide into the prostate, a syringe connected to the spinal needle is aspirated, pressure is released, and the needle is withdrawn.

3. Perirectal sample collection:
   A. The dog is sedated.
   B. The dog is placed in lateral or sternal recumbency, and the perirectal area is clipped and sterilely scrubbed. A gloved finger is inserted into the rectum and the prostate is identified and stabilized. A spinal needle is passed into the prostate lateral to the rectum, a syringe connected to the spinal needle is aspirated, pressure is released, and the needle is withdrawn (Fig. 20–7).
4. Transabdominal sample collection:
   A. The dog is sedated.
   B. The dog is placed in dorsal recumbency. If the prostate is palpable per abdomen, a needle may be introduced blindly. Ultrasound guidance allows one to visualize the exact portion of the gland being sampled, and helps to prevent accidental trauma to periprostatic structures and the prostatic urethra, and is, therefore, preferred. The prostate is identified by ultrasound. A spinal needle is passed through the guide on the transducer, a syringe connected to the spinal needle is aspirated, pressure is released, and the needle is withdrawn.
5. Transabdominal core biopsy:
   A. The prostate is identified with ultrasound. A core biopsy needle with an obturator-canula assembly (Tru-Cut biopsy needle; Travenol Laboratories Inc., Deerfield, IL) is aligned with the needle guide in the transducer. The instrument is activated; the sleeve quickly slides over the obturator, entrapping a piece of tissue within the instrument.
6. Samples collected are evaluated by cytology, microbiology, and/or histology.

Figure 20–7. Method of perirectal aspiration of the prostate in dogs (From Barsanti JA, Finco DR: Canine bacterial prostatitis. Vet Clin North Am 9:670–700, 1979, with permission.)

tatic urethra is supported by a periurethral cuff formed from the ventral portion of the capsule, decreasing postoperative urinary incontinence in one study to zero per cent.[115]

Other palliative therapies described for dogs with prostatic neoplasia include implantation of indwelling urethral catheters for dogs with urethral obstruction secondary to infiltration of prostatic adenocarcinoma,[116] and oral treatment with piroxicam at 0.3 mg/kg once daily for dogs with metastatic transitional cell carcinoma.[23]

How to Perform Retrograde Cystourethrography and Prostate Ultrasound

1. Retrograde cystourethrography.
   A. The dog is fasted for 12 hours and given a cleansing enema 2 hours prior to the procedure.
   B. The dog is mildly sedated.
   C. A 4- to 7-Fr balloon-tipped urinary catheter (Swan-Ganz catheter; Edwards Laboratory Inc., Santa Anita, CA) is passed into the urinary bladder. The bladder is emptied of urine.
   D. The bladder and urethra are distended with equal parts sterile saline and sodium iothalamate (Conray 400; Malinckrodt Inc., St. Louis, MO) or meglumine iothalamate (Conray; Malinckrodt Inc., St. Louis, MO) until the urinary bladder is palpably turgid.
   E. Lateral and ventrodorsal projection radiographs are taken, with infusion of additional increments of diluted contrast medium as needed to ensure maximal urethral distention.
2. Prostate ultrasound.
   A. This is best performed after retrograde cystourethrography without bladder evacuation to allow ready identification of the bladder and prostatic urethra.
   B. The dog is placed in dorsal recumbency and hair shaved from the ventral abdomen between the cranial aspect of the prepuce and pubic bone, from the midline to the inguinal fold on each side. Coupling medium is applied. In dogs that owners prefer not be shaved, alcohol saturation of the hair may be adequate for diagnostic ultrasonography.
   C. The transducer is placed against the body wall cranial to the pubis. Transducer used will vary with size of the patient and placement of the prostate. Transverse and sagittal views of the prostatic parenchyma are assessed for homogeneity, presence of focal or diffuse hypo- or hyperechoic regions, and cavitating lesions.

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Chapter 21

Disorders of the Canine Penis and Prepuce

Congenital Abnormalities

In the dog, formation of the penis begins 24–25 days after conception.[1] The prepuce forms as a circular plate of ectoderm invaginates at the distal tip of the developing phallus, separating into external (skin) and internal (mucosa) laminae. The balanopreputial fold is a connection of the penile and preputial mucosa, dissolution of which is androgen-dependent.[2,3]

Diphallia

Diphallia is duplication of the penis (Fig. 21–1). Three cases of canine diphallia have been reported in the veterinary literature.[1,4,5] Male dogs described were a 5-month-old pointer with diphallia and duplication of the urinary bladder[4]; a 5-month-old mixed breed with diphallia, duplication of the urinary bladder and prostate gland, a bifid scrotum, and polymelia[5]; and a 6-month-old poodle cross with diphallia, duplication of the urinary bladder, right renal hypoplasia, bifurcation of the descending colon, and bilateral cryptorchidism.[1] Karyotype was normal in both dogs in which it was evaluated.[1,5] Diphallia may be caused by duplication of the cloacal membrane early in development, with subsequent formation of two urogenital tubercles, or may be part of a more extensive hindgut defect, explaining developmental anomalies in other systems.[6]

Presenting clinical signs of diphallia were referable to the urinary tract and included hematuria, pollakiuria, and inappropriate urination.[1,5] One dog was euthanized because of right renal hypoplasia and pyelonephritis of the remaining kidney.[1] One dog was castrated and treated with antibiotics as necessary for recurrent urinary tract infections.[5]

Penile Frenulum

A penile frenulum is a thin band of connective tissue joining the ventral glans penis either to the corpus of the penis or to the prepuce (Fig. 21–2).[3,7–9] It is formed by incomplete dissolution of the androgen-dependent balanopreputial fold.[3] Penile frenulums have been described in 18 cocker spaniels (including 16 puppies from one kennel)[7,10,11] four Poodles,[8,9,11] one mixed-breed dog,[12] and in intersex dogs.[3] Despite the apparent increased incidence in cocker spaniels, no heritable basis has been identified.[11]

Age at diagnosis varies with presenting complaint and breeding status of the dog. Reported mean and range of age at diagnosis are 2.9 years and 3 months to 8 years, respectively.[7–12] Presenting clinical signs include excessive licking of the penis and prepuce, dermatitis between the rear limbs due to urine scald, phallocampsis (curvature of the penis when erect), and pain when breeding with subsequent decreased libido and inability to achieve a copulatory lock (tie).[8–12] Dogs with penile frenulums may be asymptomatic.[7,11]

Diagnosis is by visual inspection of the protruded penis. Penile frenulums in asymptomatic dogs not intended for breeding need not be corrected. Treatment in symptomatic dogs or those intended for breeding is transection of the frenulum. In many dogs, a thin frenulum may be snipped with scissors without causing pain or hemorrhage; if the frenulum appears vascular or is thick, transection under local anesthesia or a light plane of general anesthesia is indicated.[3,8–12] Two dogs described with phallocampsis and inability to breed became sexually competent after transection of the penile frenulum.[10,12]

Figure 21–1. Diphallia in a 6-month-old poodle crossbreed. (From Johnston SD, Bailie NC, Hayden DW, et al: Diphallia in a mixed-breed dog with multiple anomalies. Theriogenology 31:1253–1260, 1989, with permission.)

Hypospadias/Miscellaneous Congenital Abnormalities of the Penis and Prepuce

Hypospadias is abnormal termination of the penile urethra along the ventral surface of the penis proximal to the normal urethral opening.[3,13] Hypospadias occurs when there is incomplete or abnormal fusion of the median raphe of the penis, prepuce, and/or scrotum.[13] It is classified as glandular, penile, scrotal, or perineal, with classification determined by location of the urethral opening.[3] In general, glandular hypospadias is least severe, with the abnormal location of the urethral opening the only physical change. Severity increases with proximal location of the urethral opening, with increasing incidence of concurrent physical defects. The most common concurrent defects include cryptorchidism, shortening of the penis with underdevelopment of the glans penis, ventral deviation of the penis, and deficient development of the ventral prepuce.[13,14]

Figure 21–2. Penile frenulum in a dog.

Reported incidence of canine hypospadias is 0.003 per cent.[14] While no breed predisposition has been reported, 17 of 70 reported cases (24 per cent) have been Boston terriers.[14,15] Hypospadias also has been reported in intersex animals.[3,14] Other possible causes include administration of progestins to the pregnant dam, and feeding the dam a diet deficient in vitamin A during pregnancy.[13]

Presenting clinical signs vary with location of the urethral opening and extent of concurrent physical changes. Dogs with hypospadias may be asymptomatic.[3] The most commonly reported clinical sign is urinary incontinence with associated inguinal dermatitis.[3]

Treatment varies with extent of the anomaly and clinical state of the animal. Asymptomatic animals do not require therapy. Dogs with glandular hypospadias may require repair of the defect only[3,13]; a urinary catheter is passed, the urethral mucosa is dissected and undermined from the skin at the mucocutaneous junction, and the incised edges of the urethral mucosa are apposed and sutured. Knots in the suture material should be placed outside the lumen of the urethra so a nidus for calculus formation is not provided. An indwelling catheter may be left in place for up to 2 weeks[3]; concurrent antibiotic therapy is recommended to prevent ascending urinary tract infection. Penile hypospadias may be corrected with amputation of the penis and prepuce to the level of the urethral opening.[3,15] Postoperative urethral strictures and stranguria may occur.[15] Scrotal and perineal hypospadias generally require complete penile amputation and scrotal or perineal urethrostomy.[3,13] Castration may be performed at the same time, and is recommended.[13]

Other penile abnormalities described in the veterinary literature include penile hypoplasia and penile immaturity. Penile hypoplasia may be seen in intersex dogs, and is reported as a concurrent abnormality in cryptorchid dogs.[13,16,17] One report describes successful treatment of an abnormally short penis in a 15-month-old German shepherd by penile amputation and urethrostomy.[16]

Penile immaturity, also called infantile penis or micropenis, is presence of an abnormally small penis in relation to body size of the dog.[18] This may occur after prepuberal gonadectomy or, in adult dogs, secondary to exogenous or endogenous estrogens, as may be seen in male

dogs with functional Sertoli cell tumors.[18,19] In a study comparing penile size in 13- to 15-month-old mixed-breed dogs that had been gonadectomized at 7 weeks or 7 months of age, or left intact, it was reported that those dogs gonadectomized at 7 weeks of age had immature genitalia characterized by significantly smaller penile diameter, decreased size and radiodensity of the os penis, and immaturity of the prepuce, than male dogs gonadectomized at 7 months of age or left intact.[19] Clinical significance of these physical changes in prepuberally gonadectomized dogs was not reported. An intact 7-month-old Doberman pinscher with penile immaturity, phimosis, urinary incontinence, dysuria, and hematuria was treated successfully with surgical shortening of the prepuce and enlargement of the preputial orifice.[18] Humans with penile immaturity are treated with testosterone to increase penile size.[18]

Hypoplasia of the prepuce may be seen in intersex dogs, and has been reported as a concurrent finding in cryptorchid dogs.[3,17] A line of inbred wire-haired fox terriers with brachyury and incomplete fusion of the ventral prepuce from the scrotum to the distal extent of the glans penis has been described.[20] This defect healed spontaneously by 1 year of age in most of these dogs.[20] Traumatic loss of the distal prepuce also has been reported, with subsequent drying of the exposed portion of the penis.[21]

Dogs with preputial defects present with protrusion of the penis at the area of the defect, and may present with urinary incontinence as well.[2,21–23] Treatment is surgical reconstruction of the prepuce; creation of pedicle extension flaps, with or without oral mucosal grafts as lining, has been described.[2,22,23]

Phimosis/Paraphimosis

Phimosis is inability of the male dog to protrude the penis from the prepuce (Fig. 21–3). Phimosis was reported in 0.5 per cent of 185 dogs with 197 abnormalities of the penis and prepuce.[24] The condition may be congenital, as, for example, in intersex dogs, or may be acquired with stricture at the urethral orifice due to inflammation, edema, neoplasia, or cicatricial constriction after wound healing.[18,25–27] The penis may or may not be normal.[18,25] Treatment is surgical enlargement of the preputial orifice.[18,25–27]

Paraphimosis is inability of the male dog to retract the erect or nonerect, protruded penis into the preputial sheath. Paraphimosis was reported in 7 per cent of 185 dogs with 197 abnormalities of the penis and prepuce.[24] Causes include sexual arousal, neurologic disease including encephalitis and intervertebral disk disease, fracture of the os penis, balanoposthitis, constriction at the preputial orifice by a hair ring or scar tissue, swelling of the penis due to trauma, neoplasia or malicious strangulation of the penis with a rubber band or string, inefficiency of preputial musculature, and entrapment of the penis outside the prepuce during penile detumescence.[27–29] Paraphimosis in the dog may be idiopathic.[27]

Figure 21–3. Phimosis in a puppy. The dog has incomplete fusion of the ventral prepuce and a pinpoint-sized urethral orifice.

The exposed, entrapped penis undergoes ischemia, drying, and excoriation, and is progressively compromised with prolongation of paraphimosis. Conservative treatment, appropriate when paraphimosis is of short duration and the penis is grossly normal, includes cleaning and lubrication of the penile mucosa, and gentle replacement of the penis within the prepuce with digital pressure.[27,28] If the penile mucosa is edematous, lavage with a hyperosmolar sugar solution may facilitate replacement.[28] One report described fixation of an indwelling urinary catheter to the prepuce with stainless steel sutures for 48 hours after replacement to prevent recurrence.[28] There is one report of use of purse-string sutures at the preputial orifice after replacement to prevent recurrence; this was unsuccessful.[30] Other forms of conservative treatment include isolation of the male from estrous females or other causes of sexual excitement, and castration.[31] Castration usually is not effective, since paraphimosis is not a testosterone-dependent condition. If the penis is grossly normal and replacement cannot be achieved, even under sedation, surgical

widening of the preputial orifice should be performed.[27] Other surgical forms of treatment include myorrhaphy for shortening of inefficient preputial musculature,[31] and penile amputation for dogs with prolonged paraphimosis during which the penis has undergone severe trauma or ischemic necrosis.[30] Dogs with nonrecurrent paraphimosis that responds to conservative therapy may be used successfully as breeding animals after treatment.[28]

Urethral Prolapse

Prolapse of the distal urethra may be idiopathic or may occur secondary to sexual excitement or urethral infection in the dog.[32,33] A genetic predisposition may exist; of eight dogs reported in the literature, six were English bulldogs.[32–36] Mean age at diagnosis was 20 months, with a range of 9 months to 5 years.[32–36]

The most common presenting clinical sign of urethral prolapse in the dog is intermittent bleeding from the penis.[32–35] Increased frequency of urination also may be present.[32] The prolapsed urethral mucosa usually has a pathognomonic "red pea" appearance at the tip of the penis, which allows ready differentiation of urethral prolapse from fracture of the os penis, urethral stricture or calculi, and persistent penile frenulum.[32] The prolapse may occur only when the penis is erect.[35] In such dogs, induction of erection by manual ejaculation permits complete assessment for urethral prolapse and, coupled with the young age of animals presenting with urethral prolapse, allows differentiation of this condition from prostate disease, another common cause of intermittent bleeding from the penis (see Chapter 20).

Conservative treatment with tranquilizers, isolation from estrous females or other causes of sexual excitement, cage rest, and antibiotics usually will not effect a cure. Surgical replacement or removal of the prolapsed tissue is the treatment of choice.[32] Castration and concomitant oral estrogen therapy (diethylstilbestrol [DES], 0.1 mg per os [PO] once daily for 5 days, then twice weekly for 3 weeks) was reported as partially successful in one case, although the prolapse recurred 1 year later.[35] Manual replacement with a purse-string suture for 5 days postoperatively was reported successful in one case.[36] The most commonly recommended surgery is removal of the prolapsed urethral tissue and suturing of viable urethral mucosa to penile mucosa.[32–35] Postoperative management includes topical and systemic antibiotics, use of an Elizabethan collar to prevent licking at the penis, and use of smooth muscle relaxants to prevent urethral spasm.[32] Efficacy of concurrent castration as a means to prevent recurrence is equivocal.[32] Intact dogs with urethral prolapse that respond well to surgical treatment may be used for breeding.[33]

Fracture of the Os Penis/Penile Trauma

Fracture of the os penis in the dog is uncommon; in one study, 2 per cent of 185 dogs with 197 penile and preputial abnormalities presented with fracture of the os penis.[24] Occasionally, the dog may present with a history of known trauma,[37] but most often the cause is unknown. There is no breed or age predisposition for this disorder.

Affected dogs may exhibit clinical signs either at the time of the fracture, or months to years later, after nonunion healing of the fracture or excessive callus or fibrous tissue formation at the fracture site have exacerbated displacement of fracture fragments or caused urethral obstruction.[37–41] Clinical signs of an acute os penis fracture vary with degree of the fracture (simple or comminuted) and extent of soft tissue injury,[27] and include ventral deviation of the penis, dysuria and hematuria, pain and crepitus on penile manipulation, distention of the urinary bladder, and abdominal pain.[27,37,38,41] Clinical signs referable to excessive callus or fibrous tissue formation at the site of a healed os penis fracture include dysuria, distention of the urinary bladder, and ventral deviation of the penis.[39–42] Postrenal azotemia may be present secondary to urinary tract obstruction.[24,40]

Definitive diagnosis of fracture of the os penis is by radiography (Fig. 21–4).[37,40,41] Careful passage of a urinary catheter should be attempted and may be difficult or impossible in dogs with a comminuted fracture of the os penis or complete urinary tract obstruction.[27,37,40,41]

Treatment of an acute fracture of the os penis is surgical reduction of the fracture. If a urinary catheter can be passed and fragments of the os penis easily brought into apposition, a closed reduction may be performed with placement of an indwelling urinary catheter to act as a splint.[27,37,38,40] The catheter is left in place for 5 days, and prophylactic antibiotics are administered concurrently.[37,38] Badly comminuted

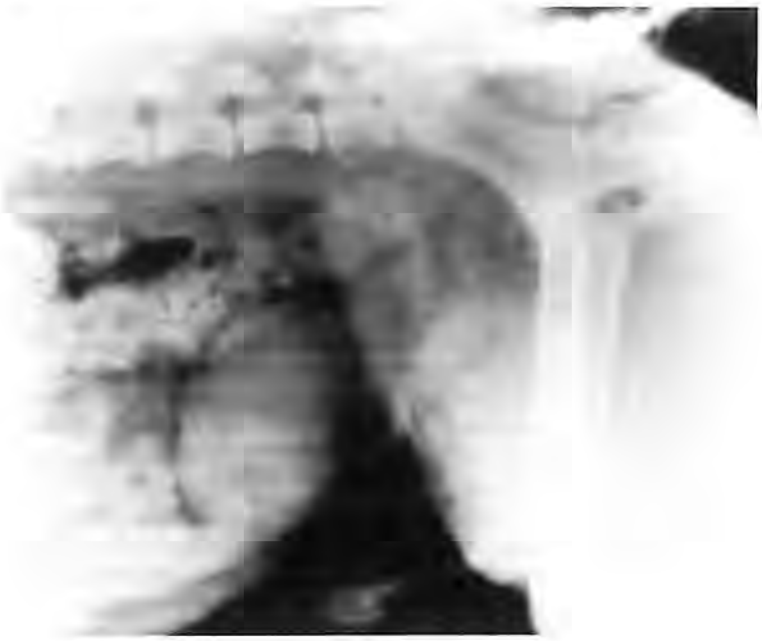

Figure 21–4. Lateral radiograph of fracture of the os penis in a 10-year-old male dog. (From Ryer KA: What is your diagnosis? [Fracture of the os penis in a dog]. J Am Vet Med Assoc 177:177–178, 1980, with permission.)

acute fractures, or nonunion or poorly healed old fractures may require open reduction, with removal of excess bone and fibrous tissue, and stabilization of the fracture with bone plates and screws.[39,41] Screws must be positioned in the dorsal surface of the os penis to avoid the ventral urethra.[41] If extensive soft tissue damage is present, urethral patency must be assessed and urethral anastomosis attempted, if indicated.[37] If urethral anastomosis is not possible, perineal or scrotal urethrostomy must be performed.[40,41] Wedge osteotomy is described as a means of straightening a ventrally deviated penis.[42]

Penile trauma was reported in 19 per cent of 185 dogs with 197 lesions of the penis and prepuce.[24] Causes of penile wounds reported in the veterinary literature include whole-body trauma, trauma at the time of breeding, bite wounds, and circumferential hair rings or maliciously placed rubber bands.[27,43,44] Clinical signs include profuse hemorrhage or intermittent bleeding as the prepuce fills and empties or penile erection occurs, penile paralysis, pain on penile manipulation, pain or lameness in the hindquarters, and dysuria.[27,43–45]

Diagnosis is by visual inspection of the protruded penis. Treatment course varies with extent of penile damage. Three dogs described with signs of penile and hindlimb pain and ultrasonographic evidence of swelling of the corpus cavernosum penis secondary to trauma at the time of breeding were successfully treated with nonsteroidal anti-inflammatory drugs.[44] If open wounds are present, debridement and suturing of the wounds are required, as are postoperative placement of an indwelling urinary catheter for 5 days to ensure urethral patency, administration of topical and systemic antibiotics, and twice-daily protrusion of the penis to prevent formation of adhesions.[27] Prevention of penile erection, with isolation of the affected male from estrous females or other causes of sexual excitement, and possible hormone therapy (DES, 1.0 to 2.0 mg PO once daily) may be beneficial.[27] If extreme damage to the penis or urethra, penile paralysis, or ischemic necrosis of the penis is present, penile amputation and urethrostomy are indicated.[27,43,44] One report describes reconstruction of the urethral orifice at the tip of a penis truncated by trauma.[45]

Balanoposthitis

Balanoposthitis is inflammation of the glans penis (balanitis) with concomitant inflammation of the preputial mucosa (posthitis). Although balanoposthitis was reported in 20 per cent of 185 dogs with 197 lesions of the penis and prepuce in one study,[24] there are few reports in the veterinary literature. No breed predisposition has been reported. Most dogs are reported to exhibit clinical signs of balanoposthitis at 4 years of age or younger.[24]

The reported cause of balanoposthitis in the dog is opportunistic infection with bacterial or viral agents that may or may not be normal preputial flora.[24,46–54] Atopic dermatitis and behavioral self-mutilation also may be causes.

Escherichia coli was the most common isolate from aerobic preputial cultures of 37 dogs with balanoposthitis.[24] Other aerobic bacteria reported from preputial cultures of male dogs with balanoposthitis include *Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus,* and *Klebsiella* sp.[46] All of these bacteria are normal preputial flora.

Mycoplasmas and ureaplasmas have been cultured from the prepuce of dogs with balanoposthitis.[47–49] Mycoplasmas and ureaplasmas also are normal flora of the preputial mucosa of dogs,[48] confounding the ability to definitively diagnose these organisms as causing balanoposthitis in a given animal. However, in two surveys, mycoplasma was cultured from the prepuce in a significantly larger percentage of dogs with balanoposthitis (92 to 95 per cent) than from reproductively normal dogs (64 to 70 per cent).[48,49] *Mycoplasma canis* is the most common isolate.[47,49] Significance of ureaplasmas as a cause of balanoposthitis is less well defined; in one study, ureaplasmas were cultured from 69 per cent of dogs with balanoposthitis and 70 per cent of reproductively normal dogs.[48]

Reported viral causes of balanoposthitis in dogs are canine herpesvirus (CHV)[50–53] and, more rarely, calicivirus.[54] CHV is spread by aerosol transmission and direct contact with oronasal secretions, and may be spread venereally, since it has been isolated both from vaginal secretions and from semen.[55] Clinical disease is more likely to occur in stressed or immunosuppressed animals.[51,56] CHV may be carried in a latent form and the infected animal may, therefore, be asymptomatic.[58] Recrudescence may occur with stress or immunosuppressive drug therapy.[56]

The predominant clinical sign of balanoposthitis due to infection with aerobic bacteria or mycoplasma in the dog is preputial discharge, varying in character from purulent to sanguinopurulent.[24,49] The discharge may have a fetid odor.[24] The penile and preputial mucosa in affected dogs is erythematous and may be ulcerated or covered with caseous material (Fig. 21–5). Penile lymphoid follicles may be prominent.[24]

Viral balanoposthitis is characterized by presence of vesicular or lymphoid nodular lesions on the preputial mucosa and near the preputial reflection on the penis.[50–54] The penile mucosa may be hyperemic, and petechial or submucosal hemorrhages may be present.[51,52] Serous preputial discharge has been reported in experimental CHV infection in the dog.[52]

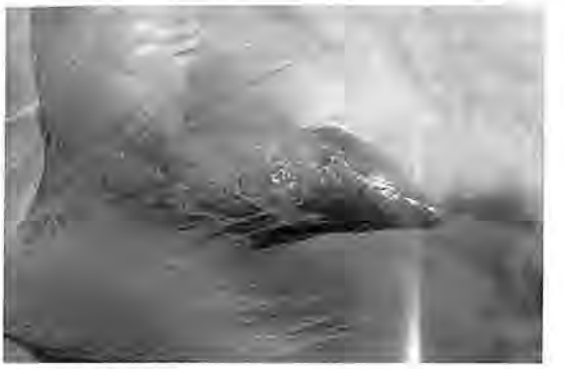

Figure 21–5. Balanoposthitis in a dog.

Dogs with balanoposthitis as a component of atopic dermatitis may show concurrent pruritis of the feet, ears, and ventrum or may show excessive licking of the penis as their only sign. The penile and preputial mucosa is erythematous and penile lymphoid follicles may be prominent. The authors are aware of one case of balanoposthitis due to behavioral self-mutilation; the penile and preputial mucosa were severely erythematous and the dog had chewed away a portion of the ventral surface of the penis.

Diagnosis of balanoposthitis is by visual inspection. Aerobic bacterial and mycoplasma cultures of the preputial and penile mucosa should be performed. *Mycoplasma* sp. and many aerobic bacteria, including *Escherichia coli, Pasteurella multocida,* and β-hemolytic *Streptococcus* sp. are normal flora of the preputial mucosa, so culture results should be evaluated with caution.[58] CHV is a poor antigen, which does not produce long-lasting antibody titers after infection. Definitive diagnosis of CHV requires demonstration of a change in titers in paired serum samples, or virus isolation.

Treatment of bacterial or mycoplasmal balanoposthitis includes administration of systemic and/or topical antibiotics based on culture and sensitivity testing. Specific antibiotic preparations intended for intramammary infusion may be dispensed into the prepuce.[24] Enrofloxacin is the antibiotic of choice for treatment of mycoplasma infection. Excessive discharge should be cleansed from the penis and prepuce by flushing of the prepuce with warm water or saline. There is a report of reconstructive preputial surgery to promote res-

olution of balanoposthitis.[59] There is no specific treatment for balanoposthitis due to CHV infection, but signs of disease generally are mild and self-limiting in male dogs.

Balanoposthitis due to atopic dermatitis may be treated with antihistamines or corticosteroids. Behavioral self-mutilation may be controlled by detecting and eliminating the trigger for the behavior, or may require therapy with antianxiety drugs.

One report in the literature describes a case of cutaneous lymphangiectasia in the dog.[60] Clinical signs were similar to those of balanoposthitis, with vesicles on the prepuce, but swelling and pitting edema of the prepuce also were present. The condition resolved with antidiuretic therapy.[60]

Persistent Erection

Persistent penile erection, or priapism, is prolonged penile erection without sexual arousal, causing discomfort and difficult urination.[61] Incidence of this disorder in the dog is not reported but is apparently very low.

Normal penile erection in the dog occurs when parasympathetic stimuli, via the pelvic nerve, actively increase arterial blood flow into the corpus cavernosum penis (CCP) and decrease venous outflow via contraction of smooth muscle fibers within the penis at the level of the tunica albuginea surrounding the CCP.[62] Sympathetic stimulation reverses the effect.[62] Normal detumescence requires relaxation of smooth muscle, allowing venous drainage into the pudendal veins.[63]

Priapism may occur secondary to prolonged or excessive parasympathetic stimulation, or due to decreased venous outflow from an occlusive thromboembolism or mass lesion.[61] Stagnation of blood with subsequent low oxygen and high carbon dioxide concentrations within the CCP causes edema, with further venous occlusion and eventual irreversible fibrosis in the main venous outflow tracts of the penis.[61] Ischemic necrosis of the penis results.

Persistent penile erection has been described in humans, horses, cats, and dogs.[61,64–70] Underlying causes described in humans include sickle cell disease, neoplasia, trauma, coagulopathy, diabetes mellitus, treatment with antihypertensive or psychotropic drugs, use of marijuana, and general anesthesia.[61,64,66] In humans, 64 per cent of cases are idiopathic.[61] Reported causes of priapism in horses include administration of phenothiazine tranquilizers, general anesthesia, and malignant melanoma.[65,66] Priapism has been reported in two cats as a postoperative complication following castration,[67,68] and in cats with penile thromboembolism.[70] Reported causes in the dog include trauma while mating, chronic distemper encephalomyelitis with distemper-associated inflammatory lesions in the spinal cord, penile thromboembolism, and administration of amphetamines.[69,70] There is one report of idiopathic priapism in the dog.[71]

Clinical signs of persistent penile erection in the dog are described in only one report.[71] The male dog exhibited dysuria and protrusion of approximately half the length of the erect penis from the prepuce. The exposed penile mucosa was hyperemic (Fig. 21–6).

Diagnosis of priapism is made by assessment of complete history and physical examination findings. Diagnosis of the underlying cause of the disorder may require extensive evaluation of the animal, to include assessment of general health with complete blood count and serum chemistry profile; coagulation profile; complete evaluation of the genitourinary tract with urinalysis, aerobic urine culture, and radiography or ultrasound; and assessment for mass lesions in the caudal abdomen. Workup also must be timely, as treatment options become limited as the penis undergoes progressive ischemic change.

In humans, treatment of the underlying cause, if known, is the treatment of choice. Described treatments for priapism itself in humans include drainage and flushing of the CCP with heparinized 0.9% sodium chloride solution and infusion of sympathomimetic drugs (e.g., phenylephrine), or surgical creation of fistulas within the penis to expand venous outflow tracts of the penis.[61] Medical therapy with the anticholinergic and antihista-

Figure 21–6. Priapism in a 6-year-old samoyed crossbreed.

minic drug benztropine mesylate (0.015 mg/kg intravenously [IV]) has been reported successful in horses if administered within 6 hours of onset of priapism.[65,66] Percutaneous drainage and flushing of the CCP with 0.9% heparinized sodium chloride solution was unsuccessful as a treatment in one horse; the penis became flaccid but had undergone irreversible damage leading to penile paralysis and paraphimosis.[65]

In the dog, the underlying cause of persistent penile erection may not be identified, and the penis usually is irreparably damaged by the time of presentation so that penile amputation usually is necessary. Castration usually is not effective. One report describes incising the penis over the bulbus glandis and pars longa glandis, through the tunica albuginea, and applying pressure to expel free blood and thrombi from the CCP.[68] Another report describes penile amputation and perineal urethrostomy as the best treatment, due to deterioration of the condition of the penis and inability to discern an underlying cause for the persistent penile erection.[71] There are no reports of male dogs mating successfully after treatment for persistent penile erection.

Penile/Preputial Neoplasia

The most common penile neoplasm, worldwide, is the transmissible venereal tumor (TVT).[24,72] Other penile neoplasms described include squamous cell carcinoma of the penile/preputial mucosa (Fig. 21–7), squamous cell carcinoma of the penile urethral mucosa, malignant mast cell tumor of the penis, and chondrosarcoma of the os penis.[73,75] Preputial tumors include TVTs and, less commonly, papillomas, carcinomas, mast cell tumors, and other tumors of the skin and subcutaneous tissue.[24,72,76]

Figure 21–7. Squamous cell carcinoma of the penis of a dog.

Transmissible Venereal Tumor

Transmissible venereal tumor also is called transmissible venereal sarcoma and venereal granuloma.[77,78] Distribution of the tumor is worldwide, with greatest incidence in tropical and subtropical urban areas with large populations of free-roaming dogs in which breeding is uncontrolled.[77,79,80] Regional outbreaks have been reported in the United States.[80]

The TVT is an allogeneic cellular transplant, with cytogenetic and cell surface antigen characteristics of the tumor suggesting that all TVTs arose from a single origin.[79,80] All TVT cells contain 59 ± 5 chromosomes, as opposed to normal diploid canine cells, which contain 78 chromosomes.[79,80]

The primary mode of transmission of TVT is exfoliation and transplantation of neoplastic cells onto damaged penile mucosa during coitus.[77,80] Transplantation of TVT cells onto nasal or oral mucous membranes also may occur as dogs lick at their own and other dogs' genitalia.[80] Experimental transmission of TVT requires intact, viable tumor cells.[81] Icosahedral electron-dense particles have been visualized in degenerating and necrotic TVT cells, suggestive of viral particles, but no etiologic agent separate from live tumor cells has been identified to date.[81,82]

TVT occurs most commonly in young, sexually mature animals.[24,79] One survey of 30 male and female dogs with TVT reported a mean age at diagnosis of 4.9 years.[83] Other surveys reported that a majority of dogs with TVT were between 2 and 4 years of age, and that 29 of 36 dogs (81 per cent) with TVT were less than 2 years of age.[84,85] No breed predisposition has been reported; mixed-breed dogs were most commonly affected, at 32.8 per cent of admissions, in one study.[84]

TVTs grow rapidly after transplantation.[80] After experimental transmission of TVT cells onto genital mucosa, tumors are visible grossly in some dogs as early as 15 days, with visible tumor growth in 37 per cent of dogs by 30 days, and in 88.7 per cent of dogs by 60 days after transfer.[81] Forty per cent of TVTs are locally invasive.[80] Rate of metastasis is 0 to 17 per cent.[79] Metastases have been reported in the regional lymph nodes (superficial inguinal and lumbar nodes), tonsil, eye, brain and meninges, pituitary gland, skin, spine, and abdominal and thoracic viscera.[80,86–91] The immune system of the infected animal controls tumor expression, with up to 19 per cent of dogs with naturally occurring disease reported

to undergo spontaneous remission.[77,85] These dogs have subsequent transplantation immunity.[77] The reported rate of spontaneous regression for experimentally induced TVT is much higher than natural exposure, at 60 per cent.[80] It is not known if spontaneous regression occurs more commonly in experimentally induced TVT than in naturally occurring disease, or if small, naturally occurring TVTs regress before inducing significant clinical signs, and therefore escape diagnosis.[79]

Of 19 cases of canine TVT reported in one study, 53 per cent were present only on the genitalia, with 32 per cent on the penis and 21 per cent on the prepuce.[91] Extragenital extension to the oral and nasal cavities has been reported.[91,92] Five dogs have been described with oronasal TVT only.[92–95]

Clinical signs of TVT vary with site(s) of the tumor. Dogs with TVT of the penis and prepuce most commonly exhibit intermittent to persistent exudation of serosanguineous to purulent discharge from the prepuce.[24,79,80,83,89,96,97] Other clinical signs of genital TVT include preputial swelling, abnormal odor, excessive licking of the genitalia, phimosis, stranguria and urinary tract infection due to obliteration of the urethral orifice by the tumor, and presence of visible tumor masses.[79,83,89,97,98] Signs referable to extragenital or metastatic TVT vary with location and extent of the tumor, and include epistaxis and sneezing, facial deformity, dysphagia, halitosis and tooth loss, hyperpnea or dyspnea, exophthalmos or sudden blindness, and lymph node enlargement.[79,86–88,90,91,93–95]

TVTs appear grossly as single or multiple, grayish cream to red, firm nodules as small as 0.5 mm in diameter, progressing to friable, cauliflower-like, pedunculated or multilobular growths greater than 10 mm in diameter, that frequently ulcerate and bleed.[24,77,80] Histologically, TVTs are round cell tumors characterized by discrete cells or sheets of cells with a high ratio of nucleus to cytoplasm, round to oval nuclei containing one or two prominent nucleoli, distinct chromatin clumping, and many mitotic figures, and pale blue or clear cytoplasm containing many distinct, clear vacuoles (Fig. 21–8).[79,80,99,100]

Presumptive diagnosis of genital TVT is by visual inspection of the penile and preputial mucosa. Definitive diagnosis is accomplished by cytologic evaluation of smears, swabs, or fine-needle aspirates from the tumor, which exfoliates readily.[77,79,97,100] Immunohistochemistry has been described as a method allowing

Figure 21–8. Cytology of oronasal transmissible venereal tumor from a nasal flush. (From Fallon RK, Swayne DE: Canine transmissible venereal tumor: A diagnostic dilemma. Compend Contin Educ Pract Vet 6:415–418, 1984, with permission.)

differentiation of TVTs from other round cell tumors.[101] Results of complete blood count, serum chemistry profile, and urinalysis usually are unremarkable in dogs without metastatic disease.[97] Changes referable to metastatic disease vary with location and extent of tumor growth. Polycythemia may be diagnosed in dogs with a large tumor burden, since TVT has been demonstrated to produce erythropoietin.[77]

A general treatment plan for TVT is to resect tumors with wide margins whenever possible, and to use chemotherapy or radiation therapy as adjunctive therapies in nonresectable, recurrent, or metastatic disease.[80]

A ventral midline approach can be made through the prepuce with minimal compromise to major preputial vessels, allowing access to the entire penile shaft.[78,102] Tumors are resected with wide margins, the penile mucosa sutured, and the prepuce closed in three layers.[78] Postoperative care includes administration of topical and systemic antibiotics and twice-daily protrusion of the penis to prevent formation of adhesions. Use of electrocautery also has been described.[97,103] In a survey of 12 dogs, those treated by surgical resection of TVT with electrocautery rather than conventional surgical therapy had shorter surgery times and less intraoperative bleeding, but longer duration of serosanguineous drainage from the prepuce, and excessive formation of scar tissue postoperatively.[103] Recurrence rate of TVT within 6 months after surgical excision

as sole treatment varies from 32 to 44 per cent.[79,104] Recurrence rate does not differ in dogs treated with conventional surgery and those treated with use of electrocautery.[103] Concurrent castration does not affect recurrence rate.[79]

The two most common chemotherapeutic regimens described are vincristine alone and combination chemotherapy with vincristine, cyclophosphamide, and methotrexate.[77,83,96,97,105–107] There is one report of successful treatment with cyclophosphamide alone as an adjunct to surgical resection of an oronasal TVT.[95]

Vincristine (0.025 mg/kg IV weekly [$n = 80$] or increasing weekly doses of 0.025 mg/kg to 0.037 mg/kg to 0.05 mg/kg IV [$n = 1$]) has been demonstrated to cause complete tumor regression after an average of 3.3 doses, with a range of 2 to 7 doses, in 95 per cent of male dogs with TVT.[77,106] Side effects reported were vomiting (7 per cent) and transient leukopenia (5 per cent).[106,107]

Combination chemotherapy with vincristine (0.0125 to 0.025 mg/kg or 0.5 mg/m^2 IV one to three times weekly), cyclophosphamide (1 mg/kg or 50 mg/m^2 per os three to four times weekly), and methotrexate (0.3 to 0.5 mg/kg IV weekly or 2.5 mg/m^2 per os four to five times weekly) has been reported in 23 male dogs with TVT.[83,96,97] Mean number of days until onset of regression was 10.2 with a range of 4 to 40 days.[83] Complete regression was noted in 86 per cent of dogs with a mean duration of treatment of 55.1 days,[83] and in 100 per cent of dogs with 4 to 6 weeks of treatment.[96] There was no recurrence for up to 2 years after treatment.[96] Side effects reported include anorexia, vomiting and diarrhea, transient neutropenia, and alopecia.[83,96]

Radiation therapy may be used as an adjunct to surgical excision of TVT in the dog. One report suggests one to three treatments of 1000 rad each to induce remission in most cases.[77] Reports of successful treatment in 6 of 10 dogs with genital and extragenital TVT with radiation therapy alone report doses of 600 rad for three treatments on alternating days,[94] and variable dosage and time between treatments depending on size and extent of the tumor.[108]

Immunotherapy has been described as an experimental treatment for TVT in the dog but is rarely used clinically.[79,80,109] Successful therapies include treatment with serum from patients that have undergone spontaneous remission, autogenous formalized vaccine, extracts of irradiated tumors, subcutaneous injection of untreated TVT cells, and the bacille Calmette-Guérin (BCG) strain of *Mycobacterium bovis*.[80,109]

Cryotherapy has been reported as a successful therapy for a small TVT on the penis of a dog.[110] A freeze-thaw cycle lasting 30 seconds was repeated three times. The lesion regressed and there was no recurrence for 1 year after therapy.

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Chapter 22

Disorders of the Mammary Gland of the Male Dog

Gynecomastia

Gynecomastia, male mammary gland and/or nipple development, is an uncommon disorder in the dog that may occur as part of a feminizing syndrome concomitant with estrogen-secreting testicular tumors. The testicular tumor most often causing this syndrome is the Sertoli cell tumor.[1-5] In one survey, 11 of 27 dogs with intra- or extrascrotal Sertoli cell tumors exhibited gynecomastia as the sole feminizing sign.[1] Besides gynecomastia, signs of feminization due to increased estrogen secretion include bilateral alopecia of the trunk and neck, hyperpigmentation of the inguinal area and ventrum, and pendulous prepuce.[2-6] Estrogen-induced bone marrow toxicity with anemia and epistaxis has been reported in one dog with a Sertoli cell tumor.[5]

Gynecomastia is identified on physical examination. Elevated serum estrogen concentrations may be inferred by presence of cornified epithelial cells collected from the preputial mucosa. Gynecomastia resolves after complete surgical removal of the functional testicular tumor; in one study, feminizing signs subsided by 21 days after castration and serum estrogen concentrations returned to normal by 60 days after surgery.[3,6]

Galactorrhea

Galactorrhea, secretion of milk from one or more mammary glands, was reported in a male dog 3 months after treatment with a synthetic progestin, chlormadinone acetate, for hypersexuality.[7] The galactorrhea resolved spontaneously within 14 days.[7] The proposed mechanism, which is similar to that of lactation in pseudopregnant bitches (see Chapter 13), is that of serum progesterone elevation stimulating mammary development, and serum progesterone decline after cessation of treatment inducing a rise in prolactin and subsequent milk production.

Mammary Neoplasia

Mammary gland neoplasia is uncommon in the male dog, compared to intact and ovariohysterectomized female dogs, with a reported incidence of 0.5 to 2.0 per cent.[8-11] In two retrospective studies of 83 and more than 500 cases of mammary neoplasia, none were identified in male dogs.[12,13] Male dogs have a 16 times higher incidence of mammary neoplasia than do male human beings.[14]

Reported mean age at diagnosis is 10.7 to 11.5 years, with a range of 6 to 16 years.[10,14] No breed predisposition has been demonstrated in the few cases reported.

Pathogenesis of mammary gland neoplasia in male dogs is unknown. While there is evidence supporting a hormonal influence on neoplastic transformation of mammary tissue in female dogs (see Chapter 13), there are conflicting reports concerning influence of estrogen in development of male mammary neoplasms.[10,15,16] One young dog developed adenoma-like mammary proliferation that resolved after castration after treatment with a synthetic progestin.[7] Sexual status of male dogs with mammary neoplasia has not been well documented.

Mammary masses are reported to occur in the first, second, fourth, and fifth mammae, with the fourth and fifth (abdominal) mammae

most frequently represented.[17,18] There is an equal distribution between left and right side.[18]

Both benign and malignant primary mammary masses have been reported in the male dog. Benign mammary masses include adenomas and mixed mammary tumors.[10,11,15,17,19] Malignant neoplasms reported include carcinomas, adenocarcinomas, cystadenocarcinomas, and malignant mixed mammary tumors.[9–11,18,19] Metastases to the regional lymph nodes and lungs have been described.[18,19] There is one report of a dog with multiple tumor types, a 14-year-old crossbreed with both benign and malignant mixed mammary tumors.[11] The two secondary tumors of the mammary gland described in the literature are metastatic osteosarcoma and chondroadenocarcinoma.[19]

Diagnosis is made by visual inspection on physical examination, followed by histologic confirmation from needle aspirate or excision biopsy specimens. A complete physical examination, including careful palpation of all mammae and the regional lymph nodes, and auscultation of the chest should be performed to look for multiple masses and clinical evidence of metastasis. Radiographs of the thorax and radiographs or ultrasound of the abdomen and axillary and inguinal regions may permit identification of enlarged lymph nodes.

Treatment is surgical excision with wide margins. Effect of concurrent castration is unknown. The mass and surrounding tissue should be submitted for histopathology to assess surgical borders and define tumor type and malignancy. Prognosis is good with benign masses, fair to good with malignant masses cleanly excised with no evidence of metastases, and poor to fair with malignant masses incompletely excised or complicated by metastatic disease. There is some evidence that male dogs with one mammary mass are at increased risk of developing subsequent mammary masses.[7] No other treatment modalities for mammary neoplasia (e.g., chemotherapy or external beam radiation) have been described in male dogs.

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Chapter 23

Clinical Approach to Infertility in the Male Dog

Demonstration of normal fertility in the male dog requires insemination of a normal bitch, either by copulation or artificial insemination, with an adequate number of motile, morphologically normal spermatozoa, fertilization of ova, and successful pregnancy and delivery of live puppies. Infertility is a clinical sign or presenting complaint that requires confirmation, characterization, and localization before an etiologic diagnosis can be achieved and treatment considered. Diagnosis of a specific etiology is difficult; 70 (n = 7273) to 74 per cent of infertile human males are reported to have idiopathic infertility.[1,2] Prognosis of male dogs with confirmed infertility is guarded. One report suggests that fewer than 10 per cent of male dogs presenting with infertility returned to fertility after diagnostic work-up and appropriate treatment.[3]

Subfertility in male dogs is defined as a whelping rate of less than 75 per cent when bred appropriately to normal bitches.[4] Infertility is defined as complete failure to impregnate normal bitches that were bred appropriately. Sterility is defined as inability to produce or ejaculate normal spermatozoa.

The following information should be collected as part of a complete work-up for subfertility or infertility in male dogs:

1. *Signalment of the dog*

Puberty in the dog refers to first appearance of a normal number of motile, morphologically normal spermatozoa in the ejaculate, and increased sexual desire and the ability to copulate. Average age of puberty onset in the dog has been described as 7 to 10 months, with a range of 5 to 12 months.[5] The age at which puberty is reached varies by breed; in general, small-breed dogs achieve physical maturity and puberty earlier than large-breed dogs, some of which reach puberty after 12 months of age. Studies evaluating semen quality through puberty in fox terriers (n = 12) and beagles demonstrated increases in scrotal size, volume of the ejaculate, percentage progressively motile spermatozoa, and concentration of spermatozoa, and decreases in percentage immotile and morphologically abnormal spermatozoa as the dogs progressed beyond puberty.[6,7] Young dogs presenting with infertility with poor semen quality should be re-evaluated when older.

2. *General health history of the dog*

Animals suffering from systemic disease, such as chronic active hepatitis, neoplasia, or renal failure, or a chronic complaint, such as chronic vomiting, may have poor semen quality. Medications the dog has received in the past or is receiving at time of admission also may decrease spermatogenesis (see Oligozoospermia). Any history of trauma or thermal injury in the area of the penis or scrotum should be recorded. The pedigree should be evaluated and the degree of inbreeding assessed. Male dogs with inbreeding coefficients of 0.125 to 0.558 (0 = completely outbred, 1.0 = completely inbred) were reported to have decreased reproductive performance compared to completely outbred male dogs, with decreased total number of pups whelped and born live.[8]

3. *Reproductive history of the dog*

The successful and unsuccessful breedings in which the dog has participated should be recorded in chronological order.[4] Other information that should be collected includes age, parity and fertility of bitches bred, type of breeding management used, if any, and dates bred. If semen was collected for artificial in-

semination, quality of semen at the time of breeding can be evaluated. Results of diagnostic tests performed, including cytology and culture of seminal fluid for aerobic and anaerobic bacteria and mycoplasma, and serology for canine brucellosis in the stud and all females bred, should be recorded

A complete physical examination, including evaluation of gait, rectal palpation of the prostate, palpation of the inguinal canals, spermatic cords, epididymes and testes, and evaluation of the flaccid and erect penis, should be performed. Blood samples for a complete blood count, serum chemistry profile, *Brucella canis* serology, and assay of thyroid hormones (free T4 by dialysis [fT4D] and canine thyroid-stimulating hormone [cTSH]) should be collected. A semen sample should be collected by manual ejaculation and evaluated (see Chapter 16). Seminal fluid samples should be submitted for aerobic, anaerobic, and mycoplasma bacterial cultures, and cytology should be evaluated on a seminal fluid sample after centrifugation. Alkaline phosphatase concentration (IU/ml) should be determined in azoospermic serum samples. A urine sample should be collected by cystocentesis after semen collection and submitted for a complete urinalysis, including examination of sediment for spermatozoa as an indicator of retrograde ejaculation.

Failure to Achieve Erection

Normal erection in the dog is mediated by the parasympathetic nervous system; stimulation of the pelvic nerve causes release of a neurotransmitter, either vasoactive intestinal polypeptide or acetylcholine, which in turn causes increased arterial flow and rapid filling of the corpus spongiosum and corpus cavernosum.[9-11] The corpus spongiosum has been shown to increase in volume within 20 seconds of pelvic nerve stimulation.[11] Concurrent active venous occlusion with subsequent decreased venous outflow further enhances filling of cavernous tissue; corpus cavernosal pressure increases from 26 mm Hg during quiescence to 5296 to 7434 mm Hg during intromission, an increase of about 250 times.[12] The pars longa glandis doubles in diameter and elongates. The bulbus glandis trebles in width and doubles in thickness when erection is complete.[13]

Failure to achieve erection may be due to psychological constraints, pain, or androgen insufficiency. Intact male dogs that have been disciplined throughout their life whenever exhibiting mounting and thrusting behavior may be unlikely to show normal breeding behavior at the desired time. Subordinate male dogs may not attempt to breed a bitch they perceive to be dominant. Males may be unwilling to mount a bitch that has been aggressive toward them in the recent past, as may occur if the dogs are introduced too early in the bitch's season. Finally, apprehension in some male dogs may prevent them from showing normal breeding behavior in an environment they find stressful. Some of these problems may be circumvented by changing the environment where copulation is attempted or where semen collection is performed. Experimentation on the part of the owner and veterinarian may allow elucidation of the most successful breeding/semen collection environment for a given male.

Pain at the time of mounting, thrusting, or ejaculation may prevent normal erection in intact male dogs. Dogs with joint or spinal pain may be unwilling to mount the bitch or unable to maintain normal breeding posture. Prostatitis and orchitis may cause pain at the time of ejaculation.

Androgen insufficiency is an uncommon cause of failure to achieve erection. Male dogs may have androgen insufficiency due to testicular atrophy, intersex states, or hypopituitarism.

Diagnosis of the cause of failure to achieve erection requires careful observation of copulation or semen collection by manual ejaculation. If the dog appears painful, localization of the pain guides further diagnostics. If the animal does not appear painful, semen collection should be attempted in the presence of an estrous, periovulatory bitch. If an estrous bitch is not present, an anestrous or spayed teaser may be used; application of the compound methyl *p*-hydroxybenzoate (Aldrich Chemical, Milwaukee, WI) to the vulva of nonestrous bitches has been shown to cause arousal in some male dogs.[14] Another diagnostic test that may define the cause of failure to achieve erection in nonpainful dogs is karyotype of blood lymphocytes (see Chapter 10).

Testosterone is secreted pulsatilely in male dogs, so assay of serum testosterone on a single, random blood sample often is not diagnostic of abnormal concentrations. Blood samples may be drawn for serum testosterone assay three times at 20-minute intervals. Mean concentration of serum testosterone from the three

samples drawn is reported to range from 2.3 to 3.0 ng/ml in normal, intact male dogs.[15] Alternatively, challange testing may be performed. A blood sample drawn 1 hour after administration of gonadotropin-releasing hormone (GnRH; 2 μg/kg intramuscularly [IM]) or 4 hours after administration of human chorionic gonadotropin (hCG; 40 IU/kg IM) should contain more than 3 ng/ml or 4.5 ng/ml testosterone, respectively, in normal, intact male dogs.[4]

Failure to Ejaculate

Normal ejaculation in the male dog requires emission (deposition of spermatozoa and seminal fluid into the prostatic urethra), closure of the bladder neck, and propulsion of the semen through the penile urethra. It is mediated by the sympathetic nervous system via the hypogastric nerve.[16–18] Sympathetic innervation causes closure of the bladder neck, allowing formation of a pressure chamber within the prostatic urethra and preventing excessive flow of semen into the urinary bladder. Rhythmic propulsion of the semen through the penile urethra requires contraction of the bulbospongiosus and ischiocavernosus muscles, which are innervated by the somatic pudendal nerve.[18]

Failure to ejaculate fluid in the presence of erection and ejaculatory behavior is called anejaculation or aspermia. Causes of aspermia in dogs and humans include lack of sexual maturity, pain, psychological factors, drug therapy, and sympathetic neuropathy, either idiopathic or secondary to causes such as diabetes mellitus or spinal cord injury.[1,7,18–20]

Failure to ejaculate may be difficult to assess in dogs attempting unassisted copulation. Assessment of vaginal cytology specimens from bitches bred may allow verification of ejaculation. In one study, spermatozoa heads and rarely intact spermatozoa were seen in about 65 per cent of vaginal cytology specimens collected within the first 24 hours after breeding[21]; absence of spermatozoa on vaginal cytology specimens does not prove that ejaculation did not occur. If there is suspicion of aspermia, semen collection by manual massage should be attempted (see Chapter 16). The average dog may begin pelvic thrusting and ejaculation of the first, pre-sperm, fraction as quickly as within 20.7 seconds of manual massage of the bulbus glandis through the prepuce.[22] Presence of an estrous teaser bitch may increase ease of collection.

Young dogs that have not reached complete sexual maturity may not ejaculate, even if capable of normal erection. In one study, intact male beagles were incapable of ejaculation, on average, until 235 days of age.[7] Small-breed dogs usually reach sexual maturity at a younger age than large- or giant-breed dogs.

Pain, especially due to contraction of an infected prostate gland, may inhibit ejaculation in the older dog.[19,20] Similarly, joint pain (especially of the hips, stifles, or vertebrae) may prevent male dogs from mounting estrous bitches and showing normal pelvic thrusting.

Some experienced stud dogs will not ejaculate during manual semen collection unless presented with an estrous teaser bitch. The occasional male requires a bitch at optimal breeding time for either semen collection or copulation.[23] Some male dogs require distinct surroundings (lighting, nonslip flooring) or operators for semen collection. Preferences of a given stud dog should be recorded to facilitate subsequent semen collections. Subordinate males may refuse to copulate with a bitch they perceive to be dominant, or which has been aggressive toward them in the recent past. Ejaculation may be effected in these dogs by treatment with GnRH (1 to 2 μg/kg subcutaneously [SC] 2 to 3 hours prior to collection or attempted breeding).[24] GnRH causes release of endogenous luteinizing hormone (LH), with subsequent release of testosterone. This method should not be used routinely in valuable stud dogs; frequent artificially enhanced serum testosterone concentrations may exert negative feedback on the pituitary, which may cause an eventual decrease in serum testosterone concentrations and decreased spermatogenesis. Use of GnRH is preferred to that of exogenous testosterone, which exerts significant negative feedback on the pituitary, with subsequent decrease in LH release, serum testosterone concentrations, intratesticular testosterone concentrations, and spermatogenesis.[4]

Drugs that have been reported to cause ejaculatory dysfunction in human beings include the tricyclic antidepressant amitriptyline, and the nonsteroidal anti-inflammatory drug naproxen.[18]

Retrograde Ejaculation

During normal ejaculation in the dog, a portion of the total volume of semen ejaculated may flow retrograde into the urinary bladder.

Blockage of sympathetic α receptors in the bladder neck, due to autonomic neuropathy as part of a disease process as may be seen in human beings with diabetes mellitus, due to genetic factors, or experimentally induced with phentolamine, causes decreased antegrade flow of semen, characterized by decreased semen volume and total number of spermatozoa in the ejaculate, and increased retrograde flow of semen.[17,18,25] Increased retrograde flow of semen was demonstrated in 12 of 15 dogs sedated with xylazine.[26]

Retrograde ejaculation has been reported to occur in the dog, although etiology of the condition in this species has not been defined.[27,28] A 19-month-old, intact male Labrador retriever with concurrent retrograde ejaculation and hypothyroidism did not demonstrate spontaneous antegrade ejaculation after normalization of serum thyroid hormone levels with thyroxine supplementation.[28] Diagnosis requires collection of a urine sample by cystocentesis after semen collection, and comparison of numbers of spermatozoa in the antegrade ejaculate and in the urine sediment.

Treatment with sympathomimetic drugs may effect antegrade ejaculation. Possible therapies include phenylpropanolamine (3 mg/kg per os twice daily) or pseudoephedrine (4 to 5 mg/kg per os three times daily or 1 and 3 hours before semen collection or attempted breeding).[28]

In human beings, spermatozoa ejaculated into the urinary bladder have been retrieved for artificial insemination. The man is instructed to drink large volumes of water, and to take sodium bicarbonate the day before and of semen collection, so as to create a positive environment for maintenance of viable spermatozoa within the bladder. Dilute, alkaline urine is desired, with a pH of greater than 7.0 and osmolality of 200 to 300 mOsm/kg of water.[18] After ejaculation, urine is retrieved with a catheter or by direct voiding. After centrifugation, the sperm pellet is suspended in extender and artificial insemination is performed. The only reported such attempt in the dog was unsuccessful.[27]

Failure to Achieve Normal Copulation

Causes of failure to achieve normal copulation in intact male dogs include sexual immaturity or postmaturity, sexual overuse, pain, psychological constraints and idiopathic poor libido. Careful evaluation of the history and physical examination findings of the bitches involved may uncover abnormalities in the female contributing to the male's apparent inability to mount and achieve intromission, such as vaginal strictures, vaginal prolapse, or improper breeding management.

Juvenile dogs may be unable to copulate even if they are capable of spontaneous ejaculation. Inexperienced males may benefit from exposure to nonaggressive, experienced females. Semen collection by manual ejaculation in young dogs will not necessarily make them less capable of copulation. Aged dogs may fail to achieve normal copulation because of decreased libido secondary to a normal decline in secretion of testosterone.[29]

Normal male dogs may be used daily for breeding with no adverse effect on libido and ability to copulate, although total number of spermatozoa in the ejaculate decrease daily as extragonadal (epididymal) spermatozoal reserves are depleted and spermatozoa numbers defined as the daily sperm output are reached.[30] Male dogs that ejaculate two or three times daily show a decline in libido, with that decline correlated with frequency of ejaculation. Semen quality and libido return to normal within 2 days after the last of these frequent collections.[30]

Dogs with joint or spinal pain may be unable or unwilling to attain or maintain the physical posture of normal copulation (Fig. 23–1). Dogs with failure to ejaculate secondary to prostatitis may appear to be unable to achieve normal copulation.[19,20] A 19-month-old intact male cocker spaniel presenting with failure to achieve normal copulation and poor libido was diagnosed with a persistent penile frenulum; 1 year after surgical correction of the penile frenulum, normal copulation and successful impregnation of a bitch were achieved.[31]

Abnormalities of sexual differentiation decrease likelihood of a phenotypically male animal's ability to copulate successfully. Prenatal and early postnatal secretion of testosterone cause neural differentiation specific for exhibition of male breeding behavior at maturity. Animals deprived of this testosterone influence at birth by neonatal castration do not exhibit normal mounting behavior at or after puberty, even if supplemented with testosterone in adulthood.[32] Female pseudohermaphrodites, animals with male external genitalia and female gonads, and true hermaphrodites, animals with both male and female gonadal tis-

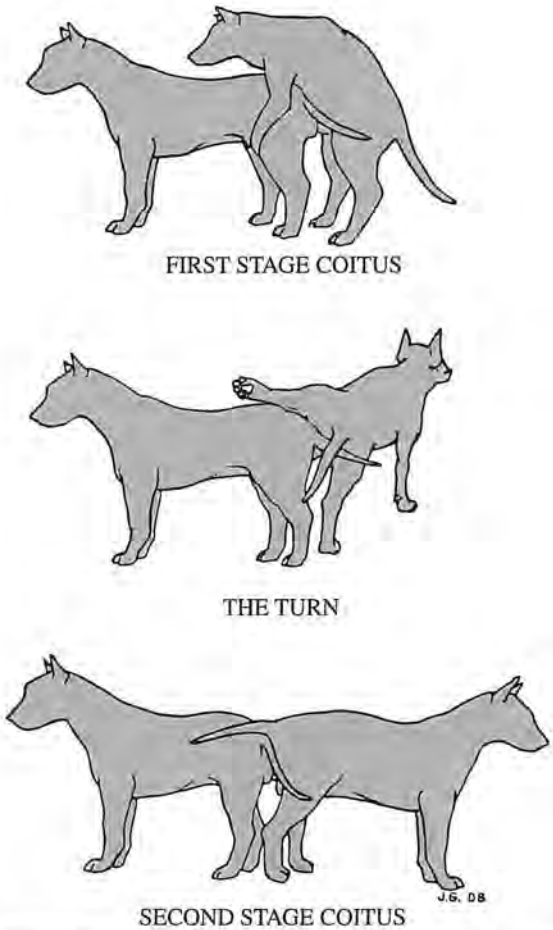

Figure 23–1. Typical coital postures in the dog. (From Grandage J: The erect dog penis: A paradox of flexible rigidity. Vet Rec 91:141–147, 1972, with permission.)

sue, are unlikely to exhibit normal breeding behavior, and usually are sterile.

Psychological constraints may prevent a physically normal intact male dog from achieving normal copulation. Normal variation exists in time between exposure of the male dog to the estrous bitch and attempted mounting and intromission. Some males attempt to mount the bitch immediately, while others engage in social and exploratory behavior before approaching the bitch.[32] If the male shows no sexual interest in the bitch, history regarding raising and housing of the male should be taken. Male dogs raised in complete isolation from other dogs from 3 to 12 weeks of age are less likely than dogs raised with littermates to show normal mounting behavior and copulation as adults.[32,33]

Male dogs with idiopathic poor libido are a diagnostic dilemma. Measurement of serum testosterone concentration rarely is rewarding, as concentrations usually are normal.[4] Administration of GnRH, as described for anejaculation (1 to 2 μg/kg SC 2 to 3 hours prior to collection or attempted breeding) may effect an increase in libido and ejaculation.[24] Administration of exogenous testosterone may cause an increase in libido and subsequent normal copulation. However, repeated treatment with either GnRH or testosterone may suppress intratesticular testosterone concentrations and spermatogenesis, and may exacerbate androgen-dependent disease, such as benign prostatic hyperplasia / hypertrophy and perianal adenoma.[4,24]

Infertility with Normal Copulation and Normal Semen Quality

No tests for function of apparently normal spermatozoa, such as the zona-free hamster egg penetration assay, are routinely used in the dog. Types of breeding (copulation or artificial insemination), semen (fresh, chilled, or frozen), and insemination route (vaginal, transcervical intrauterine, or surgical intrauterine) should be assessed. A total dose of 150 to 250 million normal spermatozoa introduced over the bitch's fertile period usually is adequate to achieve pregnancy.[34,35] In general, conception rate is better with copulation than artificial insemination, with a decline in conception rate with increasing manipulation of the spermatozoa.[36] Spermatozoa collected and used immediately have greater longevity than those placed in extender and chilled for 2 days or less, which in turn have greater longevity than those frozen and thawed[37] (see Chapter 4).

Human males with idiopathic infertility have been reported to have significantly increased fertility rates after treatment with the prostaglandin inhibitor indomethacin (75 mg per individual per day).[2]

Infertility with Normal Copulation and Abnormal Semen Quality

Semen collection and evaluation are described in Chapter 16.

Azoospermia

Azoospermia is ejaculation of seminal fluid that does not contain spermatozoa.[38] In a sur-

vey of 102 dogs with reproductive dysfunction from which 181 ejaculates were collected, 34.8 per cent were azoospermic.[39]

Artifactual azoospermia may occur in normal intact male dogs that are apprehensive at the time of collection or that require the presence of an estrous teaser bitch for optimal stimulation, since they may ejaculate only the first (pre-sperm) fraction of the ejaculate. Collection of more than one semen sample under optimal conditions is required before a diagnosis of azoospermia can be confirmed.[38] Diagnosis of incomplete ejaculation may be made by measurement of carnitine or alkaline phosphatase (AP) concentrations in seminal fluid. Concentration of carnitine in seminal fluid is much higher in intact male dogs than in those that have been vasectomized or castrated, suggesting epididymal origin.[40] AP activity has been demonstrated to be much higher in the epididymis than in the testes or prostate, with greatest activity in the cauda epididymis.[41] Seminal fluid from normal intact male dogs that have failed to provide a complete ejaculate usually contains less than 5000 U/L AP. Male dogs with true azoospermia, due to causes other than bilateral blockage of the outflow tract (see below), usually have AP concentrations of greater than 5000 U/L in seminal fluid. If AP concentrations are equivocal, greater accuracy may be achieved by collecting two ejaculates 1 hour apart, and submitting seminal fluid from the second collection for AP determination.

True azoospermia is described most commonly in purebred dogs, but may occur in crossbred dogs.[42–46] Average age at time of diagnosis is 3.7 years (n = 23), with a range of 1.5 to 8.0 years.[42–46] Male dogs diagnosed with azoospermia may or may not be proven sires; in one survey, 8 of 18 dogs (44 per cent) had sired at least one litter prior to presentation.[44] The Labrador retriever is the breed most commonly reported with azoospermia (8 of 23 reported cases).[42–44] Heritability of the condition is not reported, although related Scottish terriers[44] and Labrador retrievers[43,44] with azoospermia have been described. One report documented acquired azoospermia in a male Labrador retriever and two of his male offspring; related males were reported to become infertile anywhere from 2 to 7 years of age, but another related male had sired a litter at 12 years of age.[43] In a survey of 18 dogs with azoospermia, six (33 per cent) had male relatives with known reproductive dysfunction.[44]

Causes of azoospermia in the dog may be classified as pretesticular, testicular, or posttesticular. Pretesticular causes of azoospermia in the dog include hypopituitarism,[44,47] steroid excess in animals with hyperadrenocorticism or those receiving long-term, high-dose glucocorticoid therapy, long-term, high-dose administration of other classes of drugs that impair spermatogenesis, such as antineoplastic agents,[38] hypothyroidism,[47] inguinal or scrotal hernia,[1] and fever. In human beings, high fever (≥38°C) causes a decline in semen quality but does not cause complete azoospermia.[1]

Testicular causes of azoospermia include intersex states[1,38,48,49]; germinal cell aplasia[46,50]; bilateral cryptorchidism[1]; testicular injury due to trauma, irradiation, thermal insult, or orchitis[45]; autoimmune testicular disorders, including spermatogenic arrest[44,46,51]; and testicular neoplasia.[44]

Chromosomal or developmental abnormalities of sexual differentiation that occur in phenotypically normal male dogs that are infertile include female pseudohermaphroditism, and dogs with a 79,XXY karyotype or XX sex reversal. Female pseudohermaphrodites, individuals with male external genitalia and female gonads, are uncommon, and usually occur due to masculinization of female fetuses in utero by administration of exogenous androgens to the dam.[49] The 79,XXY karyotype in dogs is associated with hypoplastic testes and lack of spermatogenesis, and undeveloped but not ambiguous external genitalia.[38,49] In humans, only 6 per cent of men with an XXY karyotype are reported to have spermatozoa in the ejaculate.[1] The phenomenon of XX sex reversal, presence of male external genitalia and testicular and/or ovarian gonadal tissue in an individual with a 78,XX karyotype, is inherited as an autosomal recessive trait in American cocker spaniels.[49] A similar condition is reported in Kerry blue terriers, pugs, English cocker spaniels, beagles, Weimaraners, and German shorthaired pointers.[49] Phenotypically male American cocker spaniels with XX sex reversal may have bilateral testes (10 per cent) or both testicular and ovarian tissue (90 per cent), usually are bilaterally cryptorchid, and often have a hypoplastic penis and hypospadias.[49] There is some evidence that XX sex reversal in American cocker spaniels is due to translocation of a segment of the Y chromosome onto an X chromosome or autosome. Phenotypically male dogs with XX sex reversal are infertile.

Germinal cell aplasia ("Sertoli cell only" syndrome) has been reported to occur in 1.0 per cent (n = 81)[50] to 10.0 per cent (n = 10)[46] of dogs with azoospermia. Only Sertoli cells are identified on histopathology of testicular tissue of affected dogs. Histologic changes may be indistinguishable from those of testicular atrophy unless fibrotic changes also are present.

Male dogs with bilateral cryptorchidism are azoospermic. Libido is normal, since the retained testes are capable of steroidogenesis (see Chapter 18). In human beings, azoospermia may be seen at maturity in men with delayed testicular descent.[1]

Any process that damages testicular tissue with subsequent inflammation significantly affects spermatogenesis. Oligozoospermia, reduced number of spermatozoa in the ejaculate, is a more common manifestation of testicular inflammation than is azoospermia (see Oligozoospermia below). Severe or unresolved inflammation may cause progression from oligozoospermia to azoospermia; a 3-year-old intact male Shetland sheepdog with infection of the reproductive tract with *Pseudomonas aeruginosa* progressed from oligozoospermia to azoospermia over a 4-month period, despite appropriate antibiotic therapy.[45]

Autoimmune orchitis may be associated with azoospermia (see Chapter 18).[38,51] In a closed colony of 69 related male beagles, incidence of autoimmune orchitis was linked to that of autoimmune thyroiditis, and 59 per cent of the affected dogs were azoospermic.[51] In a survey of 18 dogs with azoospermia, one had evidence of orchitis and autoimmune thyroiditis, with increased serum thyroid autoantibodies.[38] Spermatogenic arrest, identified by testicular histopathology in 2 of 10 dogs with azoospermia,[46] is hypothesized to have an autoimmune etiology and may be heritable.[52]

Testicular neoplasia may be a cause of azoospermia. In a survey of 18 male dogs with azoospermia, one was diagnosed with Sertoli cell tumor of the testis.[44] Testicular neoplasms may cause azoospermia by direct destruction of testicular tissue, induction of inflammation, elevation of intrascrotal temperature, and production of estrogen or androgen that may exert negative feedback on the hypothalamus and pituitary (see Chapter 18).

Post-testicular causes of azoospermia in the dog are those causing outflow obstruction, and include epididymal segmental aplasia,[54] and spermatocele or sperm granuloma.[11,54] In human beings, obstructive causes of azoospermia account for 0.9 per cent of cases.[1] Male dogs with bilateral aplasia of any portion of the efferent ducts are azoospermic (see Chapter 18). Spermatoceles and sperm granulomas may be palpable as nonpainful unilateral or bilateral swellings of the epididymis. Obstructive azoospermia is present only in dogs with bilateral occlusion of the ducts (see Chapter 18).

There are no pathognomonic clinical signs consistent with the diagnosis of azoospermia in male dogs. The testes may be normal in size and consistency (44 per cent, n = 18), normal in size with soft consistency (33 per cent), small with firm consistency (12 per cent), small with soft consistency (6 per cent), or small with normal consistency (6 per cent).[42–44,46,54] Testicular degeneration and palpable softening of both testes was demonstrated after bilateral vasectomy[55] or bilateral ligation of the cauda epididymes[56] in dogs, suggesting that this testicular change may be more common in dogs with obstructive azoospermia. Scrotal dermatitis occasionally is reported in dogs with azoospermia (11 per cent, n = 19). Libido usually is reported as normal to excellent in affected dogs.[42–44,46,54]

Diagnosis of the etiology of azoospermia requires localization and characterization of the type of defect present (Table 23–1). Retrograde ejaculation can be diagnosed by collection of a urine sample by cystocentesis after ejaculation (see Retrograde Ejaculation).

Incomplete ejaculation can be diagnosed by measurement of AP concentration of less than 5000 U/L in seminal fluid collected from a male with poor libido collected in suboptimal conditions, as previously described. Concentration of AP in seminal fluid also is low in male dogs with bilateral obstructive azoospermia. One male dog with obstructive azoospermia was reported to have AP concentration in seminal fluid of less than 10 U/L.[44] Epididymal abnormalities may or may not be palpable or visible ultrasonographically.[42,44,54] Aspiration of the cauda epididymes may allow verification of spermatogenesis but does not allow differentiation of obstructive azoospermia and incomplete ejaculation.[38] Because aspiration of the epididymes may cause extravasation of spermatozoa and subsequent formation of sperm granulomas and antisperm antibodies, less invasive diagnostics, like epidymal ultrasound, should be considered first.[23]

If AP concentration in seminal fluid containing no spermatozoa is greater than 5000 U/L, pretesticular and testicular causes of azoospermia should be ruled out with careful history,

Table 23-1. Causes of Azoospermia in the Dog and Diagnostic Tests of Choice

Cause of Azoospermia	Diagnostic Test(s) of Choice
Retrograde ejaculation	Urinalysis by cystocentesis, after ejaculation
Incomplete ejaculation	Alkaline phosphotase (AP) in seminal fluid (<5000 U/L)
Pretesticular causes	
Hypopituitarism	LH/FSH in serum (decreased)
Hypothyroidism	Free T4 by dialysis (decreased), cTSH (increased)
Inguinal/scrotal hernia	Physical examination
Endogenous or exogenous glucocorticoid excess	History, endogenous ACTH in serum, ACTH stimulation or dexamethasone suppression test
Testicular causes	
Intersex animals	Karyotype (n = 78, XY), AP in seminal fluid (>5000 U/L)
Germinal cell aplasia	Testicular biopsy (only Sertoli cells present), AP in seminal fluid (>5000 U/L)
Bilateral cryptorchidism	Physical examination
Testicular injury (trauma, thermal injury, orchitis)	Semen culture, *B. canis* serology, seminal fluid cytology, AP in seminal fluid (>5000 U/L)
Autoimmune orchitis	AP in seminal fluid (>5000 U/L) testicular biopsy
Spermatogenic arrest	AP in seminal fluid (>5000 U/L) testicular biopsy
Testicular neoplasia	Physical examination, testicular ultrasound, testicular biopsy
Post-testicular causes	
Segmental epididymal aplasia	AP in seminal fluid (<5000 U/L), epididymal ultrasound, exploratory surgery
Spermatocele or sperm granuloma	AP in seminal fluid (<5000 U/L), epididymal ultrasound, exploratory surgery

complete physical examination, thyroid hormone testing, measurement of endogenous adrenocorticotropic hormone (ACTH), and ACTH stimulation or dexamethasone suppression testing for hyperadrenocorticism, and measurement of the gonadotropins, follicle-stimulating hormone (FSH), and LH.

Serum concentration of LH is normal to slightly elevated in male animals with gonadal failure.[38] Serum FSH concentration increases in animals with testicular pathology, with that elevation in FSH concentration in serum positively correlated with severity of altered spermatogenesis.[3,4] As spermatogenesis fails, Sertoli cells in the affected testes decrease production of inhibin, allowing excessive production and release of pituitary FSH.[3] FSH and LH are released episodically, requiring collection of three blood samples at 20-minute intervals, or challenge testing before measured concentrations can be determined accurately.[3,38] Blood samples are drawn 10 minutes after administration of GnRH (250 ng/kg intravenously = [IV]) for assay of LH and FSH. Normal serum concentrations of LH and FSH in intact male dogs post-GnRH are 32 ± 11 ng/ml and 62 to 293 ng/ml, respectively.[4] FSH assays are not routinely commercially available. Samples for LH assay can be submitted to the Rothgerber Endocrinology Laboratory at Colorado State University (303-491-5620, Fort Collins, CO).

Diagnosis of testicular causes of azoospermia begins with a complete history and physical examination. Karyotype should be evaluated in dogs with lifelong history of sterility and/or abnormal or immature external genitalia (see Chapter 18). Culture of ejaculated seminal fluid for aerobic and anaerobic bacteria and mycoplasma, cytology of seminal fluid after centrifugation and *Brucella canis* serology are indicated for diagnosis of orchitis (see Chapter 18). Testicular neoplasia may be palpable or visible ultrasonographically (see Chapter 18).[44]

Definitive diagnosis of hypo- or aspermatogenesis and germinal cell aplasia require assessment of testicular function by aspiration, or core or incisional biopsy of the testis. Fine-needle aspirate (FNA) is not an effective technique in animals with soft testes, since fewer cells are likely to be collected than in dogs with normal or firm testes. Active spermatogenesis is inferred if mature spermatozoa are collected in a testicular FNA; neoplastic and inflammatory cells also may be detected using this technique.[46] Core or incisional biopsies collect a plug or wedge of testicular tissue, and allow more complete assessment of the stages of spermatogenesis (see Chapter 18).[50,57] The gain in diagnostic

value of testicular samples collected by core or incisional biopsy compared to FNA may be offset by the greater incidence of complications after the procedure. No changes were reported in male dogs' semen quality or libido after FNA (n = 5),[46] while various gross and microscopic abnormalities, ranging from focal intratesticular hemorrhage and tubular degeneration to maturation arrest, coagulation necrosis, and formation of adhesions between testicular tunics have been described for core and incisional biopsy (n = 79).[50,57] Cost, risk to the animal, including that of anesthesia for core and incisional biopsy, and value of the data obtained to guide decisions regarding possible treatment and prognosis must be weighed in each case. In human beings, diagnostic testicular biopsy is not performed routinely if testicular volume is decreased or serum concentration of FSH is increased.[1] In dogs, with the advent of testicular ultrasound and measurement of AP in serum, need for testicular biopsy has declined.

Testicular histology reported in azoospermic dogs is extremely variable.[42,44] Most dogs have some evidence of normal spermatogenesis, with as many as 60 to 80 per cent of the seminiferous tubules appearing histologically normal.[43] In one survey, 13 of 17 (76 per cent) azoospermic dogs evaluated with testicular biopsy had some spermatozoa present.[44] Varying degrees of degeneration of seminiferous tubules are present (Fig. 23–2).

Evidence of active spermatogenesis on a testicular biopsy sample in azoospermic dogs for which no definitive etiology has been established does not guarantee a return to fertility. Treatment is dependent on etiology, if identified, and degree of testicular degeneration. Palpably soft or very firm testicles and/or those with moderate to severe degenerative or fibrotic changes identified by testicular histopathology are unlikely to become functional. Surgery may be attempted in some dogs with obstructive azoospermia, but prognosis for future fertility is guarded to poor.

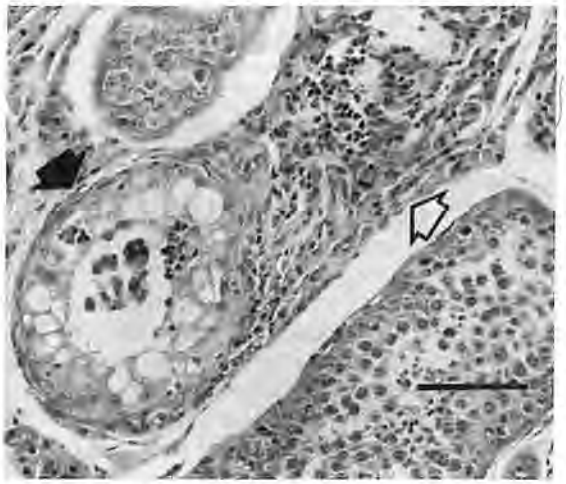

Figure 23–2. Photomicrograph of a section of testis from an azoospermic dog with a history of past fertility. Biopsy revealed degenerative seminiferous tubules, containing necrotic spermatids, multinucleate spermatids, and lymphocytes in the lumen. Vacuolar degeneration of spermatids and spermatogonia (solid arrow) and lymphocytic infiltrate around the tubule (open arrow) are evident. H&E and periodic acid–Schiff stain; bar = 100 μm. (From Olson PN, Schultheiss P, Seim HB: Clinical and laboratory findings associated with actual or suspected azoospermia in dogs: 18 cases [1979–1990]. J Am Vet Med Assoc 201:478–482, 1992, with permission.)

Oligozoospermia

Oligozoospermia is low total number of spermatozoa in the ejaculate. In a survey of 102 dogs from which 181 ejaculates were collected, 26.1 per cent were oligozoospermic.[39]

The number of spermatozoa a dog can produce daily is dependent on grams of testicular parenchyma present.[58] Testicular size is well correlated with body weight and with total scrotal width.[59,60] Measurement of total scrotal width with calipers (Fig. 23–3) was well correlated with ultrasonographic measurement of scrotal width in a survey of 30 dogs.[61] Figure 23–4 depicts correlation between body weight of male dogs and expected total scrotal width; dogs with total scrotal width measurement falling below the 95 per cent confidence limit had few to no spermatozoa in their cauda epididymes (n = 71).[60]

Normal total number of spermatozoa in the canine ejaculate is 300 million or greater.[62] Normal male dogs of very small breeds may not have enough testicular mass to produce 300 million spermatozoa, and should be considered normal if total number of spermatozoa in the ejaculate is at least 10 million per pound of body weight.

Total number of spermatozoa in the canine ejaculate is higher if manual semen collection is performed in the presence of a proestrous or estrous teaser bitch.[59,63] If semen is collected daily, total number of spermatozoa will decrease in each ejaculate as epididymal reserves are depleted.[58,63] However, normal dogs have been reported to have daily sperm output of 295 to 475 million spermatozoa, suggesting

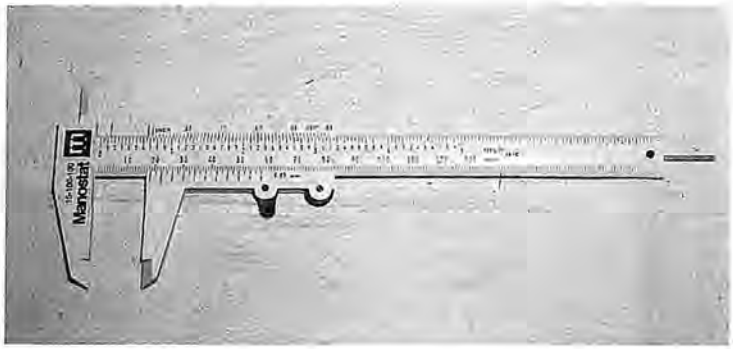

Figure 23–3. Calipers for measurement of total scrotal width in the dog.

that oligozoospermia should not occur even if the male ejaculates daily.[58]

Oligozoospermic dogs are not necessarily infertile. A total dose of 150 to 200 million spermatozoa must be introduced into the bitch during her fertile period to reliably achieve pregnancy.[34,35] In a survey of 28 dogs, a pregnancy rate of 85 per cent was reported in dogs with mean total number of spermatozoa of 333 million, with a range of 36 million to 630 million.[64]

Idiopathic oligozoospermia was diagnosed in 11.2 per cent of 7273 cases of male infertility in human beings.[1] Incidence in the dog has not been reported.

Seasonal oligozoospermia may occur in dogs; semen quality has been demonstrated to decline during the summer months in the Northern hemisphere with a decrease in total number of spermatozoa ejaculated.[65,66] No seasonal change was observed in a Malaysian study evaluating male dogs habituated to that climate.[67]

Oligozoospermia has been reported in dogs with unilateral Sertoli cell tumors.[68] Sertoli cell tumors may cause decreased spermatogenesis in the affected testis by local infiltration and in the unaffected testis by exudation of secretory products, including androgens and estrogens, which exert negative feedback on the hypothalamus and pituitary.[69] Elevation of intrascrotal temperature may cause atrophy and decreased spermatogenesis in the non-neoplastic testis.

Prostate disease may be associated with infertility; in a survey of nine male dogs present-

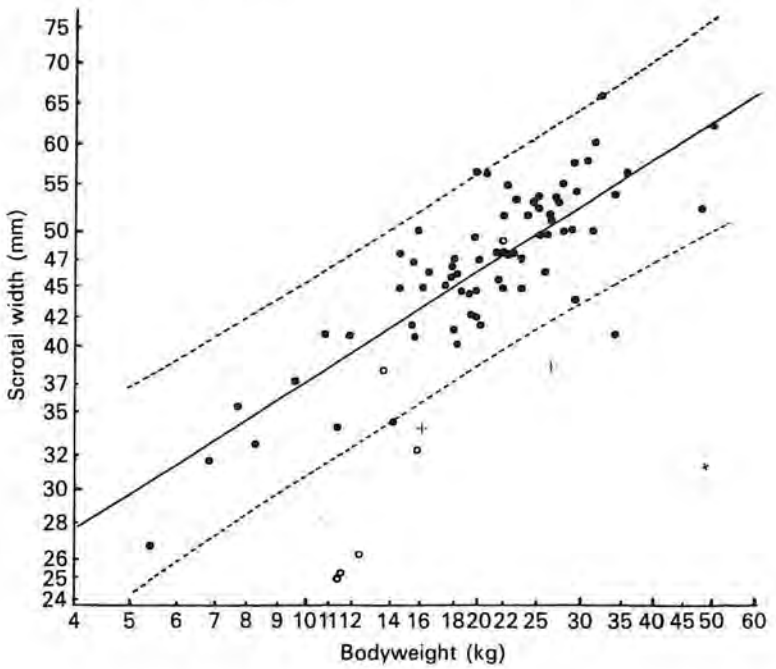

Figure 23–4. The relationship between scrotal width and body weight on logarithmic axes. The regression line for category A males is indicated by the solid line and the dashed lines enclose 95 per cent confidence limits for estimation of scrotal width from body weight. Category A males (enclosed circles) = those with many spermatozoa present in smears from the cauda epididymis. (From Woodall PF, Johnstone IP: Scrotal width as an index of testicular size in dogs and its relationship to body size. J Small Anim Pract 29:543–547, 1988, with permission.)

ing for infertility, five had prostatitis and one had benign prostatic hypertrophy / hyperplasia.[70] Complete evaluation of the prostate is recommended in all male dogs presented for infertility (see Chapter 20).

Orchitis and epididymitis cause decreased spermatogenesis both in the acute phase with associated inflammation and local hyperthermia, and in chronic conditions, with possible testicular fibrosis or atrophy. In mice, experimental bacterial infection of one testis caused inflammatory changes and aspermatogenesis in both testes.[71] Hypothesized causes were hematogenous spread of the organism to the contralateral testicle or induction of a systemic immune reaction after exposure of testicular autoantigens to the animal's immune system.[71] Reported causes of acute orchitis and epididymitis in the dog include *Brucella* sp., *Escherichia coli*, mycoplasma, and other aerobic organisms constituting normal preputial flora.[3,52,72–74]

Immune-mediated orchitis has been associated with lymphocytic thyroiditis in the dog.[51,75,76] Decreased testis size and weight and decreased fertility was reported in 41 per cent of 69 affected male beagles in one colony.[51] Colonies of borzois[75] and beagles[76] with familial lymphocytic thyroiditis have been reported with poor semen quality and decreased fertility; testes from three of four male beagles necropsied had no evidence of active spermatogenesis.[76]

The link between hypothyroidism and oligozoospermia in dogs with apparently normal testes is unclear. Three of four dogs with oligozoospermia were reported to be hypothyroid in one study.[47] Reported signs of reproductive dysfunction secondary to hypothyroidism include decreased libido, decreased ejaculate volume, and poor semen quality with hypospermatogenesis.[77,78] However, male dogs with hypothyroidism induced either by thyroidectomy and propylthiouracil therapy[3] or treatment with radioactive iodine[78] have not been demonstrated to develop changes in testicular size or daily sperm output, despite appearance of other clinical signs characteristic of hypothyroidism, such as obesity, lethargy, and bilaterally symmetrical alopecia.

Many classes of drugs have been reported to cause reproductive dysfunction in dogs, usually manifested as a rapid decrease in number of spermatozoa ejaculated (Table 23–2). Safe dose regimens generally are not defined. The negative effect of the drug on spermatogenesis usually regresses after withdrawal of the drug.

Treatment of oligozoospermia in dogs in dependent on etiology. With any treatment, no improvement is likely to be noted in the ejaculate for at least 62 days after treatment is instituted, since that is the duration of the normal spermatogenic cycle of the dog.

One of three dogs with unilateral Sertoli cell tumor was described with an increase in total number of spermatozoa in the ejaculate after unilateral orchidectomy. Dogs with oligozoospermia due to prostatitis or orchitis may

Table 23–2. Drugs Reported to Cause Reproductive Dysfunction in Male Dogs

Drug	Mode of Action	References
Glucocorticoids Prednisone, betamethasone	Decrease synthesis and release of LH	Meyers-Wallen,[4] Johnson et al.,[78] Kemppainen et al.,[79] and Freshman[80]
Anabolic steroids	Negative feedback to hypothalamus and pituitary	Goodman and Cain[37] and Kemppainen et al.[79]
Estrogens Estradiol, diethylstilbestrol	Negative feedback to hypothalamus and pituitary	Meyers-Wallen,[4] Kemppainen et al.,[79] Taha et al.,[81] and Jones and Boyns[82]
Androgens Testosterone	Negative feedback to hypothalamus and pituitary	Meyers-Wallen,[4] Bamberg-Thalen and Linde-Forsberg,[83] and England[84]
Progestogens Medroxyprogesterone acetate, delmadinone acetate	Negative feedback to hypothalamus and pituitary	Freshman,[80] Bamberg-Thalen and Linde-Forsberg,[83] and Freshman et al.[85]
Chemotherapeutic agents	Direct negative effect on germinal cells	Kemppainen et al.[79] and Paramo et al.[86]
Ketoconazole	Blocks androgen biosynthesis	Goodman and Cain,[37] Kemppainen et al.,[79] Davis,[87] and Decoster et al.[88]
Amphotericin B	Causes maturation arrest	Paramo et al.[86]
Cimetidine	Decreases responsiveness of pituitary to GnRH	Meyers-Wallen[4] and Goodman-Cain[37]
GnRH agonists/antagonists	Direct effect at hypothalamus and pituitary	Freshman et al.,[85] Willard et al.,[89] Vickery et al.,[90] Vickery et al.,[91] and Vickery et al.[92]

show improvement in total number of spermatozoa ejaculated after appropriate antibiotic therapy (see Chapters 18 and 20) and, in the case of prostatitis, improvement may be observed within days of onset of specific antibiotic therapy. If extensive inflammatory, fibrotic, or degenerative changes have occurred in the testis with orchitis, return to normal fertility is unlikely. In male dogs with unilateral orchitis, removal of the affected testis may prevent damage to the unaffected testis from local inflammation or systemic immune response. In hypothyroid dogs, appropriate supplementation with thyroxine (0.01 to 0.02 mg/kg twice daily per os) may allow a return to fertility, unless concurrent thyroiditis and orchitis are present, as with systemic autoimmune disease. Spermatogenesis and total number of spermatozoa in the ejaculate usually increase after withdrawal of drugs detrimental to reproductive function.

Medical therapy described for idiopathic oligozoospermia in dogs includes treatment with a GnRH agonist (1 μg/kg SC) with or without subsequent treatment with hCG (1600 IU IM). One of five dogs responded favorably to treatment with the GnRH agonist alone and two of four showed a transient increase in total number of spermatozoa in the ejaculate after treatment with the GnRH agonist and hCG.[94]

Management of male dogs with oligozoospermia for maximal fertility includes collection of semen no more frequently than every 2 to 4 days.[58,63] Intrauterine insemination may enhance fertility of male dogs with low total number of spermatozoa in the ejaculate (see Chapter 4).

Hematospermia

Hematospermia is blood in the ejaculate. It occurs most commonly secondary to benign prostatic hypertrophy with sanguineous prostatic fluid (Fig. 23–5) and also may occur if the penis is traumatized during semen collection or copulation.[19,23] It is seen occasionally in juvenile dogs at first semen collection. Hematospermia has been reported in human beings after prolonged sexual abstinence.[94] In a survey of 174 human beings with hematospermia, 50 (28 per cent) were diagnosed with prostate disease.[95] Malignant neoplasia of the genitourinary tract was present in 5 per cent of human beings with hematospermia.[95] One of 15 dogs diagnosed with testicular neoplasia presented with hematospermia in one report.[96] In human beings, hematospermia usually is idiopathic and self-limiting.[95]

Figure 23–5. Prostatic fluid containing blood continued to be discharged on the floor after an ejaculate had been collected from a dog with prostatomegaly. (From Olson PN, Wrigley RH, Thrall MA, et al: Disorders of the canine prostate gland: Pathogenesis, diagnosis, and medical therapy. Compend Contin Educ Pract Vet 9:613–623, 1987, with permission.)

Dogs with hematospermia are not necessarily infertile; male dogs with blood in the ejaculate have been reported to sire litters.[4] If spermatozoa are to be preserved, either as chilled extended semen or frozen semen, longevity of spermatozoa may be enhanced by centrifugation of the hematospermic sample and resuspension of the pellet containing spermatozoa in extender soon after collection.

Teratozoospermia

Teratozoospermia is decreased percentage of morphologically normal spermatozoa (Fig. 23–6). Reported percentage of morphologically normal spermatozoa (%MNS) in normal dogs is 80 per cent or greater.[82] In a survey of 67 male dogs, pregnancy rates caused by those with %MNS of 60 per cent or greater was 61 per cent, while that of dogs with %MNS less than 60 per cent was 13 per cent.[97] Although teratozoospermia is associated with infertility in dogs,[97–102] there are few descriptions of the effect of specific morphologic defects on fertility. Specific morphologic defects associated with infertility in the dog include abnormali-

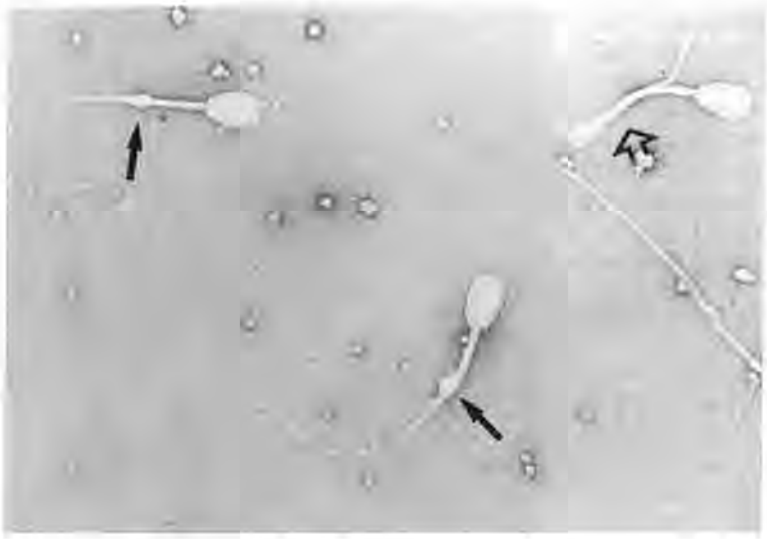

Figure 23–6. Canine spermatozoa with morphologic defects. Note spermatozoa with distal retained cytoplasmic droplets (closed arrows) and reflex midpiece (open arrow). (From Freshman JL, Amann RP, Bowen RA, et al: Clinical evaluation of infertility in dogs. Compend Contin Educ Pract Vet 10:433–460, 1988, with permission.)

ties of midpiece attachment or ultrastructure,[98,100,101] microcephalic spermatozoa,[99] and proximal retained cytoplasmic droplets.[99,101] Specific morphologic abnormalities associated with poor post-thaw motility of cryopreserved spermatozoa include coiled and bent midpieces and tails, and proximal retained cytoplasmic droplets.[103,104]

There is no reported effect of age on incidence of teratozoospermia in the dog.[97] No breed predisposition has been reported, although related male dogs with similar morphologic defects of spermatozoa have been reported.[99] There is one report of a seasonal effect, with decreased %MNS in the summer months.[65]

Teratozoospermia may be congenital or acquired. The only well-defined congenital cause reported in the dog is fucosidosis, a lysosomal storage disease affecting function of epididymal epithelial cells and causing retention of cytoplasmic droplets.[105] Acquired causes reported in the dog include testicular tumors,[65] orchitis,[74,106,107] prostatitis, high fever,[1,106] obesity with increased intrascrotal temperature due to formation of periscrotal fat,[106] and sexual abstinence or overuse.[106] Of 7273 men with infertility, 5.9 per cent were diagnosed with idiopathic teratozoospermia.[1]

Diagnosis of etiology of teratozoospermia requires a complete history and physical examination, semen evaluation and culture, and *Brucella canis* serology. Evaluation of a semen sample for teratozoospermia requires staining of spermatozoa and examination with light microscopy, or evaluation of a formalized sample with phase-contrast microscopy. Artifactual defects of varying kinds are seen with different stains used (see Chapter 16).[74,108,109]

Treatment of teratozoospermia in the dog is dependent on etiology, if known. Unilateral orchidectomy may be an effective therapy for unilateral testicular neoplasia or orchitis if atrophy of the contralateral testis due to secretion of androgens or estrogens from a functional tumor or increased scrotal temperature secondary to inflammation has not occurred. Improvement in semen quality is unlikely any earlier than 62 days after resolution of the testicular problem, since this is the duration of the normal spermatogenic cycle of the dog. In one dog with teratozoospermia due to posthitis and scrotal edema, return to fertility occurred 18 weeks, and semen returned to normal 26 weeks after therapy was instituted.[110] In general, prognosis for future fertility is guarded if no improvement is seen in 3 months, poor if no improvement is noted in 6 months, and grave if there is no change in semen quality after 12 months.[106]

Sperm Agglutination

Agglutination of spermatozoa has been demonstrated in dogs with chronic infection with *Brucella canis*.[111–113] Agglutination has been demonstrated to be due to formation of antisperm antibodies. Sperm agglutination occurred after induction of antisperm antibodies in dogs by parenteral injection of sonicated spermatozoa in Freun's incomplete adjuvant.[114] Antisperm antibodies form 3 to 6 months after experimental infection with *B. canis*[111,113] and within 40 days of testicular

trauma.[115] The blood-testis barrier formed by tight junctions between Sertoli cells normally protects testicular tissue from the immune response. The rete testis and efferent ducts are likely sites for entrance of immune reactants or exit of soluble sperm antigens across the blood-testis barrier.[116,117]

Agglutinated spermatozoa do not have normal progressive motility and are less able to traverse the zona pellucida and effect fertilization after undergoing capacitation and the acrosome reaction. In dogs with antisperm antibodies and sperm agglutination, intrauterine insemination may enhance fertility.[23] Dogs with brucellosis should not be used for breeding (see Chapter 18).

Asthenozoospermia

Asthenozoospermia, progressive motility of less than 70 per cent, often is associated with teratozoospermia, abnormal morphology. However, the correlation between %MNS and progressive motility is not exact, and both parameters should be evaluated in a complete semen evaluation. Decline in progressive motility may be the first indication of reproductive tract infection or other disease seen on a semen evaluation.[23]

Percentage of progressive motile spermatozoa is poorly correlated with fertility.[23] Lack of motility is not necessarily proof of lack of fertilizing ability, nor is it indicative of sperm cell death.[34,118]

Causes of asthenozoospermia include those of teratozoospermia, including testicular tumors, infection of the reproductive tract, contaminated collection equipment and, uncommonly, immotile cilia syndrome. Several water-soluble lubricants have been shown to decrease progressive motility of spermatozoa, as has exposure to latex.[119,120] Residues present from manufacture of latex or plastic artificial vaginas or collection tubes cause an artifactual decrease in progressive motility by killing some or all of the spermatozoa ejaculated. Several ejaculates should be collected in equipment that was rinsed in hot water and allowed to air-dry before diagnosing asthenozoospermia.

Diagnosis of etiology of asthenozoospermia in the dog requires a complete history and physical examination, semen evaluation and culture, and *Brucella canis* serology. Electron microscopy of cross sections of sperm tails are indicated in dogs suspected of having immotile cilia syndrome, in order to document morphologic absence of dynein arms in the microtubules of the sperm tail. Dogs with this disorder usually have concurrent bronchiectasis, chronic bronchial infection, and some have situs inversus of thoracic and abdominal organs. In a survey of infertile male human beings, 3.9 per cent were diagnosed with idiopathic asthenozoospermia.[1]

Treatment is dependent on etiology, if known. In dogs with poor progressive motility but good total number of spermatozoa, fertility may be enhanced by extension of the semen in a nutrient medium (see Chapter 16).

NECROSPERMIA

Eosin-nigrosin staining of canine spermatozoa may allow interpretation of percentage of nonviable spermatozoa, as normal spermatozoa do not take up stain, appearing white against a black background, while damaged spermatozoa take up stain, appearing pink. Damaged spermatozoa may be presumed to be dead, but this technique is unreliable.

In a survey of 102 dogs from which 181 ejaculates were collected, 8.7 per cent were diagnosed with necrospermia. There is one case report of reversal of necrospermia in a toy poodle by treatment with naloxone (1 mg IM twice daily for 14 days), which was hypothesized to stimulate GnRH release from the hypothalamus.[121] The semen quality improved from 3 per cent to 85 per cent progressively motile spermatozoa.[121]

IMMOTILE CILIA SYNDROME (PRIMARY CILIARY DYSKINESIA)

Immotile cilia syndrome, or primary ciliary dyskinesia (PCD), is a congenital ciliary ultrastructural abnormality causing absent or irregular, asynchronous motility of all ciliated cells in the body.[122–124] Subsequent decreased mucociliary clearance causes upper and lower respiratory disease, including sinusitis, rhinitis, bronchitis, bronchiectasis, and bronchopneumonia.[125,126] All spermatozoa ejaculated are immotile, and primary morphologic defects including coiled midpieces, tightly coiled tails, and proximal retained cytoplasmic droplets may be seen.[125] Male dogs with PCD are infertile. Conditions associated with PCD in the dog include hydrocephalus and situs inversus.[125] Heritability of PCD is unknown. Breeds in which affected littermates have been reported include the bichon frisé, springer spaniel and Old English sheepdog.[124–126] Definitive diagno-

sis of PCD as a cause of male infertility requires electron microscopy of ejaculated spermatozoa in order to document absence of dynein arms in cross sections of the microtubules of the sperm tails.[125]

Inflammatory Sediment/Infection

Canine semen is too dilute to allow accurate evaluation for inflammatory changes without centrifugation. Presence of inflammatory cells in seminal fluid is not well correlated with presence of infection. Normal intact male dogs may have as many as 2000 white blood cells per microliter in the first and second fractions of the ejaculate.[4] In a survey of 15 fertile dogs from which 464 ejaculates were collected, 75.4 per cent of semen samples containing leukocytes were negative for bacterial growth, and 9.1 per cent of semen samples positive for bacterial growth contained no leukocytes.[127] Definitive diagnosis of infection of the reproductive tract is made by growth of more than 10,000 bacteria per milliliter of semen. Absence of leukocytes in a centrifuged sample of seminal fluid does not reduce the necessity for semen culture.

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Section III

THE QUEEN

Chapter 24

Sexual Differentiation and Normal Anatomy of the Queen

Differentiation of the Reproductive Organs of the Queen

The diploid number of chromosomes in somatic cells of the cat is 38, or 19 pairs, of which 18 pairs are autosomes, and one pair consists of the sex chromosomes, XX or XY. The accepted notation for the normal chromosomal complement in the queen is 38,XX. As in the bitch (see Chapter 1), the gonads originate as undifferentiated tissue, the mesenchymal gonadal ridges, on the medial side of the mesonephros.[1] During embryonic development, primordial germ cells, which later differentiate to oogonia or spermatogonia, migrate to the gonadal ridges from the yolk-sac endoderm. Following germ cell migration, undifferentiated gonadal tissue differentiates to ovary (if the sry region of the Y chromosome is absent), or testis (if the sry region of the Y chromosome is present). In the queen, the central part of the gonadal primordium differentiates into the sex cords and rete ovarii of the ovarian medula, and the surface epithelium, containing the germ cells, differentiates as cortex. The rete ovarii, comprised of intraovarian, connecting, and extraovarian rete systems, is a system of cords and tubes that extends from the medulla into the paraovarian region.[2] Male and female duct system differentiation is governed by presence or absence of two testicular hormones, testosterone and müllerian inhibiting substance (MIS). In the normal female, in the absence of MIS, the paramesonephric (müllerian) ducts persist and differentiate into the uterine tubes (oviducts), uterus, cervix, and cranial vagina. In the normal female, in the absence of testosterone, the mesonephric (wolffian) ducts, destined to differentiate into the tubular tract of the male, regress. In the female, the paramesonephric duct system and the urogenital sinus contribute to the vagina and vestibule.[1] The genital tubercle and genital swellings that develop around the cloacal membrane become the clitoris and the labia of the vulva. The mammary glands develop embryologically as bilateral thickenings of the epithelium of the skin from the axillary to inguinal regions.[3] Epithelial buds burrow into underlying connective tissue and form cords of cells that differentiate into tubes and eventually into secretory glandular tissue. Underlying mesodermal elements of the dermis differentiate into the supporting stroma of the gland, which consists of blood vessels, lymphatics, fibrous connective tissue, and fat.[3]

Ovarian morphology, with primitive medullary and cortical regions, is fully established in the kitten fetus by 40 days or 75 mm crown-rump length.[4] Divisions of oogonia cease at

time of birth of the female kitten, with multiplication of these cells observed again in the postpartum 21-day ovary. By 23 to 35 days' postpartum, uni- and pluriovular primordial medullary follicles are developing, all of which undergo degeneration by day 60 to 65 postpartum. Ovaries of most newborn kittens and young, immature queens contain primordial ova surrounded by granulosa cells, with ovaries of occasional individuals containing mono- or polyovular (kittens) graafian follicles.[5] The ovary of the sexually mature queen is about five times greater in diameter than that of the 40-day fetus; growth appears to be mainly peripheral, in and beneath the epithelium that is the source of the ova and follicle cells.[4]

Ovaries

The ovaries of the adult queen are oval structures, approximately 1.0 × 0.3 × 0.5 cm in size and 220 mg in weight, located in the dorsal abdomen caudal to the kidneys (Fig. 24–1).[6,7] The ovary, the uterine tube, and the uterus are suspended in the peritoneal cavity by a reflection of peritoneum, the broad ligament, which is subdivided into the suspensory ligament of the ovary, the mesovarium, the mesosalpinx, and the mesometrium.[8] Each ovary is attached to the diaphragm by the suspensory ligament, to the dorsal body wall by the mesovarium, and to the end of the uterine horn by the short, thick proper ligament of the ovary. The mesosalpinx, a fold of peritoneum originating from the lateral side of the mesovarium, encloses the uterine tube and passes around the ovary to form the ovarian bursa. The bursa has a small slit-like opening on the medial side that permits communication of the bursal and peritoneal cavities.[8] The ovarian artery, originating from the aorta, supplies the ovary and cranial portion of the uterine tube.[9] The ovarian vein drains the ovary, uterine tube, and cranial portion of the uterine horn, terminating in the caudal vena cava.[9]

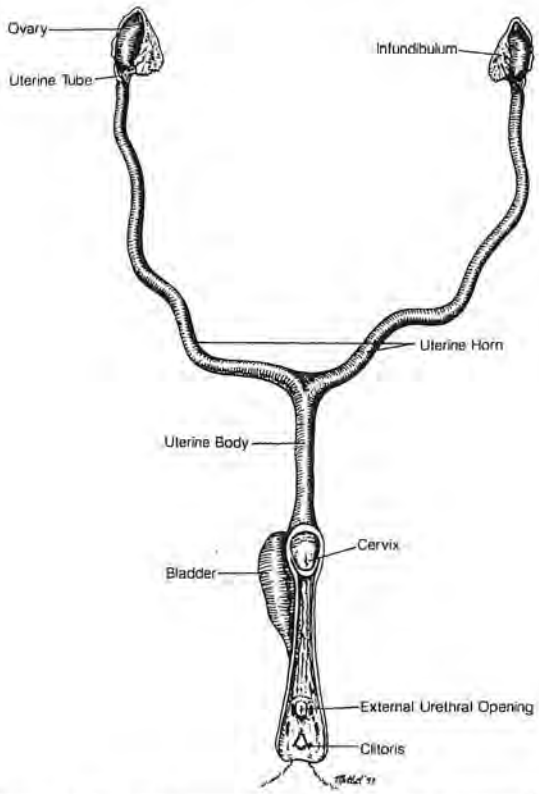

Figure 24–1. Gross appearance of the reproductive organs of the queen.

The gross and histologic appearance of the ovary varies with stage of the estrous cycle.[10] During anestrus the surface of the ovary is smooth; 0.5-mm diameter follicles are visible histologically. As the follicular phase (estrus) approaches, three to seven follicles enlarge, and the rest undergo atresia. Most follicular development (to the vesicular follicle) occurs in the 48-hour interval just prior to onset of estrous behavior, and mature follicles measure 2.5 to 3.5 mm in diameter.[11] The queen is an induced ovulator. Copulation, vaginal stimulation, or gonadotropin administration induces ovulation within approximately 24 to 32 hours.[12–17] Occasional estrous queens may ovulate spontaneously, perhaps stimulated by tactile or visual cues.[18] Corpora lutea, which form after ovulation, appear orange-yellow grossly and may reach 4.5 mm in diameter, peaking in size about 16 days after ovulation.[10,11,19] They may persist histologically for 6 to 8 months after ovulation.[20,21] In older cats the ovaries may be shrunken and nodular, but follicles and corpora lutea still are present, as true senile atrophy does not occur.[22] Polynuclear ova and polyovular follicles occur in the cat.[5,23,24]

Uterine Tube and Uterus

The uterine tube (oviduct) of the adult queen is 5 to 6 cm in length. The infundibulum, which is the cranial end of the uterine tube, is a conical enlargement lined by mucosal villi called fimbriae.[8] It lies craniomedial to the ovary; from there the uterine tube passes cranially, laterally, and then caudally in the mesosalpinx

before joining the top of the uterine horn through a low papilla with a collar of circular smooth muscle that protrudes into the lumen of the uterine horn.[25] The wall of the uterine tube is thin, and the lining is thrown up into longitudinal folds or ridges. Microscopically, the oviduct is lined by low columnar ciliated epithelium (in anestrus) and is surrounded by an inner circular and outer longitudinal layer of smooth muscle.[25]

The uterus of the adult queen is a Y-shaped organ consisting of a 2-cm-long body lying between the descending colon dorsally and the urinary bladder ventrally and two 10-cm uterine horns that extend cranially to meet the uterine tubes (oviducts) (Figs. 24–1 and 24–2).[26] The cervix is the thick-walled neck of the uterus, connecting it to the vagina.[7] The uterine body and horns are attached to the body wall by the mesometrium, which is continuous cranially with the mesovarium and which, with the mesosalpinx, comprises the broad ligament. The round ligament of the uterus, a lateral extension of the broad ligament, passes from the horn caudolaterally to attach near the inguinal ring. Size of the uterus depends on the size, age, and parity of the cat and the phase of the estrous cycle or stage of pregnancy; in nonpregnant adult cats the uterus weighs about 1.5 g.[6] The uterus is supplied by the uterine artery, originating from the vaginal artery, and located close to the mesometrial at-

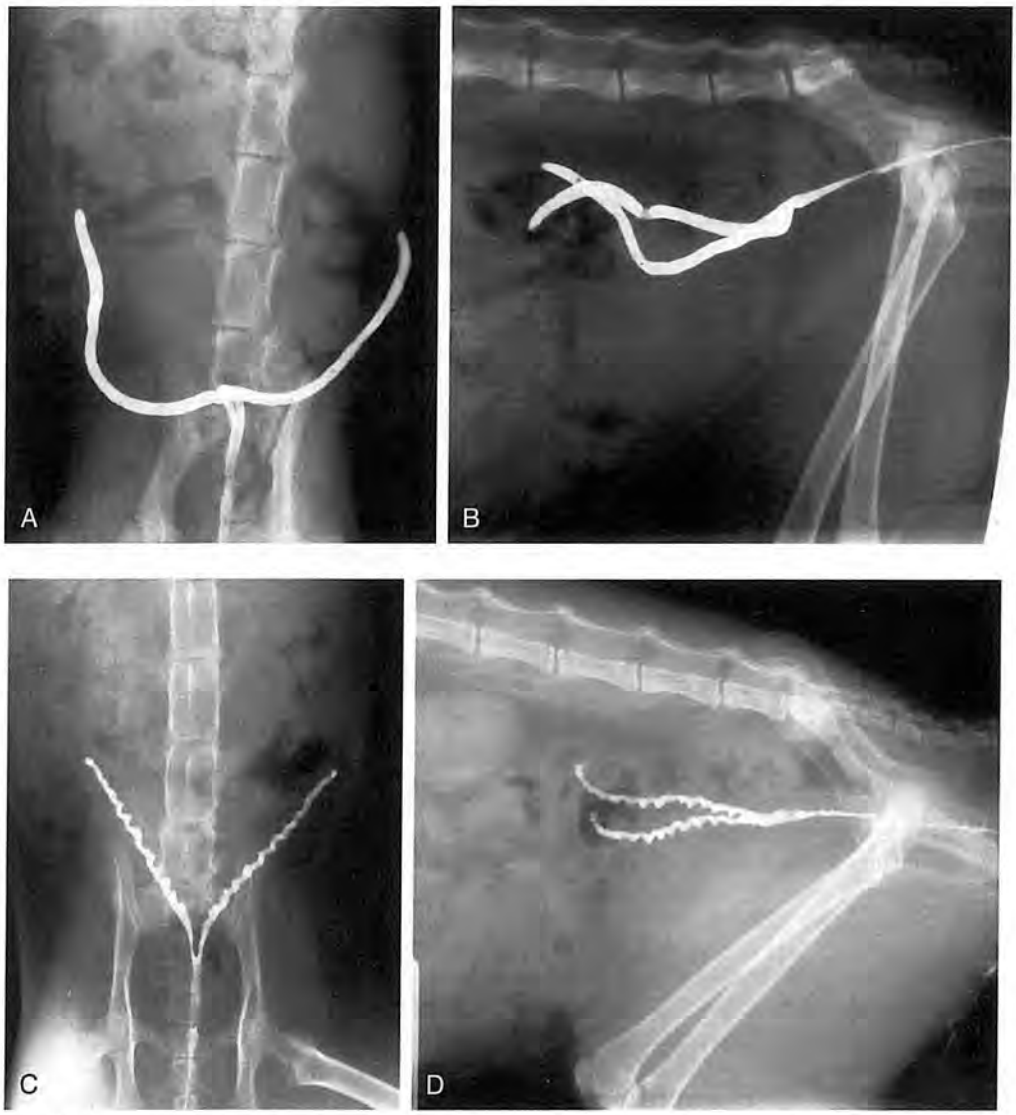

Figure 24–2. Contrast radiographic study of the feline vagina and uterus from queens in anestrus (**A** and **B**) and estrus (**C** and **D**). Ventrodorsal (**A** and **C**) and lateral (**B** and **D**) projections.

tachment of the uterus; the uterine artery terminates in a prominent branch to the caudal uterine tube.[9] The uterine vein follows the course of the uterine artery, and terminates in the internal iliac vein.[9] There are anastomoses between right and left uterine veins in the area of the feline uterus and cervix.[9]

Histologically, the uterus contains endometrium, inner circular and outer longitudinal smooth muscle layers comprising the myometrium, and the outer serosa covered by peritoneum. Histologic appearance of the feline endometrium changes with the stage of the estrous cycle.[27,28] Low columnar epithelium and poorly developed endometrial glands and crypts are present before puberty and during anestrus. With follicular growth, endometrial glands and crypts proliferate, and congestion and edema occur in the myometrium; these changes regress if pregnancy does not occur. In pregnancy, the proliferating endometrial mucosa fuses with the chorion; subsequent trophoblastic resorption of histotrophe results in presence of endotheliochorial placentation, where maternal and fetal circulations are separated by four cell layers.[29]

The uterine cervix of the cat protrudes into the vagina as a papilla directed ventrocaudally.[1] The cervical canal is open during estrus and closed during other stages of the estrous cycle.[30] The lumen is lined by a single layer of columnar mucus-secreting cells, with peak cell height and mucus-secreting activity during estrus.[30]

Vagina

The vagina of the adult queen extends caudally from the cervix to the region of the hymen, just cranial to the external urethral orifice in the vestibule or urogenital sinus.[8] The vagina and the vestibule are each about 2 cm long, so that the cervix is located about 40 to 45 mm cranial to the vulva; in pregnancy the vagina is stretched cranially by the weight of the uterus.[17] A ridge of tissue is present in the middorsal wall of the vagina caudal to the cervix.[7] The feline vagina is lined by longitudinal folds that vary in character with the stage of the estrous cycle.[22] In immature or anestrous cats, vaginal epithelium is low cuboidal with a superficial layer of flattened cells. During proestrus, the epithelium becomes stratified squamous, with 15 to 20 layers of cells. At the end of estrus, the epithelium gradually decreases in height to about a third as many layers, and changes in character to low stratified squamous. After estrus the epithelium becomes columnar, and polymorphonuclear leukocytes may be observed between epithelial cells at its lumen. Thick inner circular and thin outer longitudinal muscle layers form the vaginal wall.

Vestibule

The vestibule extends from just cranial to the external urethral orifice cranially, to the vulva caudally, a distance of about 2 cm. The external urethral orifice in the cat opens into a mucosal groove located on the floor of the vestibule just caudal to a transverse fold of mucosa that represents the hymen.[8] Feline vestibular diameter can accommodate a 4-mm-diameter probe introduced at the vulva for about 20 mm; the vagina permits passage of a 1-mm-diameter probe to the level of the cervix.[17] These data suggest that the erect feline penis, with diameter of about 5 mm, does not pass into the cranial vagina or stimulate the cervix at mating.[17] The vestibule is lined with stratified squamous epithelium. The wall of the vestibule is encircled by the striated constrictor vestibuli muscle, which attaches ventrally to the ischial arch.[8] Major lubricating mucus-producing vestibular glands (Bartholin's glands), about 5 mm in size, are irregularly situated in the lateral walls of the vestibule; these empty ventrolaterally through single ducts into the vestibular lumen.[1,8] Minor vestibular glands empty on the floor of the vestibule.[8] Nodules of lymphocytes are present in the subepithelial tissue of the vestibule, and may become hyperplastic or hyperemic in response to irritation by chemical or microbial agents.[1] Such hyperplasia is a nonspecific sign of irritation, not a manifestation of specific disease.

Clitoris

The clitoris consists of paired crura of erectile tissue (the corpora cavernosum clitoridis), originating on the ischial arch, that unite in a body becoming, distally, the erectile glans.[8] The clitoris is located in the floor of the fossa clitoridis on the ventral midline of the vestibule. The clitoris is homologous to the penis of the male; in the queen it may contain a small cartilage.[22] Clitoral hypertrophy may occur in response to exposure to exogenous or endogenous testosterone, and may be present in individuals with abnormality of sexual differentiation.

Vulva

The vulva of the adult queen consists of two small, round, lateral labia located just below the anus, which unite at dorsal and ventral commissures. The labia are smaller in spayed than in intact cats. During estrus the labia are slightly edematous and reddened; vulvar discharge is negligible.

Mammary Glands

The queen has four pairs of mammary glands, arranged in two bilaterally symmetrical rows from the ventral thoracic to ventral abdominal region.[3,31,32] They have been designated the right and left axillary, thoracic, abdominal, and inguinal mammary glands, or as right and left mammary glands 1, 2, 3, and 4 when counting from cranial to caudal. The latter designation is preferred. Each feline mammary gland consists of glandular mammary lobules drained by four to eight ducts, lined by stratified squamous epithelium, that open onto the end of each teat. The teat is composed of three layers of smooth muscle, outer and inner longitudinal layers and a middle circular layer.[3] Under the surface of the teat, each teat duct dilates into and is continuous with a teat sinus lined by a two-cell layer of columnar epithelium. Each teat sinus drains smaller branching ducts into which the secretory alveoli open.[3] Stellate myoepithelial cells that are contractile surround the glandular epithelium of the alveoli and the ducts; myoepithelial cells contract in response to oxytocin or stretching.

Arterial supply to the mammary glands is via the perforating branches of the internal thoracic arteries, cutaneous branches of the intercostal arteries, the lateral thoracic arteries, the superficial epigastric arteries, the caudal intercostal arteries, and the cutaneous branches of the deep circumflex iliac artery.[3] Venous drainage of the mammary glands occurs via the axillary veins, branches of the internal thoracic veins penetrating the pectoral muscles, the cranial superficial epigastric veins, penetrating intercostal veins, and the caudal superficial epigastric veins.[3] Small veins may cross the midline and penetrate the chest wall, serving as routes for dissemination of mammary tumor emboli.[3,31,32]

Lymphatic drainage of the two cranial pairs of feline mammary glands occurs toward the axillary lymph nodes, and that of the two caudal pairs toward the superficial inguinal lymph nodes (Fig. 24–3). Lymphatics do not connect anterior and posterior glands, nor do they cross the midline or penetrate the abdominal wall.[31]

Bacterial Flora of the Reproductive Tract of the Healthy Queen

The feline vagina contains a normal resident flora of aerobic bacteria, which may be associated with vaginal or uterine disease should host defense mechanisms become compromised. Aerobic bacteria were isolated from vaginal samples of 52 of 53 clinically normal healthy female cats ranging in age from 5 months to more than 3 years old.[33] The most common isolates were *Escherichia coli*, coagulase-negative *Staphylococcus*, *Streptococcus canis*, nonhemolytic *Corynebacterium*, and *Haemophilus* sp. (Table 24–1). Number of bacterial isolates per cat ranged from one to eight, and averaged about three in the three groups listed in Table 24–1. Numbers of colony-

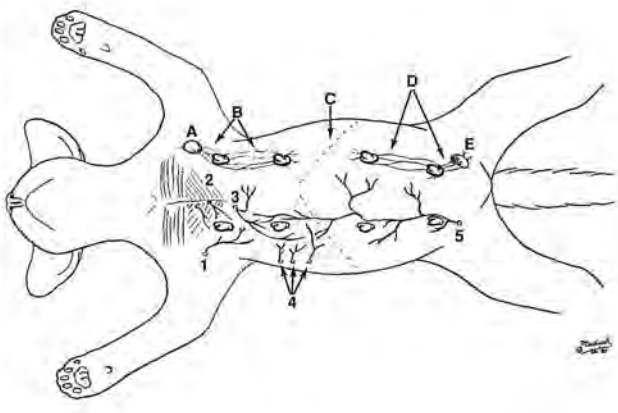

Figure 24–3. Blood supply and lymphatic drainage of the four pairs of mammary glands of the cat. The upper part of the picture depicts the lymphatics and their relationship to the diaphragm and regional lymph nodes: A, axillary lymph node; B, lymphatics connecting the two cranial thoracic mammary glands and the axillary lymph node; C, diaphragm; D, lymphatic connections between the two caudal abdominal mammary glands and the superficial inguinal lymph nodes; E, superficial inguinal lymph node. The lower part of the picture depicts the venous drainage: 1, axillary; 2, branches of the internal thoracic veins penetrating the pectoral muscles; 3, cranial superficial epigastric; 4, penetrating intercostals; 5, caudal superficial epigastric. (From Ogilvie GK: Feline mammary neoplasia. Compend Contin Educ Pract Vet 5:384–391, 1983, with permission.)

Table 24–1. Bacterial Isolates from Reproductive Tracts of Clinically Healthy Female Cats

Organism	Number of Isolates by Source of Cat		
	Breeding Colony Multiparous (>3 yr, *n* = 13)	Breeding Colony Prepuberal (5–7 mo, *n* = 10)	Client Owned Routing OHE (6–16 mo, *n* = 30)
Vaginal Aerobes			
Escherichia coli	6	2	20
Staphylococcus (coagulase negative)	7	7	10
Streptococcus canis	9	8	6
Corynebacterium sp. (nonhemolytic)	5	3	7
Haemophilus sp.-like	2	2	8
Streptococcus (unidentified)	1	0	8
Acinetobacter sp.	2	1	5
Moraxella sp.	2	2	3
Actinomyces pyogenes	0	0	4
Haemophilus paracuniculus	0	0	4
Streptococcus uberis	0	0	4
Aerococcus sp.	2	0	1
Flavobacterium sp.	1	0	2
Klebsiella ozaenae	0	0	2
Pasteurella haemolytica	0	1	1
Pasteurella multocida	2	0	0
Streptococcus agalactiae	0	0	2
Streptococcus mitis	1	0	1
Bacillus sp.	1	0	0
Gardnerella sp.-like	1	0	0
Micrococcus sp.	0	1	0
Pseudomonas sp.	0	0	1
Simonsiella sp.	0	0	1
Staphylococcus aureus	0	1	0
Staphylococcus intermedius	0	1	0
Streptococcus dysgalactiae	0	0	1
Streptococcus faecalis	1	0	0
Vaginal Anaerobes			
Peptococcus sp.	ND	ND	2
Bacteroides asaccharolyticus	ND	ND	1
Bacillus fragilis	ND	ND	1
Bacillus oralis	ND	ND	1
Uterine Flora			
Acinobacter sp.	ND	ND	1
Escherichia coli	ND	ND	1
Lactobacillus sp. (anaerobe)	ND	ND	1

ND, not determined.
From Clemetson LL, Ward ACS: Bacterial flora of the vagina and uterus of healthy cats. J Am Vet Med Assoc 196:902–906, 1990, with permission.

forming units per isolate increased as number of bacterial isolates decreased. In a study limited to staphylococcal distribution in clinically healthy cats, *Staphylococcus similans* was reported present in the vaginas of 20 of 68 queens.[34] Anaerobic bacteria (*Peptostreptococcus* and *Bacteroides*) were isolated from the vaginas of 4 of 30 cats.[33]

The normal feline uterus usually does not harbor aerobic or anaerobic bacterial flora. Aerobic (*Acinetobacter, E. coli*) and anaerobic (*Lactobacillus*) bacteria were reported present in only 2 and 1, respectively, of 29 uterine samples evaluated.[33]

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Chapter 25

The Feline Estrous Cycle

The queen is described classically as a seasonally polyestrous, induced ovulator, with ovulation induced by coitus. Spontaneous ovulation may, however, occur in some queens, perhaps triggered by visual or pheromone cues in the absence of copulation. Feline estrous cycles occur at 4- to 30- (14 to 19 modal) day intervals in queens exposed to a constant daylength (14 hours bright light per day) and not induced to ovulate (Figs. 25–1 through 25–3).[1–9] Prolonged anestrus results from decreasing or short day length. Various surveys document greatest frequency of cycling activity in queens in the Northern Hemisphere in January and February, with gradual frequency decline until September and October.[5] Data available are difficult to assess critically, however, as queens induced to ovulate early in the year would not cycle again for 2 to 3 months, and because photoperiod to which reported queens are exposed varies widely. Cats housed indoors with constant temperature but seasonal light (windows) cycle seasonally, and cats housed indoors for 12 months with 14 hours of light daily show a decrease in estrous behavior and estrous vaginal smears from April to May.[10,11] Transportation of an established feline colony 1500 miles in November did not alter reproductive cycling, and was associated with a synchronizing effect of behavioral estrus in 76.2 per cent of 21 adult queens.[12]

Puberty

The puberal estrous occurs in most queens between 4 and 12 months of age and is influenced both by time of year (photoperiod) and by condition of the queen.[5,13–15] In the Northern Hemisphere, onset of the puberal estrus in young females in good condition occurs in January and February as length of daylight increases. Queens born early in the year may not mature until the following year, while those born in summer or autumn may undergo the puberal estrus the following January.[16] Queens are reported to reach puberty at a body weight of 2.3 to 2.5 kg unless that occurs at a time of year when day length is decreasing (September through December in the Northern Hemisphere) when most cats are anestrous.[5,17] Oriental breeds (Siamese, Burmese) may show the puberal estrus when very young, whereas longhaired and Manx queens may have a later onset of puberty (11 to 21 months) than shorthaired breeds.[13,16,18] Queens have a long reproductive life, often exceeding 14 or more years, although litter size may be reduced as the queen ages.[17]

Stages of the Feline Estrous Cycle

Stages of the estrous cycle of the queen include proestrus, estrus, postestrus, diestrus, and anestrus. Figure 25–1 illustrates the relationship between these stages, and the sequential paths over which stages of the feline estrous cycle may occur.

Proestrus

Proestrus is a stage of the estrous cycle observed in only a minority of queens; most queens enter the puberal estrus directly, or enter estrus directly after preceding stages of anestrus, postestrus, or diestrus. When it oc-

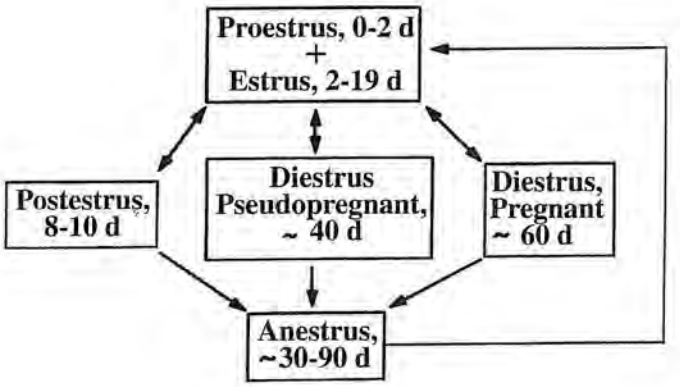

Figure 25–1. Stages of the feline estrous cycle.

curs, proestrus onset begins with the continuous rubbing of the head and neck against any convenient object by a queen that will not permit breeding by the male.[8,19] In one study, proestrus was observed in only 27 of 168 feline estrous cycles, and it lasted an average of 1.2 (±0.8, SD) days.[8]

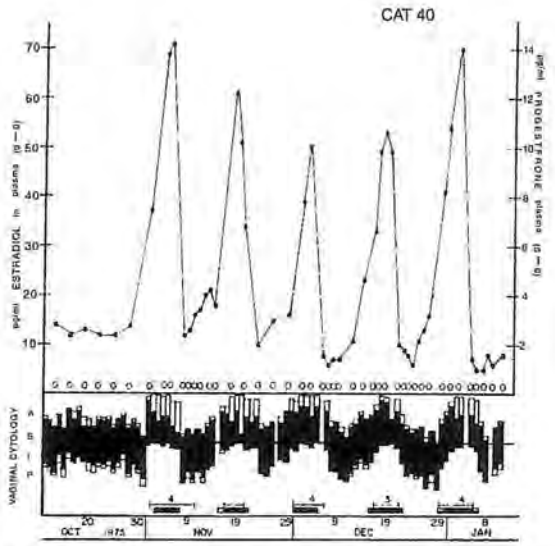

Figure 25–2. Estrous cycles in an individual cat. Coital frequency is marked by numbers above the horizontal bars, which, themselves, represent proestrus (clear) and estrus (solid). Cycle cornification of vaginal epithelium, shown by vertical bars, is indicated by the relative proportions of anuclear (A) and parabasal (P) cells (unshaded) as well as superficial (S) and intermediate (I) cells (shaded). Follicular function is indicated by concentrations of estradiol-17β (shaded dots); progesterone concentrations (clear dots) also are shown. (From Shille VM, Lundstrom KE, Stabenfeldt GH: Follicular function in the domestic cat as determined by estradiol-17β concentrations in plasma: Relation to estrous behavior and cornification of exfoliated vaginal epithelium. Biol Reprod 21:953–963, 1979, with permission.)

Proestrus in the cat is associated with rising serum estradiol concentrations secreted by granulosa cells of the ovarian follicles.[8] The effect of estradiol on vaginal epithelium of the cat (like the dog) is to cause an increased number of epithelial cell layers and to cause vaginal cornification, resulting in change in the morphologic appearance of exfoliated epithelial cells (Table 25–1). The vaginal cytology smear of the proestrus queen has been reported to consist of parabasal cells (18 per cent; range = 0 to 34, $n = 4$), intermediate cells (60 per cent; range = 57 to 67, $n = 4$), nucleated superficial cells (20 per cent, range = 6 to 40, $n = 4$), anucleated superficial cells (2 per cent; range = 0 to 6, $n = 4$), and neutrophils (8 per cent epithelial cells; range = 0 to 33, $n = 4$) (Fig. 25–4).[20]

Estrus

Estrus is the behavioral stage of receptivity to mating. Estrus occurs during peak follicular activity and estradiol secretion by the queen, with peak plasma estradiol sometimes reaching more than 70 pg/ml (Figs. 25–2, 25–4).[8] Estradiol uptake by hypothalamic neurons of ovariectomized cats causes sexual receptivity in the presence of an anestrous genital tract.[21,22]

In the queen, estrous behavior includes crouching with the forequarters pressed to the ground and hyperextension of the back causing lordosis and presentation of the vulva for mating (Fig. 25–5).[8,19,23–25] Estrous queens frequently vocalize and call to males and show restlessness and affectionate head rubbing to owners. Estrous behavior in the absence of a male includes rolling, head rubbing, treading with the hind legs, lordosis, and tail deviation.[26] Mating in-

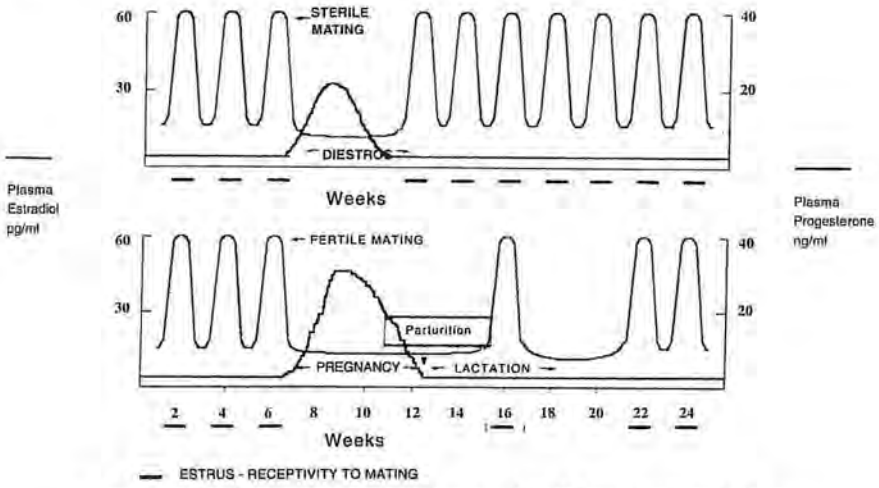

Figure 25–3. The feline estrous cycle: schematic of the follicular and luteal phases.

volves 5 to 50 seconds of mounting and grasping of the neck by the male, 0.3 to 8 minutes for positioning as the female raises her perineal area, 1 to 30 seconds for intromission and ejaculation and the queen's coital cry, 0 to 1 second for dismounting, 1 to 7 minutes for the queen's after-reaction, which is disoriented rolling, stretching, and genital licking, and 0 to 5 hours of refusing attempts to remount.[7,26] "After-reactions" of screaming (54 per cent), striking out at the tom (77 per cent), vulvar licking (92 per cent), and frantic rolling (100 per cent) were observed in a study of 120 feline copulations.[7] Copulations may number 20 to 36 over 36 hours.[7,26]

The cat is an induced ovulator, and duration of estrus has been reported, anecdotally, to be influenced by whether ovulation is induced. In a controlled study, however, duration of estrus was similar in queens that experienced coitus and ovulation (x = 8.6 days; SD = 4.1; n = 32) and queens that had coital contact but failed to ovulate (x = 8.3 days; SD = 4.3; n = 43).[8] Duration of estrus was shorter in queens that did not have coital contact (x = 6.2 days; SD = 2.9; n = 93). Duration of estrous behavior without regard for coitus in the 168 cycles was 2 to 19 days.[8] Duration of estrus and follicle numbers reported in a second study were similar in ovulating (5.8 ± 0.2 days; 5.0 ± 0.5 follicles) and nonovulating (6.4 ± 0.6 days; 5.2 ± 1.0 follicles) queens.[27] Mean duration of estrus (4.4 ± 1.6 days) in bred queens from a third study, was not statistically different than that in queens not bred (7.4 ± 4.4 days), and average duration of estrus in 14 queens (bred and unbred) was 5.8 ± 3.3 days, with a range of 2 to 19 days.[7]

Luteinizing hormone (LH) release from the pituitary gland, and subsequent ovulation, is induced by copulation in the queen. Magnitude of LH release has been demostrated to increase with increase in number of copulations in the queen, and only about half the queens bred a single time will secrete adequate LH to induce ovulation.[28–33] LH concentrations in blood are low until time of coital contact, when concentrations increase, and induce ovu-

Table 25–1. Dimensions of Feline Vaginal Epithelial Cells (Microns)

Cell Type	Cytoplasm		Nuclei		N/C Ratio
	Length	Width	Length	Width	
Superficial	68.5 (10.5)*	41.8 (11.9)	7.9 (1.2)	5.6 (1.5)	0.02
Intermediate	44.7 (8.9)	37.5 (7.7)	10.0 (2.1)†		0.06
Parabasal	18.6 (2.8)†		8.3 (1.7)†		0.20

* Parentheses enclose 1 SD of mean.
† Nuclei round, single dimension given.
From Mills JN, Valli VE, Lumsden JH: Cyclical changes of vaginal cytology in the cat. Can Vet J 20:95–101, 1979, with permission.

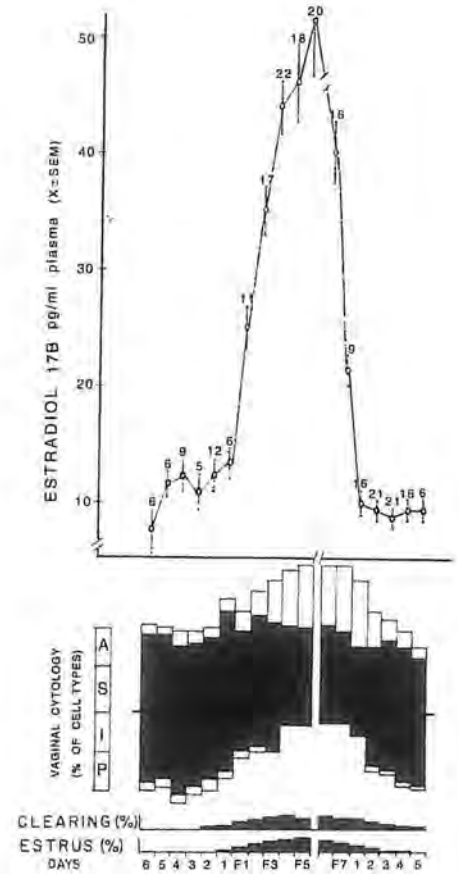

Figure 25–4. Schematic outline of events occurring during the follicular phase in the cat. Data have been arranged around the first (plasma estradiol >20 pg/ml, FI) and last (plasma estradiol >20 pg/ml) days of the follicular phase. Histogram represents mean proportions of types of epithelial cells in the vaginal smear, as anuclear (A) (clear), superficial (S) (shaded), intermediate (I) (shaded), or parabasal (P) (clear) cells. Proportion of queens showing estrous behavior is shown at the bottom. (From Shille VM, Lundstrom KE, Stabenfeldt GJ: Follicular function in the domestic cat as determined by estradiol-17β concentrations in plasma: Relation to estrous behavior and cornification of exfoliated vaginal epithelium. Biol Reprod 21:953–963, 1979, with permission.)

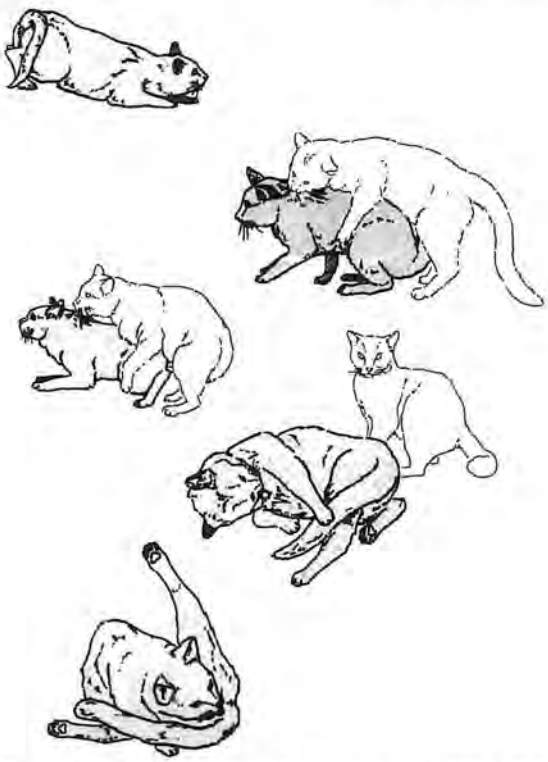

Figure 25–5. Estrous behavior in the queen (shaded) in the presence of the tom (clear). The queen displays crouching, lordosis, and deflection of the tail to the side of the perineum (top), followed by treading behavior of the hind legs while the male is mounting (middle panels). The after-reaction features loud vocalization, rapid rolling and perineal grooming (bottom), and striking out at the male.

lation 29 to 40 hours later.[27,28,34] The ability of the queen to release LH in response to coitus is dependent on duration of exposure to estrogen of the hypothalamus and/or anterior pituitary gland.[35]

In the cat, unlike the dog, peak vaginal cornification occurs at time of peak plasma estradiol concentration (Fig. 25–4).[8] Percentage of anuclear squames in the feline vaginal cytology specimen increases above 10 per cent (of cells exfoliated) on the first day of the follicular phase and increases to approximately 40 per cent by the fourth day (Figs. 25–4, 25–6, and 25–7).[8] The number of intermediate cells in the smear declines from approximately 40 per cent to 10 per cent during the first 4 days of the follicular phase. Number of superficial cells is fairly constant throughout behavioral estrus, ranging from 40 to 60 per cent of the cells present. Erythrocytes and leukocytes are rarely seen in the feline vaginal smear during proestrus or estrus, and parabasal cells comprise less that 10 per cent of the total cells at all stages of the cycle.[8] Clearing (absence of debris and mucus plus lack of cell coalescence into sheet-like aggregates) of the vaginal smear occurs in over 90 per cent of feline cycles observed during the follicular phase.

Percentages (and range) of vaginal epithelial cells in 20 estrous queens for parabasal cells was 0.3 (0 to 3), intermediate cells was 11.6 (0 to 25), nucleated superficial cells was 63.6 (5 to 90), anucleated superficial cells was 24.5 (3 to 95), and number of neutrophils per 100 epithelial cells was 4.7 (0 to 10) in Table 25–1.[20]

Ovarian follicles (3 to 4 mm in diameter) have been reported to secrete testosterone in

How to Collect and Interpret Vaginal Cytology Specimens in the Queen

- The morphology of epithelial cells exfoliated from the vagina of the queen changes in the presence of estrogen, and therefore may be used to assess stage of the estrous cycle.[8,20,68–72]
- Cells are collected using a saline-moistened cotton-tipped swab* (Fig. 25–6) to scrape cells from the ceiling of the vestibule/vagina (about ½ inch craniad from the vulva) or by flushing and then aspirating a small (0.5 ml) volume of saline into and from the vagina with a Papanicolaou pipette. Swabs are then rolled onto a clean glass slide, and allowed to air-dry. Aspirated saline with epithelial cells can be dropped onto a slide and allowed to air-dry, or stained as a wet mount with new methylene blue.
- Vaginal epithelial cell morphology can be evaluated with any of the Romanovsky stains (e.g., Giemsa stain, Wright's stain, new methylene blue, Diff-Quik [Harleco, Gibbstown, NJ]). Alternatively, one can use trichrome stains in which cornified and noncornified cells stain different colors.[73] The authors' preference is Diff-Quik stain, which is inexpensive, easy to use, permanent, and can be used rapidly.
- Epithelial cells observed in the vaginal cytology smear of the queen include parabasal cells, intermediate cells, nucleated superficial cells, and anucleated superficial cells (Figs. 25–4, 25–7: see also Table 25–1).

* Calgiswab; Hardwood Products Co., Gulford, ME.

vitro in response to administration of LH and human chorionic gonadotropin (hCG).[36]

Postestrus

Postestrus is the term used by Lofstedt for the interestrous period that follows one estrus and precedes the next in queens that have not been induced to ovulate.[5] While some texts have used the term metestrus for the stage following estrus in the queen, this term means time of corpus luteum development which, while following estrus in the cow, clearly does not occur in the queen that has not been induced to ovulate. Queens that do ovulate show morphologic evidence of corpus luteum development during estrus, and therefore the stage following estrus in the these queens should be called

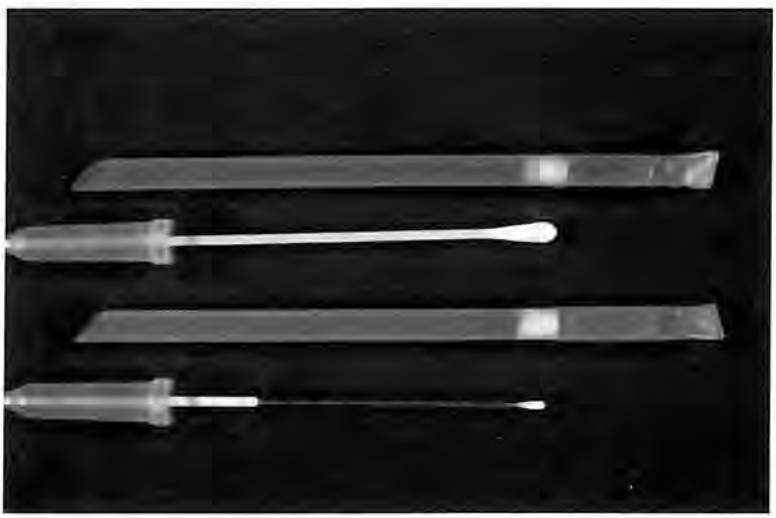

Figure 25–6. Vaginal cytology collection swab (bottom) for the cat compared to a 6-inch, cotton-tipped culture swab (top).

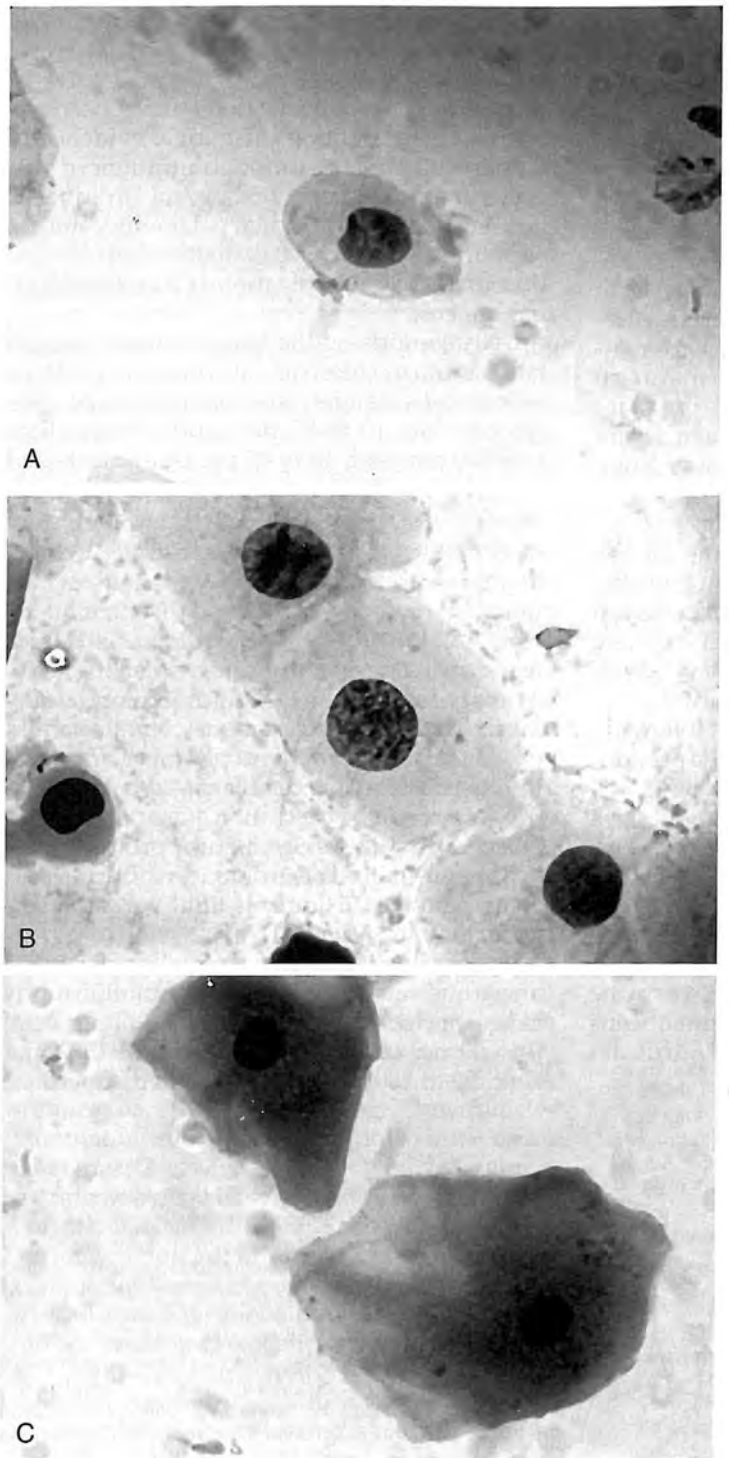

Figure 25–7. Parabasal cells (**A**), intermediate cells (**B**), nucleated and anucleated superficial cells (**C**) in the vaginal cytology smear of the queen.

diestrus (time of functional corpora lutea).[12] Plasma estradiol is less than 20 pg/ml during postestrus and no sexual behavior or receptivity is present.[12,37] Duration of postestrus is 8 to 10 days.[5,8]

Cells comprising the vaginal smear in eight postestrous cats (mean percentage followed by range) included parabasal cells (8.9 per cent, 0 to 29 per cent), intermediate cells (75.5 per cent, 50 to 100 per cent), nucleated superficial cells

(13.2 per cent, 0 to 41 per cent), anucleated superficial cells (1.9 per cent, 0 to 3 per cent), and neutrophils (32/100 epithelial cells, 1 to 78).[20]

Diestrus

Diestrus is the progesterone-dominated luteal phase of the cycle that follows estrus in the queen that has been induced to ovulate. Serum progesterone concentrations range from 1.5 ng/ml to more than 20 ng/ml in the diestrus queen (Fig. 25–8). Diestrus lasts approximately 40 days in the pseudopregnant queen and approximately 60 days in the pregnant queen, and ends with luteolysis, when serum progesterone concentrations fall below 1.5 ng/ml (Fig. 25–8).[27,38–43]

Serum progesterone concentration just before parturition (days 63 to 65) in the cat has been reported at 4 to 5 ng/ml; on the day of parturition (n = 12 queens), mean serum progesterone was 2.2 ng/ml, and immediately after parturition (n = 4 queens), mean serum progesterone was less than 1 ng/ml.[38,39]

Estrus resumes about 7 to 10 days following luteolysis in both the pregnant and the pseudopregnant queen; lactation and suckling of kittens, however, may cause lactational anestrus that persists until 2 or 3 weeks after weaning.[27,44] Estrous behavior was observed 10 to 27 days after weaning of kittens in seven of eight queens; the eighth queen had a 133-day weaning-to-estrus interval.[38] The postpartum estrus was shorter (3.8 ± 0.5 days) than the initial mated estrus, and six of the eight queens failed to ovulate at the postpartum estrus despite multiple copulations.

Spontaneous ovulation may occur in some estrous queens. Occurrence of pyometra and histologic demonstration of corpora lutea in indoor queens housed alone have been reported.[45,46] In addition, histologic evidence of corpora lutea and serum concentrations of progesterone exceeding 1.5 ng/ml in queens housed alone in nutritional palatability studies support the occurrence of spontaneous ovulation or follicle luteinization in some individual queens.[47]

Cells comprising the vaginal smear in eight late metestrus/diestrus cats (mean percentage followed by range) included parabasal cells (48 per cent, 10 to 85 per cent), intermediate cells (50 per cent, 10 to 85 per cent), nucleated superficial cells (2 per cent, 0 to 5 per cent), anucleated superficial cells (0 per cent), and neutrophils (32/100 epithelial cells, 0 to 97).[20]

Anestrus

Anestrus is a seasonal absence of cycling activity that occurs in the late autumn months (October, November, December) in queens exposed to natural (daylight) photoperiods in the Northern Hemisphere. Plasma estradiol and progesterone concentrations are at baseline levels during this stage. Serum prolactin concentration in six anestrus queens during a 7-week period of 7 hours of light per day averaged 13.2 ± 0.5 ng/ml.[48]

Cells comprising the vaginal smear in 34 anestrous cats (mean percentage followed by range) included parabasal cells (9.7 per cent, 0 to 50 per cent), intermediate cells (87.4 per cent, 50 to 100 per cent), nucleated superficial

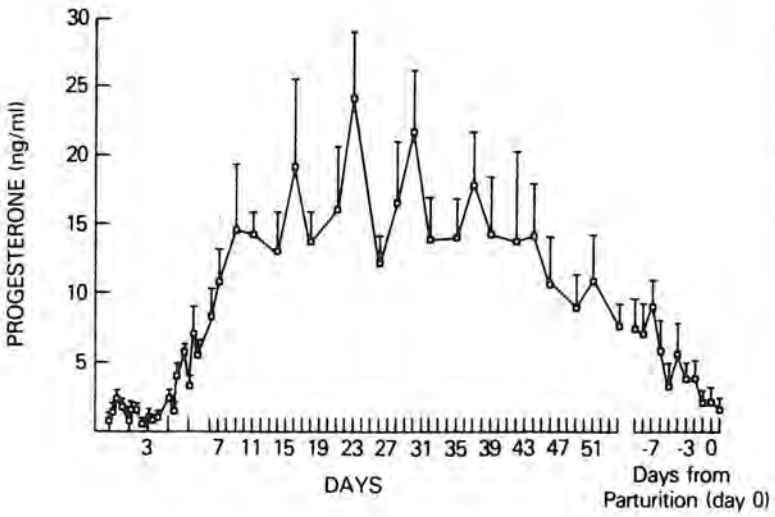

Figure 25–8. Mean ± SEM (n = 132) serum progesterone profiles during the estrus–pregnancy interval (days 1 to 54, where day 1 is the first day of mated estrus) and parturition interval (from 9 days before parturition to 1 day after parturition). (From Schmidt PM, Chakraborty PK, Wildt DE: Ovarian activity, circulating hormones and sexual behavior in the cat. II. Relationships during pregnancy, parturition, lactation and the postpartum estrus. Biol Reprod 28:657–671, 1983, with permission.)

cells (2.7 per cent, 0 to 22 per cent), anucleated superficial cells (0.2 per cent, 0 to 2 per cent), and neutrophils (3/100 epithelial cells, 0 to 50).[20]

Induction of Estrus and Ovulation in the Adult, Anestrous Queen

Queens can be induced to cycle by increasing photoperiod artificially. When queens are maintained under constant photoperiod, no seasonal influence on the cycle is present.[5,8,10,37,49–53] Increasing the photoperiod to 24 hours light and zero hours dark stimulates longer periods of estrus, increased folliculogenesis, and increased ovarian weight, but is not as effective for estrus induction as 14 hours of light and 10 hours of dark.[52] Both melatonin and short photoperiod (8 hours of light and 16 hours of darkness) suppressed cycling. Plasma prolactin concentration, which is elevated in several syndromes of ovarian inactivity in mammals and in cats, does not increase during seasonal anestrus in the cat, but is significantly elevated during the dark period (mean = 31.7 ng/ml) compared to the light period (mean = 5.5 ng/ml) in cycling queens held at 14 hours of light and 10 hours of dark for several months.[48,54] Mean plasma concentrations of prolactin in anestrous queens ranged from 12.6 to 17.2 ng/ml.[48]

A number of therapeutic regimens to induce cycling in the normal anestrous queen have been described.[10,55–62] The best of these is intramuscular (IM) injection of 2 mg follicle-stimulating hormone (FSH) daily until onset of estrus (3 to 7 days) followed by natural mating or by 250 IU hCG to induce ovulation.[9] Folliculogenesis also has been induced by administration of human urinary follicle-stimulating hormone and by human menopausal gonadotropin in concert with hCG to induce ovulation; exogenous administration of hCG, with LH-like activity, was necessary for estradiol concentrations to rise to those seen in natural estrus, suggesting a natural role for LH in this species.[63] Natural mating (three times daily for the first 3 days of estrus) followed by hCG (250 IU, IM on days 2 and 3 of estrus) synergistically enhances the ovulatory response of cats either in natural or induced estrus.[64] Glass rod stimulation of the vagina of the estrous queen also has been reported to induce ovulation.[65] Increase in serum LH and ovulation occurred in estrous queens following a single IM injection of 25 μg gonadotropin releasing hormone (GhRH).[66] It has been reported that ovulation is only triggered if the interval between the FSH-like and LH-like stimuli exceeds 88 hours.[67]

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Chapter 26

Breeding Management, Artificial Insemination, In Vitro Fertilization, and Embryo Transfer in the Queen

Prebreeding Examination

Prebreeding examination of the queen includes review of vaccination history, history of onset of puberty, interestrous intervals, previous matings and pregnancies, and photoperiod exposure. History of reproductive and nonreproductive disease and abnormal reproductive behavior (e.g., prolonged anestrus, infertility, pregnancy loss) should be assessed for etiology and impact, if any, on future pregnancy (see Chapters 25, 27, and 35).

General physical examination should include inspection of the vulva, and palpation of the uterus per abdomen to identify abnormal uterine enlargement, if present. Diagnostic tests that may be indicated prior to breeding include routine hemogram and serum chemistries as indicators of general body health, serology as a possible indicator of viral disease (e.g., feline leukemia virus, feline immunodeficiency virus, feline infectious peritonitis virus), and blood typing.

In the cat AB blood group system, blood types of A, B, and AB occur. In general, type A blood occurs in 95 to 100 per cent cats, type B occurs in 0 to 5 per cent and type AB occurs in less than 1 per cent, although these percentages vary by geographic region and by breed of cat (Fig. 26–1, Table 26–1). When a kitten with type A or AB blood is born to a mother with type B blood, neonatal isoerythrolysis can occur. These kittens are born healthy, but after ingesting maternal colostrom develop clinical signs of pigmenturia, icterus, anemia, tail tip necrosis, and sudden death.[1–6] Some breeds (such as the Abyssinian, Persian, and Devon rex) and some geographic regions (California) are associated with higher percentages of cats with type B blood. Because even low-risk breeds (such as the domestic shorthair) may include some type B queens, it is recommended that all purebred queens and toms used in a breeding program undergo blood typing in order to ensure compatible matings.

Managing Natural Breeding in the Cat

In catteries and cat colonies, mating of cats is accomplished by bringing the estrous queen to the male, as establishment of the male's territory often is important for successful breeding. A photoperiod of more than 14 hours of light per day is recommended. In research catteries, queens may run in groups in the male's territory. One active, fertile male may be able to service 15 to 20 queens in a harem, or the estrous queen may be taken to the male's territory for 24 to 48 hours to obtain dated matings.[7] This practice, and the rapidity with which copulation occurs, means that copulations may not be observed nor confirmed in this species. Queens will mate with more than one male during estrus, if available, and may become pregnant with kittens sired by different males (superfecundation).

Onset of estrous behavior in the queen may be detected by observing her for 5 to 10 minutes daily to note increased demand for attention, hind limb treading, lordosis, vocalization, or rolling.[8,9] Queens exhibiting these signs may be introduced to the tom for several hours, and receptivity to copulation and breeding behaviors observed. Breedings usually occur over 1 to 3 days.[8,9] In one study where estrous queens were exposed to fertile males for 2 hours daily when in estrus, a range of four

Figure 26–1. Map indicating the frequencies of type B blood in domestic shorthair/domestic longhair cats in the United States (n = 3785). (From Giger U, Griot-Wenk M, Bucheler J, et al: Geographical variation of the feline blood type frequencies in the United States. Feline Pract 19:21–27, 1991, with permission.)

to eight copulations per estrus (mean = 5.4) were observed.[8]

Mounting and copulation occur quickly in this species, and consist of 5 to 50 seconds of mounting and neck bite behavior, 0.3 to 9.0 minutes for positioning with the straddling tom treading along the queen's flanks, 1 to 27 seconds for intromission and ejaculation, and up to 1 second for dismounting (Fig. 26–2).[8–11] Immediately after intromission and ejaculation the queen emits a loud coital cry, leaps away from the male, and undergoes a 1- to 17-minute coital "after-reaction," which consists of disoriented rolling, stretching, and genital licking.[8–12] She will then undergo a period (up to 5 hours) of refusing copulation. Total number of copulations in queens allowed unrestricted access to males ranged from 20 to 36 over 36 hours (Fig. 26–3).[10,11] Copulation frequency decreased from 5.5 ± 0.6 in 2 hours at the beginning of estrus to 1.4 ± 0.2 in 2 hours 12 to 36 hours into estrus.[10,11]

Coitus induces luteinizing hormone (LH) release by the pituitary gland of the queen within minutes, and ovulation is reported to occur 24 to 48 hours after coitus.[9,13,14] Magnitude of LH release by the anterior pituitary has been demonstrated to increase with increase in number of copulations in the estrous queen, and 9 of 18 queens allowed to copulate only a single time failed to secrete adequate LH to induce ovulation (Fig. 26–4).[15–19] The ability to release LH in response to coitus is in part a function of duration of hypothalamic/pituitary exposure to estrogen, and sexual receptivity alone does not imply ability to respond to coitus with LH release.[20] After multiple copulations, postcopulatory LH release becomes refractory to the copulatory stimulus; there also is decreased responsiveness to gonadotropin-releasing hormone (GnRH) if given in sequential injections over 4 hours.[21] These data suggest that multiple matings over the first 2 or 3 days of estrus will optimize reproductive performance.[9]

Ovulation in cats is reported to be compromised by preovulatory anesthesia with ketamine hydrochloride/acepromazine/halothane or by laparoscopy or by both. Reduced ovulations and pregnancies were observed when queens were anesthetized for laparotomy 25 hours after administration of human chorionic gonadotropin (hCG) when compared to nonanesthetized queens.[22]

Copulation occuring on the day of, to 42 hours after, the day of hCG administration or the LH surge results in fertilization of more than 50 per cent eggs ovulated (Fig. 26–5), suggesting that the life span for fertilization of the ovulated ovum of the cat is at least 18 hours.[22,23] Healthy adult tom cats have been reported to ejaculate three times per day for 4 to 5 days without noticeable change in sperm concentration.[9]

Problems associated with copulation failure in the cat include shyness, inexperience, distraction, and mate preference. Shy males sometimes can be encouraged into mating behavior by repeated exposure to calm, experienced estrous queens for 15 to 30 minutes daily.[9] The

Table 26–1. Blood Type Frequencies in Different Feline Breeds of the United States*

Breed (Over 6000 Cats Typed)	Type B Frequency
Siamese, Burmese, ocicat, Oriental shorthair, Tonkinese	0%
DSH/DLH, Maine coon, Norwegian forest	≤5%
Abyssinian, Himalayan, Japanese bobtail, Persian, Somali, Sphinx	5–25%
British shorthair, Cornish rex, Devon rex	25–50%

* Update from Giger U, et al: Frequency and inheritance of A and B blood types in feline breeds in the United States. J Hered 82:15–20, 1991.
From Giger U, Griot-Wenk M, Bucheler J, et al: Geographical variation of the feline blood type frequencies in the United States. Feline Pract 19:21–27, 1991, with permission.

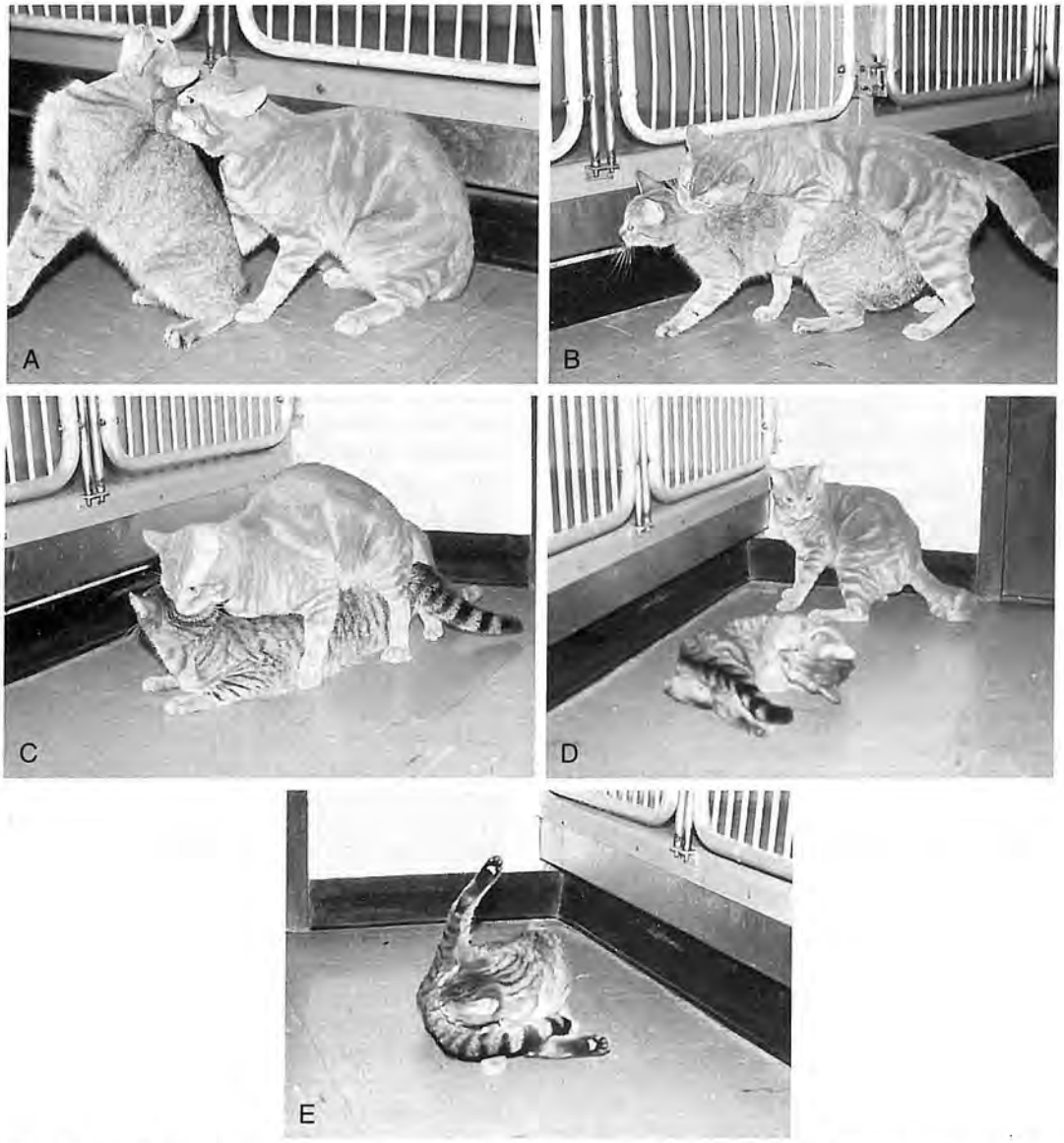

Figure 26–2. Normal copulation in the cat. Mounting and neck bite behavior (**A**) occur over 5 to 50 seconds; positioning while mounted (**B**) occurs over 0.3 to 9 minutes; intromission and ejaculation (**C**) occurs over 1 to 27 seconds, after which the male dismounts quickly.[8,10-12] The "after-reaction" (**D** and **E**) (1 to 17 minutes) consists of striking out at the male, followed by disoriented rolling, stretching and genital licking.

inexperienced female can be placed with an experienced but nonaggressive male for increasing periods of time. Some adult queens do not permit copulation with some males even when the queen is in estrus, suggesting sexual partner discrimination.[8,9] In one report of 14 queens, the females did not allow the male to breed at 15 of 38 observed estrous periods (39 per cent), despite multiple attempts during exhibiting of behavioral estrus and cornified vaginal cytology.[8]

Artifical Insemination with Fresh or Frozen Semen

Queens have conceived following artificial insemination with fresh or frozen semen depos-

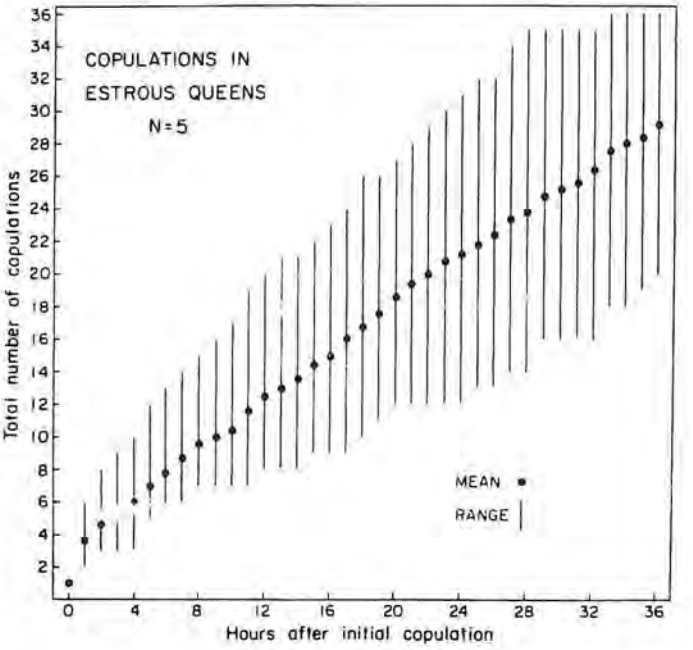

Figure 26–3. Means (and ranges) of the total number of copulations that had occurred after each hour during a 36-hour period of unrestricted copulatory activity in estrous cats, beginning 36 to 48 hours after the onset of estrus. (From Concannon PW, Lein DH, Hodgson BG: Self-limiting reflex luteinizing hormone release and sexual behavior during extended periods of unrestricted copulatory activity in estrous domestic cats. Biol Reprod 40:1179–1187, 1989, with permission.)

ited vaginally or into the uterine horn via laparoscopy (Fig. 26–6).[22-28] Ovulation can be induced with 50 or 250 IU hCG intramuscularly (IM) administered to the estrous queen on the day of or the day preceding insemination.[23,27,28] Semen is collected into an artificial vagina or by electroejaculation (see Chapter 37), and diluted with equal volumes saline or extender, or a volume of extender to bring total insemination volume to 100 μl. The extended semen is inseminated into the anterior vagina or posterior cervix using a silver abscess cannula or bulb-tipped 20-gauge needle attached to a 1-ml syringe (Fig. 26–6). Two to four kitten litters were reported in 14 of 26 queens inseminated vaginally with 5 to 50 million fresh sperm.[23] Suggested insemination dose of sperm in the cat for vaginal artificial insemination is 50 million.[27]

A pregnancy rate of 50 per cent (9 of 18) with one to four kittens per litter was reported in estrous queens that were anesthetized 31 to 50 hours after administration of 100 IU hCG, and inseminated laparoscopically into the uterine horns with $6.2 \pm 0.9 \times 10^6$ fresh sperm.[22] Only 2 of 14 (14.3 per cent) queens similarly inseminated 25 to 33 hours after hCG administration conceived, suggesting that preovulatory anesthesia or laparoscopy compromises ovulation.[22]

Chilled, extended feline semen has been used to achieve pregnancy in the cat. Feline semen diluted in an equal volume of 37°C 3% buffered citrate-egg yolk media, submerged in a 37°C water bath, then placed in a 4°C refrigerator for 4 to 5 hours, and stored at 4°C for up to 3 days, was still able to fertilize feline ova in vivo.[28] Semen stored in this manner and then used to inseminate estrous queens induced to ovulate achieved fertilization of 100, 67, 30, and 23 per cent of ova ovulated using semen stored 0, 1, 2, and 3 days, respectively.[28]

Conception and one- to four-kitten litters were reported in 6 of 56 queens inseminated vaginally with 50 to 100 million motile sperm that had been stored frozen as pellets in a diluent consisting of 20% (v/v) egg yolk, 11% (w/v) lactose, and 4% (v/v) glycerin in deionized water, then thawed rapidly.[25,26] Four of the six queens were in natural estrus, and two were in gonadotropin-induced estrus.[26]

In Vitro Fertilization and Embryo Transfer in the Cat

Assisted reproductive technologies such as in vitro fertilization (IVF) and embryo transfer (ET) with associated technologies for in vitro embryo maturation and gamete/embryo cryopreservation have been investigated in domestic cats as models for potential reproductive

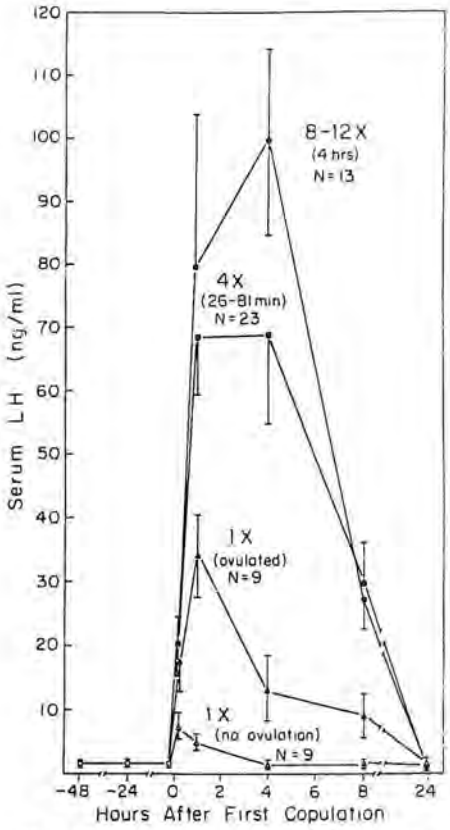

Figure 26–4. Mean ± SEM serum LH levels in cats confirmed to have ovulated following a single copulation (1×), four copulations within a 26- to 81-minute period (4×), or 8 to 12 copulations during a 4-hour period (8–12×), and in cats that did not ovulate following a single copulation. All copulations were on day 3 of estrus. (From Concannon PW, Hodgson B, Lein D: Reflex LH release in estrous cats following single and multiple copulations. Biol Reprod 23:111–117, 1980, with permission).

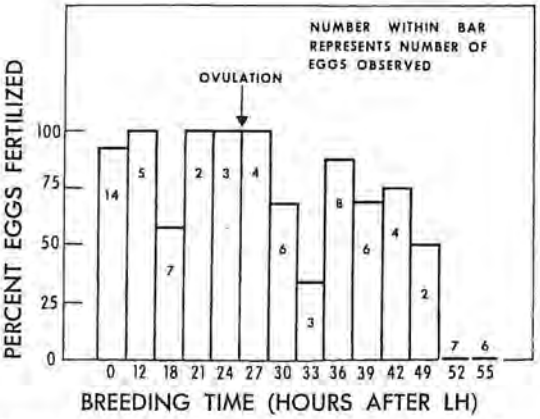

Figure 26–5. Fertilization of cat eggs by natural breeding. (From Sojka NJ, Jennings LL, Hamner CE: Artificial insemination in the cat [*Felis catus L.*]. Lab Anim Care 20:198–204, 1970, with permission).

intervention in endangered species of wild felids.[29–31]

Cat spermatozoa undergo capacitation within 1 hour of in utero exposure, or within 3 hours of incubation in vitro during "swim up" processing.[32,33] Penetration of homologous feline zonae pellucidae has been described as an index of capacitation.[33] In vitro sperm penetration of ovulated oocytes occurs 0.5 to 5 hours after exposure to sperm.[34]

Although IVF of cat oocytes was first described in 1970, in vitro oocyte maturation and IVF success in the domestic cat remains inferior to that in livestock species. Some authors ascribe the decreased IVF success in the cat to a high level of natural follicle atresia, and to presence of heterogeneous populations of cumulus-oocyte complexes that reflect differences in the oocyte's ability to mature and develop in vitro.[30–32] Forty-four of 92 oocytes (48 per cent) recovered from uterine tubal (oviductal) flushes and incubated with sperm that had been collected by artificial vagina and capacitated for 0.5 to 24 hours in utero demonstrated sperm penetration and cleavage.[32]

Feline ova have been fertilized in vitro with sperm collected from the ductus deferens of male cats; ova were flushed from uterine tubes (oviducts) collected at ovariohysterectomy from queens superovulated with pregnant mare serum gonadotropin (PMSG) and LH.[34,35] Type of culture medium and protein supplement influence oocyte maturation, fertilization, and cleavage in vitro.[36–38] Products of embryonic transcription are required for cleavage of the embryo beyond the five- to eight-cell stage.[39] Culture with growth hormone, prolactin, or gonadotropins during oocyte maturation in vitro does not enhance development of domestic cat oocytes matured in vitro.[40] Feline oocytes have been reported to survive, resume meiosis, and achieve metaphase II in vitro after slow freezing in dimethyl sulfoxide or ethylene glycol.[41]

The first successful embryo transfer in the cat was reported in 1978 using donors and recipients in natural estrus, with ovulation induced in recipient queens by sterile matings or hCG.[42,43] Embryos were recovered by uterine flush 6 days after mating. Forty-seven embryos were recovered from 17 flush attempts, transferred to 9 recipients, and resulted in 4 pregnancies and 4 live kittens.[42,43] Three pregnancies and two litters of kittens resulted from embryos recovered from naturally mated queens given 2 mg follicle-stimulating hormone (FSH) IM daily for 6 days, followed by

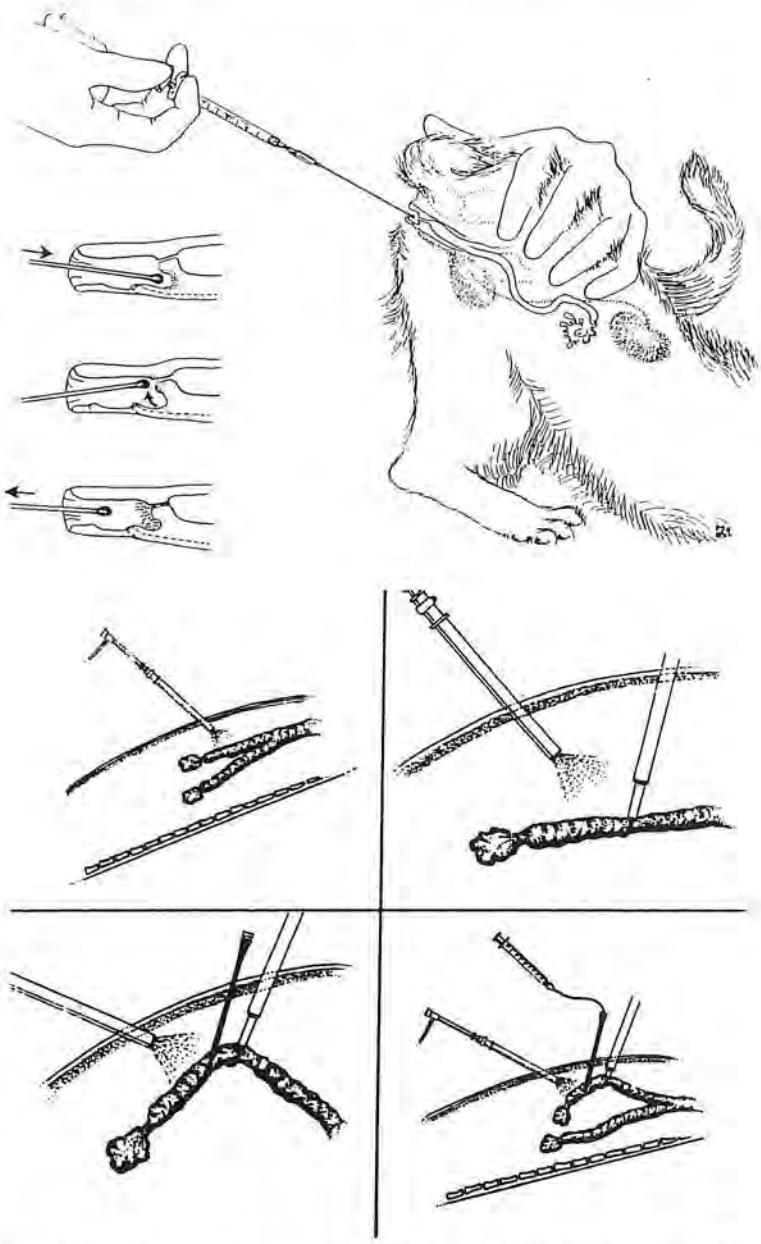

Figure 26–6. Artificial insemination in the queen using vaginal (top) or intrauterine (bottom) deposition of semen. (From Sojka NJ, Jennings LL, Hamner CE: Artificial insemination in the cat [Felis catus L.]. Lab Anim Care 20:198–204, 1970 [L]; and Howard JG, Barone MA, Donogue AM, Wildt DE: The effect of pre-ovulatory anaesthesia on ovulation in laparoscopically inseminated domestic cats. J Reprod Fertil 96:175–186, 1992 [R]).

transfer of recovered embryos to recipients induced to ovulate with hCG.[44] In another report, 12 kittens were reported from 38 embryo transfers (32 per cent) to synchronized feline recipients.[45]

Unovulated oocytes have been aspirated from ovarian follicles of the estrous queen, fertilized in vitro with ejaculated feline spermatozoa, implanted in recipients induced to ovulate with hCG, and resulted in good quality embryos and/or the birth of live kittens.[46–49]

Feline embryos can develop into transferable morulae in vitro, but, with standard culture techniques, there is partial developmental arrest from the morula to blastocyst stage.[50–52] In vivo and in vitro growth rate of cat embryos produced after natural mating is comparable to that of embryos fertilized and cultured in

vitro, but development to the blastocyst stage is superior in embryos produced in vivo by natural mating.[53,54]

First birth of kittens derived from frozen-thawed embryos transferred to recipient queens was reported in 1988.[55,56] Donor queens were stimulated with FSH injected subcutaneously (SC) over 6 days; hCG was injected IM over 2 days to induce ovulation. Surgical embryo recovery was accomplished via laparotomy and flushing of each uterine horn. Embryos were exposed to glycerol, then frozen using an adaptation of a conventional method of freezing cattle embryos using sequential incubation in Hepes/Tyrodes buffer, 1,2-propandiol, and sucrose prior to freezing.[42] Rapid thawing was followed by overnight culture and surgical transfer to recipient queens synchronized with the same regimen used in the donor queens. Eleven transfers of 137 embryos resulted in five litters of kittens with a total of 17 kittens (12.4 per cent survival rate after transfer).[55,56] Following cryopreservation, most two- to four-cell feline embryos retain their capability for in vitro development to the morula/blastocyst stage in vitro and for some embryo viability in vivo (three live kittens from 58 embryo transfers).[42]

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Chapter 27

Feline Pregnancy

Physiology and Endocrinology of Normal Pregnancy

Ovulation in the queen follows coitus by 24 to 36 hours.[1–3] Ova are fertilized in the uterine tube (oviduct), and then pass into the uterine horn as morulae by day 4 to 5 postcoitus (day 3 to 4 postovulation).[3,4] If the queen roams free, or is bred by more than one male, *superfecundation* (i.e., fetuses present in one litter that have been sired by different males) may occur.

Number of kittens per litter ranges from 1 to 13.[5,6] Average litter size in the queen has been reported as 3.3 to 5.[6–9] In a survey of 3171 kittens from 751 litters, average litter size varied by breed; in the Burmese it was 5.0, in the Siamese 4.5, in the Persian 3.9, in the Abyssinian 3.5, and in the chinchilla cat 2.8.[7] Overall sex ratio at birth was 100 males to 92 females (n = 3171), whereas a sex ratio of 47.3 per cent male and 50.7 per cent female was reported in another study of 170 kittens.[3,7] In a study of 77 litters, most (42) resulted from matings on 3 consecutive days, and the number of kittens per litter was not affected by the number of days on which mating took place.[3]

Plasma progesterone concentration in the pregnant queen increases from baseline (<1.0 ng/ml) to more than 2 ng/ml starting 1 to 2 days after ovulation, or 2 to 3 days after mating.[3] Plasma progesterone concentration in the pregnant queen then continues to increase, to peak concentrations of 15 to 30 ng/ml by 25 to 30 (range = 11 to 60) days of pregnancy, after which it slowly declines throughout the rest of pregnancy (Fig. 27–1).[3,10,11] Despite earlier reports that ovariectomy can be done after day 45 of gestation without causing abortion and that placental production of progesterone increases significantly during the latter part of gestation, recent work suggests that the feline corpora lutea are the main source of plasma progeserone in the pregnant queen, and that placental progesterone secretion is of minor or nonexistent importance.[3,9,12,13] Although progesterone declines at the end of pregnancy, low, baseline concentrations (<1 ng/ml) are not essential for the onset of parturition (Fig. 27–1).[11]

Plasma estradiol concentration, which peaks in the estrous queen at time of copulation, drops to baseline concentrations (8 to 12 pg/ml) during the first 5 days after copulation, and stays low until days 58 to 62 of pregnancy. At that time, it rises slightly to 20 to 30 pg/ml, then starts to decline just before parturition.[10]

Queens may show behavioral estrus during pregnancy. The feline ovary is not refractory to exogenous gonadotropin stimulation during midgestation.[14–16] Periods of follicle growth and regression have been observed to occur continually even during the luteal phase in the queen.[17] Estrous activity during gestation has been a suggested cause for *superfetation* (i.e., simultaneous presence in the uterus of fetuses of different gestational age), which has been assumed to occur in the cat from anecdotal reports of queens delivering viable kittens up to several weeks apart.[18–25] However, superfetation may be difficult to distinguish from arrested development unless all of the kittens are born alive and survive the neonatal period, which is not the case in many of these reports. Estrous activity during gestation also has been a suggested cause of abortion, which occurs fairly often in the queen. However, one author reports that pregnant queens showing signs of estrus do not have increased serum estradiol or demonstrate a luteinizing hormone (LH)

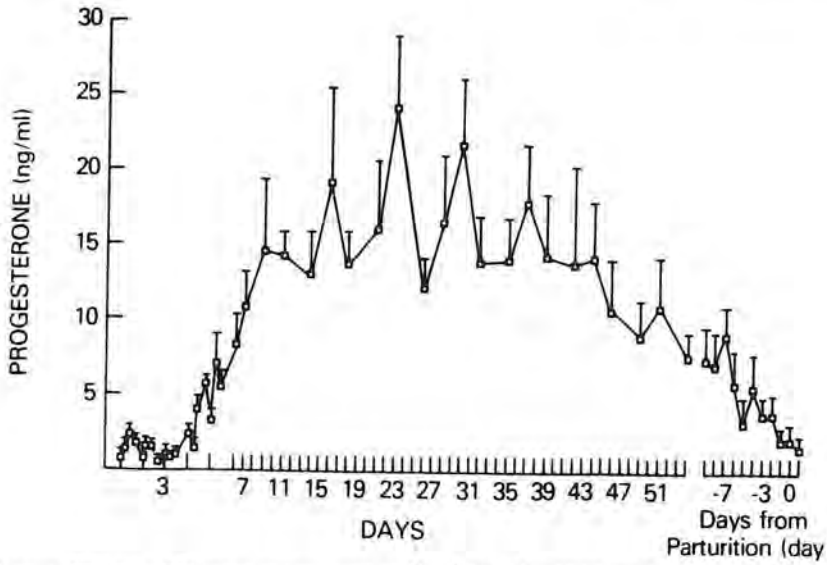

Figure 27–1. Mean ± SEM (n = 12) serum progesterone profiles during the estrus-pregnancy interval (days 1 to 54, where day 1 is the first day of mated estrus) and parturition interval (from 9 days before parturition to 1 day after parturition). (From Schmidt PM, Chakraborty PK, Wildt DE: Ovarian activity, circulating hormones and sexual behavior in the cat. II. Relationships during pregnancy, parturition, lactation and the postpartum estrus. Biol Reprod 28:657–671, 1983, with permission.)

surge if bred, despite normal estrous and copulatory behavior, suggesting that some estrous behavior during pregnancy may occur without follicle growth on the ovary.[3]

Relaxin, a peptide hormone important for softening the fibrous connective tissues of the pelvis prior to birth, is produced by the fetoplacental unit of the cat.[3] Plasma relaxin activity measured in the pregnant queen by radioimmunoassay using two different antisera was first detected at about day 25 of pregnancy; it increased rapidly, plateauing at days 30 to 35, and then declined slightly in the last 10 to 15 days prepartum (Fig. 27–2).[26] Plasma relaxin concentrations were nondetectable during estrus, pseudopregnancy, and after 24 hours postpartum, making this hormone one with potential value for pregnancy diagnosis in the cat.

Plasma prolactin increases at about day 35 of pregnancy in the cat, plateaus at about day 50, and increases suddenly just before delivery; it increases more with the suckling stimulus during lactation (Fig. 27–3).[27] Following weaning, prolactin concentrations of the queen return to basal levels within 1 to 2 weeks.

The feline fetoplacental unit and the endometrium are reported to secrete prostaglandin $F_{2\alpha}$ ($PGF_2\alpha$); concentrations begin to rise at about day 30, plateau at about day 45, and then increase greatly just before delivery.[3]

Implantation and Placentation

Feline morulae enter the uterine horn about 5 days after copulation.[3] By 8 days after copulation, blastocysts are 500 to 600 mm in diameter, and by 10 days after copulation some blastocysts have hatched (2300 × 1000 mm in size), while others have yet to hatch (1500 × 1000 mm).[3]

Transuterine migration of embryos was reported in 40.8 per cent of the embryos in 69 feline pregnancies, and appears to be correlated positively with the difference in ovulation number between right and left ovaries.[3] Transuterine migration allows fetus number in the right and left uterine horns to become evenly distributed.

Implantation occurs 12 to 13 days after ovulation.[3,28] Placentation in the cat is endotheliochorial (i.e., maternal and fetal circulations are separated by four tissue layers) and is zonary in shape.[29–33] The border is brown due to marginal hematomas.[34] Fusion of embryonic membranes from adjacent embryos resulting in synchorial littermates has been reported in rare instances in the queen, as has identical twinning.[35,36] Peak amniotic and allantoic fluid volumes in the fetal membranes of the cat have been reported as 26 and 24 ml, respectively.[37] Placental weight in the cat has been reported as 5.7 g on day 24 of gestation, 11.9 g on day

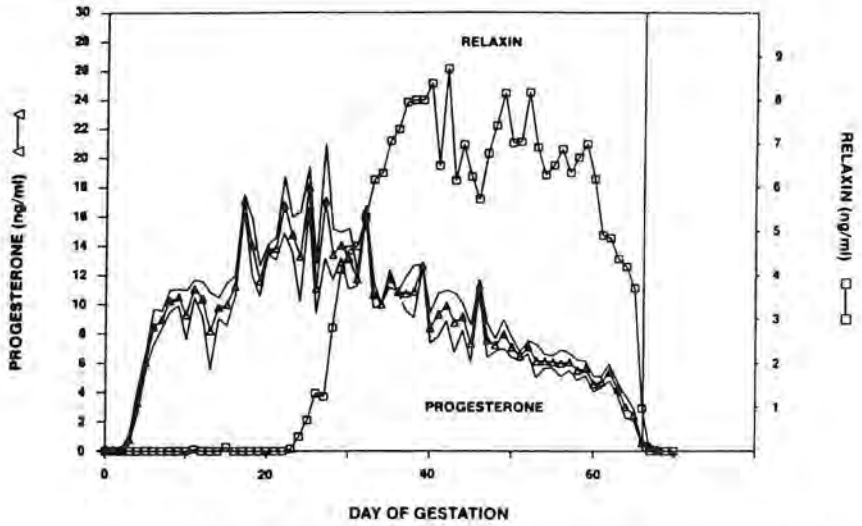

Figure 27–2. Mean concentrations of relaxin (open box) and progesterone (open triangle) in plasma reported for queens during gestation. The vertical bar toward the right indicates the day of parturition. (From Stewart DR, Stabenfeldt GH: Relaxin activity in the pregnant cat. Biol Reprod 32:848–854, 1985, with permission.)

33, and 15.0 g on day 39 before reaching a plateau at midgestation.[3] Placental transfer of maternal antibodies to the fetuses has been demonstrated in this species.[38]

Pregnancy Diagnosis

Clinical diagnosis of pregnancy in the queen may be made by abdominal palpation of fetal vesicles, by abdominal radiography, or by abdominal ultrasonography. Measurement of serum relaxin concentration is not now commercially available in the cat, but this assay may one day offer a blood test for this purpose.

Palpation of the pregnant uterus per abdomen is possible in the cat as early as day 15, when round dilations of the uterus can be detected (Fig. 27–4).[9] Pregnancy diagnosis by palpation is recommended at 21 to 25 days after breeding, at which time the fetal vesicles are approximately 2.5 cm in diameter, and identification of fetal vesicles by abdominal palpation is easiest.[16,39–41] After day 35 of gestation, the segmental fetal dilations in the uterus of the pregnant queen tend to become conflu-

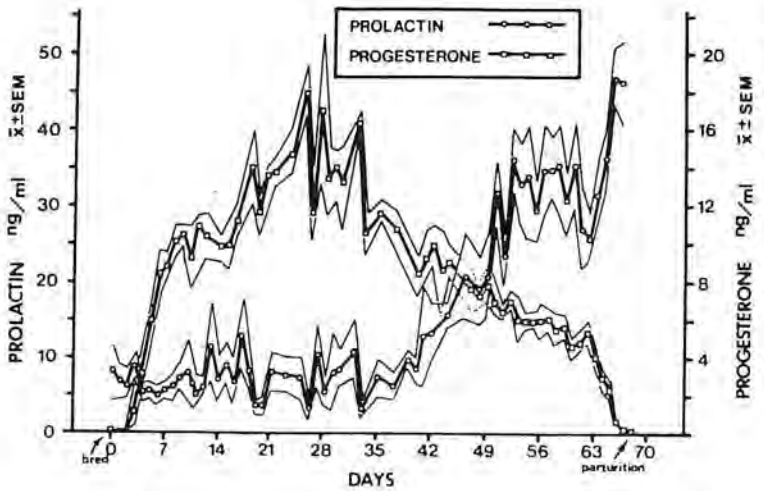

Figure 27–3. Mean concentrations of prolactin (circle) and progesterone (box) in plasma of eight queens during gestation. Day 0 is the day of copulation; P, parturition. (From Banks DR, Paape SR, Stabenfeldt GH: Prolactin in the cat. I. Pseudopregnancy, pregnancy and lactation. Biol Reprod 28:923–932, 1983, with permission.)

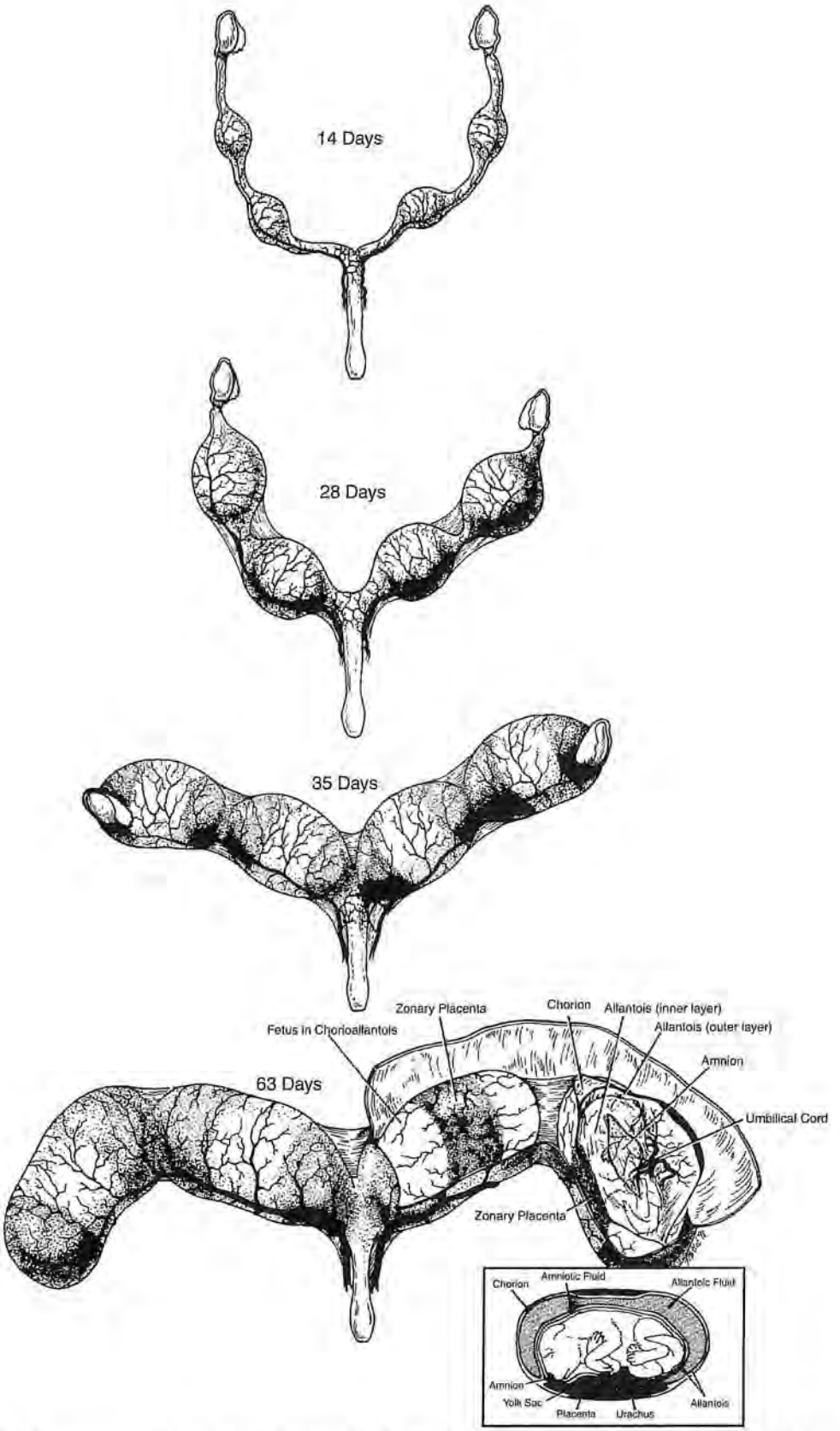

Figure 27–4. Extrauterine view of fetuses developing within the gravid uterus of the cat. Individual fetuses are evident as discrete masses through 28 days, and tend to merge by day 35. Zonary placentas are depicted by the lighter band-like areas.

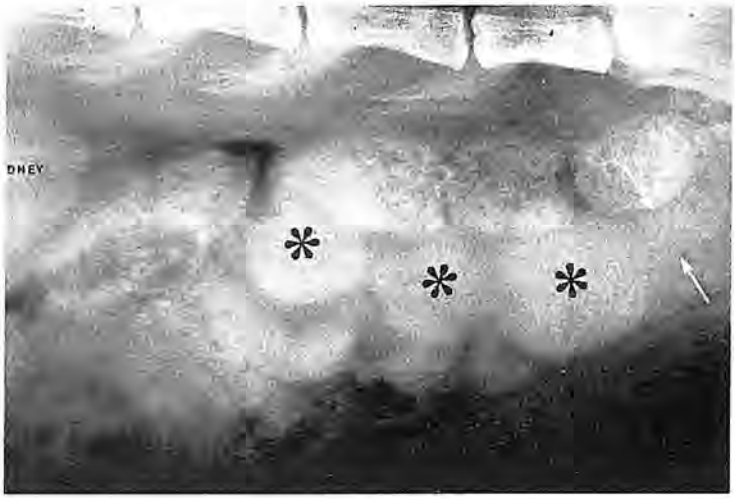

Figure 27–5. Radiograph, lateral projection, cat, 19th day of pregnancy. Three rounded uterine swellings are distinct in one row (asterisks), three other rounded shadows may be seen ventral to the above-mentioned structures. Between the arrows, an unexpanded, caudal uterine segment is visible. Original size. (From Tiedemann K, Henschel E: Early radiographic diagnosis of pregnancy in the cat. J Small Anim Pract 14:567–572, 1973, with permission.)

ent, making pregnancy diagnosis by this technique more difficult. Late in gestation (after days 58 to 60) fetal heads and bodies often can be distinguished by careful and gentle palpation of the tractable queen. Pregnancy diagnosis by palpation may be inaccurate in the queen with a segmentally dilated pyometritic uterus (see Chapter 32). Adverse sequelae to the pregnancy caused by palpation of the uterus per abdomen have not been reported in the cat, but have been reported in other species.

Use of radiographic examination for presumptive pregnancy diagnosis as early as 17 to 21 days after breeding, when segmental dilations of 1.2 to 2.5 cm may be observed, has been reported (Fig. 27–5).[40] Radiographic identification of calcification of fetal bones may occur as early as 38 to 40 days after breeding. (Table 27–1).[35,39,42] Radiographic measurement of crown-rump length of kittens may be used to estimate fetal age (Table 27–2, Fig. 27–6).[39] Because of the potential danger of radiation exposure to developing fetuses, radiographic examination of the abdomen of a pregnant queen is not recommended until after day 40. Although the authors frequently have used late pregnancy (after day 56) radiography to assess high-risk pregnancies in the queen without noted adverse effect on the fetuses, routine use of this modality is recommended only when indicated; high-risk pregnancies are suspected in queens with previous dystocias, with

Table 27–1. Methods of Diagnosing Pregnancy in the Queen

Method	Days Gestation	Findings
Palpation of uterus	15	~1 cm dilations
	28	~2 cm dilations
	35	~3 cm dilations
	>35	Difficult to identify fetuses until near the end of gestation
Radiography (not recommended for routine diagnosis due to fetal radiation exposure)	17–21	1.2–2.5 cm dilations
	22–28	1.9–3.8 cm dilations
	28–30	3.0–3.8 cm dilations
	38–40	Calcified mandible, cranium, scapula humerus, femur, vertebrae, ribs
	43	Calcified tibia, fibula, ileum, ischium
	49	Calcified metacarpals, metatarsals
	52–53	Calcified digits, sternum
	56–63	Molar teeth visible
Ultrasonography	16–25	Fetal heartbeats first detected
	26	Fetal heads, limb buds, detected
	28	Fetal movement first detected

Data from Chan et al.,[14] Markee and Hinsey,[24] and Vasseur and Feldman.[122]

Table 27–2. Radiographic Identification of Fetal Age in the Cat

Days Since Mating	Crown-Rump Length (mm)
38	58
41	75
44	84
47	94
50	106
53	114
56	121
58	130
60	136
Parturition (about 65)	145

Adapted from Boyd JS: The radiographic identification of the various stages of pregnancy in the domestic cat. J Small Anim Pract 12:501–506, 1971, with permission.

a history of pelvic fracture, with small litter size, with pre-existing metabolic disease, or with advanced (>6 years) age.

Transabdominal ultrasonography (Fig. 27–7) is the authors' recommended modality for detection and monitoring of feline pregnancy, both because ultrasonography can be used to detect fetal viability, which palpation and radiography cannot, and because ultrasound exposure may be safer than radiographic exposure for developing fetuses.[43,44] Detection of feline fetal heartbeats by ultrasonography is first possible at 16 to 25 days after breeding, and fetal morphology (e.g., fetal heads, limb buds) can be detected after day 26.[43–45] Normal fetal heart rate in the cat is stable over the duration of pregnancy, and averages 228.2 ± 35.5 beats per minute.[45] Fetal movement may be detected by ultrasound after day 28. Ultrasonography has been demonstrated to be inaccurate in determining litter size, with inaccuracy increasing as litter size increases.[44]

Use of serum progesterone to diagnose pregnancy in the domestic cat has, unfortunately, been reported, but is unreliable.[46] A serum progesterone concentration of less than 5 ng/ml taken 6 days after breeding was considered a negative indication of pregnancy, and a concentration exceeding 5 ng/ml was considered a positive indication of pregnancy. However, there is a high probability of false-positives in queens that were bred, induced to ovulate, and did not conceive (but who would have serum progesterone exceeding 5 ng/ml), or in those that ovulated spontaneously in the absence of copulation (also with serum progesterone exceeding 5 ng/ml).[3,47] Because early pregnancy is not reported to exist in the queen with low (<1 ng/ml) serum progesterone concentration, false-negatives using this technique have not been reported.

Care of the Pregnant Queen

Veterinary care of the pregnant queen usually begins at time of pregnancy diagnosis, about 3 to 4 weeks after breeding. At that time, general husbandry practices, to include isolation from outside cats, and provision of both supervision and privacy in a dry, warm queening area should be initiated. Normal pregnant females should be weighed weekly during pregnancy. Use of a queening box, to which the pregnant female is acclimated, may contribute to kitten survival postpartum. Boxes should be lined

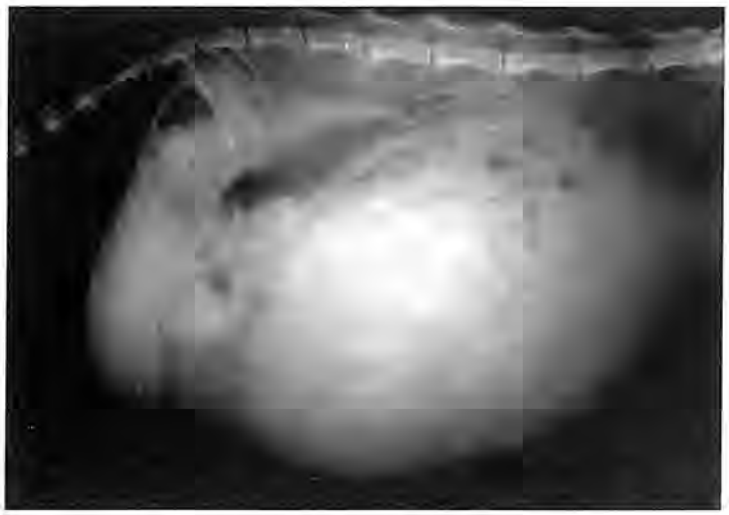

Figure 27–6. Radiograph, lateral projection, of the abdomen of a pregnant queen with unknown breeding date. Crown-rump length of one fetus (arrow) was 120 mm, predicting a gestational age of 56 days, and predicting parturition day 9 days later (see Table 27–2). The queen actually delivered her kittens 10 days following the day of the radiograph.

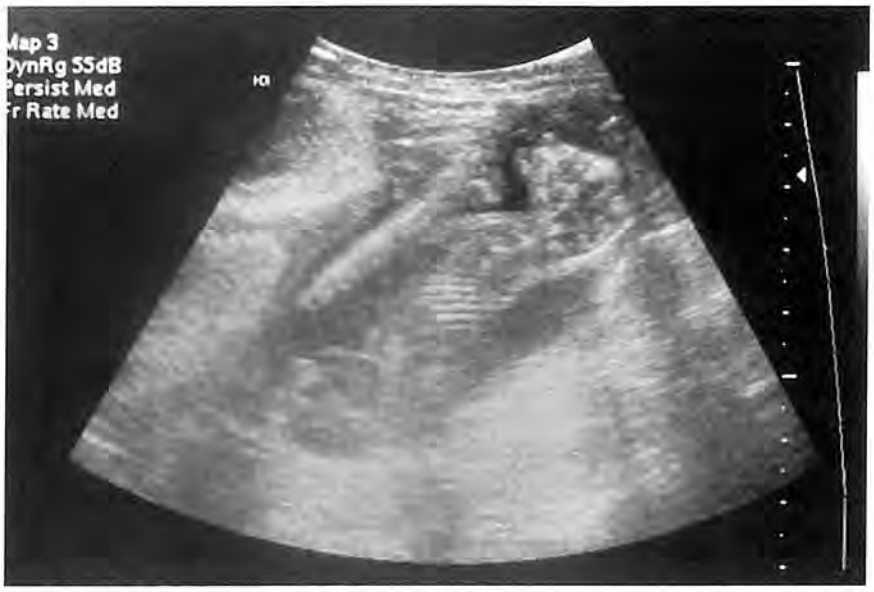

Figure 27–7. Transabdominal ultrasound of a feline fetus in utero of 38 days' gestation. Fetal limbs, organs, and blood vessels visible. 7.5-MHz transducer.

with blankets or towels that can be laundered, not with straw, hay, sawdust, or newspaper.

Queens should be fed a normal prebreeding ration (i.e., quantity and quality) for the first 3 to 4 weeks of gestation, when weight gain is minimal (Fig. 27–8).[48] Approximate caloric needs for the adult, intact, nonpregnant queen are 110 calories/kg (50 calories/lb) of body weight per day.[48] At about 3 weeks gestation, many queens undergo a short period of partial anorexia lasting 3 to 10 days that may be accompanied by mild weight loss.[48] From weeks 4 through 6 of gestation, caloric intake should be increased gradually by about 50 per cent (to 165 calories/kg or 75 calories/lb body weight per day); during the final weeks of gestation, caloric intake approaching 220 calories/kg or 100 calories/lb body weight per day may be indicated to maintain optimal body condition. The goal is to avoid both under-

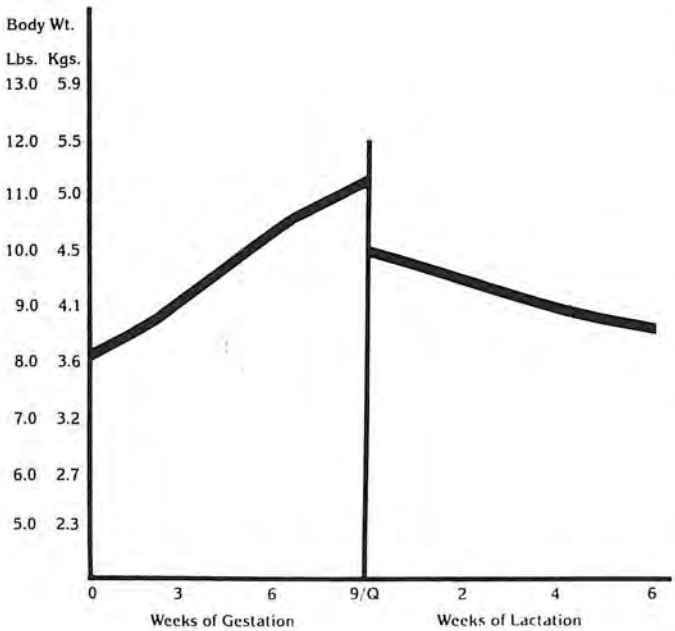

Figure 27–8. Body weight change of queens during gestation and lactation. (From Lawler DF, Babiak DM: Nutrition and management of reproduction in the cat. Vet Clin North Am 16:495–519, 1986, with permission.)

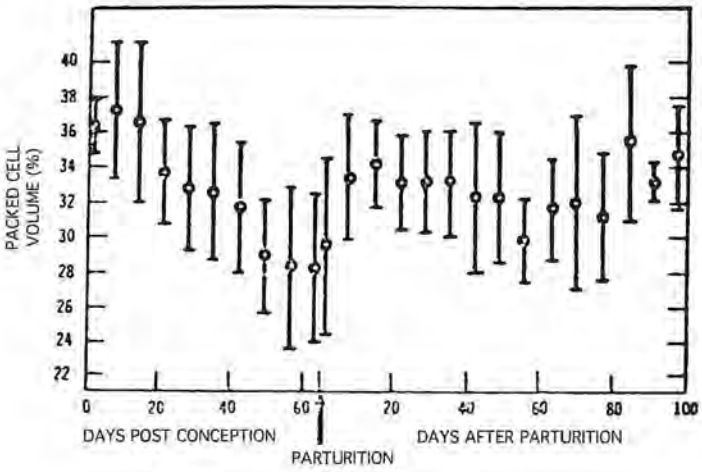

Figure 27–9. Packed cell volume in six cats during pregnancy, lactation (up to 65 days postpartum), and after lactation. Mean (circles) and 95 percent confidence limits of the standard error (vertical bars). (From Berman E: Hemogram of the cat during pregnancy and lactation and after lactation. Am J Vet Res 35:457–460, 1974, with permission.)

weight, which may contribute to difficulty maintaining lactation after delivery, and obesity, which increases risk of dystocia and kitten mortality.[48]

Plasma volume expansion may occur in the pregnant queen as it does in human and canine pregnancy, because normocytic normochromic anemia is reported during the last third of pregnancy in this species.[49,50] Physiologic anemia is manifested by relative decreased erythrocyte count, hemoglobin concentration, and packed cell volume. Reticulocytosis develops in response to the anemia. Lowest mean packed cell volume ($\bar{x}$ = 28.3 ± 4.1 SEM) occurred at 63 days' gestation in six queens (Fig. 27–9).[49] Leukocyte counts (total and differential) and total plasma protein, however, remained unchanged.[49]

Glucocorticoids, griseofulvin, and modified live virus feline panleukopenia vaccine are teratogenic in the queen, and their use should be avoided during pregnancy.[51,52] Congenital malformations in kittens born to queens treated with griseofulvin in the first half of pregnancy include cleft palate, exencephaly, hydrocephalus, spina bifida, cyclopia and anophthalmia, atresia ani, atresia coli, and abnormalities of the heart.[51,52]

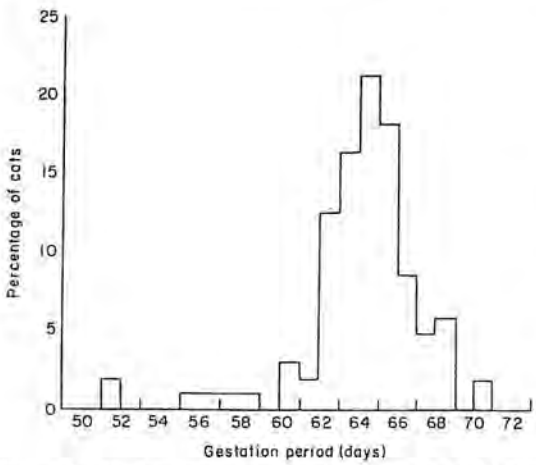

Figure 27–10. Distribution of gestation length in 104 feline litters in eight breeds, about 55 per cent shorthaired. (From Jemmett JE, Evans JM: A survey of sexual behaviour and reproduction of female cats. J Small Anim Pract 18:31–37, 1977, with permission.)

Length of Gestation in the Cat

Length of feline gestation is reported to range from 52 to 74 days, timed either from the first or last breeding (Fig. 27–10).[6,8,53–57] Reported mean gestation lengths in this species are 65 days (n = 104), 65.8 days (n = 320), and 66.9 days (n = 15).[8,53,54] In one study of 320 litters from 64 queens, gestation length was not correlated with age of queen, parity, number of kittens per litter, mean weight of kittens born, weight gain during pregnancy, or genetic background.[8] There was a high degree of variation both within and between individuals.

Pregnancy Loss

Pregnancy Resorption, and Abortion

Physiologic ovulation failure, fertilization failure, and embryo loss occur in normal cats, in which only 86 to 88 per cent of mature follicles become corpora lutea, and only about 67 per

cent of corpora lutea result in kittens.[58] In one report, 48 of 52 cats (92.3 per cent) mated three times ovulated [evidenced by the presence of ovarian corpora lutea at ovariohysterectomy (OHE)], and of these, 38 (79.2 per cent) produced normal embryos or had normal implantation sites; 21 per cent failed to produce any fertilized or viable embryos.[59] Aberrant histologic changes of the endometrium and corpora lutea are not reported to be primarily responsible for the failure of the natural estrous, mated queen to produce good-quality embryos.[60] Age and parity of the queen are related to successful production of kittens, with smaller litter size reported in primiparous and aging queens; although queens in breeding colonies can be productive for 8 to 10 years, replacement after 6 years of age is reported to maintain peak colony performance.[58]

Pregnancy loss that occurs after fertilization and implantation is common in the queen, and may be associated with infection, nutritional insufficiency, fetal chromosomal defect, or a maternal hormonal environment that is inadequate to maintain pregnancy. Pregnancy loss may be associated with resorption of embryos in the first half of pregnancy, and/or abortion of fetuses in the second half of pregnancy; with infectious etiologies, the outcome of resorption or abortion may depend on time during the pregnancy that infection occurs.

INFECTIOUS CAUSES

Infectious causes of spontaneous feline pregnancy loss may be viral, bacterial, or protozoal.

Feline herpesvirus (FHV) (feline viral rhinotracheitis virus) infection in pregnant cats may produce abortion, intrauterine fetal death and maceration, placental necrosis, and congenital fetal infection.[61,62] Experimental inoculation of FHV administered intravenously or intranasally to pregnant queens between 42 and 50 days of gestation caused abortion of both live and dead fetuses, or fetal death in utero with partial maceration and autolysis.[62] Abortion or fetal death occurred 6 to 26 days after experimental infection was induced. Other than presence of a sanguineous vaginal discharge associated with impending abortion, other signs of illness were minimal and limited to mild serous nasal discharge and transient fever. Uterine lesions in affected cats included coagulation necrosis in the placental labyrinth, thrombosis of maternal vessels in the placenta, degeneration of the trophoblast and endometrial epithelium in the junctional zone of the placenta, and separation of the placenta from the endometrium at the junctional zone.[62]

Feline immunodeficiency virus (FIV), first recognized in 1986, is a lentivirus similar to the human immunodeficiency virus (HIV).[63,64] FIV has been isolated from cell-free seminal plasma and spermatozoa from experimentally infected cats.[65] FIV can be transmitted horizontally to estrous queens by artifical insemination with fresh semen from asymptomatic males chronically infected with the virus.[66] At one time, transmission of the virus to kittens in utero was reported as uncommon or nonexistent, and transmission in the saliva or milk of infected queens was considered rare, perhaps depending on phase of infection of the dam at the time kittens are born.[67] In one report, experimental transmission of FIV to a seronegative pregnant queen 6 weeks before parturition was associated with elevated antibody titers and virus isolation from peripheral blood lymphocytes in one of her five kittens, but mode of transmission (via colostrum or maternal saliva) was not determined.[67] More recent studies, using molecular techniques for demonstration of fragments of the FIV genome or gene antigen secretion, have demonstrated that pregnant queens acutely infected with FIV can transmit the virus to their offspring via both prenatal and postnatal routes.[68,69] In utero transmission resulted in arrested fetal development, abortion, stillbirth, subnormal birth weights, and birth of viable, virus-infected kittens. Queens infected prior to conception transmitted the virus to 71 per cent of their kittens, and 51 per cent of these kittens were virus positive on the day of birth; late in utero transmission occurred in approximately 20 per cent of the kittens, and intrapartum transmission also was demonstrated.[70]

Feline infectious peritonitis virus (FIPV), a coronavirus, is reported to be transmitted to kittens in utero, and reproductive failures in cats, particularly resorption, have been reported in catteries where feline coronavirus is endemic.[71–73] However, pathogenesis of FIPV is poorly understood, and its role in the pregnant queen may be more significant in causing kitten infection than in causing pregnancy loss. Pedigrees from some purebred cats with susceptibility to FIPV can be traced to a few lineages, and a polygenic threshold model of inheritance has been reported to describe the pattern observed, with a heritability of about 50 per cent.[74] This suggests that suspect cats should be removed from breeding programs.

Feline leukemia virus (FeLV) is transmitted by in utero infection, which results in fetal or neonatal death in up to 80 per cent of kittens born to infected queens; 20 per cent of kittens born to infected queens may carry the infection into later life.[63] FeLV infection has been associated with fetal resorption and abortion, but the effect of the virus on the fetal-placental unit has not been reported.[75,76] Ten of 11 cats (91 per cent) with complaints of infertility, fetal resorption, or abortion were found infected with FeLV.[75] Five of six FeLV-recovered queens conceived from breedings to non–FeLV-exposed toms and delivered kittens of normal size (4.2 kittens), which included at least one kitten infected in utero.[77] None of the five queens became detectably viremic during pregnancy or lactation.[77]

Feline panleukopenia virus (FPLV) infection of the pregnant queen includes viral passage across the placenta and fetal pathology that depends on stage of gestation at time of infection. Abortion, stillbirth, neonatal death, or fetal cerebellar hypoplasia may occur in pregnancies of infected queens.[78]

Bacterial causes of pregnancy loss or abortion in the cat that have been reported include *Brucella* organisms or *Salmonella cholera suis* ingested from exogenous sources (such as farm animal abortions in early reports), or aerobic bacteria and mycoplasma that are vaginal flora of the normal queen (*Escherichia coli, Staphylococcus* sp., *Streptococcus* sp., and others; see Chapter 24) that ascend into the uterus from the vagina causing uterine infection, fetal infection, or pyometra.[79–82] Unilateral pyometra was described in the left horn of a queen carrying two viable fetuses in her right horn.[82] Experimental inoculation of feline T-strain mycoplasma into three pregnant queens resulted in kitten death ($n = 2$) or abortion ($n = 1$); mycoplasma was recovered from heart blood of kittens aborted from an infected dam.[83]

Infection with the protozoan *Toxoplasma gondii* is reported as a cause of abortion in the domestic cat. Abortion in infected queens followed maternal signs of emaciation, lymphadenopathy, dyspnea, lethargy, diarrhea, and central nervous system disturbance.[84] Abortion was reported in 1 of 12 cats acutely ill with systemic toxoplasmosis.[85] The affected queen aborted four partially formed fetuses on the second day of her illness; she died 6 days after the abortion, and was confirmed histologically to have generalized toxoplasmosis. However, there were no uterine lesions of the systemic disease. Abortion also occurred in 17 cats chronically infected with toxoplasmosis.[85] Experimental trials failed to demonstrate transplacental transmission and infection of kittens following oral infection of eight queens with *T. gondii* before or 16 queens during pregnancy.[86,87] In one report, fatal neonatal toxoplasmosis in three kittens that died at 16, 18, or 32 days of age was assumed to have been acquired transplacentally because of the histologic finding of well-developed *T. gondii* cysts (known to take 4 weeks to form in experimentally infected animals) in the kittens' tissue.[88] Still, toxoplasmosis does not appear to be an important cause of abortion or pregnancy loss in the cat, except perhaps by its causing debilitating systemic disease in the pregnant queen.

NUTRITIONAL INSUFFICIENCY

Nutritional insufficiency of taurine in the mother is reported to result in resorption, abortion, and stillbirth of fetuses from affected queens fed taurine-free diets or diets containing up to 0.01 per cent taurine.[89–92] Queens fed a diet containing 0.05 to 1 per cent taurine resulted in normal breeding performance.[90–92] Increased resorption of fetuses, reduced litter size, and increased incidence of stillborn kittens was observed in queens while on taurine-deficient diets, as well as after refeeding a taurine-enriched diet for 6 months.[89] Thirty per cent of the ovulatory cycles of taurine-deprived queens resulted in delivery of kittens, with mean live litter size of 2.2 ± 0.4 kittens and mean stillborn litter sizes of 0.8 ± 0.4 kittens (mean $\pm$ SEM). Remaining ovulatory cycles resulted either in pregnancies with fetal resorption (38 per cent) or pseudopregnancies (32 per cent). Pregnancy loss in affected queens was associated with normal relaxin secretion by day 20, followed by relaxin decrease by day 25 associated with the pregnancy loss, which suggests presence of a post-ovulatory defect within the first 10 days after implantation not reversible upon refeeding a taurine-enriched diet for 6 months.[89]

FETAL CHROMOSOMAL ERROR

Fetal chromosomal error or defect is a reported cause of abortion or fetal death in many species. In the cat, trisomy of autosome D-2 was reported in a small macerated feline fetus present in the uterus with three normal live kittens.[93] Abnormal karyotypes were detected in tissue from 4 of 25 (16 per cent) kittens examined after spontaneous stillbirth or exploratory

laparotomy during pregnancy of consistently subfertile queens; three were mosaics (one 38,XX/37,XX, and two 38,XX/39,XX), with normal cell lines coexisting with aberrant ones, and the fourth kitten (a stillbirth) was a 37,XO individual.[94,95] Macerated feline fetuses are encountered occasionally, but rarely karyotyped.[96]

MATERNAL ENVIRONMENT INADEQUATE TO MAINTAIN PREGNANCY

Abnormal maternal environment may include endocrine imbalance (e.g., estrus during pregnancy, inadequate progesterone to maintain pregnancy) or placental disease of noninfectious origin (including umbilical cord aplasia).[97] Support for endocrine imbalance comes from observation of estrous signs during pregnancy, reported cases of superfetation, and demonstration that the feline ovary is not refractory to exogenous gonadotropin stimulation during midgestation. Many cases of feline abortion occur around day 40 of pregnancy, which, at one time, was thought to be the approximate time that ovarian (luteal) progesterone was being replaced by placental progesterone; more recent studies, however, suggest that feline corpora lutea are the main source of plasma progesterone in the pregnant queen throughout gestation, and that placental progesterone secretion is of minor importance or nonexistent.[3,13] Most reported cases of feline abortion are not associated with a recognized infectious cause, which may suggest presence of maternal endocrine abnormality.[98] Treatment of recurrent aborting queens with 1.0 to 2.0 mg/kg repositol progesterone intramuscularly (IM) every 7 days until 7 days before parturition has been recommended.[99] Such treatment should not, however, be used in impending or first-time spontaneous abortion where infection and fetal defect have not been ruled out. Masculinization of female fetuses may occur in queens treated with progesterone during pregnancy.[98]

Multifocal placental necrosis and subsequent fetal autolysis were observed in five pregnant female cats with a history of habitual abortion in the third to fourth week of pregnancy.[100] The pattern of histologic change suggested presence of primary placental disease; no bacterial, mycoplasmal, or viral etiology was identified.

Clinical signs of pregnancy loss in the cat depend on the cause of the problem, and are highly variable, ranging from no external evidence of systemic illness to a 1 to 2 day course of sanguineous or purulent vaginal discharge (with or without abortion) to severe maternal compromise with toxemia or septicemia. Systemic infectious disease is associated with a variety of clinical signs depending on the etiologic agent and affected organs.

Diagnostic and therapeutic intervention should be attempted concurrently. Diagnosis is based on observation of abortion or nonpregnant status after a pregnancy has been confirmed (Table 27–3). Fetal culture, histology, and cytogenetic evaluation, if available, are indicated. Maternal hemogram, serology (e.g., FHV, FIV, FIPV, FPLV, FeLV, toxoplasmosis), and cytology and culture of the vaginal discharge (if any) should be monitored and the dam treated supportively if necessary. In general, pregnancy loss is not life threatening to the queen, and prognosis for subsequent normal pregnancy is guarded to good. Occasionally, hemorrhage into the uterus at time of abortion will cause maternal death.[101] Progesterone therapy (1.0 to 2.0 mg/kg repositol progesterone IM) every 7 days starting 5 to 7 days prior to previous time of abortion and continuing until 7 days before parturition should be instituted only after recurrent pregnancy loss when infectious causes of abortion have been ruled out.[99]

Extrauterine Mummified Fetuses ("Ectopic Pregnancy")

The presence of mummified fetuses outside the uterus has been reported many times in the queen.[102–123] Some authors refer to these kittens as ectopic pregnancies, implying that extrauterine fetal growth and placental attachment to a tissue other than endometrium can occur. These have been classified as primary if there is no evidence of uterine rupture and secondary if uterine rupture can be demonstrated. There is, however, no documented evidence that fertilized eggs can grow and develop placental attachment to omental mesentery and abdominal viscera in the queen. Absence of a grossly visible uterine scar does not preclude previous uterine rupture; one free-floating mass containing two aseptically necrotic fetuses was determined histologically to be surrounded by uterine wall that apparently ruptured segmentally from the left horn (Fig. 27–11).[112] Some reports of extrauterine mummified fetuses can document trauma, supporting possible uterine rupture. Two cats reported to have primary ectopic pregnancy had mummified fetuses removed from the

Table 27–3. Diagnostic Approach to Fetal Loss in the Cat

Test	Purpose of Test		
	To Confirm Presence of Fetal Loss	To Establish Cause of Fetal Loss	To Evaluate Presence of Maternal Compromise
Maternal:			
History of -			
Vaccinations		+	
Previous reproductive problems		+	
Trauma, drug use (steroids)		+	
Concurrent systemic illness		+	+
Abortion	+		
Dystocia, if intrapartum	+	+	+
Physical examination			
Abdominal palpation of uterus	+		
Vaginal palpation (after culture and cytology)	+		
Auscultation of fetal heart	+		
General physical examination			+
Abdominal radiography (to confirm pregnancy, determine fetal crown-rump length, and uterine size)	+		+
Uterine/fetal ultrasonography	+		+
Blood count, chemistries, urinalysis			+
Serology/agent identification		+	
Feline herpesvirus		+	
Feline immunodeficiency virus		+	
Feline infectious peritonitis virus		+	
Feline leukemia virus		+	
Feline panleukopenia virus		+	
Toxoplasma gondii		+	
Serum progesterone assay	+		
Vaginal discharge cytology (to look for evidence of infection)		+	
Vaginal discharge culture (bacteria, mycoplasma, ± virus)		+	
Hysterotomy at subsequent gestation to confirm pregnancy and consider fetal karyotype (days 25 to 30)	+	+	
Fetal/placental:			
Karyotype		+	
Gross pathology		+	
Histopathology		+	
Culture (bacteria, mycoplasma, ± virus of fetal stomach, lung, liver)		+	+

Adapted from Johnston SD, Raksil S: Fetal loss in the dog and cat. Vet Clin North Am 17:535–554, 1987, with permission.

peritoneal cavity 18 and 11 months after OHE.[124,125] These authors did not, however, rule out uterine rupture and fetal loss into the peritoneal cavity prior to OHE with mummified fetuses that went unnoticed at the subsequent abdominal surgery.

Clinical signs of illness usually are absent in queens with extrauterine mummified fetuses unless hemorrhage or infection compromise uterine rupture. Most mummified feline fetuses are bacteriologically sterile and are discovered incidentally at OHE or other abdominal surgery. Fetuses may be found encapsulated in uterine tissue, wrapped in omental adhesions, or fetal bones denuded of tissue may be found free in the peritoneal cavity or protruding from an old uterine tear.[126] Treatment is surgical removal of fetuses at celiotomy.

Uterine Torsion

Torsion of the pregnant uterus of the queen (Fig. 27–12) has been described in many case reports.[127–155] Age of 24 affected queens ranged from 1 to 10 ($\bar{x}$ = 3.5) years. Twelve queens were known to have had previous normal parturitions; three queens were reported to have been in their first pregnancy, and no information on previous pregnancies was available for the others. Stage of pregnancy at time of diagnosis ranged from 4 weeks' gestation through 2 weeks after expected due date and was, in some cases, associated with signs of dys-

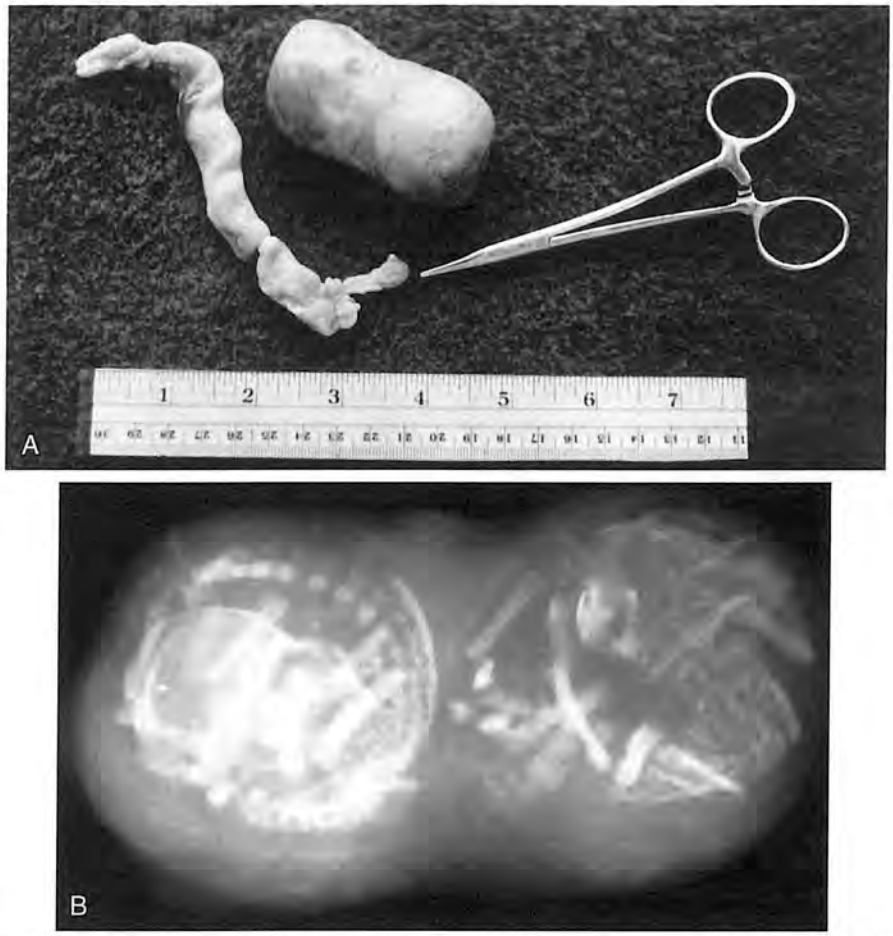

Figure 27–11. Ectopic mass, right ovary, and right uterine horn (a) and radiograph of ectopic mass (b) showing two fetal skeletons, taken from the abdomen of a 2 ½-year-old female domestic shorthair cat admitted for routine ovariohysterectomy. (From Johnston SD, Harish G, Stevens JB, Scheffler HG: Ectopic pregnancy with uterine horn encapsulation in a cat. J Am Vet Med Assoc 183:1001–1002, 1983, with permission.)

Figure 27–12. Intraoperative photograph demonstrating the left uterine horn twisted 540 degrees on its long axis. The horn (solid arrow) contains two fetuses, and is engorged because of obstructed venous return. The right uterine horn is visible, and apparently normal. (From Freeman LJ: Feline uterine torsion. Compend Contin Educ Pract Vet 10:1078–1082, 1988, with permission.)

tocia.[154,155] Clinical course ranging from 2 hours to 3 days ($\bar{x}$ = 1.2 days, n = 9) was reported, and one affected queen was found dead within 6 hours of onset of labor.[155] Clinical signs included depression (n = 17), anorexia (n = 10), collapse (n = 8), painful abdomen (n = 6), pale mucous membranes (n = 6), hypothermia (n = 5), presence of a sanguineous vaginal discharge (n = 4), shallow respiration (n = 3), tachycardia (n = 3), and straining. Diagnosis was made at exploratory surgery or at necropsy following death or euthanasia. Eight queens were reported with 360- to 1080-degree torsions of the left horn, 10 queens were reported with 180- to 540-degree torsions of the right horn, three queens were reported with 180-degree torsions of the uterine body, one queen torsed both the uterine body and the left horn, one torsed the uterine body and right horn, and in one queen the torsion of a horn

was undesignated as to side. Twenty-one of these queens were reported treated with exploratory surgery, and 16 survived; one was found dead, one queen died, and two were euthanized without surgery.

Presumptive diagnosis of uterine torsion in the pregnant queen is based on presence of signs of acute abdomen (i.e., shock, collapse, painful abdomen); abdominal ultrasonography of these queens is recommended in order to assess viability of fetuses. Treatment is exploratory celiotomy to confirm the diagnosis and perform an OHE. Correction of the torsion alone with or without cesarean section cannot be advocated due to the frequency of tissue devitalization with this problem and the risk of recurrence. Status of the affected queen at time of diagnosis dictates use of supportive (i.e., fluid, corticosteroid, antibacterial) therapy.

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Chapter 28

Feline Parturition

Physiology and Endocrinology of Onset of Parturition

Duration of gestation from first day of breeding to delivery may range from 62 to 71 days (average = 66 to 67 days) in the cat.[1] No strong correlation exists between litter size and gestation length in the cat.[1] Serum progesterone declines to 4 to 5 ng/ml just before parturition (days 63 to 65), to about 2 ng/ml on the day of parturition, and to less than 1 ng/ml immediately after parturition (n = 4).[2,3] Although serum progesterone concentration declines near term in this species, baseline concentrations (<1 ng/ml) are not a prerequisite for onset of parturition.[3] Feline serum progesterone concentrations may be measured using enzyme-linked immunosorbent assay (ELISA) test kits available for use in the dog.[4]

Mean serum estradiol-17β is reported to peak at nearly 100 pg/ml 8 days before parturition, and then to drop to 17 to 36 pg/ml by the day of parturition.[3] Mean serum luteinizing hormone concentrations are reported as low (1.9 to 5.8 ng/ml) during the 9 days prior to parturition.[3] Plasma relaxin immunoreactivity declines from a plateau of 3 to 9 ng/ml in the last half of gestation to undetectable concentrations within 24 hours of delivery (see Fig. 27–2).[5,6] Feline serum prolactin concentration is about 40 ng/ml at parturition (see Fig. 27–3).[7]

Seasonal distribution of parturitions of queens in the Northern Hemisphere includes deliveries throughout the year, with greater frequency in early spring and again in late summer (Fig. 28–1).[8] In the Southern Hemisphere, deliveries peak in September to October (early spring) and January to March (late summer).[9,10]

Milk may be observed in the queen's mammary glands just prior to parturition, although this is uncommon, and she may seek a secluded place for delivery. The prepartum rectal temperature drop that precedes parturition in the bitch by about 8 to 12 hours usually is not observed in the pregnant queen. At onset of uterine contractions the queen becomes restless and may pant. Some Siamese queens will vocalize and call to their owners at onset of a first parturition.[11]

Normal Parturition

At time of parturition in the queen, pacing alternates with purring behavior. When contractions begin, the queen may assume a semi-squatting position, with the calcaneus bones pointed almost straight up and wide apart.[11] When contractions subside the queen may lay down on her side and continue purring. Near the time of delivery the queen may appear to make a "nest" by turning around in the box while standing with her head lowered, smelling various parts of the floor of the box, and pawing at the bedding. Contractions of the skeletal musculature of the abdomen, delivery of fetuses, and delivery of placentas occur in variable orders and over variable lengths of time.[12] Fluid generally is expelled from the vulva prior to delivery of the first kitten. Once the head or the caudal portion of the fetus appears at the vulva, contractions may become slower and stronger, and 3 to 5 minutes may elapse until the kitten is completely expelled.[11] The mother usually cleans the kitten vigorously by licking at the allantoamnion, which still covers the kitten. Posterior presentation is common, and does not predispose to dystocia. The allantochorion of each kitten usually is ruptured prior to or during delivery, and the

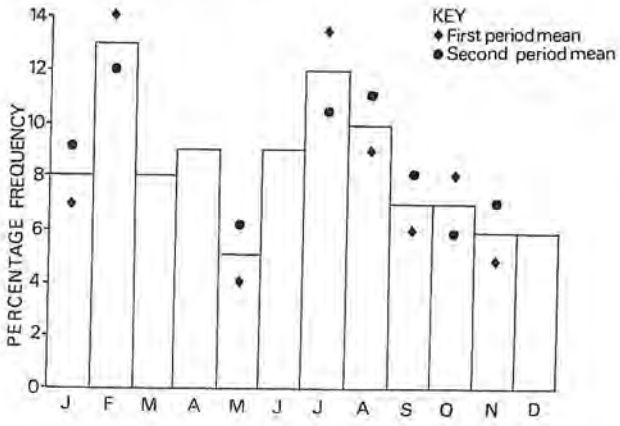

Figure 28–1. Monthly distribution of total litters born throughout the year in English catteries. First period, 1958–1963; second period, 1964–1968. (From Robinson R, Cox HW: Reproductive performance in a cat colony over a 10-year period. Lab Anim 4:99–112, 1970, with permission.)

queen will rupture the allantoamnion and sever the umbilical cord while cleaning and stimulating the kittens.[13] Placentas usually are ingested wholly or in part by the queen, although there is no known advantage or disadvantage to her ingesting them.[14]

Most queens deliver kittens with ease over a period of several hours. Average length of parturition in seven queens was 16.1 ± 14.3 (SD) hours, with a range of 4 to 42 hours.[1] Occasionally, a queen will deliver live healthy kittens over 2 to 3 calender days, which stresses the importance of understanding the wide range of normal parturition lengths in this species before intervening.[1]

First nursing by the kittens may not occur until 30 to 40 minutes following birth, by which time the kittens are dry and able to pull themselves up toward the nipples.[11] Kittens should ingest colostrum within the first 24 hours of birth, and should be weighed daily for the first few weeks of life.

Litter size in the cat ranges from 1 to 10 kittens, with a modal litter consisting of 4 kittens (Fig. 28–2).[8] In a survey of 189 female cats having a total of 370 litters and 1329 kittens, 11.6 per cent of the kittens were stillborn and 27.3 per cent of the kittens died before 8 weeks of age.[15] In a mortality study of 294 domestic shorthair kittens, low-birth-weight kittens and one-kitten litters had greatest mortality.[16] Feline stillbirth also has been associated with mixed *Salmonella typhimurium* and leptospira infection.[17] Feline kitten mortality is reported lowest in litters from queens that are not overweight, that are in their fifth parity, and that deliver (large) litters of five kittens.[16]

The sex ratio for live kittens has been reported as 99.3 males per 100 females (n = 3357),[8] and one male per one female (n = 56).[1] The sex ratio in stillborn and neonatal death kittens has been reported as 65.6 (n = 106) and 79.7 (n = 208) males per 100 females, respectively.[8]

Diagnosis and Management of Feline Dystocia

Humphreys reports an incidence of 134 dystocias in 4077 (3.3 per cent) intact female cats (from a total of 19,355 cats seen in veterinary practice) during a 4½-year period.[18] Gunn-Moore and Thrusfield reported dystocia in 5.8 per cent of 2928 litters from 735 queens, and ranged from 0.4 per cent in mixed-breed cats to 18.2 per cent in litters in the Devon rex.[19]

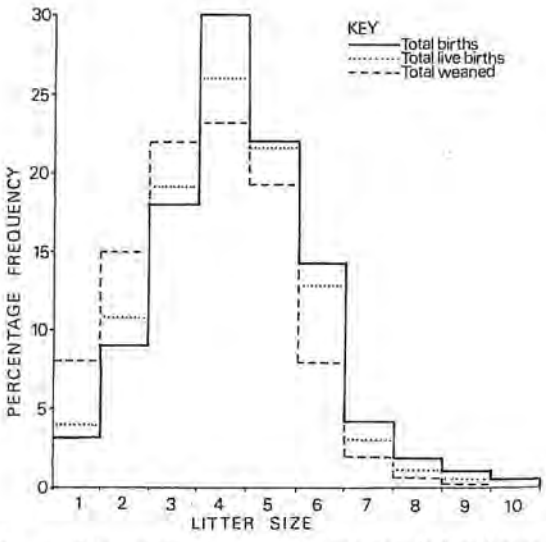

Figure 28–2. Histograms indicating distributions for total kittens born (4088), number of kittens born alive (3911), and number of kittens weaned (3352) in 973 feline litters. (From Robinson R, Cox HW: Reproductive performance in a cat colony over a 10-year period. Lab Anim 4:99–112, 1970, with permission.)

Pedigreed litters were at significantly higher risk than litters of cats of mixed breeding. Dolicocephalic and brachiocephalic types were found to have a higher incidence of dystocia than mesocephalic breeds.

Causes of feline dystocia include obstruction to fetal egress through the birth canal and insufficient or ineffective contractions by the uterus (uterine inertia). Obstructive causes may be maternal or fetal in origin (Table 28–1),[19–21] whereas uterine contraction failure (inertia) is solely maternal in origin. Uterine inertia may be primary (failure to start synchronous uterine contractions) or secondary (uterine fatigue). Of all causes identified, the most common cause of feline dystocia reported in the literature is primary uterine inertia (Table 28–1). The importance of this cause must be interpreted with caution, however, as criteria for diagnosing primary inertia are usually not reported in manuscripts on feline dystocia; since normal feline gestation may last as long as 71 days, it is unknown whether affected queens really had primary inertia, or whether veterinarians interceded in some queens that would have gone on to deliver normally if left alone.

Obstructive dystocias may result from inadequate size of the maternal birth canal due to bony or soft tissue impingement on the canal, from intrapartum uterine torsion or rupture, or from large fetal size (often associated with small litter size) or fetal deformity/monstrosity (Fig. 28–3).

Diagnosis of feline dystocia is based on failure to start labor at term (primary inertia), failure to progress normally through labor (obstruction or secondary inertia), or intrapartum maternal compromise (depression, shock). Intrapartum fetal compromise also is an indication of dystocia; at present, however, use of real-time ultrasonography to monitor fetal heart rate is the only tool available to detect intrapartum fetal compromise objectively in this species. Normal fetal heart rate in the cat, which is stable over the duration of pregnancy, averages 228.2 ± 35.5 beats per minute (see Chapter 27).

Diagnostic evaluation of all cases of dystocia should include collection of a history of previous pregnancies, breeding dates at this pregnancy, time of labor onset, and time of delivery of previous kittens, if any. Physical examination should include confirmation of pregnant status by abdominal palpation, digital examination of the vagina if possible, rectal assessment of pelvic diameter if possible, and good general physical examination. Laboratory evaluation should include a hemogram, serum calcium, and plasma glucose determination to monitor maternal status; and abdominal radiographs to document fetal size, number, and position (Fig. 28–3). Need for other serum chemistries (blood urea nitrogen [BUN], electrolytes, blood gases) is indicated by the physical status of the queen.

Diagnosis of primary uterine inertia is based on length of pregnancy from breeding, which is complicated by the fact that many queens are bred over several days. Most queens deliver kittens 65 to 66 days from a single breeding, although multiple breedings over several days

Table 28–1. Reported Causes of Dystocia in the Queen

Cause	Reference			
	Ekstrand and Linde-Forsberg[20]	Gunn-Moore and Thrusfield[19]	Robbins and Mullen[21]	Total
Obstructive causes—maternal				
Uterine prolapse	1	1		2
Uterine torsion	1	1	2	4
Uterine rupture		2		2
Birth canal too narrow	8	11		1
Obstructive causes—fetal				
Malpresentation	24	26		50
Malformation	12		8	20
Fetal oversize	3	18		21
Fetal death	7	11	1	19
Maternal uterine inertia*	94	32	9	135
Total	150	102	20	272

* Diagnosis of maternal uterine inertia in these studies were not, generally, rigorously distinguished from normal parturition in the queen, which may include normal onset of parturition up to 71 days after breeding, and duration of parturition up to 42 hours. Therefore, if these authors intervened in some pregnant queens that normally would have gone on to normal parturition, the numbers represented under this category of dystocia above would be artificially high.

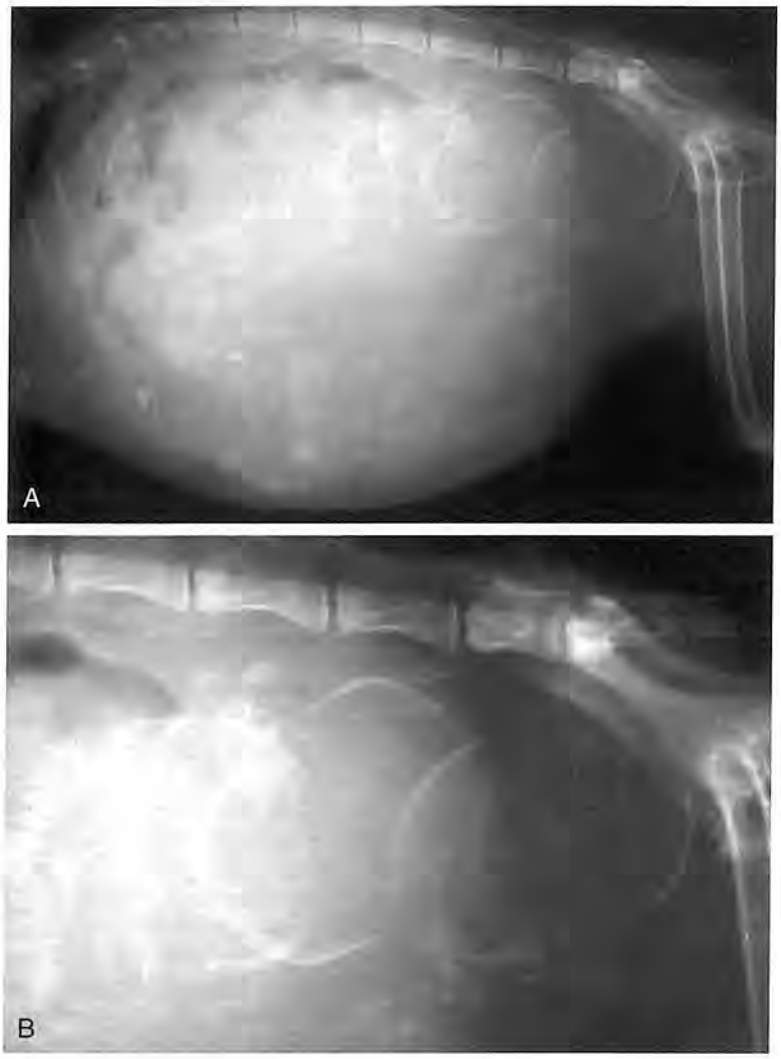

Figure 28–3. Lateral abdominal radiographs of a 4-year-old pregnant domestic shorthair queen, depicting presence of hydrocephalic fetuses.

of estrus coupled with early refractoriness to luteinizing hormone (LH) secretion and late refractoriness to repeated gonadotropin-releasing hormone (GnRH) stimulations when in estrus make precise calculation of parturition date difficult. Primary inertia can be confirmed only in the presence of gestation length exceeding 71 days from first breeding, or in the presence of serum progesterone concentration less than 2 ng/ml.

Diagnosis of failure to progress while in labor is based on duration of the first and second stages of parturition. Because most queens deliver their kittens over a period of several hours, a 4-hour course of labor before the first kitten and 2-hour course between kittens should be allowed. Partial expulsion of a fetus is a common problem experienced by queens during parturition; digital manipulation and delivery following vaginal lubrication usually are successful.[22,23] Occasionally, however, queens deliver healthy live kittens over a 2- to 3-day period. Obstructive dystocias often are associated with uterine torsion[24] or fetal oversize (Fig. 28–3) in the cat, which reinforces the importance of pretreatment radiographic or ultrasonographic imaging of the dam. Hydrocephalus was detected in two kittens born to a pregnant queen vaccinated with live feline parvovirus vaccine 6 weeks earlier; parvoviral DNA was detected in the brain of both kittens.[25]

Diagnosis of intrapartum maternal compromise often is associated with failure to progress in labor when uterine rupture or uterine torsion is present.[18,26–38] Signs of maternal compromise have been reported in pregnant queens aged 9 months to 8 years, and include depres-

sion, anorexia, weak vocalization, prolonged capillary refill time, tachycardia, and sometimes collapse.

In the absence of maternal compromise or known obstruction, a trial course of oxytocin (3 to 5 U intramuscularly [IM]) is indicated; injections may be repeated at 20- to 30-minute intervals. This treatment is recommended in queens that have already delivered at least one kitten, and are known (from radiographs or ultrasound) to contain more in the absence of fetal oversize or malposition. Oxytocin treatment of the pregnant queen with a closed cervix (or the preterm queen) is risky, as it may cause placental detachment, fetal compromise, and death prior to delivery. In 97 cases of feline dystocia treated with oxytocin and/or calcium, only 29 (29.9 per cent) of the cases were relieved by medical treatment alone.[20] Because of the danger that oxytocin will cause placental separation and fetal compromise, this regimen is indicated only when facilities for cesarean section are available for queens that fail to respond. Use of intravenous (IV) calcium (0.5 to 1.0 ml 10% calcium gluconate IV) in feline dystocias is controversial, because some queens show strong uterine contractions following treatment despite presence of normal total serum calcium concentrations prior to treatment.

Dystocia patients that fail to respond to oxytocin or patients showing maternal compromise should undergo immediate cesarean section with appropriate fluid therapy. Ovariohysterectomy (OHE) is indicated if uterine infection, decomposing fetuses, or gangrene is present. Shock, if not already present, should be anticipated when a large gravid uterus is removed from a compromised queen, and appropriate therapy instituted.

Prognosis depends on the cause of dystocia, treatment used, and on time from onset of parturition until veterinary intervention. Brandariz reported survival of 90 per cent of 20 queens seen less than 30 hours after onset of labor; whereas only 75 per cent, 30 per cent, and none of eight, 10, and one queens seen 30 to 40 hours, 40 to 60 hours, and more than 60 hours after labor onset, respectively, survived.[39]

Anesthetic and Surgical Technique for Cesarean Section in the Queen

Anesthetic techniques reported effective for cesarean section in the cat include: isoflurane by mask administration with postoperative butorphanol (0.1 mg/kg IV) for analgesia[21]; glycopyrrolate (5 μg/lb preanesthetic), followed by mask administration of isoflurane[40]; atropine (0.04 mg/kg IM), thiamylal sodium (4.4 mg/kg IV to effect), succinylcholine (0.5 mg/kg IV), intubation, controlled ventilation with 50% nitrous oxide and isoflurane (0.75 per cent)[41]; and lidocaine (1 ml 2% lidocaine per 3 to 5 kg) epidurally.[41] Ketamine should be avoided because of the depressive effect it has on the kittens.

Speed usually is an important consideration in cesarean section, so preoperative preparation (placement of IV catheter, shaving and preparation of the surgical site) should be completed before induction of anesthesia. Routine surgical technique for cesarean section in the cat includes ventral midline incision from umbilicus to pubis, incision of the linea alba, and hemostasis, followed by exteriorization of the gravid uterus. The exteriorized tissues are covered with moist laparotomy sponges.[41] Usually, a single incision is made through an avascular region of the dorsal uterine wall at the cranial aspect of the body of the uterus, and kittens are "milked" down the uterine horns to the incision for delivery. Alternatively, if litter size is great, if fetal compromise is suspected, or if cesarean section is performed prior to the end of gestation and detachment of the placentas is anticipated to be difficult, incisions can be made in the dorsal aspect of each of the two uterine horns in order to facilitate removal of all of the kittens and placentas. The amniotic sac is opened, and gentle traction is placed on the umbilical cord to facilitate removal of the placenta. The umbilicus is clamped, and the neonate, with placenta attached, is passed in a sterile towel to an assistant. If the uterus does not begin to contract following removal of the kittens, oxytocin (1 to 2 U/kg IM or IV) is administered to facilitate uterine involution. The hysterotomy incision is closed with absorbable suture in a continuous inverting pattern, the uterus is returned to the abdomen, abdominal lavage is performed if fetal fluids entered the peritoneal cavity, and the incision site is closed routinely.

En bloc OHE, which is removal of the uterus followed by hysterotomy and removal of the neonates, was reported in 26 cats with complete or partial uterine inertia refractory to medical treatment.[-1] Criteria for confirming the diagnosis of inertia were not presented. Forty per cent of the queens were reported to have

partial primary inertia, and forty per cent had oversized, malpositioned, or maldeveloped fetuses. General anesthesia was induced by administration of isoflurane in a chamber or by mask. Lactated Ringer's solution (10 ml/kg/h, IV) was administered from induction until extubation, and analgesia for postoperative pain was provided when needed by administration of IV butorphanol (0.1 mg/kg). The ovaries and body of the uterus were exteriorized through a caudal ventral midline incision; vascular pedicles containing the ovarian artery and vein were isolated by breaking down the mesovarium and ligated. Double clamps were placed on each ovarian pedicle and on the body of the uterus adjacent to the cervix. The ovaries and uterus were removed by dividing between the clamps and were given to a team of assistants who opened the uterus and resuscitated the neonates. The ovarian and uterine pedicles were double ligated with chromic gut, and the abdomen was closed routinely.[21] None of the animals died during surgery, although one cat bled excessively from the cut surfaces of her skin, subcutis, and abdominal musculature. That cat had prolonged prothrombin time, and partial thromboplastin time, low platelet count, and positive fibrin degradation products consistent with disseminated intravascular coagulopathy. Postoperative complications included anemia in two cats and anorexia in two. One cat with hemoabdomen and anemia was explored after whole blood transfusion; although no source of hemorrhage was identified, the cat died 9 days after the first surgery. Rate of stillbirths in these cats was 58 per cent (44 of 76 kittens). OHE at time of cesarean section is very controversial, with opponents claiming increased morbidity in the dam, and proponents arguing that a single anesthetic experience is safer for the dam than a second experience for later OHE. En bloc OHE of the pregnant uterus is controversial, and has been associated with high neonatal morbidity and mortality; its advocates claim that the minimal duration of anesthesia and the minimal potential for peritoneal contamination with uterine contents are positive reasons for its use.

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Chapter 29

The Postpartum Period in the Cat

Care of the Normal Queen

Most queens deliver their kittens easily, and require little more than privacy during both the time of parturition and the postpartum period. Within 24 hours after parturition, the queen's appetite returns, and her food consumption increases above prepartum amounts of food.[1] The lactating queen should be fed commercial cat food and fresh water ad libitum during lactation. Demand for milk by nursing kittens will increase for 20 to 30 days, and some queens will consume two to four times their normal adult maintenance ration while nursing. Food should not be restricted during this time (Table 29–1). When the kittens reach 3 to 4 weeks of age, and weaning begins, the queen's food intake may be gradually reduced, down to adult maintenance levels when weaning is completed at about 6 to 8 weeks.

Colostrum is produced by the queen for 24 to 72 hours postpartum, after which lactation gradually changes from colostrum to milk.[1] In the first 1 to 3 days of life, the newborn kitten can absorb whole dietary proteins, such as immunoglobulins, which are present both in the colostrum and in queen's milk, and it is recommended that kittens receive colostrum or milk during this time to obtain optimal serum antibody titers and adequate passive immunity.[2]

Feline colostrum contains about 88 per cent water, 4 per cent protein, and 3.4 per cent fat. Feline milk contains about 82 per cent water, 6.6 per cent protein, and 5.5 per cent fat.[3] Mean immunoglobulin G (IgG) concentration is higher in colostrum than in milk.[4] Neither IgG nor IgA was detected in precolostral serum samples obtained from 46 kittens.[4] Transferred IgG was reported to have a half-life in suckling kittens of 4.4 ± 3.57 and 4.15 ± 1.29 days, respectively; transferred IgA had half-lives of 1.93 ± 1.94 and 2.03 ± 0.33 days, in two separate studies, respectively.[4,5] Foreign IgG, given orally up to 12 hours after birth, was detected in kittens' serum, whereas IgG given at or after 16 hours was not found in any kittens' serum.[4]

During the first 24 hours postpartum, the queen may not leave her kittens to eat, drink, defecate, or urinate.[6] Food, water, and a litter pan should be provided adjacent to her kittens' box. After 7 to 8 days she will stay away from her kittens for longer intervals, and may come into estrus, mate, and conceive. During lactation, cyclic elevations in serum estradiol have been reported, even when sexual behavior is not observed, until 1 to 4 weeks after removal of the suckling kittens.[7]

Postpartum lochial discharge from the queen is scant, and often is not even noticed by the owner, as the postpartum queen cleans the vulva frequently. Postpartum uterine involution in the queen is impacted by duration of lactation.[8] Although the early phase (weeks 1 and 2) of uterine involution are the same in nursing and non-nursing postpartum queens, and consist of intense phagocytosis in the endometrium and decrease in myometrial thickness, by week 3 and thereafter, uterine involution differs between these groups. The non-nursing queen shows ovarian follicular activity, which is associated with return to normal (prepartum) uterine size and tone by the fourth week postpartum. The nursing queen shows retrogressive changes in the uterus by 4 weeks postpartum, with continued involution (hyperinvolution) and changes resembling those of queens that have been neutered for comparable periods.[8]

The postpartum queen should be monitored frequently for signs of purulent vaginal or

Table 29-1. Approximate Caloric Needs of the Female Cat

Life Stage	Food Source	Calories*
1 year +	Cat ration	50
Gestation	Cat/kitten ration	50–75
Parturition	Cat/kitten ration	75
Lactation	Cat/kitten ration	75–100
Weaning	Cat ration	50
Maintenance	Cat ration	50

* Per pound of body weight per day.
From Lawler DF, Bebiak DM: Nutrition and management of reproduction in the cat. Vet Clin North Am 16:512, 1986, with permission.

mammary discharge, fever, inappetance, or neglect of the kittens. The mammary glands should be examined daily for signs of mastitis (i.e., swelling, redness, heat, pain). Some nursing queens have no expressible milk; this is not indicative of agalactia if the kittens are showing normal activity and weight gain.

Care of the Normal Neonates

Birth weight for normal kittens is reported as 100 ± 10 g; low birth weight (<90 g) increases risk of neonatal mortality.[9] Kittens are born with closed eyelids and external ear canals. The eyelids open at about 5 to 14 (average = 8) days, and the external ear canals open between 6 and 14 (average = 9) days of life.[10] Sexing of neonatal kittens can be difficult, as both the preputial opening and the vulva are slit-shaped openings directed caudally, and the testes may not be descended at birth.[11] Measurement of anogenital distance may be used to distinguish the sexes, as 11 to 16 mm distances in males and 6 to 9 mm distances in females at birth have been reported.[12]

The newborn kitten crawls slowly and irregularly toward the warmth of the mother's body, moving its head side to side in a "scanning" manner until it comes into contact with its mother.[13] Kittens climb onto the mother's body and nuzzle into the fur until they find a nipple. Normal kittens may begin to suckle as early as an hour after birth or as late as after delivery of the last kitten. The mother's licking behavior and cleaning of her kittens may move them into position near a nipple. After 2 or 3 days, some kittens take specific nipple positions with some regularity, while others nurse from any available space.[13] After about day 30 postpartum, when the kittens are ambulatory and can see, nursing behavior may be initiated almost entirely by the kittens, who follow the mother to attach to her nipples. By this time, the mother may try to evade some attempts at nursing by her kittens. Even if the mother does not evade her kittens, the young show a decline in nursing duration at this time as they continue to explore their environment. Normal kittens should be weighed every day, and should gain a minimum of 7 to 10 g/day while nursing.[14] Frequent, gentle handling of neonatal kittens is reported to improve physical and social development.

Weaning of the kittens and provision of a litter pan for them can be started at 3 to 6 weeks of age, or when kittens weigh over 500 g. Canned, semimoist, or dry kitten food moistened with water all are suitable weaning rations. These should be provided in small amounts in a shallow dish; the young kittens will walk and fall into the dish, until they begin to learn normal eating behavior. Kittens weaned at 2 weeks of age are reported to be slower to learn and more suspicious, cautious, and aggressive than kittens weaned at 6 or 12 weeks of age.[13]

Kitten mortality within the first year of life was reported as 27.1 per cent (of kittens born alive) in a survey of 3116 kittens.[15] Most neonatal (<3 days of age) mortality in kittens is caused by stillbirth (125 of 267 neonatal deaths); other causes include inadequate body weight, abnormal gross anatomy, and cannibalization by the mother or other adult cats in the environment, which may include kittens with low birth weight or those with abnormal gross anatomy.[9] Ninety-three of 125 (74 per cent) stillborn kittens had normal gross anatomy at necropsy, but 46 of the 93 (49 per cent) had lower than normal body weight.[9] Overweight queens experienced increased mortality of kittens in this study.

Neonatal isoerythrolysis is a cause of neonatal death in kittens with A or AB blood type born to, and nursing from, queens with type B blood.[2,16–22] Since all type B queens have high anti-A antibody titers, even primiparous queens can deliver affected kittens.[2] Affected kittens usually die acutely within the first 48 hours of life, with hemoglobinuria (red to brown urine), anemia, weakness, gasping, icterus, and tail tip necrosis. Although percentage of purebred domestic shorthair queens with type B blood in the United States is reported to be less than 5 per cent, percentage of type B queens reported in other breeds is higher, with Abyssinian (n = 194) at 19 per cent, Birman (n = 216) at 18 per cent, British shorthair (n = 85) at 59 per cent, Devon rex

(n = 100) at 43 per cent, Himalayan (n = 35) at 20 per cent, Persian (n = 170) at 24 per cent, Scottish fold (n = 27) at 15 per cent, and Somali (n = 27) at 22 per cent.[22] Because of these incidences, blood typing prior to breeding is recommended in these species. Kittens at risk for neonatal isoerythrolysis should not be allowed to nurse from their mothers for the first 24 hours of life, and can be fostered onto a lactating, type A queen or supplemented as orphans for 24 to 48 hours before being placed back with their mothers. Even if kittens are removed from the dam at first clinical signs, mortality is high.[2] Normal packed cell volume (PCV), hemoglobin, red blood cell count, and white blood cell counts of kittens from birth through 17 weeks of age are depicted in Figure 29–1.[23]

Care of Orphan Kittens

Orphan kittens must be provided a warm, moist environment free of drafts, must be provided with milk or milk replacer delivered in appropriate amounts and at appropriate frequency, and must be stimulated to urinate and defecate. Although orphan kittens can be hand-raised successfully to normal adult animals, it is always to the kitten's advantage to be grafted onto a lactating queen, if one is available, as long as there is not a risk of inducing neonatal isoerythrolysis, as the queen most efficiently provides the warmth, care, and nutrition needed. Queen's milk obtained any time during lactation is a good source of antibodies for neonatal kittens if given during the first 1 to 3 days of life.[4] Orphan kittens should be weighed daily to monitor weight gain and measure food intake.

Commercial incubators, if available, are preferred for the raising of orphan kittens, as temperature can be maintained at 88° to 92°F for the first week of life, at 80° to 85°F for the second, and at 75° to 80°F for weeks 3 to 5, with relative humidity set at 50 per cent.[24] In the absence of an incubator, a box with soft,

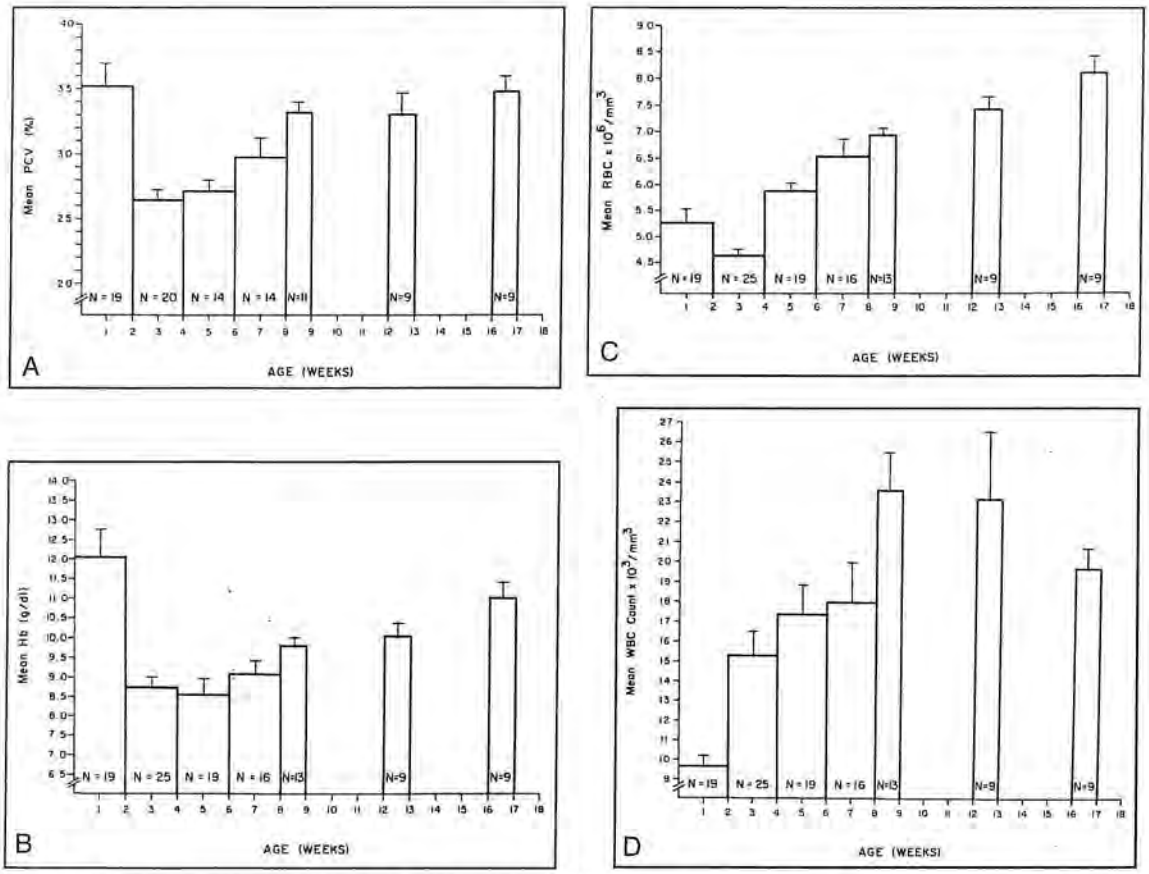

Figure 29–1. Normal packed cell volume (PCV), hemoglobin (Hb), red blood cell count, and white blood cell counts of kittens from birth through 17 weeks of age. (**A**) The PCV of kittens from birth to 17 weeks of age. (**B**) The Hb concentration of kittens from birth to 17 weeks of age. (**C**) The RBC count of kittens from bith to 17 weeks of age. (**D**) The WBC count of kittens from birth to 17 weeks of age. Bars indicate ± 1 SEM. N = No. of kittens. (From Meyers-Wallen VN, Haskins ME, Patterson DF: Hematologic values in healthy neonatal, weanling, and juvenile kittens. Am J Vet Res 45:1322–1327, 1984, with permission.)

Table 29–2. Experimental Milk Replacer for Kittens

Ingredients	Quantities: As Fed (%)	Quantities: Dry Matter (%)	Common
Egg, whole, fresh	7	23	1
Protein supplement*	4	43	25 g
Milk, sweetened, condensed	3	22	17 ml
Corn oil	1	12	7 ml
Water	85	0	250 ml

* Feline Probalance, Smith Kline Beecham Animal Health, Inc., Exton, PA.
From Remillard RL, Pickett JP, Thatcher CD, Davenport DJ: Comparison of kittens fed queens milk with those fed milk replacers. Am J Vet Res 54:901–907, 1993, with permission.

clean bedding, a constant heat source (such as a heating pad covering part of the bottom of the box) and pans of water placed nearby to provide humidity, all in a small space, may be used. A thermometer should be hung inside the box. Other reported heat sources for raising kittens include use of hot water bottles and heat lamps; but a disadvantage of the former is that they begin to cool as soon as they are placed in the box, and of the latter that they sometimes dehydrate or burn the kittens and preclude provision of natural light/dark cycles.[24]

Orphan kittens, or neonatal kittens that fail to gain weight, should be supplemented with milk or milk replacer. If another lactating queen to which the kitten(s) could be grafted is not available, commercially available queen's milk replacer* or a homemade experimental milk replacer (EMR) similar in content to queen's milk is preferred to the use of cow's or goat's milk (Tables 29–2 and 29–3).[25] Recommended amount of milk replacer to be fed varies with age and with kitten growth, but, as a general rule, is started at 5 ml/100 g body weight per day at birth; increased by 1 to 2 ml/day; and increased to approximately 20, 25, 30, and 35 ml/100 g body weight per day during weeks 2, 3, 4, and 5 of life, respectively.[24,25] Milk replacer should be warmed to 25° to 37°C prior to administration. It is important not to overfeed, especially in the first several days of life. The daily amount of milk replacer should be divided into four feedings given at 6-hour intervals or at regularly spaced feedings between 7:00 AM and 10:00 PM. If nursing animals are being supplemented in order to maintain weight gain, they should be handfed 2 to 3 hours after the last nursing, in order to help alleviate any incompatibility between the dam's milk and the milk replacer.[24]

Milk may be provided to orphan kittens using a pet nurser (a smaller version of a human baby bottle) available at pet stores, or by a feeding tube directed through the esophagus into the stomach. The nurser is preferred by some, as this encourages the suckling reflex of the neonate, and the gradual delivery of milk to the stomach; in addition, the nurser is less likely than the feeding tube to compromise the kitten by the inadvertent delivery of milk to the lungs instead of the stomach. However, use of a pet nurser is very labor intensive and time consuming with multiple-kitten litters, and some veterinarians and trained technicians prefer use of an orogastric feeding tube. The orogastric tube for kittens should be a 15-inch, No. 8-Fr catheter. The tip of the tube should be placed on the outside of the body at the costal arch and the tube directed toward the mouth, where a mark or tape is affixed to

Table 29–3. Composition of Feline Milk, Compared to Milk of Other Species

Nutrient	Queen's Milk	KMR Queen's Milk Replacer	Experimental Milk Replacer (see Table 29–2)	Cow's Milk	Goat's Milk
Per cent solids of mature milk	18.2	—	13.6	11.9	13
Per cent protein—solids basis	40	42.2	46.7	25.6	27.4
Per cent fat—solids basis	28	25	25.1	29.9	31.9
Per cent lactose—solids basis	27	26.1	21.3	38.7	34.2
Gross kilocalories per gram solids*	5.2	5.0	4.55	5.26	5.34
Per cent calories from protein	30.8	33.9		19.5	20.5
Per cent calories from fat	48.5	45.2		51.1	53.8
Per cent calories from lactose	20.8	20.9		29.4	25.7

* Gross kilocalories calculated on the basis of 4, 9, and 4 calories per gram of protein, fat, and lactose, respectively.
Adapted from Monson WJ: Orphan rearing of puppies and kittens. Vet Clin North Am 17:567–576, 1987; and Remillard RL, Pickett JP, Thatcher CD, Davenport DJ: Comparison of kittens fed queens milk with those fed milk replacers. Am J Vet Res 54:901–907, 1993, with permission.

* KMR, Kitten Milk Replacer; Pet-Ag, Inc., Elgin, IL.

the tube to designate appropriate length from the mouth to the stomach. The tip of the tube is then introduced to the dorsal aspect of the oropharynx with the head extended, and, as the kitten swallows, gently directed down the esophagus to the level of the mark. A small amount of milk is injected by syringe into the tube to confirm that the tube is in the stomach (no reaction to the test milk) and not the bronchi (which elicits a cough). The rest of the measured amount of milk may then be injected, and the tube removed. Records should be maintained of amounts fed and weight gain per day (see Fig. 8–13).

In studies of puppies fed a commercially available milk replacer, (reversible) lens opacities have been reported, and diets deficient in amino acids were reported to induce lens opacities in other species.[25] In one report of 15 2-week-old kittens randomly assigned to treatment groups receiving sole nutrition from queen's milk, commercially available kitten milk replacer (KMR*) or an experimental milk replacer (EMR; Table 29–2), the EMR diet was shown to support normal growth without development of lens opacities.[25] The KMR and EMR were fed during treatment weeks 2 through 5 of life. KMR supported normal kitten growth, but resulted in diarrhea and cataract formation, perhaps due to low arginine concentration. Subsequent to that study, the commercial kitten milk replacer had L-arginine added to the ingredient list.

Stimulation of urination and defecation should be done for orphan kittens after every feeding by stroking the anogenital area with a warm wet cloth, which elicits the anogenital reflex.[10] The anogenital reflex disappears between 23 and 39 days of age, and kittens can voluntarily eliminate by 21 days of age.[10]

Postpartum Disorders in the Queen

Retained Placenta

Retained placenta occurs occasionally in the queen. It may be diagnosed historically based on the owner's observation of parturition (although identical twin kittens that share a placenta may occur occasionally), and confirmed by abdominal palpation, ultrasound, or radiology of a placental mass in utero. Affected queens should be treated with 3 to 5 U oxytocin intramuscularly (IM); treatment with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (0.25 mg/kg IM) should be instituted in queens that do not respond to three doses of oxytocin administered at 30-minute intervals. Queens failing to respond to either treatment that are bright, eating, and nursing kittens (hence releasing their own oxytocin) can be sent home with instructions to watch carefully for signs of metritis (e.g., fever, anorexia, purulent vaginal discharge). Retained placentas usually break down and pass in the lochial discharge of the nursing queen without causing metritis. If the retained placenta is associated with signs of metritis, and if medical therapy is unsuccessful, hysterotomy or possibly hysterectomy may be indicated.

Uterine Prolapse

Prolapse of part or all of the feline uterus has been reported in queens ranging in age from 10 months to 6 years.[26–35] Eight of nine affected queens prolapsed within 2 hours of delivering their last kitten; one queen (an 18-month-old domestic shorthair) had never delivered a live litter, but may have aborted several weeks prior to prolapse. Eight of nine queens prolapsed both horns and part of the uterine body; one queen prolapsed only the right horn. With one exception (the queen that may have aborted), severity of clinical signs varied directly with time from initial prolapse until veterinary assistance was sought.[27] Queens seen immediately were clinically normal except for presence of the prolapse, while those seen 6 to 48 hours after prolapse were anorexic, depressed, and exhibiting signs of shock. Uterine prolapse may occur after a queen's first pregnancy or it may occur in a queen with multiple prior normal parturitions. One queen, treated with manual reduction and intrauterine infusion of an antibiotic/estrogen solution, had a normal pregnancy and delivery (without recurrence of the prolapse) 4 months later.[35]

Diagnosis is made by inspection of the prolapsed uterus. Treatment selected depends on the owner's wish for future reproduction in the queen, and on tissue devitalization, if any. Treatment options include amputation of the everted uterus (with ligation of the prolapsed ovarian and uterine vessels); manual reduction and repositioning by abdominal palpation and use of infusions; manual reduction followed by ovariohysterectomy (OHE); and partial manual reduction with final positioning via celiotomy. Prognosis improves if treatment is instituted rapidly.

Metritis

Metritis in the queen usually is caused by ascent of vaginal bacteria to a compromised uterus postpartum. Uterine compromise may be caused by prolonged delivery, trauma at delivery, retained placental tissue, or breakdown of local host defenses. Signs of metritis in the queen include depression, anorexia, fever, neglect of kittens, and presence of a sanguineous or purulent vulvar discharge. Uterine enlargement can be detected by abdominal palpation or radiography. The hemogram usually reveals presence of an immature leukocytosis, and culture and cytology of the vulvar discharge usually reveal presence of *Escherichia coli*, staphylococci, or streptococci and polymorphonuclear leukocytes (Fig. 29–2).[36]

Therapeutic goals are to evacuate the uterus and provide a 4-week course of specific antibiotic therapy. Uterine evacuation, if indicated by enlarged uterine size, can be accomplished with 0.25 mg/kg $PGF_{2\alpha}$ IM; this therapy is contraindicated if a thin-walled, friable uterus is suspected based on uterine radiography or ultrasound. Occasionally, a large friable infected postpartum uterus will need to be removed surgically, but more often prognosis is good for complete recovery and future pregnancy if uterine evacuation and specific antibacterial therapy are used.

Mastitis

Inflammation of the mammary glands of the queen is caused by bacterial infection of the lactating gland postpartum or during pseudopregnancy.[37,38] Enterobacteria, streptococci, and staphylococci are commonly cultured from mastitic milk; routes of infection have not been well examined but are assumed to occur by ascending or hematogenous routes. There is no evidence to indicate that mastitis occurs with greater frequency in previously affected animals.

Clinically, the queen shows one or more warm, painful mammary glands (Fig. 29–3) and elevated rectal temperature (103.5° to 106.0°F); maternal anorexia, vomition, or lethargy, and/or acute illness in the suckling kittens may be present.[37,38] Because elevated temperature may be an early sign of both mastitis and metritis, owners of lactating companion animals should be encouraged to monitor rectal temperature daily throughout lactation if the queen permits. The affected queen may show depression, anorexia, and reluctance to care for her offspring. Her hemogram reveals an immature leukocytosis. Cytology of milk from affected glands shows presence of degenerative neutrophils and bacteria (Fig. 29–4).[38,39] Quantitative culture of the mastitic milk usually reveals a pure culture of a large number of bacteria that are susceptible to commonly used antibiotics.

Therapeutically, the affected queen should be given broad-spectrum antibiotics, such as amoxicillin with clavulonic acid immediately; specific antibiotics are selected when milk culture results become available. If signs of dehydration, septicemia, or septic shock (i.e., tachycardia, hypotension, increased capillary refill time, muscle weakness, hyperventilation) are present, immediate intravenous fluid, antibiotic, and steroid therapy are warranted.

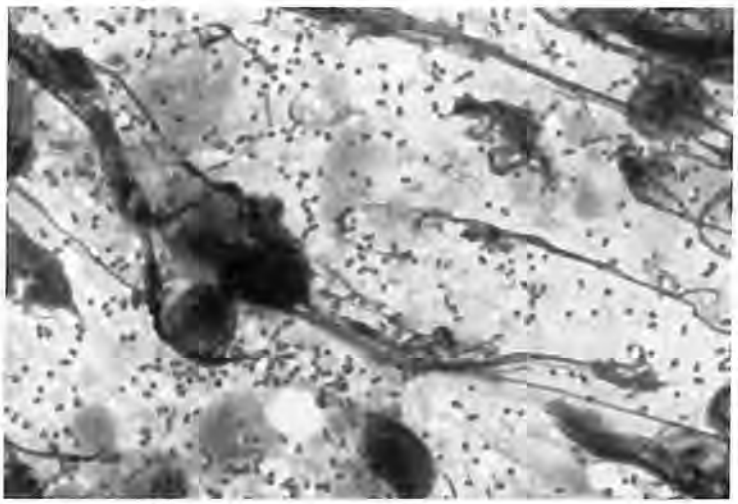

Figure 29–2. Microscopic appearance of the vulvar discharge from a queen with β-hemolytic streptococcus metritis.

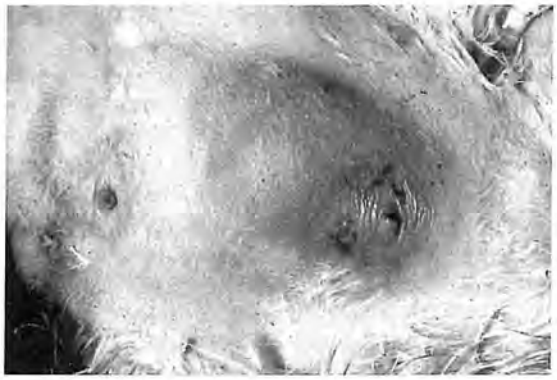

Figure 29–3. Gross appearance of mastitis in the queen.

When acute mastitis is diagnosed and treated early, and when mammary abscessation is absent, the offspring may be allowed to continue nursing. Offspring ingest antibiotics in milk, and frequent nursing helps drain the mastitic gland, preventing galactostasis. In addition, nursing offspring may themselves be the source of the mammary infection, as has been documented in the human disease. Human mothers under therapy for acute mastitis in the absence of abscessation continue to nurse without difficulty or infant illness; our experience with queens indicates that early and aggressive treatment of acute mastitis need not interrupt lactation.

Acute mastitis may progress to abscessation or gangrenous mastitis or both. In both conditions, surgical drainage followed by immersion of the glands in a dilute povidone-iodine solution and warm water three times daily is effective. Specific parenteral antibiotic therapy should be given, and offspring should not be allowed to nurse from the affected glands.

Very little glandular tissue is present in nonlactating glands, so inflammation of them due

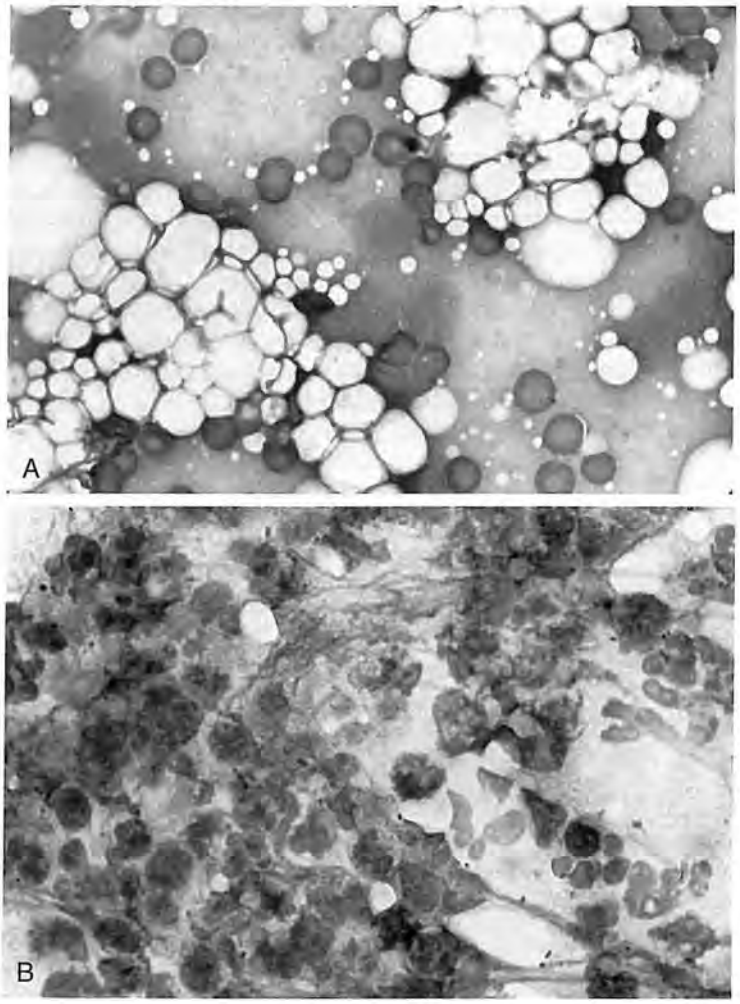

Figure 29–4. Microscopic appearance of milk from a normal feline mammary gland (**A**) and a mastitic gland (**B**).

to trauma or laceration may be treated in the same way as simple skin wounds.

Postparturient Hypocalcemia

Hypocalcemia during lactation has been reported in queens from 14 months to 5 years of age.[39–52] Affected queens usually have had two or more previous litters and are nursing five to seven kittens, although occasional cases in first-litter queens with three kittens or less have been reported. Clinical signs include incoordination, stiff gait, and vomiting; these may progress to muscle fasciculation, tonic/clonic convulsions with extensor rigidity, hyperthermia, hyperpnea, and dilated fixed pupils. Diagnosis is based on physical findings in a lactating queen and presence of total serum calcium less than 8 mg/dl. Treatment should be instituted after blood is drawn and before calcium results are known if clinical signs are present. Treatment is slow intravenous administration of a calcium solution to effect. Two to 5 ml of 10 per cent calcium gluconate is recommended as initial treatment, given over 3 to 5 minutes, which is repeated as needed until remission occurs. Treatment regimens reported effective in the literature include 3 to 10 ml of 5 to 50 per cent calcium gluconate or borogluconate sodium given intravenously (IV). The queen then may be discharged with an oral calcium supplement (250 to 500 mg calcium gluconate daily). Need to wean the kittens should be determined by their age and the mother's response to therapy; recurrence is an indication for weaning. Although some authors advocate the use of glucocorticosteroids in treating postparturient hypocalcemia in the queen, these drugs are calciuretic, and their value in postparturient hypocalcemia has not been documented.

Hypocalcemia also has been reported in pregnant queens 3 to 17 days prior to parturition.[53] Clinical signs included rapid onset of lethargy and anorexia, and affected queens exhibited trembling, muscle fasciculations, dehydration, weakness, hypothermia, pallor, tachypnea, dyspnea, bradycardia, and/or panting. All affected queens had delivered kittens prior to this event. All responded to IV or subcutaneous (SC) calcium, and were maintained on oral calcium (100 mg/kg per os [PO] twice daily) until one month after parturition. All queened and lactated normally.

Lactation Failure

Lactation failure is poorly understood in the queen. Delayed lactation may occur if a cesarean section was performed too early, and low milk production may occur due to inadequate intake of calories and fat postpartum; or inhibition of milk letdown in the nervous queen may play a role. The postpartum queen should be encouraged to eat, and tempted, if necessary, with high-calorie, canned food. Oxytocin injection or nasal spray may be used to induce milk letdown while kittens are held up to the nipples of nervous, first-time mother queens.

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Chapter 30

Prevention and Termination of Feline Pregnancy

Pet Overpopulation

In 1988, 5.9 to 9.8 million cats were euthanized in animal shelters in the United States, according to estimates by the American Humane Association's Animal Shelter Reporting Study.[1,2] These numbers represent 10 to 20 per cent of the U.S. cat population. Such magnitude of euthanasia is associated with tremendous fiscal cost to municipal animal shelters and emotional cost to persons performing euthanasias of healthy animals. The U.S. cat population is projected to increase from about 58 million in 1990 to 66 to 72 million by the year 2000 (Table 30–1).[3] Practicing veterinarians must promote cooperative solutions to the pet overpopulation problem, to include sterilization, prevention and/or termination of unwanted pregnancy, and animal behavioral counseling to decrease pet relinquishment.

Surgical Sterilization of the Queen

Ovariohysterectomy: Early Spay/Neuter

Ovariohysterectomy (OHE) is a surgical means of controlling fertility. OHE causes permanent estrus suppression, prevents subsequent ovarian and uterine disease and, if performed in the queen younger than 2 years of age, confers protection against later mammary neoplasia.[4] Possible complications of OHE in adult female queens include anesthetic complication, infection, and/or wound dehiscence. In a retrospective study of 66 cats undergoing OHE, 22 (33 per cent) showed some adverse reaction to suture material, including swelling, inflammation, abscessation, seromas, edema, or dehiscence; there was no association between type of adverse reaction and type of suture material (e.g., chromic gut, polydioxanone, nylon) used.[5] Undesirable sequelae of OHE in queens are rare, and, in general, are limited to increased weight gain associated with decreased caloric requirements.[6] Occasional cases of acquired vaginoureteral fistulae with incontinence, or fibrous connective tissue colonic stricture with obstipation, both attributed to development of post-OHE adhesions, have been reported.[7,8] Urethral closure pressure and urethral sphincter pressure have been reported to be the same in spayed and in intact female cats.[9] Incomplete ovariectomy may result in recurrence of estrual cycling, starting as soon as several weeks to as long as several years after OHE (see Chapter 29). Ovariohysterectomy at time of parturition or in lieu of cesarean section is discussed in Chapter 26.

Early spay/neuter means ovariohysterectomy or castration of animals at 6 to 14 weeks of age instead of at the more conventional 6 to 9 months of age. Early spay/neuter has been investigated as a partial means of alleviating the pet overpopulation problem. Owners of more than half of the pets adopted from shelters do not comply with spay/neuter contracts, which leads to repopulation of shelters by animals placed. As puppies and kittens are more desirable for adoption than are adult animals, there is need to sterilize these animals prior to placement, despite their young age. In addition, pediatric sterilization of purebred pet-quality animals sold by breeders might prevent later violation of agreement not to reproduce these animals.[6,10]

Several anesthetic regimens for early spay/neuter in 6- to 14-week-old kittens have been

Table 30–1. Projections of Dog and Cat Populations (in Millions) at the National Level, Based on Three Series of Projections for the United States Human Population Size, As Presented By the Bureau of the Census

	Populations by Year		
	1990	1995	2000
Middle series			
Dogs	55.70	60.55	66.18
Cats	58.38	63.28	69.00
Low series			
Dogs	54.83	58.75	63.25
Cats	57.47	61.41	65.95
High series			
Dogs	56.70	62.55	69.54
Cats	59.42	65.37	72.50

From Nassar R, Mosier J: Projections of pet populations from census demographic data. J Am Vet Med Assoc 198:1157–1159, 1991, with permission.

reported safe and effective. These include preanesthesia with atropine (0.045 mg/kg subcutaneously [SC]) followed by induction and maintenance of isoflurane inhalation anesthesia via mask.[6] Induction of anesthesia with midazolam (0.22 mg/kg intramuscularly [IM]) and ketamine hydrochloride (11 mg/kg IM) followed by intubation or mask isoflurane inhalation anesthesia is reported as an effective anesthetic combination for neutering 6- to 14-week-old female kittens, because of its ease of administration and rapid, calm recovery of treated animals.[11] Food is withheld for 3 to 8 hours prior to anesthesia. Heat loss before and during surgery should be minimized by using minimal hair clipping, warm surgical scrub, and warm water blankets during surgery and recovery.

A 3- to 4-cm ventral midline skin incision is made 1 cm caudal to the umbilicus, which is followed by extension with scissors and exteriorization of the urinary bladder.[10] Ovarian and uterine pedicles are clamped with hemostats and ligated with hemoclips or with 2-0 suture material after which the uterine body and ovaries are removed. After examination of the abdomen for bleeding, the ventral midline fascia is closed with a continuous pattern in 3-0 nylon, and the abdomen is closed with a continuous intradermal pattern using 3-0 nylon.[10] Kittens are given a small meal within 1 hour of standing in order to maintain normal blood glucose concentrations. Forty-eight female kittens neutered with anesthetic and surgical techniques described had negligible morbidity (minor incisional swelling in 2) and no mortality.[10,11] The surgical wound infection rate was zero per cent, and antibiotics were not used. Care must be taken, when performing early spay/neuter in the cat, to perform thorough preoperative examinations, to keep kittens warm, to handle delicate pediatric tissues carefully, and to minimize/control hemorrhage and hypoglycemia.

Gonadectomy in female cats 7 weeks or 7 months of age results in final long bone length about 10 per cent greater than intact littermates, because distal physeal closure is delayed 5 to 7 months in these animals (Figs. 30–1 and 30–2).[6] In a study of urethral diameter in 2-year-old queens, those gonadectomized at 7 weeks of age had smaller prepelvic urethras (2.0 ± 0.3 mm) than did those left intact (3.2 ± 0.8 mm).[6] Urethral diameter of 2-year-old queens gonadectomized at 7 months of age was (2.3 ± 0.9 mm) not different than that of animals neutered at 7 weeks (Fig. 30–3).[6] Heat production, a measure of basal metabolic rate, was about 33 per cent higher in the intact queens than in cats gonadectomized at 7 weeks or 7 months of age.[6] These data suggest that gonadectomy of female cats at 7 weeks of age is not associated with significant physical differences compared to female cats gonadectomized at 7 months of age. Both groups, however, are slightly taller than intact queens, have smaller prepelvic urethras, and have lower heat production or metabolic rate, and therefore require about 30% fewer calories than intact queens.[6]

Uterine Horn Occlusion/Ligation

Uterine tube or uterine horn occlusion by electrocautery or ligation has been performed via laparotomy or laparoscopy in the queen.[12,13] Laparoscopic examination of the uterus 1 year after electrocoagulation occlusion of one uterine horn midway along its length revealed an enlarged, thin-walled, fluid-filled uterine segment cranial to the midcornus occlusion site.[13] Laparoscopic uterine horn occlusion is reported to be a rapid, safe, and effective procedure, but it does not prevent estrual cycling, or pyometra, nor does it offer protection against mammary cancer as does OHE of the young queen.

Estrus Suppression

Progestogens

Estrus suppression in the queen traditionally has been accomplished medically with proges-

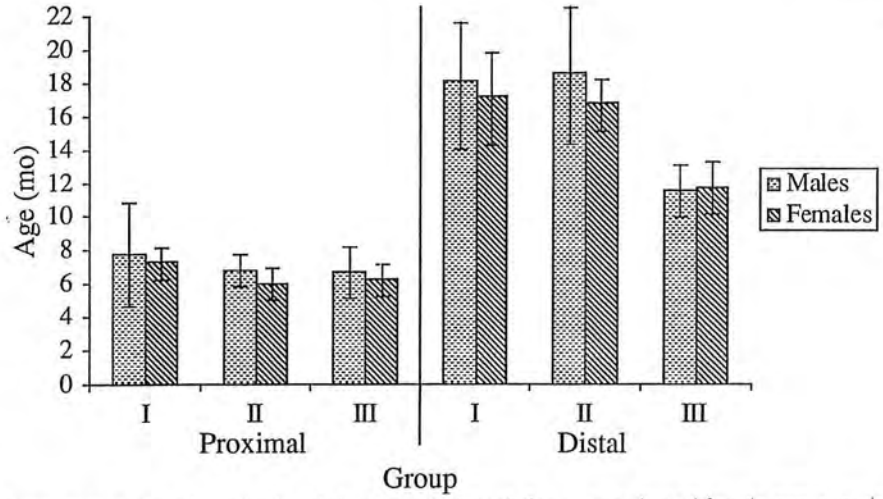

Figure 30–1. Mean age at time of proximal and distal radial physeal closure in male and female cats gonadectomized at 7 weeks of age (Group I) or 7 months of age (Group II) or left intact (Group III). (From Root MV: The effect of prepuberal and postpuberal gonadectomy on the general health and development of obesity in the male and female domestic cat. PhD Thesis, University of Minnesota, 1995, p 57, with permission.)

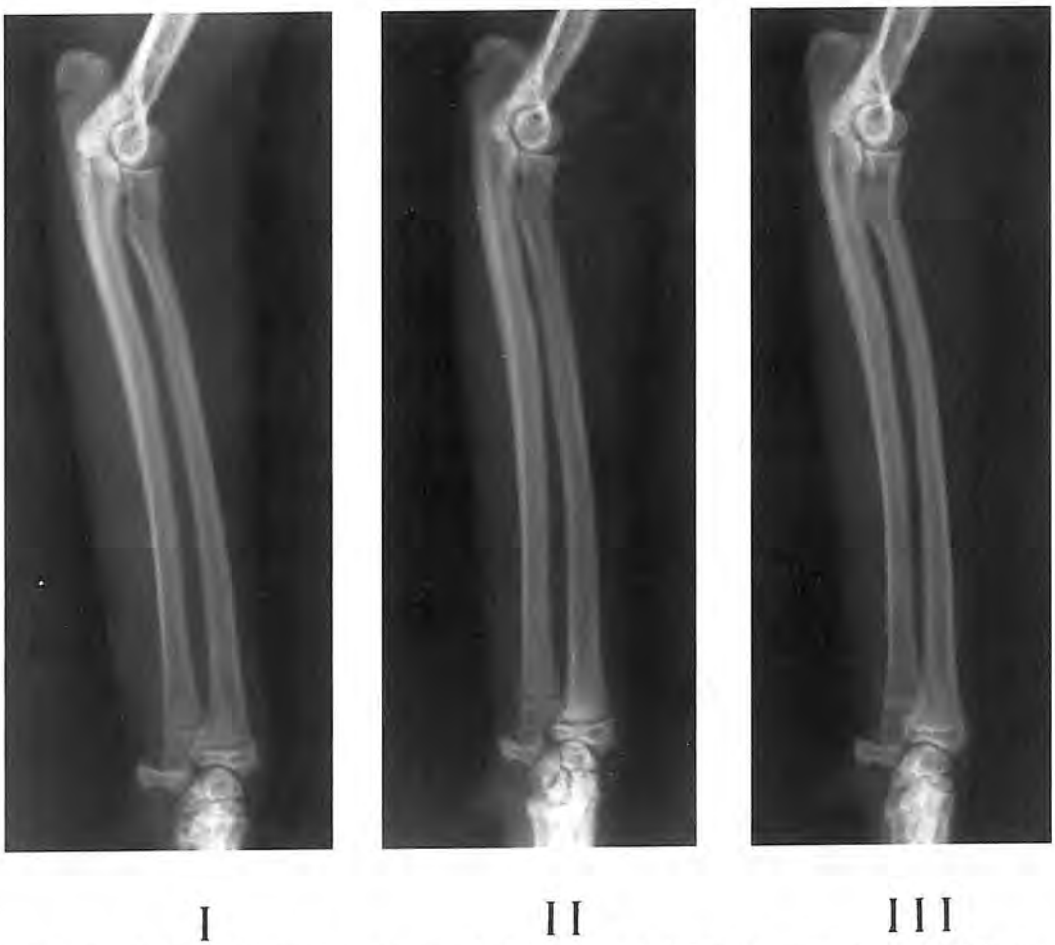

Figure 30–2. Lateral radiographic view of the right forelimb of three 11-month-old feline litter sisters. Group I (gonadectomy at 7 weeks of age) and Group II (gonadectomy at 7 months of age) animals show closed proximal radial physes and open distal radial physes. Group III (intact) animals show closure of both radial physes. (From Root MV: The effect of prepuberal and postpuberal gonadectomy on the general health and development of obesity in the male and female domestic cat. PhD Thesis, University of Minnesota, 1995, p 60, with permission.)

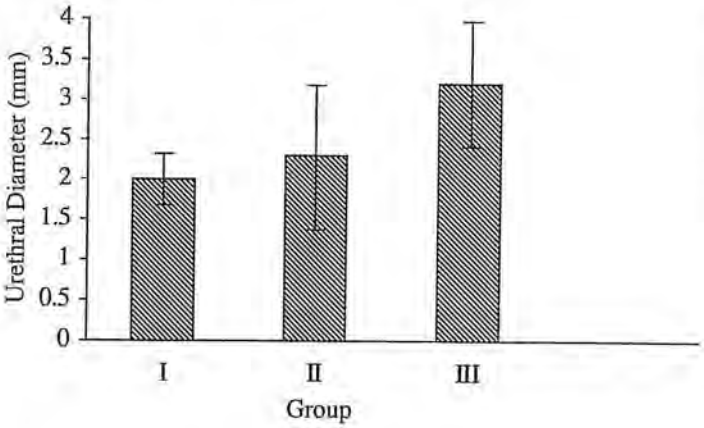

Figure 30–3. Mean diameters of the prepelvic urethra in 24-month-old female cats gonadectomized at 7 weeks of age (Group I), 7 months of age (Group II), or left intact (Group III). (From Root MV: The effect of prepuberal and postpuberal gonadectomy on the general health and development of obesity in the male and female domestic cat. PhD Thesis, University of Minnesota, 1995, p 79, with permission.)

togens or synthetic androgens. Megestrol acetate* is effective at a dose of 5 mg by mouth once daily for 5 days and then once weekly.[14–17] Medroxyprogesterone acetate† is reported effective at a dose of 25 to 100 mg IM every 6 to 12 months as required to suppress heat.[18] Although the queen does not appear as susceptible as the bitch to pyometra after progestin therapy, undesirable side effects associated with use of these drugs include cystic endometrial hyperplasia, mammary hypertrophy, mammary neoplasia, and diabetes mellitus.[19–22] Hyperglycemia, glucosuria, polydipsia, and polyuria may occur within 1 to 2 weeks after onset of megestrol acetate therapy.[23] Blood sugar in affected animals usually returns to normal following insulin administration and cessation of megestrol acetate treatment. Two weeks of therapy with megestrol acetate (5 mg per os [PO] once daily) caused significant suppression of plasma adrenocorticotropic hormone (ACTH) concentrations and elevation in plasma insulin concentrations during treatment and for 2 to 4 weeks following treatment.[24] Progestogens, though effective for estrus suppression, are not Food and Drug Administration (FDA)-approved for estrus suppression in the queen, and are not recommended by the authors because of their undesirable side effects. If used for estrus suppression, the progestogen of choice is megestrol acetate by mouth because its half-life is shorter than that of medroxyprogesterone acetate.

Androgens

Mibolerone‡ is a synthetic androgen that has been used for long-term estrus suppression in queens at a dose of 50 μg (0.5 ml) by mouth once daily.[25] Mibolerone is associated with undesirable side effects that include clitoral hypertrophy (two- to threefold increase in size), thickening of the cervical dermis, and urine spraying behavior with tomcat-scented urine.

Induction of Ovulation

Induction of ovulation by mechanical stimulation of the vagina using a teaser tom or glass rod, or by pharmacologic stimulation with 250 IU human chorionic gonadotropin (hCG)§ IM, which has luteinizing hormone–like action, or 25 μg gonadotropin-releasing hormone (GnRH)‖ IM, which causes endogenous luteinizing hormone release (see Chapter 23), may prevent normal return to estrous behavior by inducing a 45-day luteal phase (pseudopregnancy). It will not, however, shorten behavioral estrus for that season. Repeated pseudopregnancy induction may predispose the queen to pyometra.[26,27]

Pregnancy Termination

Mismate Injection

Administration of parenteral estrogen (2 mg repositol diethylstilbestrol [DES] IM, or 0.25 mg estradiol cypionate [ECP] IM) 2 to 3 days after coitus has been reported effective at preventing pregnancy in the queen.[28] Mechanism of action is reported as retardation of tubal transport of the fertilized egg.[28] These products extend behavioral estrus in the queen, however, and they may have other undesirable or dangerous side effects; therefore, estrogen

* Ovaban; Schering-Plough Corporation, Kenilworth, NJ.
† Depo-Provera; Upjohn Company, Kalamazoo, MI.
‡ Cheque; Upjohn Company, Kalamazoo, MI.
§ Chorulon; Intervet Incorporated, Millsborough, DE.
‖ Cystorelin; Sanofi Animal Health, Overland Park, KS.

mismate injections are not recommended in the cat.

Induction of Abortion

Abortion may be induced in cats with natural prostaglandin $F_{2\alpha}$[¶] ($PGF_{2\alpha}$). Doses of 0.5 or 1.0 mg/kg SC given twice, 24 hours apart after day 40 of pregnancy, or five daily IM injections of 2 mg per cat after day 33 of pregnancy, have been reported to result in complete abortion within 1 to 6 days of the first injection.[29,30] At the 2-mg dose, side effects started 10 minutes after injection, and lasted for about an hour. Side effects included prostration, nausea, vomiting, diarrhea, and listlessness, which continued for up to 3 hours.[30] Plasma progesterone concentrations declined to concentrations less than 1 ng/ml within 24 hours of the first injection. Plasma progesterone concentrations may be monitored to evaluate effectiveness of treatment. The authors do not recommend this treatment, because of the severity of side effects.

In the pseudopregnant queen, neither $PGF_{2\alpha}$ (220 to 440 μg/kg SC twice daily on days 11 to 15 or 21 to 25 postcoitum or 0.5 to 5.0 mg/kg SC on days 4 and 5 or 12 and 13) nor an ester-containing synthetic prostaglandin analogue (TPT-methyl ester) (20 μg/kg on day 11 or 21 postcoitus) was reported effective in inducing luteolysis and plasma progesterone decline.[31,32]

Induced abortion also has been reported in queens treated with the antiprolactin drug cabergoline, available in Europe.[30] When administered SC at 1.65 μg/kg divided twice daily for 5 days to five queens starting 30 days after mating, cabergoline induced abortion in four of the five. Queens aborted 7 to 10 days after the start of treatment with no adverse side effects or adverse sequelae. The single treatment failure cat showed only transient plasma progesterone decline after the start of cabergoline treatment. In another study, cabergoline administered orally at 5 to 15 μg/kg/d for 5 days to 34 pregnant queens starting 25 to 48 days from mating resulted in complete abortion in all queens at 34 to 53 days gestation.[33]

Induced abortion also has been reported in two queens given feline ovarian antiserum on days 36 to 40 or 49 to 51 of gestation; these queens did carry some of the litter to term and to normal delivery.[34]

[¶] Lutalyse; Upjohn Company, Kalamazoo, MI.

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Chapter 31

Disorders of the Feline Ovaries

Anomalies and Congenital Abnormalities

Anomalies of the feline ovary include unilateral agenesis, gonadal dysgenesis, true hermaphroditism (presence of an ovotestis), and presence of three ovaries (supernumerary/accessory ovary).[1-4] Absence of an ovary, and often of the ipsilateral uterine horn as well, is encountered occasionally. In one report, the right ovary, right uterine tube, right uterine horn, right half of the body of the uterus, and the broad and round ligaments on the right side were absent in a queen with unilateral vascular anomalies; the left uterine horn and ovary were normal in appearance and location, and the left uterine horn contained three embryos.[4] Left ovarian aplasia was reported in an 11½-year-old Siamese queen with a tendosynovial sarcoma of the right ovary.[5]

Ovarian dysgenesis refers to underdevelopment of the ovary; it may refer to ovarian hypoplasia or to hermaphroditic and streak gonads, which consist of a thin, white ridge of tissue in place of the normal ovaries.[2] In the cat, gonadal dysgenesis usually is associated with an abnormal sex chromosome complement, such as XO monosomy or mosaicism.[6] Bilateral ovarian dysgenesis was reported in a 2½-year-old Burmese cat with primary anestrus and a 37,XO chromosome complement (Fig. 31–1).[7] The ovaries and uterus were small (Fig. 31–2), and ovarian histology consisted of fibroblastic stromal elements and large aggregates of hypertrophied interstitial cells; follicles or corpora lutea were not observed (Fig. 31–3). No somatic abnormalities were present in the cat. Other 37,XO cats have been described with coarctation of the aorta and with spina bifida, and in one report where evaluation other than karyotype was not included.[8-10] These were kittens that died or were euthanized by 3 days of age, and gonadal histology was not reported. The XO female is an example of an abnormality of sexual differentiation due to numerical abnormality of the sex chromosomes. In one report of the presence of fused placentae between two adjacent feline embryos of different sexes, the author suggested that female sterility might therefore occur in this species as it does in the bovine freemartin; gonadal histology of the kittens was not, however, presented.[11]

Unilateral ovarian dysgenesis was observed in two unrelated, pregnant, stray, domestic shorthair cats presented to the veterinary clinic of an animal shelter for ovariohysterectomy (OHE).[12] Both cats were estimated to be less than 6 years of age. Cat 1 was orange-white with some tabby spots at the base of the tail; at OHE a uterus was removed that contained four fetuses, two in each horn. The average crown-rump length of the fetuses was 50 mm, corresponding to a stage of pregnancy of less than 38 days.[13] The right ovary was normal size, and corpora lutea were visible on the surface. The left ovary appeared to be a hypoplastic, 1-mm-diameter nodule which, histologically, consisted of a network of small sex cords embedded in connective tissue, and lacking ovarian structures. Karyotype of lymphocytes revealed a 37,XO chromosome complement; karyotype of cultured fibroblasts from cat 1 revealed two cell lines, the major line containing 37,XO chromosomes, and the minor line containing 39,XXX chromosomes. Cat 2 was tabby and white; at OHE a uterus was removed that contained five fetuses, two in each horn and one in the uterine body. The average crown-rump length of the fetuses was 90 mm,

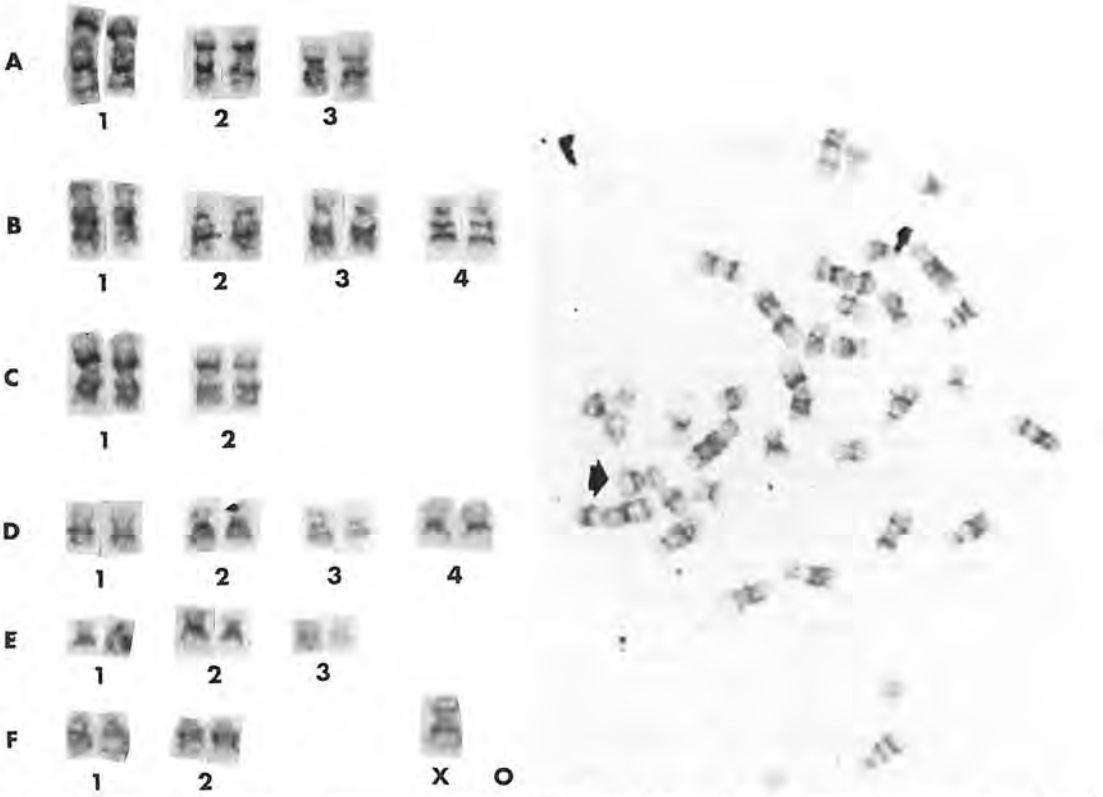

Figure 31–1. G-banded karyotype and chromosome spread of 37,XO cat, illustrating the characteristic banding pattern of the X chromosome of the domestic cat (arrow). (From Johnston SD, Buoen LC, Madl JE, et al: X-Chromosome monosomy [37,XO] in a Burmese cat with gonadal dysgenesis. J Am Vet Med Assoc 182:986–989, 1983, with permission.)

corresponding to a stage of pregnancy of about 47 days.[13] The left ovary was normal size, and corpora lutea were visible on the surface. The right ovary appeared to be a hypoplastic, 2 × 1-mm nodule which, histologically, consisted of ovarian tissue with several small antral and preantral follicles, predominantly containing two oocytes of different sizes. No large follicles, corpora lutea, or luteinized interstitial tissue were observed, but remnants of atretic follicles, which often contained hyalinized zonae pellucidae, were present. Karyotype of lymphocytes revealed two cell lines: the major one had a normal 38,XX chromosome complement, and the minor one had a 37,XO chromosome complement; karyotype of cultured fibroblasts from cat 2 revealed the normal, 38,XX complement. Three of the fetuses from cat 2 were karyotyped, and were normal (38,XX or 38,XY); one of the five fetuses showed palatoschisis, and the other four were grossly normal, including grossly normal gonads.[12]

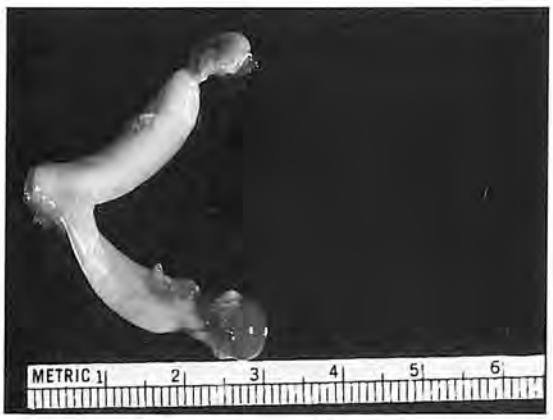

Figure 31–2. Gross appearance of the uterus and ovaries of a 37,XO Burmese cat described in Figure 31–1.

True hermaphroditism requires presence of both ovarian and testicular tissue in the same individual; these tissues may be present in a single ovotestis or in two separate gonads within the same animal. In other species, ovotestes have been detected in phenotypic female individuals with XX/XY, XO/XY, or XXY sex chromosome complements.[14] True

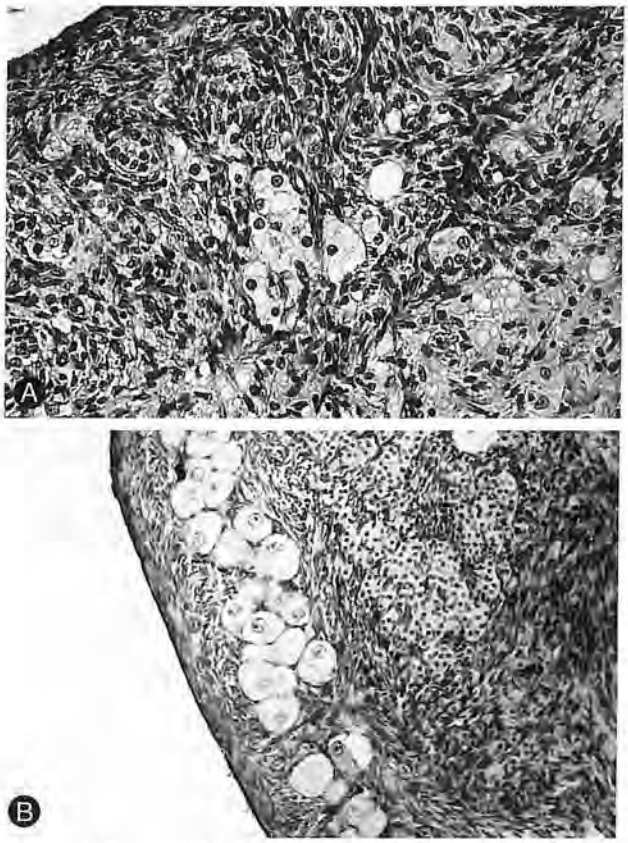

Figure 31–3. Histologic appearances of the ovary of a 37,XO cat (**A**) consisting of fibroblastic stromal elements and an absence of follicles or corpora lutea; and of the ovary of a normal queen (**B**), showing primordial follicular aggregates and interstitial cells. Hematoxylin and eosin stain; 125×. (From Johnston SD, Buoen LC, Madl JE, et al: X-Chromosome monosomy [37,XO] in a Burmese cat with gonadal dysgenesis. J Am Vet Med Assoc 182:986–989, 1983, with permission.)

hermaphroditism is rare in cats, and has not been reported in phenotypic females where both gonad histology and chromosome complement are known. True hermaphroditism has been reported in cats that are phenotypically male, where both gonad histology and chromosome complement are known, suggesting that presence of testicular tissue in the embryo induces development of (male phenotype) secondary sexual characteristics, regardless of presence of ovarian tissue or karyotype. Ovotestes were reported in two phenotypic male calico–tortoise-shell cats with 38,XX/38,XY or 38,XX/57,XXY karyotypes.[15] Presence of a left testis outside the body cavity and a right abdominal ovotestis was reported in a dissected laboratory cat in which secondary sexual characteristics were not reported.[16] Another author reported removing a scrotal testis from a phenotypic male and later finding a rudimentary uterus, two normal uterine horns, and ovaries at exploratory laparotomy; histologic examination was not reported, and karyotype was unknown.[17] Sheppard reported having seen one hermaphrodite cat—a Siamese—in over 20,000 cases, but did not report gonadal histology.[18] Presence of a cryptorchid testis was reported to the left of the vulva of a phenotypic female calico cat that had undergone routine OHE 6 weeks earlier.[19] The cat had a 38,XX/57,XXY karyotype; the testis contained tubules lined by Sertoli cells with a few spermatogonia but no evidence of spermatogenesis. Presence of bilateral ovotestes was reported in a 4-year-old American shorthair cat examined for incontinence; on physical examination the cat had a normal penis but no scrotal testicles. The cat also had a thin-walled uterus that terminated at the urethra and acted as a urine reser-

voir; hysterectomy resulted in cessation of signs of incontinence.

Pseudohermaphroditism, or presence of phenotypic sex opposite that of gonadal sex, also is rare in the cat. Presence of abdominal testes was reported at the tip of the uterine horns of a 1-year-old phenotypic female blue tabby (male pseudohermaphroditism).[20] The testes contained seminiferous tubules lined with Sertoli cells; no spermatogonia were seen. The authors examined 400 cells from the oral mucosa looking for Barr bodies (sex chromatin) and found none, suggesting that the cat was not a normal XX female. A 4-month-old kitten presenting with routine OHE at Angell Memorial Hospital was similarly found to have abdominal testes and an underdeveloped uterus.

Testicular feminization was reported in a 6-month-old orange tabby cat with a normal female phenotype (vulva and clitoris), 38,XY chromosome complement, abdominal testes but no uterus, uterine tubes, epididymides or vasa deferentia, and absence of androgen receptors in cultured fibroblasts from genital skin.[21] Findings in this cat are identical to those in humans with testicular feminization, who are XY males with bilateral testes and normal testosterone production, but who develop as phenotypic females because the androgen receptor is nonfunctional. Testes, found in the abdomen, the inguinal canal, or in the labia majora, have the histologic appearance of undescended testes. Müllerian duct derivatives (the female tubular reproductive tract) are absent. In humans, testicular feminization is inherited as an X-linked trait, attributed to a mutation that blocks the function of the cytosolic androgen receptor.

Female pseudohermaphroditism has not been confirmed in the cat and is rare in other species in the absence of virilizing drugs or hormones given to the mother during pregnancy.

The single report of a supernumerary (left) ovary in a cat described three ovaries found during routine OHE.[1] The two left ovaries were about 1 cm apart, and were individually smaller than the single right ovary, but their total mass was greater than that of the right ovary. Absence of ovarian histology in this report, and the documentation of paraovarian nodules (described in the section that follows) call this report into question. There is one report that documents presence of a granulosa cell tumor at the upper end of the left uterine horn in a 5-year-old Siamese queen.[22] Right and left ovaries in this cat appeared normal, and the tumor was not connected with the left ovary, suggesting the possibility of the tumor arising in a supernumerary ovary. The not uncommon observation of "ovarian remnants" that cause reproductive cycling in spayed female cats from which two ovaries were thought to have been removed may represent, in some queens, presence of a supernumerary ovary, or else presence of a normal mass of ovarian tissue which, embryonically, develops into two lobes or two separate structures.

Diagnosis of ovarian anomalies is based on history of primary anestrus (ovarian agenesis or dysgenesis) on careful gross evaluation of internal and external genital organs, on histologic examination of the ovary, and on karyotype of affected queens (see Chapter 10 for karyotype procedure).

Ectopic Adrenocortical Paraovarian Nodules

Ectopic adrenal gland nodules have been recognized in many species. In the cat, they occur in the broad ligament of the ovary, within 1 to 4 cm of the ovary, as single, unilateral nodules, as bilateral nodules, or as two nodules on a single side.[23] A survey of 499 female cats undergoing routine OHE revealed 11 (2.2 per cent) with paraovarian adrenocortical nodules.[23] These nodules ranged in size from 2 to 5 mm in diameter, and were smooth, well delineated, firm, and gold to tan. Paraovarian adrenocortical nodules are presumed to be islands from the adrenocortical anlagen that migrate abnormally, embryologically, with genital tissue from the urogenital ridge to the location of the ovary. They are reported to be of no clinical significance in the cat.[23]

Ovarian Cysts

Ovarian cysts have been reported to be common in the cat, and their frequency is reported to increase with age; follicular cysts that arise from mature or atretic follicles are reported to be the most common type.[2,24–27] Their cause in spontaneous disease is unknown, but they can be produced experimentally in immature cats treated with pregnant mare serum gonadotropin.[28] Affected queens may be asymptomatic or may exhibit prolonged estrus if cells lining the cyst secrete estrogen.[24] Prolonged estrus may be hard to distinguish from normal estrus,

because the normal queen may cycle in and out of the follicular phase as frequently as every 4 to 7 days. In the authors' experience, some owners describe a complaint of prolonged estrus in a cat that is demonstrated to be cycling normally when serial vaginal cytology specimens and hormone levels are examined carefully.

Figure 31–4 depicts bilateral ovarian cysts in an 8-year-old Birman cat presented for infertility associated with (prolonged) estrus of 6 weeks' duration. The queen had had two normal litters more than 3 years earlier, and had been bred four times without conceiving; on physical examination she had a cornified vaginal smear, and two abdominal masses, determined to be septate and fluid filled on abdominal ultrasonography. Administration of 500 IU human chorionic gonadotropin (hCG) intramuscularly (IM) did not result in luteinization of these structures, as determined by subsequent vaginal cytology, serum progesterone concentrations, and ultrasound. Surgical resection of the cysts was performed, sparing both ovaries, which resulted in the queen going out of estrus and exhibiting a noncornified vaginal cytology smear by 2 weeks postoperatively. Cyst fluid contained more than 6000 pg/ml estradiol, and no detectable progesterone or testosterone. The cat subsequently conceived and delivered a normal litter of kittens.

Figure 31–5 depicts a unilateral ovarian cyst in an asymptomatic domestic shorthair cat presenting for routine OHE. A large ovarian cyst containing more than 1400 ml dark fluid was reported in a 17-year-old female cat with abdominal distention, a left ovarian remnant after previous OHE, and multiple scirrhous adenocarcinomas on the omentum.[26]

Figure 31–4. Gross appearance of bilateral, functional ovarian cysts in an 8-year-old Birman queen with prolonged estrus and infertility. Surgical resection of the cysts, sparing the ovaries, resulted in resolution of clinical signs. The cyst fluid contained more than 6000 pg/ml estradiol. The queen later became pregnant and delivered a normal litter.

A subset of feline ovarian cysts are those that arise from the rete ovarii, a convoluted system of cell cords and tubes which, at birth, occupies most of the ovarian medulla and extends into the periovarian tissue. Granulosa cells may develop, in part, from cells of the rete ovarii. Grossly visible cysts of the feline rete ovarii have been reported in 20 cats ranging in age from 7 months to 13 years (x = 6.7 years, n = 13; age unknown in 7).[25] There was no common reproductive history among the cats examined; 19 were domestic shorthairs and one was a Siamese. Cysts were visible grossly, ranged from 1 to 2.5 cm in diameter, and were unilateral in 16 cats and bilateral in four.[25] Histologically, the cyst lining varied from single to several layers of cuboidal (n = 12) to ciliated columnar (n = 8) epithelium. The significance of these cysts, or their relationship to functional follicular cysts, is unknown.

Ovarian cysts are diagnosed histologically; observation of signs of estrus, a cornified vaginal smear, elevated plasma estradiol concentrations, and presence of an abdominal mass are contributory diagnostic findings if cysts are of follicular origin and are functional. Real-time ultrasonography also is indicated, if available, to image large cysts. Differential diagnoses are normal cycling behavior and a functional granulosa cell tumor of the ovary. Treatment options include attempts to induce ovulation and follicular luteinization (500 IU hCG or 25 μg gonadotropin-releasing hormone [GnRH] IM), surgical resection of the cysts if medical treatment is unsuccessful, and ovariectomy if future reproduction is not desired.

The Ovarian Remnant Syndrome

The ovarian remnant syndrome describes presence of ovarian tissue and signs of estrus in a female cat after OHE.[29-31] The cause may be failure to remove all of a normal ovary at OHE or presence of a partial or complete separation of a portion of normal ovary during development (the fragment may be located near the ovary or in the broad ligament) that is not detected at OHE.[24,30] Rarely, presence of a supernumerary ovary may be considered as the cause of estrus signs after bilateral OHE. Excision of the ovarian cortex from its vascular

Figure 31–5. Gross appearance of an ovarian cyst in an asymptomatic domestic shorthair cat presented for routine OHE.

supply and subsequent suturing of the cortex to the lateral abdominal wall in experimental cats resulted in signs of estrus 4 months after the surgery, suggesting that ovarian tissue may be active even after ligation of the ovarian artery.[29] Ovarian hypertrophy occurs following unilateral or partial ovariectomy in cats.[24,32]

Affected queens demonstrate normal signs of estrus, and may allow copulation, but do not become pregnant if bred. The estrous cycles usually show normal periodicity, but there may be a delay of months to years after OHE was performed before the estrous cycles appear.[31] In 11 cats described in one report, the mean age at time of diagnosis was 3 years, with a range of 1 to 10 years.[31] The time interval from OHE to return to estrus was 17 days to 9 years (mean = 2 years, $n = 6$). In nine of the cats, the interval from recurrence of estrous cycles to time of veterinary diagnosis ranged from 4 days to 2.5 years. Vaginal cytology was consistent with estrus in six of eight of the cats, and mean serum estradiol concentration in seven cats at time of behavioral estrus (11.5 to 43 pg/ml) was not as contributory as the cornified vaginal cytology specimens in making a diagnosis. Serum estradiol concentrations exceeding 20 pg/ml are reported present in the follicular phase of the estrous cycle of normal queens.[33] In four affected cats, stimulation of ovulation and luteinization (using 25 μg GnRH IM during behavioral estrus) followed by measurement of serum progesterone concentration 2 to 3 weeks later resulted in serum progesterone concentrations of 3.3 to 39.8 ng/ml. Poststimulation serum progesterone concentrations exceeding 2 ng/ml strongly support presence of ovarian luteal tissue.

Diagnosis is based on confirmation of estrus (using observation of mating behavior and vaginal cornification), and on detection of serum progesterone concentrations exceeding 2 ng/ml 2 to 3 weeks after induction of ovulation at estrus in a neutered cat.

Treatment is exploratory laparotomy and examination of both ovarian pedicles for retained or accessory ovarian tissue. Exploratory surgery is recommended within 3 to 6 weeks of induction of ovulation, at which time presence of corpora lutea in a "grape cluster" appearance on the surface of the ovarian remnant may make small remnants easier to identify (Fig. 31–6).[33] Eleven of 11 cats explored for ovarian remnants in one study had remnants at the ovarian pedicle. The ovarian remnants were bilateral in three cats, and unilateral remnants were located at the right ovarian pedicle in three of the four cases in which the surgeon recorded the affected side. One remnant was

Figure 31–6. Gross appearance of an ovarian remnant with corpora lutea removed from a 1-year-old Siamese cat showing estrous behavior 1 week following ovariohysterectomy. (From Johnston SD, Root MV, Olson PNS: Ovarian and testicular function in the domestic cat: Clinical management of spontaneous reproductive disease. Anim Reprod Sci 42:261–274, 1996, with permission.)

found in the omental fat near the ovarian pedicle. One cat had histopathologic evidence of a granulosa cell tumor in excised ovarian remnant tissue.

Ovarian Neoplasia

The incidence of primary ovarian neoplasia in the cat is low. Surveys of 328, 571, and 254 feline neoplasms revealed the presence of 0.4, 0.3, and 0.2 per cent ovarian tumors, respectively.[34–36] Occurrence and behavior of ovarian tumors are influenced by the tissue of origin within the ovary (Table 31–1).[37] The granulosa cell tumor of sex cord-stromal origin is the most common primary ovarian tumor in the cat.

Most feline ovarian tumors, regardless of histologic type, are associated with a palpable mass in the cranial or midabdomen. Abdominal and thoracic radiographs and abdominal ultrasonography are indicated in all cases to assess tumor size and location, and evidence, if any, of the presence of metastases. Metastases occur by extension to abdominal surfaces or by lymphatic/hematogenous spread to regional lymph nodes and the thoracic cavity. A vaginal cytology specimen should be examined for cornification as evidence of estrogen secretion in suspect queens. Measurements of serum estrogen, testosterone, and progesterone are of interest if functional tumors are suspected based on clinical signs of prolonged estrus, virilization, or pyometra. Evaluation of the hemogram and serum chemistry profile is indicated prior to exploratory surgery.

Epithelial Origin Feline Ovarian Tumors

ADENOMA/CYSTADENOMA

A cystadenoma described in a 9-year-old cat occurred with a lipoma (from neoplastic transformation of luteinized stromal cells of the ovarian cortex); clinical signs included persistent estrus, virilization, and cystic endometrial hyperplasia.[2,36,38]

ADENOCARCINOMA

A bilateral ovarian adenocarcinoma with spindle cell transformation was reported in a 5-year-old cat with a palpable abdominal mass, ascites, and hair loss; metastases were present throughout the pelvis and in the peritoneum, liver, and lungs. The endometrium was histologically normal.[38]

Germ Cell Origin Feline Ovarian Tumors

DYSGERMINOMA

Dysgerminomas are malignant tumors arising from undifferentiated germ cells of the ovary; they are comparable to the seminoma of the male.[2,36,39–42] Dysgerminomas constitute about 20 per cent of feline ovarian tumors. These

Table 31–1. Ovarian Tumor Types Reported in the Queen

Tumor Type	Number Reported	Metastasis (Y/N)*	Functional (Y/N)*	References
Epithelial origin				
Adenoma	1			Nielsen[36]
Cystadenoma	2		Y	McEntee[2] and Norris et al.[38]
Adenocarcinoma	2	Y		Nielsen[36] and Norris et al.[38]
Germ cell origin				
Dysgerminoma	13	Y	Y	McEntee,[2] Nielsen,[36] Norris et al.,[38] Andrews et al.,[39] Dehner et al.,[40] Gruys et al.,[41] and Sbernardori and Nava[42]
Teratoma	3	N		
Sex cord origin				
Granulosa cell tumor	20	Y	Y	Arnbjerg,[22] Nielsen,[36] Norris et al.,[38] Baker,[44] Gelberg and McEntee,[46] and Fukushima and Konishi[48]
Interstitial cell tumor	6	N	Y	Norris et al.[38] and Gelberg and McEntee[46]
Androblastoma/ Sertoli cell tumor	4	N		McEntee[2] and Hofman et al.[49]
Mesenchymal origin				
Leiomyoma	1			McEntee[2]
Metastatic origin				
Uterine adenocarcinoma	1	(Y)		Nielsen[36]

* Presence (Y) or absence (N) of metastasis or steroid-induced clinical signs has been reported.

tumors usually are slow-growing and unilateral, and range in diameter from 2 to 30 cm; grossly they appear as nodular masses with bulging, tan cut surfaces with hemorrhage and necrosis.[40] Dysgerminomas generally occur in cats over 6 years of age, although bilateral dysgerminomas weighing 40 g each were reported in a 1-year-old shorthaired cat presented for routine OHE.[39] Ages of six queens with dysgerminomas from a series of 22 feline ovarian neoplasms were 1, 2, 5 (two animals), 7, and 18 years.[2] Metastasis by extension to adjacent omentum was reported in a 7-year-old affected Siamese queen with signs of masculinization and in a 2-year-old cat from which the ovaries, the tumor, and the uterus had been removed at an earlier OHE.[2,40]

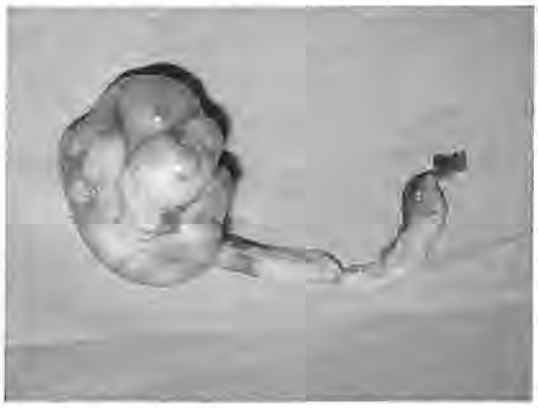

Figure 31–8. Gross appearance of a unilateral granulosa cell tumor in the right ovary of a 4-year-old domestic longhair cat presenting for OHE because of hemorrhagic vulvar discharge and straining.

TERATOMA (DERMOID CYSTS)

Teratomas are germ cell tumors that show somatic differentiation beyond the primordial germ cell stage into masses with three germ layers (ectoderm, mesoderm, entoderm) (Fig. 31–7).[38,41] They have been called dermoid cysts, because the masses typically include cysts lined by hair and keratinized squamous epithelium; they often contain sebaceous fluid, sweat glands, cartilage, fat, muscle fiber, bone, nervous tissue, connective tissue, teeth, and glandular epithelium. Feline teratomas have been described ranging form a 1.5-cm benign cystic teratoma to a 1000-g unilateral solid ovarian teratoma.[2] Teratomas have been reported in cats from 2 to 6 years of age.[38,41,43] In one case, a solid teratoma was present with a unilateral dysgerminoma. In another, an extragonadal teratoma was present between the adrenal glands in a domestic shorthaired cat from which a dysgerminoma had been removed 3 months earlier; metastases of the dysgerminoma were present in abdominal and thoracic lymph nodes, lung, liver, adrenals, and kidneys at the time the teratoma was removed. Human teratomas have been described in metastases of dysgerminomas.[41] A 2-year-old domestic shorthaired cat died suddenly of intra-abdominal hemorrhage from rupture of blood vessels associated with a 1.5-cm-diameter teratoma.[40]

Figure 31–7. Gross appearance of a teratoma in a feline ovary.

Sex Cord Stromal Origin Feline Ovarian Tumors

GRANULOSA CELL TUMOR

The granulosa cell tumor arises from ovarian sex cords; it is the most common primary ovarian tumor in the cat.[2,22,36,38,44–48] Granulosa cell tumors in female cats (Fig. 31–8) usually are functional, producting signs of prolonged estrus or cystic endometrial hyperplasia, and occur in cats between 3 and 16 years of age.[2,22,44,45,47] Reported clinical signs include abdominal enlargement due to a palpable mass, ascites, vomiting, and alopecia. Tumor size may reach 5 × 5 × 5 cm. Metastases to regional lymph nodes, omentum, liver, spleen, kidneys and/or lungs were reported in six of eight cases.[22,38,44,45] One bilateral and one extraovarian granulosa cell tumor (located at the upper end of the left uterine horn with no connection to the left ovary) have been reported in the cat.[22,38] Some granulosa cell tumors produce estrogen, progesterone, or both. These tumors therefore may occur with paraneoplastic syndromes of prolonged estrus (vulvar swelling, occasional sanguineous vulvar discharge, attraction of

males), estrogen-induced aplastic pancytopenia, and/or progesterone-induced endometrial hyperplasia and pyometra. In a review of nine cats with granulosa cell tumors, at least four were reported to have signs of abnormal estrus, and four of the tumors had metastasized.[46]

INTERSTITIAL CELL TUMOR

Six cases of interstitial cell ovarian tumors have been reported in the queen.[38,46] The tumors consisted of solid, orange-yellow to brown masses of tissue up to 5.1 × 3.6 cm. One of these occurred in a 9-year-old cat with prolonged estrus.[38]

ANDROBLASTOMA/SERTOLI CELL TUMOR

Ovarian tumors arising from sex cord stroma that demonstrate a histologic pattern similar to that of Sertoli cell tumors of the testes have been reported in four female cats.[2,49] One occurred in the right ovary of a 6-year-old domestic shorthaired queen with cystic endometrial hyperplasia; metastases were not reported.[49] Three of these cases also were described as granulosa cell tumors with interstitial gland transformation.[2]

Excision biopsy is the treatment of choice with all ovarian tumors of the queen. Complete OHE is recommended, because occasional tumors occur bilaterally, occasional extension to the uterus may occur, and cystic endometrial hyperplasia leading to pyometra may be present. Care should be taken not to rupture fluid-filled cysts in the tumor, as abdominal implantation of tumor cells from such fluid may occur. Prognosis is good if metastasis has not occurred and poor if it has.

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Chapter 32

Disorders of the Feline Uterus and Uterine Tubes (Oviducts)

Congenital Anomalies

Uterine and uterine tube anomalies that have been described in the cat include segmental aplasia (uterus unicornis, segmental aplasia of the uterine horn, aplasia of the caudal uterine body and/or cervix), uterine hypoplasia, partial fusion of the uterine horns, and duplication of a uterine horn.[1-8] Uterus unicornis was reported in 21 cases from approximately 21,000 admissions.[8] In all cases, one ovary, uterine tube, and uterine horn were normal, and the others were absent except for a thread-like remnant of the uterine horn; right and left horns were affected equally.[8] Uterus unicornis and unilateral renal agenesis were reported in a 7-month-old female domestic shorthair cat with two normal ovaries.[7] Three embryos were found in the left uterine horn of the cadaver of an adult queen during routine laboratory dissection; the right ovary, uterine tube, uterine horn, right half of the body of the uterus, right broad and round ligaments, right kidney, and right ureter were absent, and the right half of the vesicular trigone was undeveloped.[9] The right renal, ovarian, and uterine arteries and veins also were absent. The ipsilateral kidney often is absent in unilateral uterine agenesis in the cat.[2,7,9] This condition is diagnosed as an incidental finding at ovariohysterectomy (OHE) or necropsy.

Segmental aplasia of part of one uterine horn or of the uterine body and cervix has been reported in the cat; signs include failure to conceive when bred or abdominal distention with a fluid-filled uterus (Fig. 32–1).[3,5] Surgical correction of these defects has not been described in this species; usual treatment is OHE.

Bloom reported myometrial fusion of uterine horns that began at the bifurcation and extended cranially for several centimeters.[1]

Uterine hypoplasia was present in a 37,XO Burmese cat and also in a 1-year-old cat with primary anestrus and rudimentary ovaries that was not karyotyped.[6,10]

Congenital cysts of the mesosalpinx that arise from mesonephric duct remnants (wolffian rests) were reported in five cats ranging from 1 to 15 years of age.[11] These are simple tubular structures lined by low columnar to cuboidal cells, encountered incidently at ovariohysterectomy. Although these remnants may occur in concert with hyperplastic or inflammatory changes in the uterus, they are not known to predispose to such changes.

Hyperplasia of the Uterus and Uterine Tube

Multiple, broad-based or pedunculated hyperplastic endometrial polyps have been reported in 14 cats ranging in age from 4 to 15 years.[12] The polyps protruded into the uterine lumen, and were lined by endometrial epithelium varying from columnar to squamous, depending on stage of the estrous cycle. Two of the 14 cats had concurrent pyometra.

Bilateral papillary hyperplasia and stromal edema of the uterine tube was observed in an infertile 3-year-old Himalayan queen with bilateral occlusion of the tubes at the uterotubal junction (Fig. 32–2). Villous hyperplasia of the oviduct and "blocked" uterine tubes have been reported in the queen, and uterotubal junction obstruction also has been de-

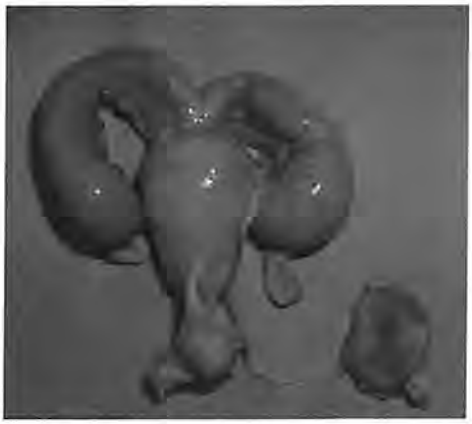

Figure 32–1. Uterus with imperforate cervix and distended uterine body and horns from a 1½-year-old domestic shorthair cat presenting for routine ovariohysterectomy. The cat had been cycling regularly prior to surgery, and had never been pregnant. The distended uterus was palpated as a caudal abdominal mass prior to surgery.

scribed.[13,14] Diagnosis is based on histologic examination of the tissues involved.

Hydrometra/Mucometra

Hydrometra, and mucometra, the accumulation of noninflammatory, clear to slightly cloudy, watery to viscid, sterile fluid in the uterine lumen, occurs occasionally in the cat.[1,15–17] Causes include impatency of the vulva, vagina, cervix, or uterus resulting from congenital anomaly, neoplasia, inflammation, and scarring or accidental ligation. Fluid volume in the uterine lumen may reach 500 ml, and distention of the uterine body and/or horns may be diffuse or segmental (Fig. 32–3).[16] The uterine wall becomes thin as the uterus distends and the endometrium atrophies.[18,19] Clinical signs of illness may be absent or may be related to abdominal distention. Diameter of the affected uterine horn may reach 4.5 cm. Endometrial polyps ranging from 1.1 to 2.0 cm in length have been reported lining the uterus of a queen with hydrometra.[15] An enlarged uterus may be detected by palpation, abdominal radiography, or ultrasonography. Treatment is OHE. Hydrosalpinx has been reported in the presence of hydrometra.[1]

Figure 32–3. Segmental hydrometra of the uterine horn in a 1-year-old female cat.

Cystic Endometrial Hyperplasia/ Pyometra Complex

Feline pyometra is a uterine inflammatory disorder characterized by cystic endometrial hyperplasia (CEH), a sequel to progesterone

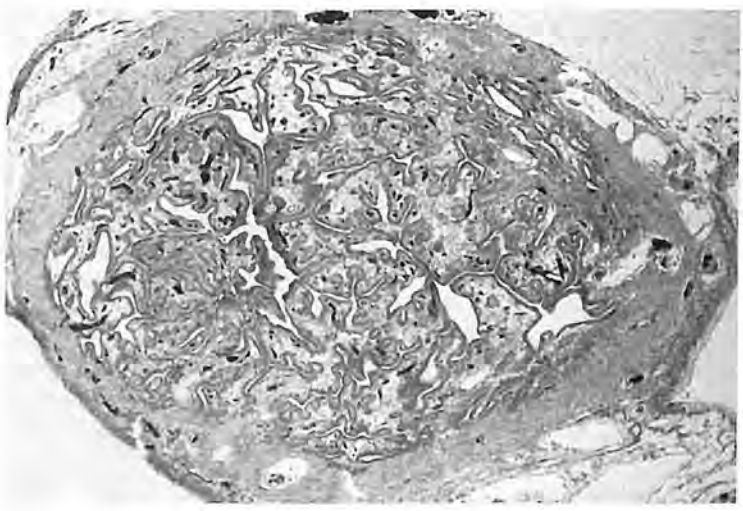

Figure 32–2. Histologic appearance of bilateral papillary hyperplasia and stromal edema of the uterine tube in an infertile 3-year-old Himalayan queen with bilateral occlusion of the tubes at the uterotubal junction. Hematoxylin and eosin stain.

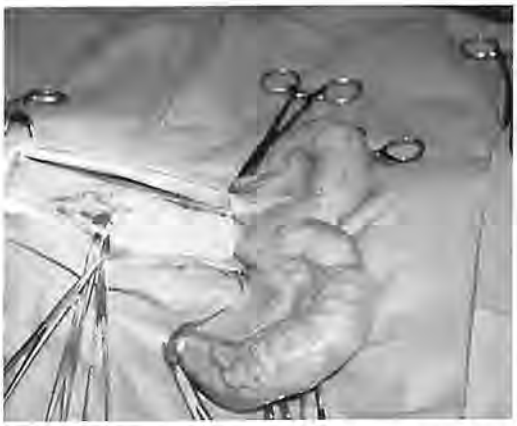

Figure 32–4. Diffuse enlargement of the feline uterus due to pyometra.

stimulation of the endometrium, and by ascending uterine infection with vaginal bacteria.[20–23] It is one of the most common and important reproductive disorders of the queen. Feline pyometra may involve diffuse or segmental enlargement of the uterus (Figs. 32–4 and 32–5).

Pathogenesis

Dow described 91 cases of the CEH/pyometra complex in the cat, and categorized affected animals as belonging to one of four groups based on increasing severity of histologic lesions of the endometrium (Table 32–1).[24] Pathologic lesions ranged from simple CEH (Fig. 32–6) to endometrial atrophy (Fig. 32–7). Because seventy-seven per cent of the affected queens with known breeding history were nulliparous and because many cats in the study were reported to live in single-cat households without opportunity for copulation, presence of corpora lutea in the ovaries of at least 42 of the cats suggests that spontaneous ovulation may occur in this species. Ovulation results in secretion of progesterone by the corpora lutea; over time, progesterone stimulation of the endometrium may lead to cystic endometrial hyperplasia. In a study of 44 cats with pyometra, 19 had follicular phase ovaries, and 25 had luteal phase ovaries; some cats housed alone without opportunity for copulation had corpora lutea present, suggesting presence of spontaneous ovulation.[25] In a report of 79 cats with pyometra from a single colony, 43 (54 per cent) had never been bred.[26] Cats with pyometra may have elevated (i.e., >2 ng/ml) serum progesterone concentration, or may have low/baseline (i.e., <2 ng/ml) serum progesterone concentration. Cystic endometrial hyperplasia and superimposed inflammatory changes of the uterus may persist beyond the end of the luteal phase.

CEH/pyometra also has been observed in intact and ovariectomized queens treated with exogenous progestogens, such as medroxyprogesterone acetate or megestrol acetate.[27–31] Histologic features of the feline uterus with pyometra include cystic endometrial hyperplasia, endometritis, uterine polyps, and endometrial atrophy.[25,32]

The bacterium most often isolated from fluid in the pyometritic uterus of the cat is *Escherichia coli*, which also is one of the most commonly isolated organisms from the vagina of healthy cats.[21,24–26,33–37] *Streptococcus* sp., *Staphylococcus* sp., *Pasteurella* sp., *Klebsiella* sp., *Moraxella* sp., and *Pseudomonas* sp. also have been cultured from feline pyometritic uteri, and from the vaginas of normal cats.[37] Uterine tuberculosis and brucellosis acquired from infected bovine tissues also have been reported in the cat, but these are rare in the United States.[38–40]

Dow attempted to induce pyometra in ovariectomized queens by intrauterine injection of uterine contents from spontaneous cases; infection was established in 5 of 12 queens pretreated with progesterone, but not in eight cats pretreated with estrogen.[41]

These data support a pathogenesis of CEH in the luteal phase uterus of the middle-aged nulliparous queen followed by ascending bacterial infection and signs (e.g., depression, anorexia, purulent vaginal discharge, abdominal distention) referable to duration of illness and degree of cervical patency. While *E. coli* and

Figure 32–5. Segmental pyometra of the feline uterus, following hysterectomy. (From Bradley RL, Olson PN: Feline pyometra. Feline Pract 9:17–22, 1979, with permission.)

Table 32–1. Clinical and Pathologic Features of the Cystic Endometrial Hyperplasia/Pyometra Complex in the Cat, As Described by Dow Based on Histologic Appearance of the Uterus

Parameter	Dow Stage I (n = 20)	Dow Stage II (n = 39)	Dow Stage III (n = 10)	Dow Stage IV (n = 22)
Histologic appearance of reproductive organs	Cystic endometrial hyperplasia (CEH)	CEH; acute endometritis in superficial half of endometrium; corpora lutea (CL) in 33; cystic follicles in 4; normal ovaries in 2.	CEH; subacute endometritis with plasma cell and macrophage infiltration of superficial half of endometrium; focal scattered endometrial abscesses and myometrial hypertrophy; recent or regressing CL in 9.	Chronic endometritis with thin, atrophic endometrium; cervical fibrosis.
Clinical signs/history	None in 16; vulvar hemorrhage in 4	All were clinically ill; depression; anorexia; vulvar discharge (green, brown or red); 18 in estrus <2 mo prior to presentation; 3 to 28 d duration.	Illness less marked and of longer duration than in Stage II; depression; anorexia; vulvar discharge (green, brown or red); weight loss; 5 in estrus <2 mo prior to presentation; >2 mo duration.	Varied inversely with degree of cervical patency; inappetance, weight loss, intermittant vulvar discharge, and abdominal distention in 14; more serious illness in 8, with abdominal enlargement, scant (2) or no (6) vulvar discharge, vomiting, and collapse, and/or death (2).
Laboratory findings	Regenerative anemia (4)	WBC = 23,000 to 74,000/μl	WBC = 21,000 to 32,000/μl	WBC = 24,000 to 61,000/μl
Uterine fluid findings	Sterile in 19; *E. coli* in 1	Volume = 3–1500 ml; *E. coli* in 32; β-hemolytic streptococci in 6; sterile in 1	*E. coli* in all; β-hemolytic streptococci in 1	*E. coli* in 18; β-hemolytic streptococci in 6; sterile in 1

From Dow C: The cystic hyperplasia-pyometra complex in the cat. Vet Rec 74:141–147, 1962, with permission.

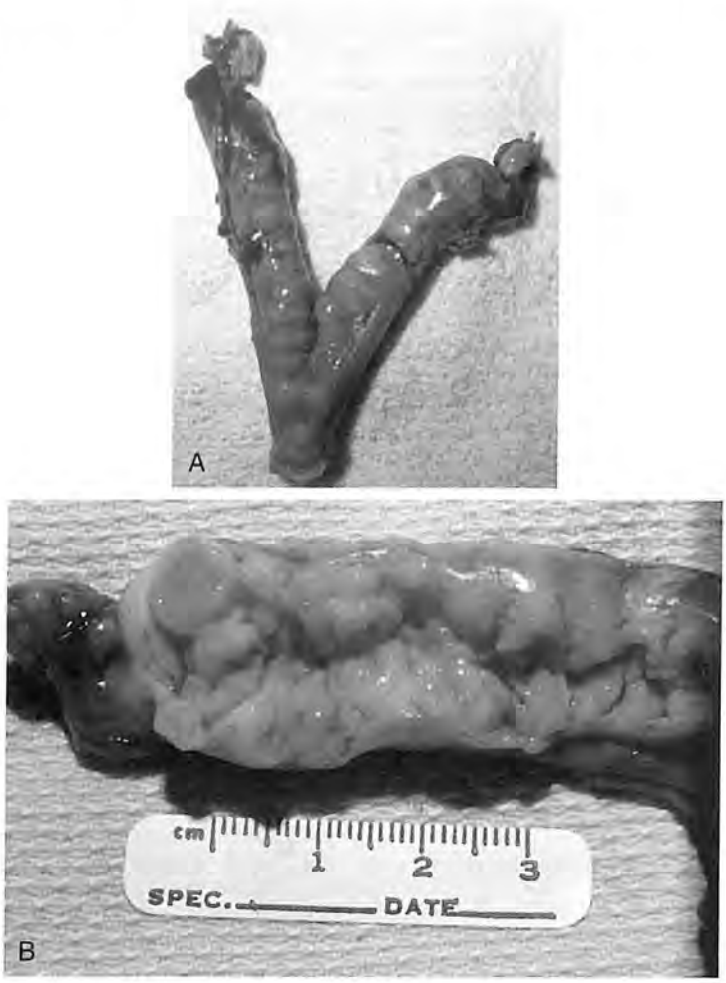

Figure 32–6. Gross appearance of cystic endometrial hyperplasia (Dow Stage I) of the feline uterus, viewed from the serosal (**A**) and mucosal (**B**) surfaces.

β-hemolytic *Streptococcus* sp. are the bacteria most commonly isolated from the cat with pyometra, other bacteria may occur alone or as mixed bacterial populations in the affected uterus. Some feline pyometra uterine fluids have been reported as sterile; some of these have been cultured for aerobic bacteria alone. Mycoplasma may be normal feline vaginal flora incriminated in ascending uterine infection in the cat; some feline pyometra cultures historically reported as sterile may, in fact, have contained mycoplasma organisms that are not readily identified on routine aerobic bacterial culture. The role of anaerobic bacteria in feline pyometra is unknown; infection with anaerobic bacteria has been observed in cases of canine pyometra. Extragenital lesions that may occur in affected queens with pyometra include myeloid metaplasia of the liver, kidneys, spleen, and adrenals; degenerative lesions in the kidney, liver, and spleen; and congestion of the lungs and lymph nodes.[1]

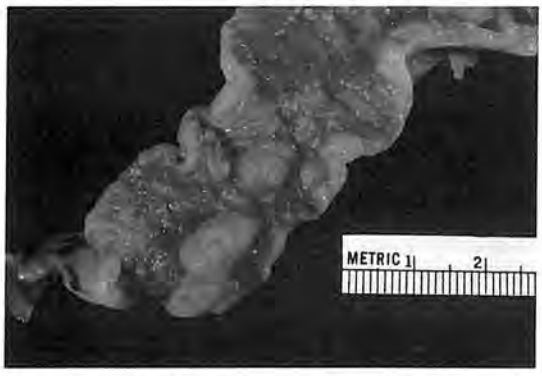

Figure 32–7. Atrophic endometritis (Dow Stage IV) in a 14-year-old Persian cat.

In neutered queens, or those with ovarian remnants, uterine stump tissue remaining after subtotal hysterectomy can be affected by progestogen therapy and undergo histologic changes of CEH and inflammation. Clinical signs are similar to those in the intact queen

with pyometra, and may include presence of a purulent or sanguineous vulvar discharge with variable depression, anorexia, weight loss, and a palpable caudal abdominal mass.

History and Clinical Signs

Age of affected animals ranges from 1 to 20 years, with average age of about 7.5 years.[24,26,32] Estrus occurred within the 4 weeks preceding presentation in 49 of 63 (78 per cent) affected cats.[26] Clinical signs of pyometra in 183 cats included purulent vulvar discharge (68 per cent), anorexia (40 per cent), abdominal distention (40 per cent), dehydration (33 per cent), lethargy (32 per cent), pyrexia (20 per cent), vomiting (16 per cent), polyuria/polydipsia (9 per cent), and weight loss (3 per cent).[26] The uterus was palpably enlarged in 39 per cent.[26] Leukocytosis was present in 66 per cent (109 of 163) of affected cats examined, leukopenia was present in 5 per cent, and a normal white blood cell count was present in 28 per cent. Most common serum chemistry abnormalities included hyperproteinemia, hypokalemia, and azotemia.[26] Serum progesterone exceeding 1 ng/ml is present in affected cats with functional corpora lutea.[25,26]

Diagnosis

Presumptive diagnosis of pyometra in the intact queen is based on signalment, history of previous estrus and clinical signs, physical examination, hemogram, and presence of a purulent vulvar discharge and/or enlarged uterus in the nonpregnant animal (Table 32–2). Vulvar discharge, if present, should be cultured for presence of aerobic bacteria and mycoplasma, and sensitivity to antibiotics should be determined for organisms isolated. Abdominal radiography or ultrasonography is indicated to define uterine size and shape for initial diagnosis, to rule out pregnancy (ultrasonography, after 21 days following estrus), and to establish a baseline for uterine size for the patient destined for medical management. Measurement of serum progesterone concentration may be indicated in the patient managed medically, in order to assess whether she is in the luteal phase at time of treatment onset, and in order to assess response to treatment.[42]

Treatment

Recommended treatment for CEH/pyometra in the queen is OHE with concurrent fluid and antibiotic therapy. Recommended treatment for uterine stump granuloma is surgical removal of the granuloma and examination of the ovarian stumps for presence of retained ovarian tissue. The progressive and recurrent nature of this disorder in the cat, the absence of a safe and effective cervical dilating medication for use in the patient with a closed cervix, and presence of a ruptured uterus (7 of 183), or uterine torsion in some affected animals are indications for complete OHE as treatment for this disorder.[33] Cats with closed-cervix pyometra should be considered emergency patients requiring immediate surgery. Cats with open-cervix pyometra (diagnosed due to presence of a purulent vulvar discharge) are not emergency patients, and can undergo surgery as soon as is convenient following initiation of antibiotic therapy. In one study of 183 cats with pyometra confirmed at surgery, 8 per cent (15 of 183) died or were euthanized postoperatively.[26] Twenty per cent (38 of 183) had postoperative complications, which included anorexia (n = 20), lethargy (n = 6), anemia (n = 4), pyrexia (n = 3), vomiting (n = 3), icterus (n = 3), subcutaneous emphysema (n = 1), or sponge foreign body (n = 1); clinical signs resolved in all of these cats.[26]

In females with reproductive value and an open-cervix pyometra (diagnosed by the presence of a purulent vulvar discharge), uterine evacuation with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a smooth muscle contracting drug, may be used concurrently with antibiotic therapy. Recommended doses of the tham-salt of $PGF_{2\alpha}$ reported to be effective in treatment of feline open-cervix pyometra range from 0.05 to 0.5 mg/kg subcutaneously (SC) once or twice daily for 2 to 5 days until uterine size decreases to normal.[34,43–47] Prostaglandin analogues should not be used in the cat, because safe and effective doses have not been established. The authors recommend 0.25 mg/kg natural $PGF_{2\alpha}$* administered SC twice daily for up to 5 days or until uterine size is normal, whichever occurs first. Within 1 to 60 minutes of drug injection the treated queen may show vocalization, panting, restlessness, grooming, tenesmus, salivation, vomition, defecation, or diarrhea.[34] Reactions diminish in severity following subsequent treatments. Amount of vulvar discharge may increase during the first few days of treatment.

When administered early in diestrus, $PGF_{2\alpha}$ is not luteolytic in the queen.[48] Doses of 0.5 or

* Lutalyse; Upjohn Company, Kalamazoo, MI.

Table 32-2. Diagnostic and Therapeutic Plan for Pyometra in the Cat

History

1. Obtain general health history.
2. Obtain history of time of last estrus, previous pregnancies, previous reproductive disorders.

Physical

1. Perform general physical examination; assess pulse rate and character, hydration status, capillary refill time, mucous membrane color, body temperature.
2. Inspect vulva. Note quantity, consistency, and color of vulvar discharge, if present.
3. Assess uterine size and consistency per abdominal palpation; note whether palpation of uterus is followed by increased vulvar discharge.

Clinical Evaluation

1. Examine vulvar discharge cytologically for presence of inflammatory cells.
2. Culture vulvar discharge for aerobic and mycoplasma bacteria; request sensitivity information for aerobes.
3. Confirm assessment of uterine size with radiology or ultrasonography.
4. Draw blood for complete blood count, serum chemistries profile, and serum progesterone concentration.

Treatment

1. Initiate antibiotic therapy with any broad-spectrum antibiotic effective against gram-negative rods; change antibiotic if needed when culture results return.
2. Correct fluid/electrolyte imbalance, if any.
3. Choose medical or surgical treatment:
   a. Choose surgical treatment (ovariohysterectomy) for all cases of closed-cervix pyometra (no vulvar discharge); surgery should be performed quickly once the diagnosis is confirmed, because closed-cervix pyometra may be a life-threatening emergency.
   b. Choose surgical treatment (ovariohysterectomy) for most cases of open-cervix pyometra (vulvar discharge), because of the likelihood that pyometra will recur following medical therapy. Open-cervix pyometra usually is not a life-threatening emergency in the queen, so surgery should be performed as quickly as is convenient.
   c. Choose medical treatment for:
      i. the young to middle-aged valuable purebred queen in which the owner wishes to attempt future breeding, and
      ii. the occasional older queen that is a very poor surgical risk in which owner understands the risk of recurrence of pyometra and potential need to retreat following a later estrus.
   d. If indicated, administer medical treatment as 0.25 mg/kg tham-salt of prostaglandin $F_{2\alpha}$ SC twice daily for up to 5 days, depending on response of uterine size to myometrial contraction and uterine evacuation induced by prostaglandin.

1.0 mg/kg SC given twice, 24 hours apart after day 40 of pregnancy, or five daily intramuscular (IM) injections of 2 mg per cat after day 33 of pregnancy, have been reported to result in complete luteolysis and abortion within 1 to 6 days of the first injection, suggesting that such treatment also may be effective for inducing luteolysis in pyometra.[49,50] At the 2-mg dose, side effects started 10 minutes after injection and lasted for about an hour. Luteolysis in some but not all queens has been reported following administration of multiple injections up to 0.25 mg/kg each.[44] Because of the small amount of information available on induction of luteolysis in the cat with pyometra, the authors strongly caution clinicians not to use this potentially dangerous drug for luteolysis in this species unless indication is clear and the owner understands the risks. Uterine evacuation alone usually is very beneficial to the cat.

Prognosis for successful reproduction following medical treatment of pyometra in the queen depends on severity of endometrial pathology at and following treatment. The majority of cats reported in the literature treated with $PGF_{2\alpha}$ have been able to conceive and deliver kittens following treatment.[44,46,47] In one study, 18 of 20 queens (90 per cent) bred following prostaglandin therapy for pyometra conceived and delivered live kittens; these 20 came from a study population of 21 cats ranging in age from 10 to 96 months (mean = 32.4 months).[34] Older affected queens might be suspected of having more severe endometrial pathology and might, therefore, have a poorer prognosis for future fertility than younger queens.

Inflammation of the feline uterine tube, salpingitis, usually is purulent, and usually occurs secondary to uterine inflammation.[1,11] *E. coli* pyosalpinx has been observed in a 1-year-old cat, and bilateral pyosalpinx has been observed in a 4-month-old kitten with purulent uterine infection.

Neoplasia of the Uterus/ Uterine Tubes

Uterine tumors constitute 1 to 2 per cent of tumors of the female reproductive organs of the cat (including mammary glands), or 0.2 to 0.4 per cent of all feline tumors.

Both epithelial (e.g., adenoma, adenocarcinoma) and mesenchymal (e.g., fibroma, fibrosarcoma, leiomyoma, leiomyosarcoma, lipoma, and lymphosarcoma) tumors have been reported in the feline uterus; these may range in diameter from less than 1 cm to greater than 10 cm.[4,51–56] Uterine leiomyomas and leiomyosarcomas are the most common.[53,57] Metastatic uterine adenocarcinomas have been described in the adrenals, brain, diaphragm, eyes, heart, kidney, liver, lung, ovary, and regional lymph nodes.[53,58,59] A mixed mesodermal tumor, with both carcinomatous and sarcomatous elements, was described in a queen with metastases to the abdominal and thoracic lymph nodes and lungs; other mixed histologic types have not been reported in the cat.[60] Primary uterine tumors occur in cats between 5 and 12 years of age. Uterine tumors often are diagnosed incidentally at OHE or postmortem.

Clinical signs of uterine adenocarcinomas depend on tumor size and pattern of metastasis. Signs include ascites, anorexia, weight loss, purulent or hemorrhagic vulvar discharge, vomiting, constipation, dysuria, and presence of a palpable abdominal mass.[55,61] Cats with thoracic metastases may have periodic cough; those with tumor spread to the central nervous system may have blindness or motor incoordination.[55,58,62] Diagnosis is based on uterine palpation, abdominal and thoracic radiographs, surgical exploration, and histopathologic examination of tumor tissue. Ultrasonography has been used to detect uterine neoplasia in the diffusely enlarged uterus with pyometra (Fig. 32–8).

The recommended treatment for primary uterine neoplasia without metastasis is OHE. Abdominal and thoracic radiographs are indicated prior to surgery in order to rule out presence of metastatic disease. Wide surgical excision without ovariectomy may be considered with a single leiomyoma if maintenance of the queen's reproductive capacity is essential. Successful treatment regimens for metastatic uterine neoplasia in the cat have not been reported. Prognosis is good for benign tumors (such as leiomyoma) and poor for malignant tumors such as adenocarcinomas when metastases are present.

Figure 32–8. Gross appearance of a uterine leiomyoma in a 9-year-old queen; note distention of uterine horns with fluid.

Tumors of the uterine tubes have not been reported in the queen.

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Chapter 33

Disorders of the Feline Vagina, Vestibule, and Vulva

Congenital Anomalies

Anomalies of the vagina and vulva that have been described in the cat include segmental aplasia of the cranial vagina (müllerian duct system), presence of a common vulvovestibular-anal opening, and rectovaginal fistula.[1] Segmental aplasia causes signs similar to those of uterine body aplasia, such as conception failure in the cycling queen, and abdominal distention with an enlarged, fluid-filled uterus. Impatent vagina has been observed in a 4-year-old queen with pyometra and an imperforate hymen, and in a 3-year-old queen with vaginal distention and pyometra subsequent to one normal litter (J. Holzworth, personal communication, 1982). The latter queen was presumed to have developed adhesions and impatency following the first delivery.

Common vulvovestibular-anal openings (Fig. 33–1) and rectovaginal fistulae may be corrected surgically.[2]

Vaginal Prolapse

Vaginal prolapse (nonobstetric) is rare in the queen, but may occur during estrus or anestrus. A prolapsed vagina removed from a 14-year-old anestrous domestic shorthair cat at ovariohysterectomy had histologic evidence of marked edema with surface ulceration and inflammation; prolapse did not recur following excision. Treatment is surgical excision via episiotomy.

Vaginitis

Primary vaginitis is rare in the cat. Clinical signs include pollakiuria, dysuria, frequent cleaning of the vulva, and vulvar discharge. Diagnosis is made by inspection, culture, and biopsy of the vaginal mucosa using an otoscope speculum in the anesthetized cat. Primary vaginitis is reported to be rare and self-limiting, with no treatment indicated; secondary vaginitis may occur following obstetric or coital trauma, pyometra, or viral rhinotracheitis infection.[3–6]

Differential diagnoses include urinary tract disorder, pyometra, or uterine stump granuloma, which are ruled out on the basis of urinalysis (cystocentesis sample) and palpation, radiographic and ultrasonic imaging of the urogenital tract. Perivulvar inflammation may be caused by injury, urine scald, or primary bacterial infection.

Neoplasia

Vaginal tumors are uncommon in cats, and reports in this species are of single occasional cases in spayed or intact older females (Fig. 33–2).[7–10] The most common primary vaginal tumor type in the cat is the leiomyoma, which may measure up to $7 \times 7 \times 8$ cm.[9,10] No vaginal tumors were observed in series of 464, 395, and 256 feline neoplasms from both sexes.[11–13] Two vaginal leiomyomas were observed in a series of 165 feline tumors (34 of the reproductive organs, including mammary glands).[9] The feline vagina occasionally may serve as a site for metastatic neoplasia.

Clinical signs of vaginal tumors include bulging of the perineal region, prolapse of tumor tissue from the vulva, dysuria, pollakiuria, and constipation. Intraluminal tumors may become infected and cause sanguineous or purulent vaginal discharge. Initial diagnosis

Figure 33–1. Congenital common vulvovestibular-anal opening in a kitten; the urinary catheter has been placed in the external urethral orifice.

Figure 33–2. Vaginal neoplasm in a cat. (From Wolke RE: Vaginal leiomyoma as a cause of chronic constipation in the cat. J Am Vet Med Assoc 143:1104, 1963, with permission.)

is based on palpation and on retrograde vaginography and/or cystourethrography to characterize size and extent of the mass. Abdominal and thoracic radiography to look for tumor metastasis should be performed prior to surgical excision. Exfoliative cytology may be diagnostic and should be performed on accessible masses of the vagina and vestibule. Final diagnosis is based on histopathologic examination after core or excision biopsy.

Vaginal tumors should be treated by surgical excision following thoracic and abdominal radiography to look for metastatic disease. Need for episiotomy depends on the tumor size and location within the vagina or vestibule. Prognosis for cure is good for leiomyomas.

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Chapter 34

Disorders of the Mammary Glands of the Queen

Anatomy of the Normal Feline Mammary Gland

The queen has four pairs of mammary glands. These have been identified by various authors as thoracic, cranial abdominal, caudal abdominal, and inguinal glands, or as two pairs of thoracic and two pairs of abdominal glands, or as paired glands numbered one through four, from cranial to caudal.[1,2] The authors prefer the latter terminology. The nonlactating mammary gland in the intact or neutered queen is occupied by a very small amount of dense stroma and fat, not by glandular tissue, and, except for the teat, usually cannot be distinguished from adjacent subcutaneous tissue.[1] The teat contains a relatively large amount of smooth muscle arranged in three layers; the distal third of the teat is occupied by four to eight teat ducts, lined by squamous epithelium and opening in a roughly circular pattern on the end of the teat. Proximally, each teat duct connects with a teat sinus, which continues into a lobule of the gland. Outside the lining of the sinus, and continuing into smaller branching ducts from which secretory alveoli open, are contractile, myoepithelial cells.[1]

Figure 34–1 depicts the lymphatic drainage and blood supply of the four pairs of mammary glands of the cat.[2] Pairs 1 and 2 have a common lymphatic system, and drain into the axillary lymph nodes. Pairs 3 and 4 also have a common lymphatic system, and drain into the superficial inguinal node. The lymphatics infrequently cross the midline, and rarely cross between glands 2 and 3.[2] Small veins draining the mammary glands do cross the midline, however, and may be responsible for spread of malignant mammary tumors in pairs of glands.[3,4] Glands 1 and 2 are served by the axillary vessels, the intercostal vessels (laterally), and the internal thoracic vessels (medially); direct drainage of glands 1 and 2 through the chest wall via the internal thoracic or intercostal veins may lead to mammary tumor spread to the thoracic cavity. Glands 3 and 4 are supplied by the cranial and caudal superficial epigastric vessels.[1,2]

Mammary Hypertrophy

Mammary hypertrophy (i.e., mammary hyperplasia, mammary dysplasia, fibroadenoma complex, mammary fibroadenomatosis, fibroepithelial hyperplasia, fibroglandular mammary hypertrophy) was first reported in 1973 as a benign fibroglandular proliferation of one or more mammary glands of the young queen (Figs. 34–2 and 34–3).[5–17] Later reports documented presence of similar mammary changes in male and female cats of any age that were treated with progestogens.[11,13,14,18–21] Affected mammary glands may be diffusely enlarged (Fig. 34–2) or may contain localized masses of variable size that cannot be distinguished grossly from mammary neoplasms.

The condition usually occurs in young (13 weeks to 2 years of age), pregnant or nonpregnant intact queens under the influence of luteal progesterone; one case was reported in a 5-month-old queen, never observed to be in estrus, that aborted two fetuses shortly after examination for the mammary enlargement.[11] Occasionally, the condition is seen in older (9 to 12 years) pregnant queens.[11,13] In most of these cases, the condition is characterized by rapid (over 2 to 5 weeks) diffuse enlargement of all of the mammary glands (Figs. 34–2 and

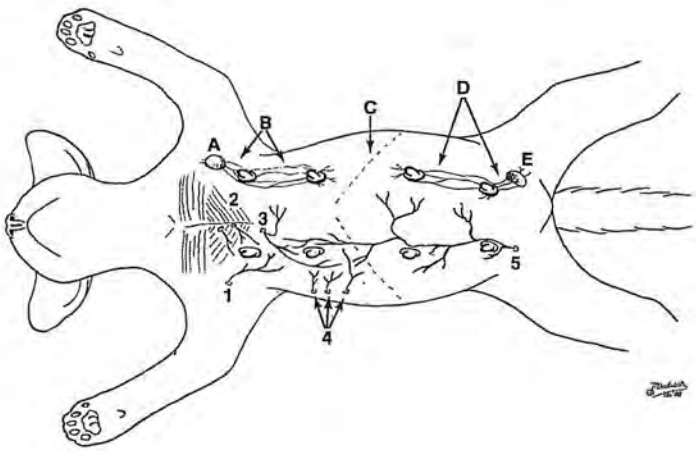


Figure 34–1. Blood supply and lymphatic drainage of the four pairs of mammary glands of the cat. The lower part of the picture depicts the venous drainage: 1, axillary; 2, branches of the internal thoracic veins penetrating the pectoral muscles; 3, cranial superficial epigastric; 4, penetrating intercostals; 5, caudal superficial epigastric. The upper part of this picture depicts the lymphatics and their relationship to the diaphragm and regional lymph nodes: A, axillary lymph node; B, lymphatics conecting the two cranial thoracic mammary glands and the axillary lymph node; C, diaphragm; D, lymphatic connections between the two caudal abdominal mammary glands and the superficial inguinal lymph nodes; E, superficial inguinal lymph node. (From Ogilvie GK: Feline mammary neoplasia. Compend Contin Educ Pract Vet 5:384–391, 1983, with permission.)

34–3); occasionally, single localized hypertrophic masses may occur.[8,11,15] In one report of mammary hypertrophy in 21 intact queens with no history of exogenous progestogen therapy, 4 were known pregnant and 1 had been in estrus 2 to 3 weeks before onset of mammary enlargement; historic information was not available for 7.[11] Nonpregnant affected queens may have been bred and failed to conceive, or may have ovulated spontaneously (see Chapter 25). Spontaneous remission and complete regression of the mammary enlargement usually follow luteolysis, ovariectomy, spontaneous abortion, or parturition.

Mammary hypertrophy also has been observed in male and female cats of any age (intact or neutered) receiving exogenous progestogens, such as medroxyprogesterone acetate (MPA) or megestrol acetate (MGA); in these cats, one or more nodules, ranging in size from 1 to more than 50 cm^3, may be detected, and, occasionally, transformation of hypertrophic to neoplastic tissue has been suspected.[11,18] In one report of 11 queens (4 intact, 7 spayed)

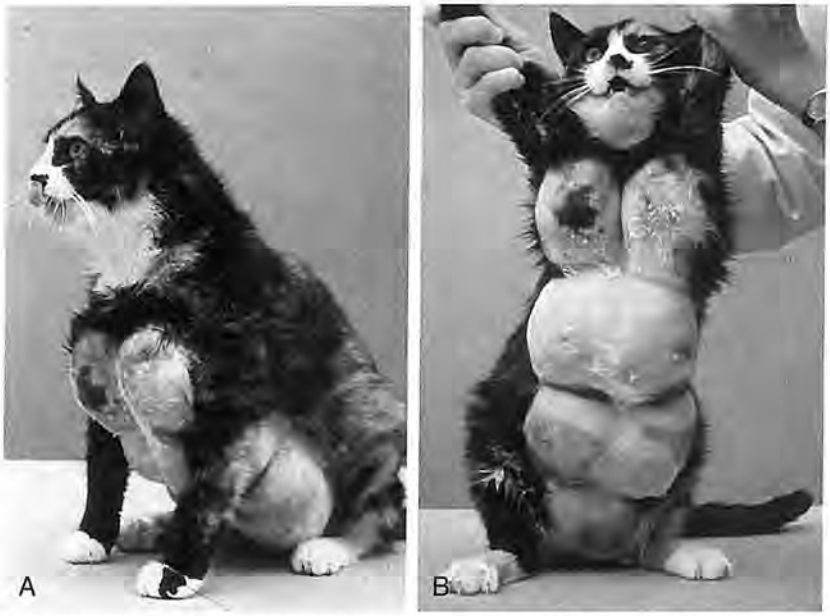


Figure 34–2. A: Gross appearance of diffuse mammary hypertrophy with ulceration in a 10-month-old pregnant domestic shorthair cat. Dimensions of the largest of these glands were 7 × 6 × 4 cm. In **B,** necrosis of the right first mammary gland can be seen. (From Center SA, Randolph JF: Lactation and spontaneous remission of feline mammary hyperplasia following pregnancy. J Am Anim Hosp Assoc 21:56–58, 1985, with permission.)

Figure 34–3. Gross appearance of diffuse mammary hypertrophy in a 10-year-old intact female domestic shorthair cat.

with hypertrophic mammary nodules following MGA therapy, 6 had single nodules, 2 had nodules in two glands, and 3 cats had involvment of three or more glands.[18] Size of the mammary nodules ranged from 1 × 1 × 1 cm to 5.2 × 4.2 × 2.5 cm. The predominant histologic change in these nodules was fibroepithelial hyperplasia (seven), lobular hyperplasia (two), and duct ectasia (two).[18] In these cases, remission usually does not follow cessation of progestogen therapy, and the nodule(s) should be removed surgically in order to confirm the diagnosis and distinguish hypertrophic from neoplastic nodules.

Histologically, masses consist of well-demarcated, unencapsulated, benign fibroglandular proliferation (Fig. 34–4).[3,5,11,12,18,20,22] There usually is no evidence of necrosis or inflammation except in surface epithelium when ulceration occurs in greatly enlarged tissue (Fig. 34–2B), and there usually is no evidence of lactation. In one report, however, of mammary hypertrophy in a 10-month-old pregnant domestic shorthair cat, the cat lactated normally after parturition despite persistence of the hypertrophic mammary enlargement; this queen became pregnant again while lactating, showed some persistence of the hypertrophy during the second pregnancy, lactated normally following parturition, and underwent complete mammary regression after the kittens were weaned.[8] In animals that have received MGA therapy, gross and histologic appearance differs slightly from that of the luteal phase disease, in that papillary outgrowth into the ducts is present.

Progesterone receptors, but not estrogen receptors, have been detected in cytosols or nuclei of affected mammary tissue, and the occurrence of this disorder in luteal phase queens and cats receiving progestogen therapy indirectly supports an endocrine etiology.[11] Occasionally, however, the disorder is reported in the neutered male or female cat in which no progestogen therapy is known to have been administered, in which case etiology is unknown. In the case of spayed female cats with mammary hypertrophy, the possibility of an ovarian remnant should be investigated (see Chapter 31).

Evidence that progestogen therapy can lead to neoplastic transformation of hypertrophic mammary tissue over time is indirect, but is supported by reports of mammary carcinoma developing in female cats given progestogens for 4.5 months to 5.5 years, and by evidence that progestogens may enhance the growth of mammary tumors in this species.[19,23–25] In addition, several reports document the diagnosis of mammary tumors in spayed female and cas-

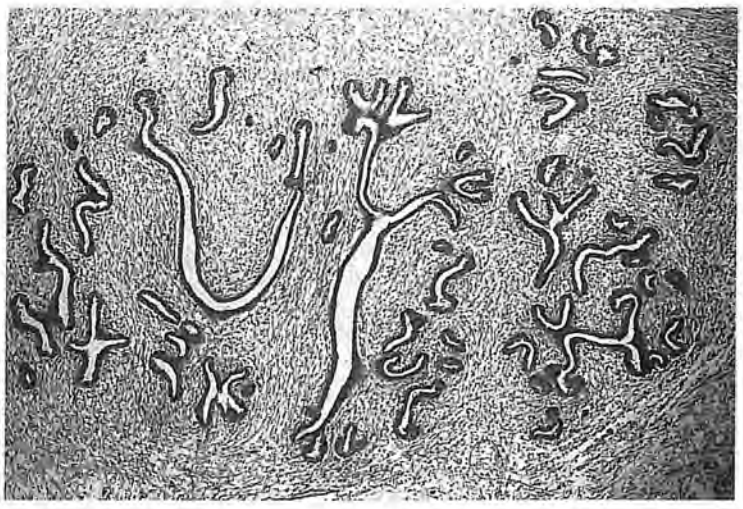


Figure 34–4. Histologic appearance of feline mammary hypertrophy, demonstrating fibroepithelial hyperplasia with abundant stroma surrounding mammary ductules. Hematoxylin and eosin stain.

trated male cats following long-term administration of MGA, whereas males and neutered females usually have greatly reduced risk of spontaneously developing this cancer.[18,25]

Tentative diagnosis of mammary hypertrophy in the cat is based on gross appearance, patient signalment, and history. Because of the highly malignant nature of most feline mammary neoplasms, excision biopsy of all localized swellings is recommended, especially in older animals, following thoracic and abdominal radiography to look for evidence of metastasis (see Mammary Neoplasia below). Biopsy of diffuse mammary enlargement in young queens (Fig. 34–2) usually is not recommended, because signalment, history and physical examination confirm the diagnosis.

Mammary hypertrophy in pregnant or nonpregnant luteal phase queens regresses spontaneously after parturition or luteolysis if untreated, so surgical removal of diffusely affected glands in young queens is not recommended. Ovariohysterectomy also will effect a cure. In progestogen-treated male and female cats, cessation of steroid therapy and complete surgical removal of the mass(es) are recommended, because these masses may not regress spontaneously following removal of the progestogen source.[5–7,10–13,15–17,20,21] Surgical removal also permits histologic confirmation of the diagnosis and distinction from mammary neoplasia. In addition, surgical removal and removal of progestogen therapy may decrease risk of neoplastic transformation of this tissue.

Mammary Neoplasia

INCIDENCE

Mammary tumors are the third most common neoplasm in the female cat, following tumors of the skin and lymphoid tissue.[26] The incidence is 25.4 in 100,000 queens per year. Mammary tumors have been reported to comprise 56 of 395 (14 per cent) of all tumors, and 52 of 68 (76 per cent) tumors of the female reproductive organs, of the queen.[27–29] Less than 2 per cent of feline mammary tumors have been reported in the male cat (see Chapter 42). Intact female cats, like dogs, have a sevenfold greater risk of mammary cancer when compared with neutered females, although mammary tumors have been diagnosed in queens ovariohysterectomized at less than 1 year of age, so that early neutering does not completely eliminate the risk of this tumor.[30,31] There is no evidence of an association between parity and risk of tumor development.[32,33] Incidence increases most dramatically after 6 years and peaks (at 200 in 100,000 queens) at 10 to 11 years, after which it declines.[30,34] In one survey of 136 mammary tumors in 132 cats, Siamese ($n = 52$) were over-represented and had twice the risk of developing mammary tumors as the other breeds (e.g., domestic, Persian, mixed) combined.[25] All four pairs of mammary glands are equally at risk of developing mammary tumors in the queen.[28,29,34]

Feline mammary tumors usually are malignant carcinomas, and the adenocarcinoma is the major type.[3,25,29,35–37] In reports of 236 mammary tumors in cats, 202 (86 per cent) were carcinomas, 2 (0.8 per cent) were sarcomas, 4 (1.7 per cent) were carcinosarcomas, and 28 (12 per cent) were benign tumors.[3,25,29] Of the 202 carcinomas, 179 (89 per cent) were adenocarcinomas, and 23 (11 per cent) were carcinomas (i.e., spindle cell carcinoma, anaplastic carcinoma, and squamous cell carcinoma). Carcinomas usually present as multiple, large, poorly demarcated nodules; they readily invade lymphatics and blood vessels.[32]

TUMOR BEHAVIOR

Metastasis of malignant mammary tumors in the cat is common, and is reported, in various studies, to occur in 50 to 90 per cent of affected animals.[2,22,32,34,38] Metastases were reported in 339 of 657 (51.6 per cent) cases in a literature review of nine studies.[38] Metastases were detected at necropsy in 120 of 129 cats with malignant mammary tumors and were found in the lungs (83.6 per cent) (Fig. 34–5), regional lymph nodes (82.8 per cent), pleura (42.2 per cent), and liver (23.6 per cent).[34] Metastases also have been reported in the spleen, omental fat, pancreas, adrenals, kidneys, ovaries, pericardium, heart, brain, vertebrae, rib, tibia, and tibiotarsal bone.[3,4,29,34,39–41] Despite the highly malignant nature of this tumor, and the frequent metastasis of mammary cancer to bone in dogs and humans, only five cases of skeletal metastasis have been reported in mammary carcinoma in the cat.[38,40,42] In a survey of 132 cats with mammary tumors, 8 had other concurrent neoplasia (i.e., lymphosarcoma [2], thyroid carcinoma, squamous cell carcinoma, endometrial adenocarcinoma, cervical leiomyoma, intestinal adenoma, and malignant granulosa cell tumor).[25] Mammary tumors were described in two cats with single thoracic lesions interpreted radiographically as metasta-

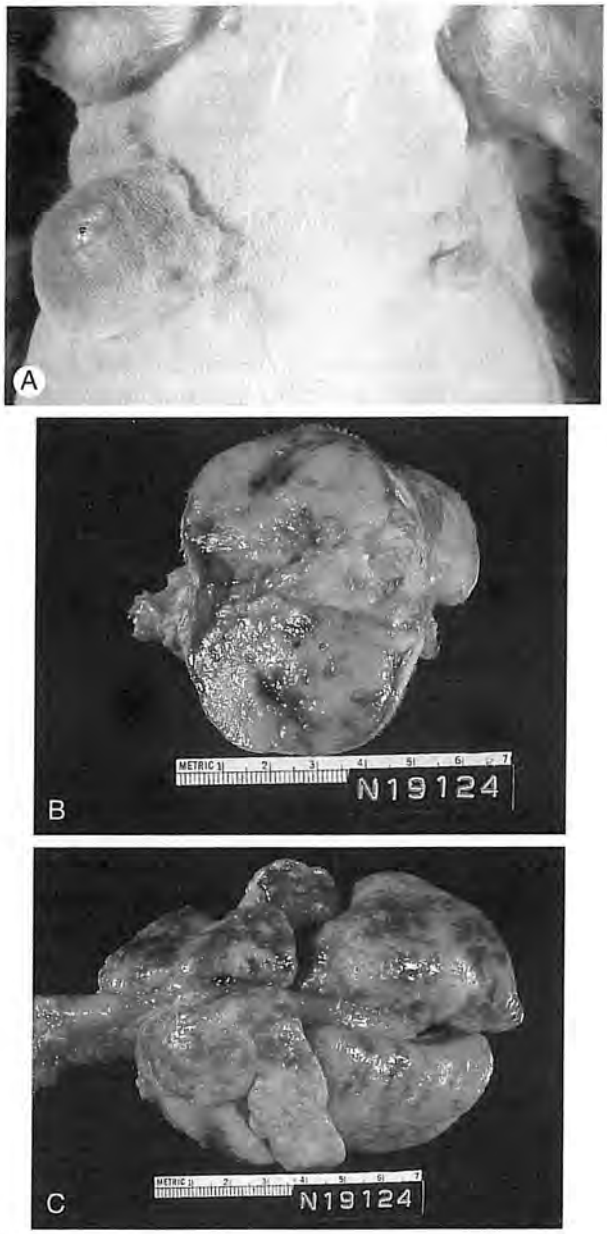


Figure 34–5. Gross appearance of a feline mammary adenocarcinoma in a 10-year-old intact female cat (**A**) and gross appearance of cross section of the tumor after excision (**B**). Gross appearance of the lungs showing infiltration by the mammary adenocarcinoma (**C**) in the affected cat at necropsy.

ses that turned out to be a pulmonary abscess in one and a primary bronchoalveolar adenocarcinoma of the lung in another.[43] Mammary tumors of different histologic type (i.e., mixed mammary tumor and adenocarcinoma or carcinoma and adenoma) have been reported to occur in the same cat.[25,44]

ETIOLOGY

Reproductive hormones have long been suspected of having a role in the etiology of feline mammary tumors, because (1) the great majority of these tumors occur in females, (2) early ovariectomy significantly decreases the risk of acquiring such tumors, and (3) administration of exogenous progestogens is suspected to cause tumor development and growth in this species. Although a hormonal etiology has not been proven, several reports document presence of mammary adenocarcinomas in neutered or intact queens treated with repositol progesterone, megestrol acetate, or medroxyprogesterone acetate.[18,23,28,31,45] In one report, 39

female cats (34 neutered, 5 intact) were treated with oral progestogens for treatment of refractory eosinophilic granuloma; 6 of the 39 (15 per cent) developed pathology of the mammary glands, which included mammary hypertrophy (4), mammary carcinoma (1), and mammary adenocarcinoma (1).[31] Three of seven intact female cats that received semiannual injections of repositol progesterone developed mammary adenocarcinoma.[23,28] Two of 17 megestrol acetate–treated cats (1 neutered female, 1 neutered male) that developed morphologic changes in the mammary glands had carcinomas; the other 15 developed hypertrophic or benign lesions.[18] Progesterone has been reported to augment epidermal growth factor–induced proliferation in feline mammary adenocarcinoma cells cultured in vitro.[46]

Steroid receptor proteins have been measured in the cytosol of mammary tumor tissue in the cat.[47–54] Identification of estrogen receptor (ER)- and progesterone receptor (PR)-rich tumors is clinically important in humans, because these tumors regress after additive (such as antiestrogen) or ablative (e.g., ovariectomy, adrenalectomy, hypophysectomy) endocrine therapy.[55] In the first reports of receptor assay of feline mammary tumors, ER-rich tissues were detected in only 3 of 70 tumors, and PR-rich tissues were detected in 7 of 17 tumors.[47–51] A later study reported ER-positive tissue in 9 of 17 malignant feline mammary tumors and PR-positive tissue in 5 of the 17, although in lower concentrations than those detected in receptor-rich human tumors.[52,53] Although the veterinary literature states that ovariectomy at time of mammary tumor excision is not associated with increased survival time, the appropriate study to demonstrate survival after ovariectomy in estradiol and progesterone receptor–rich patients has not been reported. The presence of progesterone receptors, but not estradiol receptors, in some feline tumors is puzzling, as induction of both estradiol and progesterone receptors is dependent on circulating estradiol; the presence of the progesterone receptor, then, in the absence of the estradiol receptor is unexplained. A small number of feline patients tested have not shown tumor regression after antiestrogen (tamoxifen) therapy, suggesting that these patients are not really estradiol and progesterone receptor–rich with a masked estradiol receptor undetected by assay (S.D. Johnston, unpublished observations, 1982).

Although virus particles have been detected in some feline mammary tumors, little proof exists that viruses are causative agents in this species.[2] Electron microscopic studies have indicated that at least 30 per cent of tumors studied were positive for intracisternal type A viral particles, and C-type virus particles have been detected in electron microscopic examination of feline but not canine mammary tumors.[2,25,56–59] Feline mammary tumor tissue has been reported positive for feline leukemia virus (FeLV) core antigens, and the immunosuppressive effect of FeLV and feline immunodeficiency virus may play a role in mammary tumorigenesis in this species. Some cats have lymphoma concurrently with mammary neoplasia.[56] Viral significance is unknown; one author speculates that these viral particles may be passenger viruses and not the cause of mammary tumors in the cat.[31]

CLINICAL SIGNS

The major clinical sign of mammary gland neoplasia in the cat is presence of one or multiple mammary mass(es) in the middle-aged to older female. More than half of affected cats have multiple gland involvement, and many cats have simultaneous involvement of right and left mammary gland chains.[2,25,31,60] Mammary tumors are firm, well- or poorly demarcated nodules that vary in diameter from several millimeters to 10 cm. They may adhere to the overlying skin, but rarely are adhered to the underlying abdominal wall, even when malignant.[2] Involved nipples may be swollen, and may exude a tan or yellow fluid. At least one quarter of affected patients are reported to have ulcerated tumors.[2] Involved lymphatics may be palpated as thick cords in the subcutaneous tissues, and lymphedema of a limb or limbs may be present.[2,32] Metastasis to regional lymph nodes may or may not be detected by palpation of increased size or abnormal consistency. The wide variety of metastatic patterns with malignant mammary tumors in the queen leads to a variety of clinical signs. These include dyspnea (pulmonary metastases are the most frequent cause of death), cough, lymphedema of one or more limbs, lameness, hindquarter paresis, and emaciation.

DIAGNOSIS

Diagnosis of feline mammary tumors is based on palpation of masses in the gland and on tumor histology. Although fine-needle aspiration, scraping of ulcerated lesions, or cytology of fluid expressed from affected glands may

yield diagnostic cytologic samples from malignant tumors, an absence of neoplastic cells in the sample does not rule out malignancy; and examination of an excision biopsy may be necessary for definitive diagnosis. In general, tumors that are smaller (<2 cm diameter), more highly differentiated (with tubule or acinar formation), with fewer mitotic figures and more regular nuclear size and shape are associated with a more favorable prognosis. Diagnostic evaluation should include thoracic and abdominal radiographs to look for metastases; these may be circular, well-defined radiodensities or a diffuse interstitial pattern with lymphatic infiltration by tumor cells. Bony metastases are visualized radiographically as irregular osteolytic foci. Radioreceptor assay of estradiol and progesterone receptor in tumor tissue may be of interest if available.

Differential diagnoses for feline mammary neoplasia include retention cysts in the postlactational gland and mammary hypertrophy lesions (often nodular) in the female treated with progesterone.[5,11,13,18] Mammary hypertrophy (diffuse or nodular) also may be seen in the luteal-phase cat, but affected patients generally are younger than those in the mammary tumor population.[5,11,13,18] Occasionally, inflammation and necrosis at the tumor site in a lactating gland may be confused with mastitis, although the former is not usually seen with the fever, leukocytosis, and systemic illness seen in the queen with bacterial mastitis alone.[61]

TREATMENT

Treatment of feline mammary neoplasia has been reported using surgical excision, chemotherapy, immunotherapy, and endocrine therapy. The average time between detection of the primary tumor and death in untreated cats is 12 months.[32] Radical surgical excision is recommended in all but the most advanced cases, in which case chemotherapy is a therapeutic option. Incidence of recurrence and metastasis following surgical treatment is high.[25]

Six kinds of surgical excision have been reported in cats with mammary carcinoma: (1) removal of the tumor alone; (2) removal of the gland bearing the tumor; (3) removal of affected gland(s), intervening lymphatics, and regional lymph nodes (en bloc dissection); (4) removal of the gland and adjacent glands if glands 1 and 2 or 3 and 4 are affected (half chain removal); (5) removal of the entire chain of four mammary glands plus or minus regional lymph nodes (unilateral mastectomy); and (6) bilateral mastectomy with removal of axillary and inguinal lymph nodes (radical mastectomy). The most radical of these surgeries, bilateral mastectomy with removal of axillary and inguinal lymph nodes in a two-stage procedure (one chain removed at each surgery), with a 2-week interval between surgeries is recommended for three reasons.[62–64] First, removal and histologic examination of the lymph nodes provides prognostic information (see Prognosis below).[64] Second, radical surgery was associated with a significantly ($p < 0.01$) longer remission duration in 44 affected cats when compared to that of 46 affected cats treated with removal of affected and adjacent glands only; survival time also was longer, though not significantly longer, in the cats treated with the radical surgery.[62] Third, serial histologic examination of all mammary glands and lymph nodes from 45 cats with mammary tumors revealed microscopic evidence of neoplasia in grossly normal glands of eight affected cats.[63] In addition, 22 (49 per cent) of the 45 tumor-bearing cats had metastasis to the regional lymph node(s), although nodes were clinically palpable in only 10. These findings support the use of radical mastectomy and lymphadenectomy in cats with mammary lesions.

Chemotherapy of feline mammary adenocarcinoma has been reported, but, in general, response to treatment has been poor, due to the advanced nature of the disease in treated cats. In addition, effect of treatment has been difficult to assess when various methods of pretreatment surgical excision and staging (such as regional lymph node histology) were used.[32] However, cell lines derived from primary feline malignant mammary tumors are sensitive to antineoplastic drugs (such as 5-fluorouracil, methotrexate, cyclophosphamide, prednisone, vincristine, and doxorubicin) and increased survival time has been reported in some affected cats treated with chemotherapy when compared to nontreated affected cats. Doxorubicin (25 to 30 mg/m^2 intravenously [IV] slowly) and cyclophosphamide (50 to 100 mg/m^2 orally on days 3, 4, 5, and 6 following doxorubicin administration), repeated at 21-day intervals, was reported to induce short-term partial or complete response in 50 per cent of cats with metastatic or nonresectable local disease.[65,66] Median survival times of responders and nonresponders were 283 and 57 days, respectively, and longest survival time was 344 days. Adverse side effects

included profound anorexia and moderate myelosuppression, which were managed by pretreatment with atropine (0.022 mg/kg) IV, reduced doxorubicin dose (20 mg/m^2), and increasing the interval between treatments to 5 weeks.[65]

Immunotherapy of cats with mammary neoplasia has been reported as ineffective in several studies.[32,67–69] Addition of the immunostimulant levamisole, per os, to surgical excision was ineffective in altering recurrence rate or survival time in treated, compared to control, cats treated with surgery alone.[67] Use of the biologic response modifier liposome-encapsulated muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE) to attempt to stimulate monocyte cytotoxic activity following radical mastectomy did not improve the disease-free interval or survival time when compared to placebo-treated cats.[68] In a randomized trial of 47 cats treated with radical mastectomy and lymphadenectomy with and without adjuvent immunotherapy, there was no statistically significant benefit conferred by adding bacille Calmette-Guérin (BCG) alone or BCG plus autochthonous tumor cell vaccine to the surgery; median survival times for cats treated with surgery alone or surgery and BCG was 17.5 months, and for cats treated with surgery, BCG, and tumor cell vaccine was 10.5 months.[69]

Antiestrogen (such as tamoxifen) therapy is of therapeutic value in women with estrogen receptor–rich tumors. Tamoxifen was not,

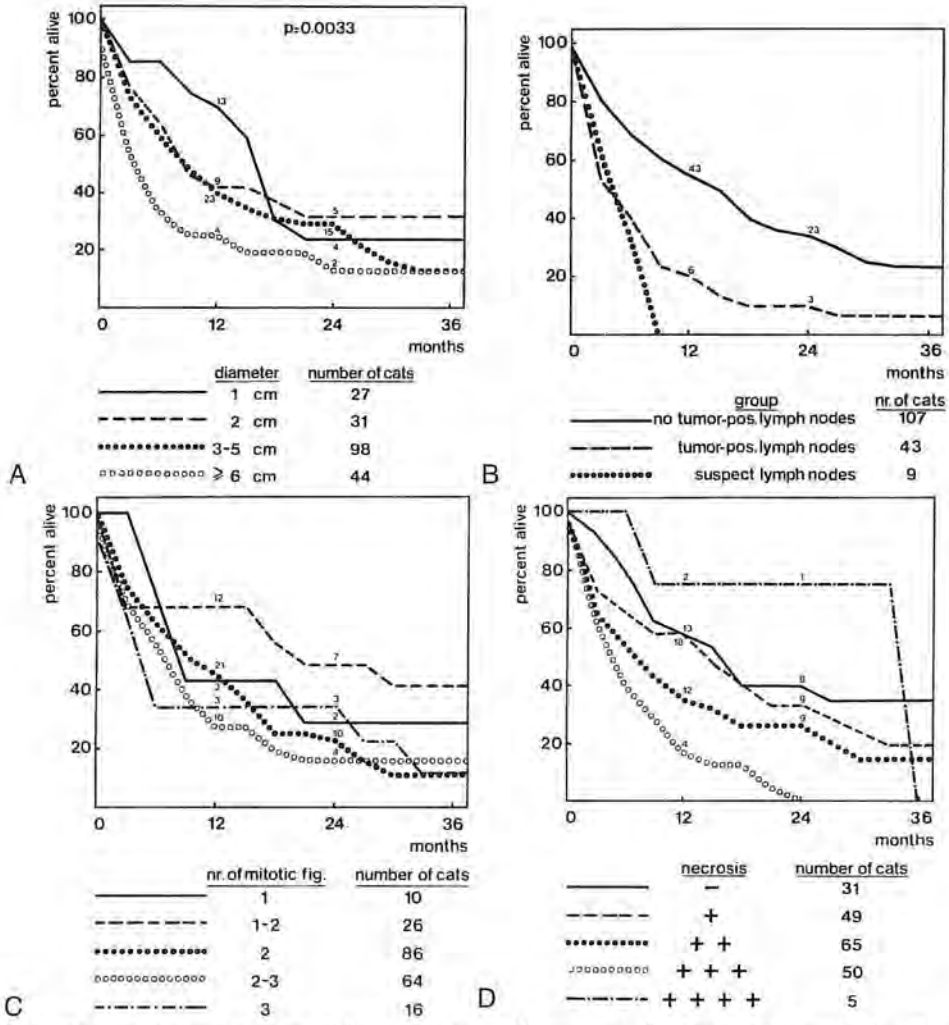
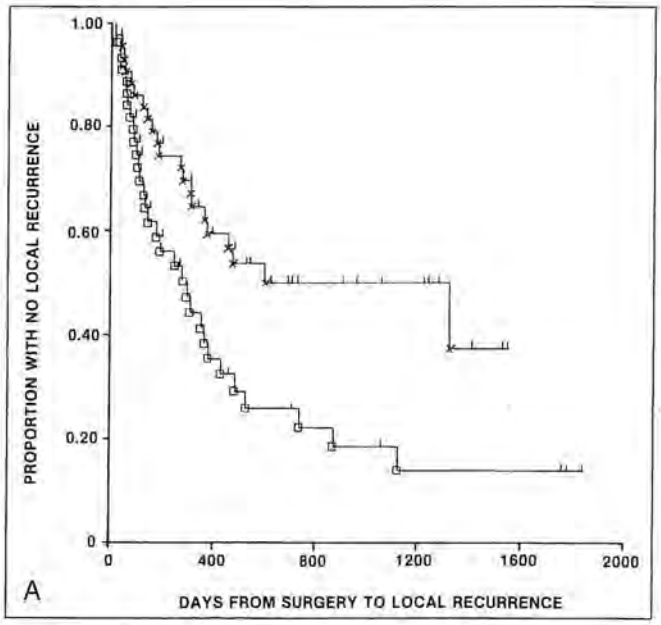


Figure 34–6. Relationships between diameter of the primary tumor (**A**), presence of tumor-positive lymph nodes (**B**), number of mitoses (**C**) and necrosis of the primary tumor (**D**) and survival of queens with mammary carcinoma. (From Weijer K, Hart AM: Prognostic factors in feline mammary carcinoma. J Natl Cancer Inst 70:709–716, 1983, with permission.)

Table 34–1. Correlation of Tumor Size to Survival Time in 91 Cats with Mammary Adenocarcinoma

No. of Cats	Tumor Size (cm^3)		Median Survival Time
54	1–8	(2 cm diameter)	>3 yr
19	9–27	(2–3 cm diameter)	2 yr
18	>28	(>3 cm diameter)	6 mo

From MacEwen EG, Hayes AA, Harvey J, et al: Prognostic factors for feline mammary tumors. J Am Vet Med Assoc 185:201–204, 1984, with permission.



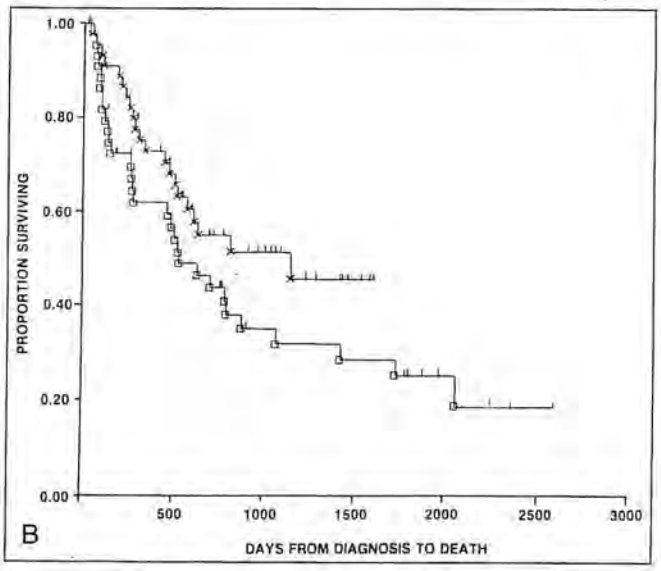


Figure 34–7. Remission duration (disease-free interval) (**A**) and survival time (**B**) in 90 cats with malignant mammary tumors, based on year and type of surgery. □ = 1972 to 1975 series—conservative surgery (removal of involved and adjacent gland[s]), 46 cats; X = 1976 to 1980 series—radical surgery (bilateral radical mastectomy including the inguinal lymph node in a two-stage procedure; one chain removed at each surgery), 44 cats. Type of surgery was significantly ($p < 0.01$) related to disease-free interval, but not to survival time. (From MacEwen EG, Hayes AA, Harvey J, et al: Prognostic factors for feline mammary tumors. J Am Vet Med Assoc 185:201–204, 1984, with permission.)

however, associated with tumor regression in three cats with advanced mammary adenocarcinoma (S.D. Johnston, unpublished observations, 1982). There is no evidence that ovariohysterectomy is beneficial in intact female cats with mammary neoplasia.[25,52]

PROGNOSIS

Prognosis for cats with malignant mammary cancer was reported related to seven independent factors in a prospective follow-up study of 202 cats, of which 157 were treated surgically by mastectomy (105) or block dissection (52); cats with more than one malignant mammary tumor were excluded.[64] These factors were (1) age of the queen at time of diagnosis, with shorter survival times in older cats; (2) diameter of the primary tumor, with longer survival when diameter is less than 2 cm (Fig. 34–6A); (3) presence of tumor-positive lymph nodes, with longer survival if nodes are negative (Fig. 34–6B); (4) presence of metastasis at first clinical examination associated with shorter survival time; (5) number of mitotic figures in tumor tissue, with longer survival with less than two mitotic figures per high-power field (Fig. 34–6C); (6) necrosis of the primary tumor, with longer survival when necrosis is minimal (Fig. 34–6D); and (7) histologic completeness of surgical treatment.[34,62,64] Other factors, such as breed, treatment delay, multiple localization, tumor volume, ulceration, infiltrating growth, pleomorphism, chronic inflammatory infiltrate, and type of surgical treatment (block vs. local dissection) that appeared related to survival before correction for the seven independent factors above were found to be unrelated after correction for these factors.[64] For example, longhaired cats had a shorter survival time ($p = 0.030$) because they more frequently had tumor-positive lymph nodes at first examination; treatment delay was associated with larger tumor diameter; and block dissection was associated with a worse prognosis than simple mastectomy, because the block dissection was done when regional lymph nodes were involved.

In a study of 100 cats with malignant tumors, tumor size was the most significant prognostic factor (Table 34–1), and type of surgery (conservative vs. radical) was significantly ($p < 0.01$) related to disease-free interval, but was not significantly related to survival time (Fig. 34–7A,B).[62]

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Chapter 35

Clinical Approach to the Complaint of Infertility in the Queen

Infertility in the queen is a nonspecific, historical complaint that relies on historical reproductive information for consideration of appropriate cause(s) and appropriate diagnostic plan(s). Infertile queens can be assigned to one of four groups: those with persistent anestrus, those with persistent estrus, cycling queens that refuse copulation with the male, and cycling queens that fail to conceive after copulation with a fertile male.

Minimum Database for Queens with Infertility

Elements of the reproductive and management history and some physical examination findings to be evaluated in the queen with a complaint of infertility are presented in worksheet form in Tables 35–1 and 35–2. In some cases, when complete historical information may not be available, findings listed on the worksheet may be used prospectively as part of a diagnostic plan to evaluate, for example, interestrous interval, evidence of ovulation, or evidence of spermatogenesis by the male. Blank copies of the worksheet may be provided to clients with fertile or infertile queens for record keeping in their catteries to assist in the compilation of useful data on reproductive performance in individual queens.

Complete history (including vaccination history and the presence of viral diseases, if any, in the cattery), physical examination, and routine clinical pathology screening (e.g., complete blood count, serum chemistry profile and urinalysis, feline leukemia virus [FeLV] and feline immunodeficiency virus [FIV] testing) are indicated in all queens at the beginning of the infertility work-up.[1]

Infertility in the Queen With Persistent Anestrus

Causes of persistent anestrus in the queen include inadequate photoperiod or season of the year, previous ovariohysterectomy (OHE), abnormality of sexual differentiation, progesterone-secreting ovarian cyst, progesterone-secreting functional ovarian neoplasm, and ovarian aplasia. Other possible causes, either reported rarely or reported in other species, include drug-induced (e.g., megestrol acetate or medroxyprogesterone acetate) anestrus, immune-mediated oophoritis, and malnutrition or systemic illness associated with ovarian failure.[2] Some queens, housed in colonies with other females that are dominant, may show lordosis behavior but fail to show outward signs of estrus even though they have normal waves of ovarian follicles, as evidenced by serum estradiol concentrations.[3,4]

Inadequate Photoperiod

Inadequate photoperiod should be suspected if the queen's environment does not provide enough light exposure to read a newspaper for at least 14 h/d. Seasonal persistent anestrus is normal when associated with decreasing (starts after June 21 in the Northern Hemisphere) or short day length. Diagnosis is confirmed by response (onset of estrus) to 14 to 16 hours of bright light per day; this response sometimes is enhanced by housing the affected queen with another, cycling, queen. Queens usually exhibit estrus within 1 to 2 months of onset of increasing photoperiod, and one author reports that 75 per cent of queens will develop synchronized estrus if housed with normally cycling queens for 3 to 4 months.[1]

Table 35-1. Worksheet for the Evaluation of Infertility in the Queen—Blank

1. Signalment: Name/Case No:
   Breed:
   Color:
   Date of Birth:

2. Reproductive History:

Date of Estrus Onset (Interestrous Interval)	Date of Receptivity (No. of Days)	Dates and Numbers of Copulations	No. "After-Reactions"* Observed? Y/N	Male ID and Age	Has Male Sired Other Litters? Y/N	Ovulation Confirmed?† Y/N	Date(s) of Parturition	No. of Female Kittens‡ (L:D)	No. of Male Kittens (L:D)

3. Comments:

* After-reaction includes screaming (54%), striking out at tom (77%), vulvar licking (92%), frantic rolling (100%).
† Ovulation confirmed by observation of serum progesterone >1.5 ng/ml 2 to 6 weeks after breeding.
‡ L:D, Born live or born dead.

Table 35–2. Worksheet for the Evaluation of Infertility in the Queen—Example

1. Signalment: Name/Case No: Jasmine/19-17-02
   Breed: Persian
   Color: White
   Date of Birth: 4-14-96

2. Reproductive History:

Date of Estrus Onset (Interestrous Interval)	Date of Receptivity (No. of Days)	Dates and Numbers of Copulation's	No. "After-Reaction" Observed?* Y/N	Male ID and Age	Has Male Sired Other Litters? Y/N	Ovulation Confirmed?† Y/N	Date(s) of Parturition	No. of Female Kittens‡ (L:D)	No. of Male Kittens (L:D)
1-27-97	1-27 to 1-31 (5)	—	—	—	—	—	—	—	—
2-18-97 (23 d)	2-18 to 2-26 (8)	2-24 (3)	Y-Y-Y	Khan (3)	Y	N	4-26-97 (67d)	2:0	1:1
5-6-97 (79 d)	5-6 to 5-7 (2)	—	—	—	—	—	—	—	—
1-03-98 (237 d)	1-03 to 1-08 (6)	1-05 (1)	Y	Max (2)	N	N	Not pregnant	—	—
1-30-98 (28 d)	1-30 to 2-05 (7)	2-01 to 2-02 (4)	Y-Y-Y-Y	Max (2)	N	N	4-08-98 (66d)	2:0	3:0

3. Comments:
   4-20-99: Treated for open-cervix pyometra.

* After-reaction includes screaming (54%), striking out at tom (77%), vulvar licking (92%), frantic rolling (100%).
† Ovulation confirmed by observation of serum progesterone >1.5 ng/ml 2 to 6 weeks after breeding.
‡ L:D, Born live or born dead.

Previous Ovariohysterectomy

Previous OHE usually is ruled out by the history in purebred queens, but may be a difficult diagnosis to confirm if history is unknown. Identification of a ventral midline scar, palpation of nonabsorbable sutures (if present), palpation of the uterus per abdomen (if present), ultrasonography of the abdomen to look for uterus and ovaries, and elevation in serum gonadotropin concentrations (not generally commercially available) are contributory findings. In the absence of abnormal karyotype (see Abnormality of Sexual Diffentiation below), the investigating clinician may consider either attempting to rule out this diagnosis by inducing estrus with follicle-stimulating hormone (FSH) (see below) or by exploratory laparotomy and examination of the region of the ovarian pedicle(s).

Table 35–3. Case Example: Queen with Persistent Anestrus

Signalment:	2.5-year-old female, sable Burmese
Presenting Complaint:	Persistent anestrus
History:	Housed all of her life in a cattery with adequate photoperiod and exposure to estrous queens
Physical exam:	Small (weight 3.2 kg; weighed 70 g at birth, compared to littermates at 90 to 100 g); vulva appeared normal; uterus palpable per abdomen
Initial problem list:	Persistent anestrus
Diagnostic plan:	Karyotype of heparinized, venous blood
Results:	37,XO karyotype (normal queen is 38,XX) Later ovariohysterectomy revealed gonadal dysgenesis (see Figs. 31–1 through 31–3)
Refined problem list:	X-Chromosome monosomy with gonadal dysgenesis
Therapy:	None
Comment:	Phenotypic queens with persistent anestrus caused by abnormalities of sexual differentiation may have karyotypes of 37,XO, 38,XX/38,XY, or 38,XY (testicular feminization)

Abnormality of Sexual Differentiation

Abnormality of sexual differentiation in the queen is described in Chapter 31.[5–10] Ovarian dysgenesis or presence of abdominal testes or ovotestes may occur in phenotypic queens with abnormality of sexual differentiation. Abnormalities of sexual differentiation reported in the queen with persistent anestrus include abnormality of chromosomal sex (37,XO [Table 35–3], mosaicism), abnormality of gonadal sex (such as true hermaphroditism), and abnormality of phenotypic sex (male pseudohermaphrodites and individuals with testicular feminization). Bilateral ovarian dysgenesis was reported in a 2½-year-old Burmese cat with primary anestrus and a 37,XO chromosome complement (see Fig. 31–1).[6] The ovaries and uterus were small (see Fig. 31–2), and ovarian histology consisted of fibroblastic stromal elements and large aggregates of hypertrophied interstitial cells; follicles or corpora lutea were not observed (see Fig. 31–3). No somatic abnormalities were present in the cat. Other 37,XO cats have been described, one with coarctation of the aorta and one with spina bifida.[6,8,10] These were kittens that died or were euthanized by 3 days of age, and gonadal histology was not reported. The XO female is an example of an abnormality of sexual differentiation due to numerical abnormality of the sex chromosomes. The most common numerical abnormality of the sex chromosomes in the cat, however, is that of mosaicism or chimerism, where different cell lines are present in the body. Mosaicism, such as 38,XX/57,XXY, has been reported in both male and female phenotype cats, including queens with persistent anestrus and queens that cycle and conceive kittens, depending on the gonadal tissue present.

True hermaphroditism is rare in cats, and has not been reported in phenotypic females where both gonadal histology and chromosome complement are known. True hermaphroditism has been reported in cats that are phenotypically male, where both gonadal histology and chromosome complement are known, suggesting that presence of testicular tissue in the embryo induces development of (male phenotype) secondary sexual characteristics, regardless of presence of ovarian tissue or karyotype. In other species, ovotestes have been detected in phenotypic female individuals with an XX/XY, XO/XY, or XXY sex chromosome complement.[5]

Testicular feminization was reported in a 6-month-old orange tabby cat with a normal female phenotype (vulva and clitoris), 38,XY chromosome complement, abdominal testes but no uterus, uterine tubes, epididymides or

vasa deferentia, and absence of androgen receptors in cultured fibroblasts from genital skin.[11] This disorder, which is an inherited defect in humans, may be suspected in queens with the XY karyotype, abdominal testes, and absence of the female (müllerian duct) tubular tract. Male pseudohermaphroditism, which also has been reported in the cat, usually occurs with the XY karyotype, abdominal testes present at the tip of the uterine horns, and presence of derivatives of the müllerian duct system.[5] Prognosis is grave for future fertility in affected queens.

Progesterone-Secreting Ovarian Cyst or Functional Ovarian Neoplasm

Progesterone-secreting ovarian cysts or functional ovarian neoplasms are possible, if uncommon, causes of persistent anestrus in this species. Presumptive diagnosis is based on serum progesterone concentrations exceeding 1.5 ng/ml in nonpregnant queens that persist beyond normal, or nonpregnant diestrus (40 to 45 days), and on presence of an ovarian mass (see Chapter 31). Diagnosis is confirmed at exploratory laparotomy, and treatment is surgical excision.

Ovarian Aplasia

Ovarian aplasia that has been reported in the queen usually has been unilateral, not bilateral and, therefore, should be considered an uncommon cause of persistent anestrus.[12,13]

Induction of Estrus

Induction of estrus may be attempted in the queen with persistent anestrus when a cause of this sign cannot be identified, and response to estrus induction treatment is used both as a diagnostic and as a therapeutic tool. Recommended treatment is intramuscular (IM) injection of 2 mg FSH daily until onset of estrus (3 to 7 days) followed by natural mating or by 250 IU human chorionic gonadotropin (hCG) IM to induce ovulation.[14] Natural mating (three times daily for the first 3 days of estrus) followed by hCG (250 IU), IM on days 2 and 3 of estrus) synergistically enhances the ovulatory response of cats either in natural or induced estrus.[15,16] FSH has been intermittantly commercially available in the United States.

Infertility in the Queen With Persistent Estrus

Causes of persistent estrus in the queen include normal ovarian follicular function that either is misinterpreted as persistent estrus by the owner or is associated with prolonged sexual receptivity (into postestrus or diestrus), functional follicular cysts of the ovary, and functional ovarian neoplasia.

Normal Ovarian Function and Estrous Behavior

Normal ovarian function and estrous behavior are described in Chapter 25. The first step in investigating any complaint of persistent estrus in the queen is to rule out the possibility that normal estrus is being misinterpreted as persistent by the owner, or that normal ovarian function is associated with sexual receptivity that extends beyond the time of the follicular phase. Feline estrous cycles may occur at 4- to 30- (modal = 14 to 19) day intervals, and estrus durations of 4 to 12 days with very short postestrus periods, when serum estradiol is less than 20 pg/ml, may appear, to the owner, to be persistent estrus. Duration of continuous, true estrus in the normal cat should not exceed 19 days.[4] Queens with a history of persistent estrus should be examined every 2 to 3 days for 3 to 4 weeks with vaginal cytology (to look for persistent cornification) and, if available, serum estradiol concentrations to determine whether the queen is, in fact, cycling in and out of estrus. Examination of the vaginal smear is preferred, but smears may be difficult to collect from the estrous queen, and if the clinician is unsure of the cytology site obtained (vestibule vs. vagina), persistent serum estradiol concentrations exceeding 20 pg/ml also support the diagnosis of true, persistent estrus. If examination or serum estradiol concentrations support normal cycling, the queen may be exposed to an experienced male for mating at time of peak serum estradiol concentrations, or induced to ovulate and go into the luteal phase by the administration of gonadotropin-releasing hormone (GnRH) (25 μg, IM) or hCG (250 IU, IM). In some queens, manipulation of the photoperiod to less than 10 hours of light exposure per day may induce seasonal anestrus.

A variation of normal estrous behavior involves ovarian follicle growth patterns in which follicular waves overlap so that serum

estradiol concentrations between follicular waves exceed 20 pg/ml (Fig. 35–1).[4] This is an unusual type of ovarian follicle activity in the cat, but conception is possible if mating occurs during time of peak estradiol secretion.[17]

Functional Follicular Cysts of the Feline Ovary

Functional follicular cysts of the feline ovary (see Chapter 31) should be suspected in the queen with true persistent estrus.[18-20] Follicular ovarian cysts, which arise from mature or atretic follicles, have been reported to be common in the cat, and their frequency is reported to increase with age.[18]

Figure 32–4 depicts bilateral ovarian cysts in an 8-year-old Birman cat presenting with infertility associated with (prolonged) estrus of 6 weeks' duration caused by functional ovarian cysts (Table 35–4). The cysts did not respond to an attempt to induce ovulation with administration of hCG, and were resected surgically. Following treatment, the queen cycled normally, conceived, and delivered a normal litter. Functional ovarian cysts are diagnosed histologically after observation of signs of estrus; cornified vaginal cytology; elevated plasma estradiol concentrations; and palpation, imaging, or laparotomy evidence of an abdominal mass. Differential diagnosis includes presence of a functional ovarian neoplasm. Treatment options for follicular cysts in the queen include attempts to induce ovulation and follicular luteinization (250 to 500 IU hCG or 25 mg GnRH IM), surgical resection of the cysts if medical treatment is unsuccessful, and ovariectomy if future reproduction is not desired. Success of induction of ovulation is based on elevation of serum progesterone to a concentration exceeding 1.5 ng/ml by 1 week after attempted induction of ovulation (mating or medical treatment), and decline in percentage cornification of exfoliated anuclear squame vaginal epithelial cells to less than 10 per cent of the specimen by that time.[4]

Table 35–4. Infertility Case Example: Queen with Persistent Estrus

Signalment:	8-year-old female, Birman
Presenting complaint:	Infertility associated with persistent estrus
History:	Two normal litters more than 3 years ago; signs of estrus for 6 weeks; bred four times to a fertile male with no conception during that time; FeLV negative; FIP in cattery
Physical exam:	Normal TPR; cornified vaginal smear; palpable abdominal mass caudal to right kidney
Initial problem list:	Persistent estrus; palpable left abdominal mass
Diagnostic plan:	Abdominal ultrasound to rule out ovarian cyst or neoplasia
Results:	Bilateral, septate, nonechogenic masses in the region of the right and left ovaries
Refined problem list:	Functional ovarian (follicular) cysts
Therapy:	500 IU hCG IM; no effect on serum progesterone concentrations or vaginal cornification; followed by exploratory laparotomy and bilateral cyst resection; cyst fluid contained >6000 pg/ml estradiol; vaginal smear noncornified by 2 weeks postoperatively; subsequently cycled and delivered one normal litter

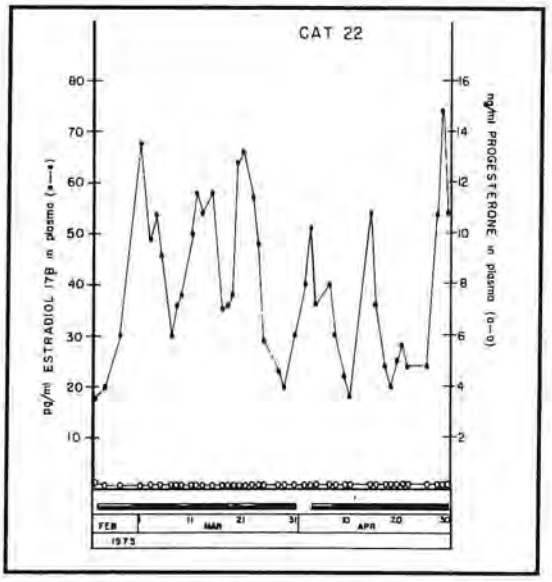

Figure 35–1. Continuous estrous behavior in a queen with ovarian follicle waves that overlap.[4] Follicle activity is depicted by plasma estradiol concentrations. The overlap was accentuated during the month of March. Copulation was allowed only once, on April 12. Ovulation did not follow this mating because copulation occurred between two follicle waves. The solid horizontal bars indicate sexual acceptance. The partially solid bars indicate proestrus-like activity. (From Stabenfeldt GH, Pedersen NC: Reproduction and reproductive disorders. *In* Pedersen NC [ed]: Feline Husbandry: Diseases and Management in the Multiple Cat Environment. Goleta, CA, American Veterinary Publications, Inc, 1991, pp 143–153, with permission.)

Functional Ovarian Neoplasia

Functional ovarian neoplasia is less common than follicular ovarian cysts in cats with persis-

tent estrus, but functional granulosa cell tumors have been reported in queens between 3 and 16 years of age (see Chapter 31). Although the incidence of primary ovarian neoplasia in the queen is low, the granulosa cell tumor is the most common of these tumors, and the most common functional ovarian tumor, secreting estradiol, progesterone, or both.[19] About half of these tumors are functional and are associated with persistent estrus (with estradiol secretion) or cystic endometrial hyperplasia (with progesterone secretion) or both. Queens with types of ovarian tumors other than granulosa cell tumors (e.g., cystadenoma, dysgerminoma, and interstitial cell tumor) occasionally have been reported to show persistent estrus (see Table 31–1). Excision biopsy is the treatment of choice with all ovarian tumors of the queen; the guarded prognosis associated with the granulosa cell tumor in the cat supports the removal of both ovaries in the infertile queen with persistent estrus, rather than attempt at surgical resection that preserves reproductive function.

Infertility in the Cycling Queen that Refuses Copulation with the Male

Refusal of copulation by a normal estrual queen may be caused by abnormal reproductive behavior and/or mate preference by the queen, or by vestibular/vaginal anomaly that precludes normal intromission.[20] A male cause of copulation failure that has been reported in the domestic cat is presence of a penile hair ring that prevents complete penile protrusion and intromission (see Chapter 41); therefore, if copulation failure is suspected, the male also should be examined.

Copulation failure is supported by observation of mating(s) where the female "after-reaction" is not displayed, by measurement of low (<1.5 ng/ml) serum progesterone in the queen following mating attempt(s), and by failure to detect sperm in a postcoital vaginal wash. However, none of these observations provides absolute evidence of absence or presence of copulation. After-reactions of screaming (54 per cent), striking out at the tom (77 per cent), vulvar licking (92 per cent), and frantic rolling (100 per cent) were observed in a study of 120 feline copulations.[22] Even in the presence of these reactions and normal copulation, about 50 per cent of queens fail to ovulate after only a single copulation, so that the after-reaction alone does not confirm ovulation.[23] Serum progesterone concentration exceeding 1.5 ng/ml by about a week after breeding supports, but is not absolute evidence of, successful copulation, as some queens ovulate spontaneously during the follicular phase, even in the absence of a male.[24] Finally, presence of spermatozoa in the postcoital vaginal wash supports completion of normal copulation, but absence of spermatozoa does not rule it out.

Some normal adult queens reject copulation with some male cats even when in estrus, suggesting sexual partner discrimination.[22,25] In one report of 38 observed estrous cycles in 14 queens, the females did not allow the male to breed at 15 of the 38 (39 per cent), despite multiple attempts during exhibition of behavioral estrus and cornified vaginal cytology.[22] Breeding strategy in these queens is to try different males, if available, or to introduce the queen and tom when she is not in heat, and try again at a later estrus.

Vestibular/vaginal congenital abnormalities reported in the cat include segmental aplasia of the cranial vagina, presence of a common vulvovestibular-anal opening, imperforate hymen, and rectovaginal fistula[18] (J. Holzworth, personal communication, 1982). These abnormalities are reported rarely, and the small size of the feline vestibule and vulva, which precludes digital examination of these organs, may prevent detection of most anomalies of this organ. One of the authors (MVRK) has diagnosed presence of a vulvar stricture preventing normal copulation in a queen that delivered a single, large kitten with vulvar tearing a year earlier.

Infertility in the Cycling Queen that Accepts Copulation with a Fertile Male

Infertility in the cycling queen that accepts copulation with a fertile male may be caused by impatency of the tubular tract; infection of the cervix, uterus, and/or uterine tubes; or anovulatory cycles. In one report of 33 queens subjected to laparotomy after continuing infertility, 13 (39 per cent) had one or more pathologic conditions of the reproductive tract.[26] Other possible causes include subfertility associated with spontaneous chromosome anomalies of

the conceptuses, immune-mediated infertility, in-breeding, stress, and hypoluteoidism.

Impatency of the Tubular Tract

Impatency of the tubular tract secondary to segmental aplasias, tubal hyperplasia (see Fig. 32–2), endometrial hyperplasia, or endometrial scarring may occur in the queen.[26–31] Three of 33 queens explored for infertility from the same colony had villous hyperplasia of the oviducts (uterine tubes) and three had infantile tracts; there was one case each of cervical and uterotubal junction obstruction.[26] Table 35–5 describes an infertile cat with normal copulation and normal ovulation that did not conceive due to bilateral obstruction of the lumen of the uterotubal junction. This cause should be suspected in queens that have never conceived despite good breeding management. Diagnosis is confirmed at exploratory laparotomy and gross inspection of the uterus and uterine tubes.

Table 35–5. Infertility Case Example: Infertility in the Cycling Queen that Accepts Copulation with a Fertile Male

Signalment:	3-year-old female, Himalayan
Presenting complaint:	Infertility
History:	Bred at four different seasons with two different fertile males between ages 2 and 3; never pregnant; breedings were not observed; female and male were housed together; Last breeding occurred 2 weeks prior to presentation
Physical exam:	Normal; uterus palpable per abdomen
Initial problem list:	Infertility DT unknown
Diagnostic Plan	CBC/UA/chemistries normal; FeLV negative; serum progesterone = 26 ng/ml; wait 2 weeks for pregnancy diagnosis = negative; Exploratory laparotomy for uterine biopsy, uterine culture, saline uterine flush for patency
Results:	Bilateral occlusion of the uterotubal junction; ovariohysterectomy and histologic examination revealed bilateral papillary hyperplasia of the uterine tube at the uterotubal junction
Refined problem list:	Bilateral papillary hyperplasia of the uterine tube
Therapy:	None

DT = Due to.

Infection of the Cervix, Uterus, and/or Uterine Tubes

Infection of the cervix, uterus, and/or uterine tubes is a common cause of infertility in the cycling queen that accepts normal copulation.[1,18,26,32–36] Infection usually is superimposed on underlying cystic endometrial hyperplasia (see Chapter 32). In one survey of 33 infertile queens examined at laparotomy, seven (21 per cent) had cystic endometrial hyperplasia, and one had cystic polypoid hyperplasia of the endometrium.[26] In the cat, as in the dog, hyperplastic and inflammatory changes of the uterine lining are common and appear to increase with age.[33] These changes also may be more prevalent in intact, aged nulliparous females than in multiparous females.[37] Infections of the tubular tract are suspected in the middle-aged to older queen that may have previously delivered a normal litter. Presence of an elevated white blood cell count and imaging evidence of thickening of the uterine wall are contributory findings, if present. Bacteria isolated from fluid in the infected uterus are described in Chapter 32 and include flora that normally reside in the feline vagina.[38] Infection is confirmed using exploratory laparotomy for uterine biopsy and uterine culture. Alternatively, breeding attempt while the queen is treated with broad-spectrum systemic antibiotics may result in successful pregnancy in some queens.

Anovulatory Cycles

Anovulatory cycles occur in about half of queens bred a single time when in estrus, as multiple stimulations of the vagina appear necessary to elicit a luteinizing hormone surge of adequate magnitude to induce ovulation (see Fig. 26–4).[23] Anovulatory cycles are diagnosed by measuring serum progesterone in queens 2 to 3 weeks following breeding; ovulation is associated with serum progesterone exceeding 1.5 ng/ml, and ovulation failure is associated with serum progesterone less than this level.

Other Causes

Other causes of infertility in the cycling queen that accepts copulation include inbreeding, stress, and estrus suppression with progesta-

tional drugs.[21,35] In a study of 33 infertile laboratory queens, 20 lacked pathologic abnormality of the reproductive organs.[26] If impatency and infection of the reproductive tract and ovulation failure have been ruled out, selection of an unrelated male cat and sexual rest or stress reduction as possible should be attempted. Hypoluteoidism, or premature lysis of the corpora lutea with resulting pregnancy loss, has not been reported in the queen, but may occur in this species; diagnosis is based on evaluation of serial serum progesterone concentrations following breeding.

Subfertility in the cycling queen caused by fetal chromosomal error has been described in queens with consistently small litter size accompanied by erratic and irregular estrous cycles.[39-41] Because early embryonic death with resorption was suspected, laparotomy was performed in three queens at about 4 weeks after mating. Fourteen conceptuses were karyotyped, and autosomal mosaicism (38,XX/37,XX and 38,XX/39,XX) was detected in two.[40] In another study, 4 (16 per cent) of 25 kittens that died prenatally, were stillborn, or were liveborn with congenital defects, exhibited abnormal karyotypes, most with autosomal mosaicism, similar to karyotypes reported in human abortuses and pregnancy loss in other species.[41]

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Section IV

THE TOM

Chapter 36

Sexual Differentiation and Normal Anatomy of the Tom Cat

Differentiation of the Reproductive Organs of the Tom Cat

The reproductive organs of the tom cat include the testes, epididymides, ductuli (vasa) deferentia, prostate, bulbourethral glands, penis, and prepuce (Fig. 36–1). The process of sexual differentiation of the male cat begins at time of fertilization, when a Y-chromosome-bearing sperm penetrates an X-chromosome-bearing oocyte. The chromosomal complement of the normal tom cat is recorded as 38,XY. Embryologically, the gonads of the tom cat originate as undifferentiated tissue, the mesenchymal gonadal ridges, on the medial side of the mesonephros.[1] Following the migration of primordial germ cells from the yolk-sac endoderm to the gonadal ridges, undifferentiated gonadal tissue differentiates to testis in the presence of a normal Y chromosome, which contains an sry (sex determining region of the Y) region. Testosterone secreted by the interstitial cells of the fetal testes causes persistence of the mesonephric (wolffian) duct system, from which arise the epididymides and vasa deferentia. Müllerian inhibiting substance (MIS) from the fetal Sertoli cells induces regression of the paramesonephric (müllerian) duct system which, in the female, develops into the tubular reproductive organs.

The prostate arises from endodermal buds of the urethra and adjacent urogenital sinus.[1] The bulbourethral glands arise from the penile urethra.[1] External genitalia form from the genital tubercle and genital swellings under the influence of dihydrotestosterone, converted from testosterone by the enzyme 5α-reductase. The genital tubercle becomes the penis, and the ventral urethral groove on the penis develops into the urethra following fusion of the urethral folds under the influence of dihydrotestosterone. During its early formation, the penis adheres to the prepuce via the balanopreputial fold; dissolution of this fold is an androgen-dependent event that occurs postnatally in most male mammals.[2,3] In the cat, breakdown of this fold, and resulting ability to completely protrude the penis from the prepuce, occurs at about 7 to 12 months of age, unless the cat is castrated prior to that time, in which case the adhesion persists.[4] At about 9 weeks of age, elongated pits become evident on the surface of the glans penis, from which androgen-dependent penile spines erupt between 9 and 13 weeks of age.[5] Diameter of the preprostatic and penile urethra of 2-year-old cats are not different in males castrated at 7 weeks, 7 months, or left intact.[4]

In the cat, as in many mammals, each testis descends from the abdominal cavity through

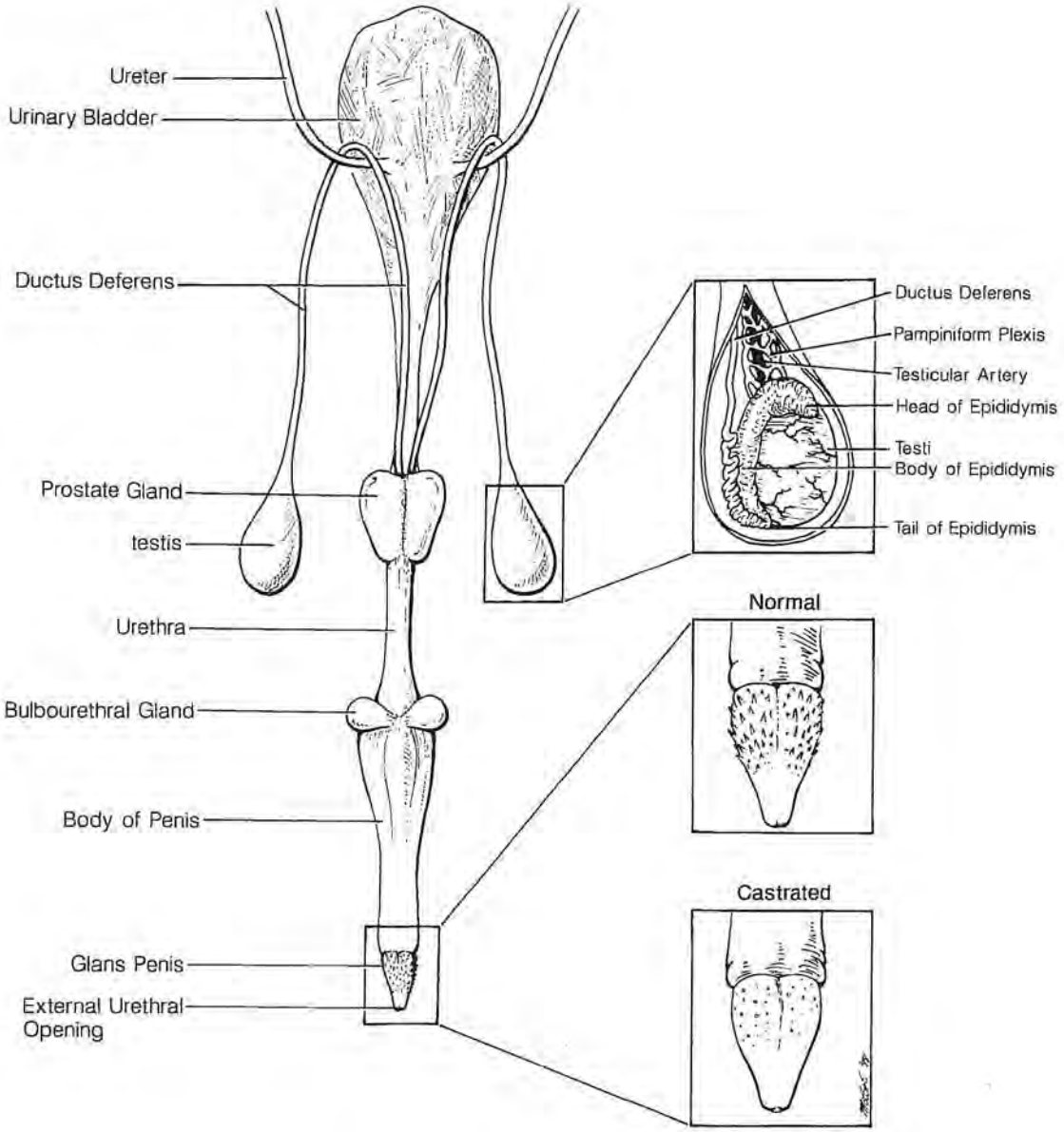

Figure 36–1. Gross anatomy of the male reproductive organs of the cat.

the inguinal ring into the scrotum prior to birth. The testis is enclosed by the tightly adherent fibrous covering, the tunica albuginea, and also by layers of visceral (lamina parietalis tunica vaginalis) and parietal (fascia spermatica interna) peritoneum drawn out of the abdominal cavity with it.[6–10] A potential space, the vaginal cavity (which is continuous with the peritoneal cavity), lies between the lamina parietalis tunica vaginalis and fascia spermatic interna. An incision through the scrotal skin, therefore, passes through the fascia spermatica interna (tunica vaginalis communis), the vaginal cavity, the lamina parietalis tunica vaginalis (tunica vaginalis propria), and the tunica albuginea before entering testicular parenchyma.[6–9] Testes of normal kittens usually are descended at birth, but, prior to puberty (7 to 10 months of age), cat testes move freely up and down in the inguinal canal.[10,11]

Combined weight of both testes of male kittens at birth ranges from 20 to 58 mg.[9,10] Combined testes weight in older kittens is reported as 100 mg at weaning, as 130 mg at 12 weeks of age, and as 500 mg at 20 weeks of age.[10] Thereafter, the testes increase in proportion to

body weight, with maximal combined testes weight of 4 g recorded in a 5-kg cat.[10] Total seminiferous tubule length in immature cat testes weighing 800 to 940 mg has been reported as 22.8 to 25.8 m.[12] Diameter of feline seminiferous tubules is 60 to 90 μm at birth, 110 μm at 20 weeks of age, and 130 to 175 μm at 30 to 36 weeks of age, when spermatozoa are identified histologically.[10,13] Mean diameter of seminiferous tubules of 10 tubules each during stage 7 in the testes of 2- to 3-year-old cats was reported as 182 to 210 μm.[14]

Postnatal growth of the epididymides is rapid in the intact male kitten; combined weights of both epididymides of 14 mg at birth, 200 mg at weaning, and 500 mg at 20 weeks of age have been reported.[10]

Testes

The testes of the adult male cat are spherical to ovoid structures approximately 1.5 × 1.0 × 1.0 cm each in size, with a 2- to 4-g combined weight, and are located in the scrotal sac ventral to the anus.[10,15–17] Adult feline testicular weights were reported higher in June than in December and March in one study of male cats in North America; however, sperm were present in the seminiferous tubules during all seasons.[18] The skin of the scrotum is covered by hair. The scrotal sac is distinguished externally by a median groove corresponding to an inner fibrous septum that divides the sac into two halves.[9]

Spermatogenesis

Histologically, the testis of the domestic cat is composed of closely massed and convoluted seminiferous tubules and islands of interstitial cells (of Leydig) that almost fill the intertubular spaces (Fig. 36–2).[19] The three major cell types of the testis are the interstitial cells, which secrete testosterone in response to stimulation by luteinizing hormone (LH); the Sertoli cells, which are estrogen- and inhibin-secreting cells lining the seminiferous tubules that act as nurse cells to elongating spermatocytes; and the germ cells (i.e., spermatogonia, primary and secondary spermatocytes, spermatids, spermatozoa), which are present within the seminiferous tubules (Fig. 36–2).[20,21] Interstitial cells change in appearance and number from the neonatal (i.e., testosterone-secreting fetal cells) to infant (i.e., no androgenic activity) to pubertal/adult (i.e., mature, testosterone-secreting) periods in the cat.[22] Sertoli cells decrease in number per transverse seminiferous tubular section from about 25 at 1 day to 1 month of age, to 17 to 18 at 4 to 5 months of age, to 10 to 13 at 5 to 7 months of age, to 8 to 10 in adults.[13] Mature Sertoli cells in the cat have elongated nuclei, irregular outlines, and prominent nucleoli.[13]

Testicular Steroid Regulation and Production

Testicular function is mediated by LH and follicle-stimulating hormone (FSH) secretion from the anterior pituitary gland, under the influence of gonadotropin-releasing hormone (GnRH) released from the hypothalamus. LH stimulates testosterone secretion by the interstitial cells of the testis; LH secretion is inhibited by high circulating concentrations of LH or testosterone.[23] FSH stimulates secretion of, and is itself inhibited by, estradiol and inhibin secretion by the Sertoli cells of the testis. Electrical stimulation of the medial preoptic region and the median basal hypothalamus increases LH secretion in the cat.[24] Serum LH concentrations are reported to vary widely (2.2 to 29.2 ng/ml) in the cat, depending on assay system used[23]; episodic LH activity has been reported in gonadectomized males at 20- to 30-minute intervals.[25] Serum FSH concentrations have not been reported in the domestic cat.

Resting plasma testosterone concentrations in intact adult male cats range from nondetectable (<0.5 ng/ml) to more than 20 ng/ml.[14,26–32] Mean plasma testosterone concentrations in blood collected from testicular veins of normal male cats are reported to range from 23.2 to 36.8 ng/ml.[14] Feline testosterone secretion is episodic, without diurnal rhythm.[14,30] Both presence[30] and absence[14,18] of a positive effect of breeding season on circulating concentrations of testosterone in the cat have been reported. Serum testosterone concentrations decline in some male cats during manual restraint and/or anesthesia.[26,33]

Because single resting concentrations of testosterone are so variable, due to the episodic nature of its secretion, stimulation of maximal testosterone secretion by administration of human chorionic gonadotropin (hCG), which has LH-like activity, or GnRH, which stimulates endogenous LH release, permits clinical assessment of maximum testicular capacity to secrete testosterone.[27] Resting serum testosterone concentration in six cats ranged from non-

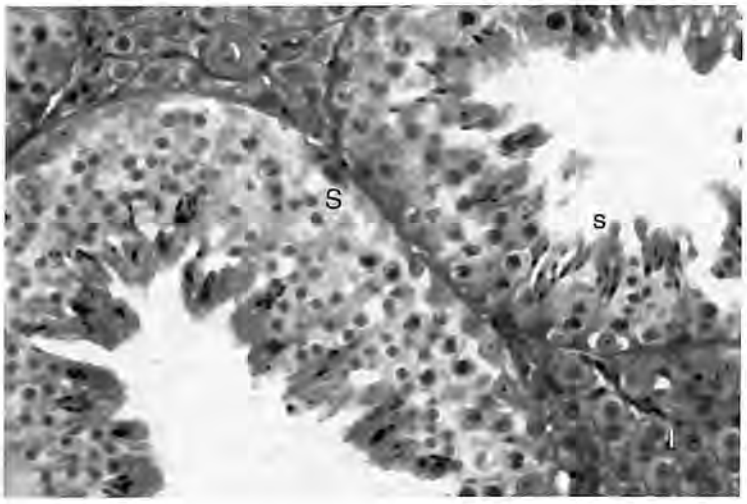

Figure 36–2. Histologic appearance of the adult cat testis, showing a cross section of a seminiferous tubule containing Sertoli cells (S) and development of germ cells to spermatids (s), with adjacent interstitial cells (I).

detectable (<0.05 ng/ml) to 3.0 ng/ml; the range 4 hours after 250 IU hCG administered intramuscularly (IM) was 3.1 to 9.0 ng/ml, and 1 hour after 25 μg GnRH IM was 5.0 to 12.0 ng/ml (Fig. 36–3).[29] Human chorionic gonadotropin or GnRH stimulation tests may be used to demonstrate presence of a hidden, abdominal testis in animals in which cryptorchidism is suspected (see Table 39–2). Castration causes an immediate decrease in plasma testosterone to baseline concentrations (0 to 0.5 ng/ml), suggesting that plasma levels of this hormone in the intact male are testicular in origin.[30,41]

Mean plasma concentrations of androstenedione in the intact male cat are reported at 0.31 to 0.69 ng/ml in peripheral blood and 7.8 to 25.7 ng/ml in blood from the testicular veins.[14] Plasma androstenedione is not suppressed completely by castration, suggesting another source (such as the adrenal glands) for this hormone.[30] Androstenedione is released in episodic bursts, but no diurnal rhythm of secretion has been observed.[30]

Mean plasma concentrations of 5α-dihydrotestosterone in the intact male cat are reported at 0.03 to 0.70 ng/ml in peripheral blood and 6.4 to 25.7 ng/ml in blood from the testicular veins.[14]

Plasma estradiol concentrations in normal intact adult male cats range from 12.1 to 16.1 pg/ml.[30,31]

Epididymides, Ductuli (Vasa) Deferentia, and Spermatic Cord

The epididymides are tightly coiled, contiguous, efferent ducts that adhere closely to the surface of the testes. The epididymides are the sites of sperm maturation and of storage of extragonadal reserves of sperm.[9,34] The head of the epididymis is located at the dorsal cranial medial aspect of the testis; the epididymal

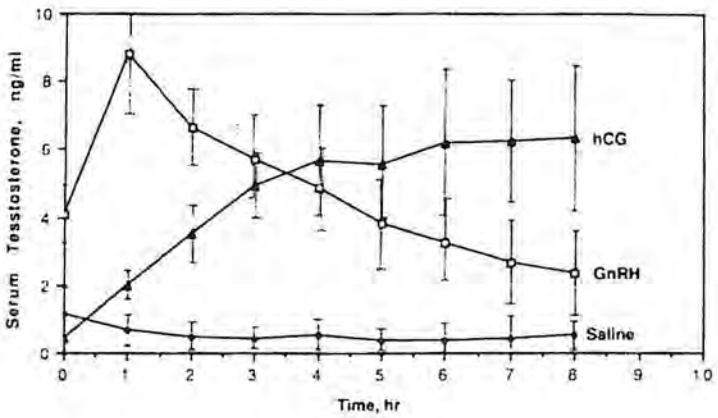

Figure 36–3. Serum testosterone concentrations (mean ± 1 SEM) in six normal male cats before, and at hourly intervals after IM administration of 0.5 ml saline, 250 IU human chorionic gonadotropin (hCG), or 25 μg gonadotropin-releasing hormone (GnRH).

body curves around the dorsolateral aspect of the testis and becomes continuous with the tail of the epididymis at the dorsal caudal pole of the testis. The tail of the epididymis then passes medial to the surface of the testis and becomes the ductus deferens, which passes, with the rest of the spermatic cord, into the abdominal cavity via the inguinal ring and then over and down into the dorsal surface of the prostate, which is the site of its entry, at the colliculus seminalis, into the urethra (Fig. 36–4).[35] There is no dilation of the ductus deferens (ampulla) at its entrance to the prostate.[36]

The epididymides are composed of convoluted tubules similar to those of the testes, except that sperm maturation (i.e., shedding of cytoplasmic droplet, acquisition of motility and fertilizing capacity) occur here rather than sperm cell formation. At the end of the tail of the epididymis, the convoluted tubules empty semen into the ductus deferens, or vas deferens. Histologically, the ductus deferens is composed of a mucosal surface at its lumen, a tunica muscularis and an outer serosal surface; it is a convoluted tube at its beginning and becomes straight as it approaches the inguinal ring. The ductuli deferentia of the cat lack ampullae, and lose their muscular tunic before terminating at the prostatic urethra.[37] Epithelial cells of the terminal vas deferens of the cat may phagocytize spermatozoa and other foreign particles; this phagocytic ability is independent of presence of testicular androgen.[38,39]

Characteristics of spermatozoa recovered from the feline epididymides include about 50 per cent progressive motility and about 50 per cent normal morphology.[40] Epididymal sperm cells from the cat have the ability to capacitate and undergo first stages of fertilization with zona-free hamster oocytes.[40]

The spermatic cord of the cat consists of the ductus deferens; the testicular artery, vein, and lymphatics; and a testicular nerve plexus on the artery.[36] The testicular vein arises from a venous plexus, the pampiniform plexus, that surrounds the coiled testicular artery distally in the cord; the pampiniform plexus, containing cool blood from the testis and epididymis, cools arterial blood flowing toward the testis, which contributes to a cooler testicular temperature in the scrotum than the core body temperature.[36] The cord is enveloped in visceral and parietal layers of the vaginal tunic, which are separated by the vaginal cavity.[36]

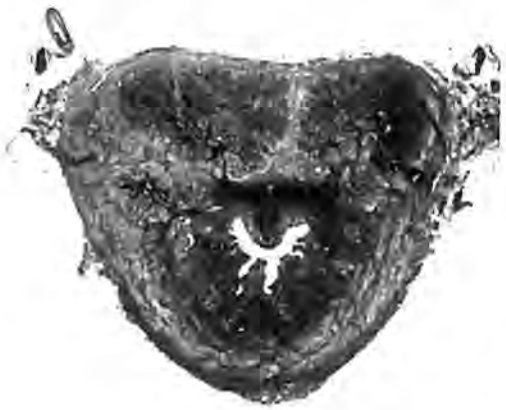

Figure 36–4. Transverse section through the caudal prostatic urethra of the cat. The colliculus seminalis indents the urethra dorsally, and glandular tissue is dispersed in the submucosa. Triple stain; reduced from 12×. (From Cullen WG, Fletcher TF, Bradley WF: Morphometry of the male feline pelvic urethra. J Urol 129:186–189, 1983, with permission.)

Prostate

The prostate of the domestic cat is an organ that consists of four flattened spherical lobes (two cranial, two caudal) approximately 1 cm in length that covers the urethra dorsally and laterally 2 to 3 cm from the neck of the urinary bladder (Fig. 36–4).[35] Histologically, the feline prostate is a compound tubular gland with tubules of varying diameter lined by secretory epithelium.[37,41] Small lobules extend into the urethral muscle.[37] Histochemical evaluation suggests that the feline prostate secretes fluid containing a protein-lipid complex, zinc, and acid phosphatase.[37,41,42] Prostatic fluid collected by electroejaculation of cats following bilateral prescrotal vasectomy and excision of the bulbourethral glands averaged 0.04 ml in volume, with mean specific gravity of 1.007, pH of 7.8, and osmolality of 331 mOsm/L (n = 6); this prostatic fluid contained albumin (mean ± SD, 0.115 ± 0.009 g/dl), acid phosphatase (7.0 ± 1.8 U/L), alkaline phosphatase (281 ± 164 U/L), sodium (151 ± 17 mEq/L), chloride (159 ± 0 mEq/L), and potassium (15.5 ± 0.5 mEq/L).[43] The feline prostate is an androgen-dependent organ that atrophies following castration. Estrone has been reported to cause hypertrophy of the glandular epithelium of the prostate in immature male cats.[44]

Bulbourethral Glands

The bulbourethral glands are two 5-mm-diameter, pea-shaped glands located dorsolat-

eral to the bulb of the penis at the ischial symphysis that drain by simple ducts into the urethra at the root of the penis.[1,9] These are tubuloalveolar glands completely enclosed by striated muscle, with histochemical staining reactions consistent with production of sulfated mucopolysaccharides.[37,45,46] Disseminate tubuloalveolar glandular tissue is reported present in the periurethral connective tissue of the pelvic urethra extending from the region of the prostate to the posterior aspect of the bulbourethral glands.[37] Some authors suggest that this tissue is disseminate prostate gland, while others suggest that it resembles bulbourethral gland based on the presence of mucin secretory granules in both the bulbourethral and disseminate gland but not prostate gland tissue.[35,37]

Penis

The penis is located ventral to the scrotum; it is composed of two corpora cavernosa penis, one on either side, and the corpus spongiosum penis, which lies in the middle.[6–9,47] In the adult intact male, its free end, the glans, is a 5- to 10-mm-long conical structure directed caudally that contains a band of 120 to 150 androgen-dependent penile spines in six to eight circular rows (Fig. 36–5).[5,48–50] These spines, which are directed away from the tip of the glans, are 0.1 to 0.7 mm both in length and in diameter at their base. Histologically, penile spines of the cat are composed of a connective tissue core covered by a heavily cornified epithelium, much like that of the spiny papillae of the cat's tongue.[48,51] The surface of the distal 4 mm of the glans is smooth, as is the entire glans of the prepuberal and adult castrate cat. Following castration of the adult cat, spines regress rapidly, reaching hair-like structures in 5 to 6 weeks.[48] In castrate male cats, which lack penile spines, spines recur under the influence of exogenous androgen administration, and regress in size when androgens are discontinued (Fig. 36–5D,E).[48] The physiologic role of these spines in copulation is unknown; they have been proposed to provide sexual stimulation for the male or female, to impede withdrawal of the penis from the vagina (as they are directed toward the base of the penis), or to increase vaginal stimulation, therefore favoring successful induction of ovulation. An os penis, which is 3 to 5 mm long, may be present inside the tip of the glans in the intact adult male cat. The os penis was present in three of nine medium-sized cats, and in 10 of 12 large-sized cats; no os penis was present in seven kittens or five small adult cats.[47] All of the cats examined were intact males.

The mean (±SD) length of the erect penis of five male cats during sexual arousal was 21.2 ± 2 mm, and the mean diameter (±SD) of the shaft was 5.1 ± 0.5 mm.[50]

The cat does not have a cremaster muscle, but does have a striated levator scroti muscle that originates from the external anal sphincter muscle and inserts on the scrotal septum.[36]

Bacterial Flora of the Feline Preputial Mucosa

In a study of normal aerobic bacterial flora of the feline prepuce, 29 samples were collected

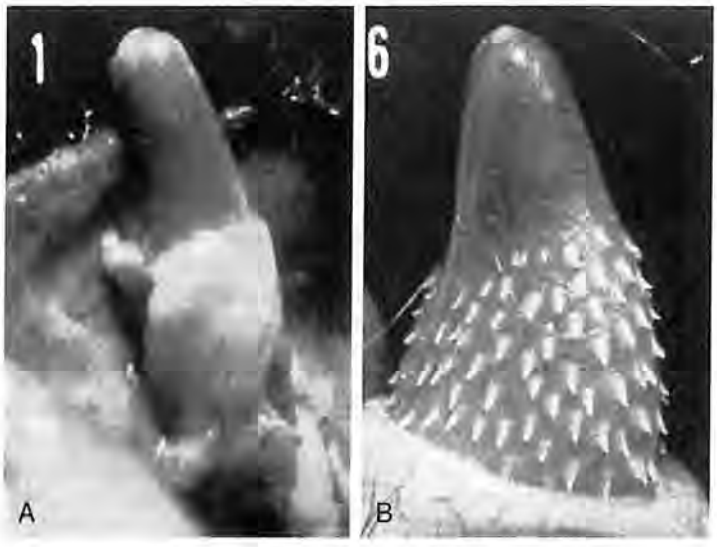

Figure 36–5. Gross anatomy of the cat penis. **A:** The glans penis of a 4-week-old kitten, with prepuce dissected away (4×). **B:** The glans penis of the adult male cat, showing penile spines (4.7×).

Illustration continued on opposite page

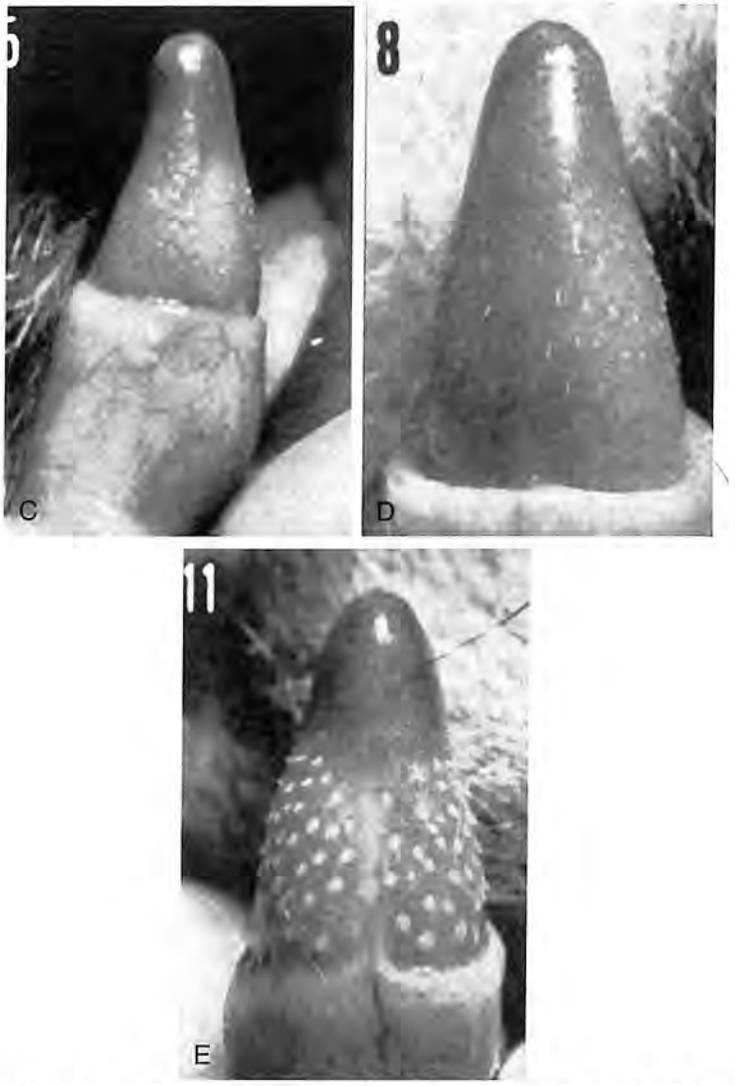

Figure 36–5. *Continued.* **C:** The smooth glans of a 5-year-old male cat, castrated at 4 months of age (4×). **D** and **E:** The glans penis of an adult male 6 weeks following postpuberal castration (**D**), and 14 days later (**E**), following implantation of a pellet of testosterone propionate, which induced reappearance of penile spines (4.7×). (From Aronson LR, Cooper ML: Penile spines of the domestic cat: Their endocrine-behavior relations. Anat Rec 157:71–78, 1967, with permission.)

at 1- to 2-week intervals from six young fertile male cats.[29] Collection technique was to press a sterile cotton-tipped swab around the circumference of the glans penis one time and then clip off the end of the swab into a transport tube containing 3 ml lactated Ringer's saline (LRS) solution. Twenty-six of the 29 samples contained a total of 67 isolates of aerobic bacteria. Ten of the 67 cultures contained more than 100,000 bacteria per milliliter (using described dilution in LRS) and 5 contained less than 10,000 bacteria per milliliter. Aerobic bacteria cultured, in decreasing frequency, included *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxytoca, Streptococcus* sp., *Enterococcus, Bacillus* sp., and *Staphylococcus* sp. In a study of *Staphylococcus* species in the cat, culture of *Staphylococcus simulans* (n = 14), *S. intermedius* (n = 4), *S. epidermidis* (n = 3), and *S. xylosus* (n = 1) was reported from the prepuce of clinically normal cats; 22 of 45 cats cultured had staphylococcal species present on the prepuce.[52]

Reproductive Behavior of the Normal Tom

Puberty

Puberty in the male cat is variably described as the time of first spermatogenesis, or the time

of first ability to ejaculate. Earliest histologic evidence of spermatogenesis in the cat occurs at approximately 20 weeks of age, and spermatozoa are present when the testes exceed 1 g in combined weight.[10] Sperm may be ejaculated in semen of male kittens as young as 7 months of age, but the age at which mating begins varies with physical condition, body size, and season, with average onset of puberty occurring at 8 to 10 months of age or at body weight of 2.5 kg, whichever occurs first.[11,23,53] Male cats maintained under controlled illumination (12-hour light/dark cycle) do not exhibit seasonable breeding behavior.[54]

Copulatory Behavior

Reproductive behavior in the male cat has been studied both by observing copulation in normal animals and by observing reproductive behavior in animals compromised by additive or ablative endocrine and neurologic treatment. The free-roaming estrous queen may be courted by several males for 12 to 96 hours before she selects and accepts one.[55] A normal male cat will approach an estrous female, touch her nose with his nose, and then investigate her perineal region.[56,57] He may exhibit a flehmen reaction, by opening his mouth slightly and closing his eyes while sniffing, an action associated with reception of pheromones of the female by the male's vomeronasal organ.[58-60] The flehmen reaction in the cat is influenced by presence of testosterone in the male, as well as by fluid-borne chemical stimuli in urine and vaginal secretions of the female.[60] He will then grasp the skin over the dorsal part of her neck with his teeth, mount her, and engage in treading activity with his hind legs while sliding down over the back of the female so as to position his penis appropriately for intromission (Fig. 36–6).[56,57] Males grip estrous females an average of 16 seconds after being exposed to them, and achieve first intromission after an average of 107.5 seconds.[61] Pelvic thrusting by the male is then followed by intromission and ejaculation, which occur in less than 20 seconds; the male then pulls away quickly to avoid being struck by the female in her after-reaction. As that reaction subsides the male usually will mount and breed her again. Mating continues until approximately seven intromissions have occurred, and a sexually exhausted male can be rearoused by a sexually fresh female.[61] Copulation may not be completed if the male is not yet acclimated to his environment (a process that may take 1 to 2 months), if he does not grasp the female's neck firmly (aberrant neck grip) or if he releases her too soon, or if a penile hair ring precludes intromission, resulting in long bouts of unsuccessful pelvic thrusting.[57] Most cats will mate during the night instead of the day if allowed to do so under natural conditions. An experienced, receptive, and patient female often is necessary to train young stud cats.

Normal and abnormal types of aggressive behavior occur in male cats as in other male mammals. Intermale aggression, which is normal in the cat, subsides in 80 to 90 per cent of male cats castrated for this reason; castration may not be an effective treatment with other types of aggression in male cats.[62]

Mating behavior usually ceases following castration of the male cat, but it may persist for as long as several years following castration of the experienced male cat. Mating behavior has been observed in castrates with and without superimposed bilateral adrenalectomy, performed to rule out an influence of adrenal androgens.[2,63,64] Prepuberally castrated male cats may or may not show sexual behavior and copulation following testosterone replacement therapy as adults; environmental stimuli of estrous queens were necessary to elicit sexual behavior after replacement therapy.[63]

Normal copulatory behavior in the male cat is reported to result from sensory reception and cortical processing of visual, auditory, and olfactory stimuli from the estrous female and from the presence of functional testes as well as from an intact gonadal-hypothalamic-pituitary axis. At one time, experimental cats were subjected to induced lesions of different parts of the brain in order to study the role of the brain in sexual behavior. Early studies of the effect of sensory deprivation on sexual behavior revealed that ablation of the olfactory bulbs alone was not associated with decreased mating behavior in the male cat.[65,66] Lesions of the frontal cortex caused interference with the motor pattern of mounting and copulating (i.e., delayed mounting, decreased frequency of intromissions, increased mountings without intromission, increased neck grips not followed by mounting), and lesions of the parietal or temporal cortex produced little change in mating behavior. Occipital cortex destruction sufficient to cause visual deficit prevented the male from easily locating and following the female, but did not interfere with copulation per se.[67-69] Bilateral lesions of the medial preoptic/anterior hypothalamic area in combination

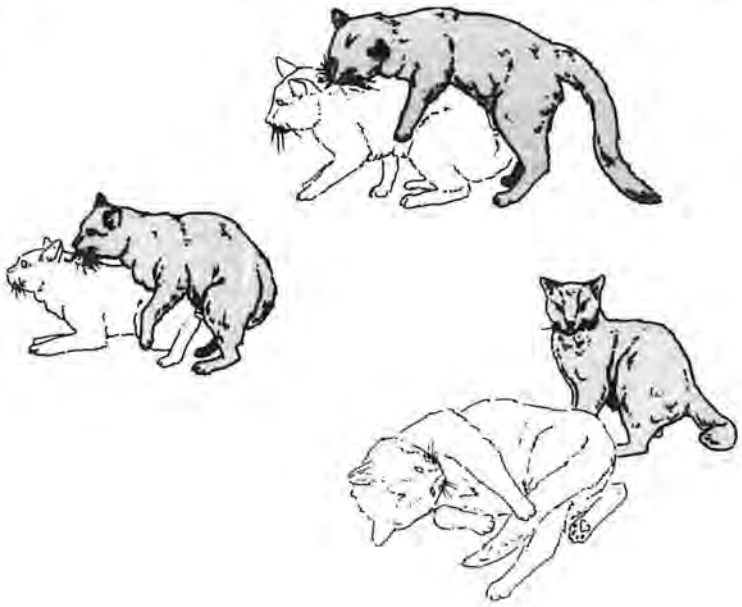

Figure 36–6. Mounting behavior in the male cat.

with olfactory bulbectomies eliminate male sexual behavior in the cat.[59] Adult male cats with lesions of the caudate nuclei show estrous-like behavior consisting of lordosis, deflection of the tail to the side of the perineum, rear limb treading, and vocalization; these changes are not associated with abnormal plasma concentrations of testosterone or estradiol (suggesting that the behavior is observed neurally) nor are they hormonally mediated.[31,69]

Aberrant sexual activity (i.e., mounting and copulatory behavior with anestrus, nonreceptive female cats, other male cats, kittens, or inanimate objects, such as small stuffed animals) has been observed in males with lesions of the temporal lobe of the cerebral cortex, particularly the amygdala and pyriform cortex; such behavior also has been observed in normal male cats without histopathology of the temporal cortex.[70]

Normal sensory innervation of the glans penis is necessary for normal copulation, as sexually experienced male cats with surgically desensitized glans penes become disoriented and cannot achieve intromission; there is a subsequent nonseasonal decline in sexual behavior with some recovery at onset of female cycling with increased photoperiod.[3,71–74] Firing of cutaneous mechanoreceptors in the dermal core of the cat's penile spines may be necessary for oriented pelvic thrusting in this species.[73]

Erection and Ejaculation

Penile erection, emission of semen into the urethra, and ejaculation of semen from the tip of the penile urethra of the male cat are mediated by parasympathetic, sympathetic, and somatic nerve fibers.[75,76] Stimulation of parasympathetic fibers of the second sacral nerve roots produces erection. Subsequent stimulation of the sympathetic trunk at L1–2 or of the hypogastric (sympathetic) nerve causes emission of seminal fluid into the urethra, and stimulation of the internal pudendal (i.e., parasympathetic, sympathetic, somatic) nerve results in ejaculation. Subsidence of erection is a result of subsequent excitation of a sympathetic pathway.[75,76] Sympathetic stimulation either centrally or peripherally does not have a visible effect (via cystoscope) on the opening to the urinary bladder.[76] Dooley et al. found more sperm in the urinary bladder of domestic cats following electroejaculation than in the antegrade ejaculate; they also report the presence of variable, but often large numbers of sperm in the urine of cats following natural mating or ejaculation into an artificial vagina, suggesting that retrograde flow of sperm and seminal plasma may be a normal phenomenon in the cat.[77]

Prebreeding Examination

Prebreeding examination of the tom includes review of vaccination history, and history of

reproductive and nonreproductive disease. General physical examination should include palpation of the testes for normal size and texture, and protrusion of the penis to demonstrate presence of a normal preputial orifice, and presence of normal penile spines, and absence of a penile hair ring. Diagnostic tests that may be indicated prior to breeding include serology to rule out viral disease (e.g., feline leukemia virus, feline immunodeficiency virus), and blood typing of both the male and female to rule out AB mismatch, which may cause feline neonatal isoerthrolysis (see Chapters 26 and 29).

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Chapter 37

Semen Collection and Evaluation in the Cat

Feline semen characteristics in samples collected by various collection methods are listed in Table 37–1.

Semen Collection

Feline sperm have been collected using an artificial vagina with conscious ejaculation of the male cat, by electroejaculation of the anesthetized male, by vaginal lavage of the postcoital queen, and by collection of urine by cystocentesis from the tom following ejaculation.

Toms must be trained to ejaculate into an artificial vagina (AV) (a 2-ml rubber pipette bulb with the bulb cut off and the cut end attached to a 3 × 44-mm test tube; the AV and test tube are placed in a 60-ml polyethylene bottle filled with 52°C water to provide an internal working temperature of 44° to 46°C) (Fig. 37–1).[12,16] Training is accomplished by repeated gentle handling of the tom in the presence of an estrous female. Three of five randomly selected laboratory cats could be trained to ejaculate into the AV after 2 weeks of handling and exposure to estrous queens.[12,17,18]

Weekly semen collections of two toms for 3 weeks using the artificial vagina varied greatly in volume and total sperm count, with relatively little change in percentage of abnormal sperm or those with progressive motility.[12] When the same toms were collected three times weekly for 1 week the semen volume and sperm numbers were more constant and nearly equaled numbers in the weekly collections. Daily semen collection from these two males caused a drop in sperm number by the fourth day to less than one half of the first day's count, but numbers then leveled off and held constant (14 to 45 million sperm per ejaculate) for the rest of the 11-day period.[12] This suggests that extragonadal reserves were exhausted after 4 days of daily ejaculation, and that sperm numbers obtained thereafter were equivalent to the daily sperm output. Although this study was small, it suggests that male cats can be used at stud three times weekly without showing a drop in numbers of sperm per ejaculate.

Electroejaculation was first reported in cats anesthetized with ketamine hydrochloride by Platz et al.; ejaculates were obtained during application of 180 2- to 8-V (volt) stimuli applied via a Teflon and stainless steel rectal probe (Fig. 37–2).[19] The effect of sequential and long-term ejaculation studies and effect of voltage and order of voltage application on semen quality of the ketamine hydrochloride–anesthetized male cat using an automatic stimulus delivery electroejaculator have been described.[5,7,20,21] When four sequential ejaculates were obtained at single seminal collections performed weekly for 22 weeks there was a significant effect of cat and sequence of ejaculate for both semen volume and number of sperm per ejaculate; repeated weekly anesthesia and electroejaculation did not alter ejaculate quality significantly, although there was a trend for ejaculate volume to increase with time.[5] In voltage application studies, number of sperm per ejaculate was shown to be affected by cat and by magnitude of voltage application, with more sperm collected at 4 or 8 V than at 1 or 2 V; stimulation with 8 V resulted in urine contamination of some ejaculates.[7] These authors propose that separate voltage thresholds for emission (epididymides, vasa deferentia) and ejaculation (urethral musculature) between cats may account for

Table 37–1. Feline Semen Characteristics

Reference	No. of Cats	No. of Ejaculations	Volume Semen, ml			Sperm Concentration Per ml × 10^6			No. of Sperm Per Ejaculate, × 10^6			Progressively Motile Sperm, %			Sperm Morphology, %				Seminal Plasma	
			$\bar{X}$	(Range)	SD	$\bar{X}$	(Range)	SD	$\bar{X}$	(Range)	SD	$\bar{X}$	(Range)	SD	N	1°	2°	Abn	pH	(Range)
Semen Collected by Electroejaculation																				
Platz et al.[1]	6	45	.2240		.0500				29.7		9.00	70.40		2.6						
Platz and Seager[2]	17	303	.2330	(.140–.738)					28.00	(9–153)		60.00	(47–81)							
Wildt et al.[3]	16	16				147.0		39.50				77.00		3.0	70.9	5.8	23.3	29.1		
Johnstone[4]	4	38	.0640	(.018–.202)					11.30	(3.1–34.4)										
Pineda et al.[5]	9	788	.1000	(.010–.400)					3.50	(0–104.5)										
Dooley et al.[6]	8	8	.2380	(.100–.390)	.0870	60.3	(.50–190)	66.75	15.46	(0.5–49.4)	17.51									
Pineda and Dooley[7]	14	32	.2300	(.100–.370)					50.51	(.55–153.9)	40.57									
Dooley and Pineda[8]	4	8	.2600	(.100–.490)	.1300				42.70	(11.1–65.96)	20.51	65.00	(44–85)	14.0					8.6 ± 0.09	
Johnstone et al.[9]	6	60	.1250		.0110														6.6 ± 0.10	
Howard et al.[10]		18	.1240		.0100	167.6		43.60				84.40		5.9	71.6					
Dooley et al.[11]	8	8	.2000		.0700				33.50		35.90									
Semen Collected by Ejaculation into Artificial Vagina																				
Sojka et al.[12]	6	24	.0400	(.01–.12)		1730.0	(96–5101)		57.00	(3–143)		78.00	(35–100)					1.6	7.4	(7.0–8.2)
Platz et al.[1]	6	183	.0338		.0048				60.70		12.90	82.50		2.7						
Dooley and Pineda[8]	4	8	.0600	(.03–.09)	.0200				60.97	(21.5–117)	31.05	58.00	(4–87)	27.0					8.3 ± 0.15	
Pope et al.[13]	3	17	.0380		.0260	541.2		660.60				90.10		5.6						
Dooley et al.[11]	5	5	.0200	(.01–.05)	.0200				30.15		27.64									
Sperm Collected from Epididymides																				
Niwa et al.[14]	Epid*						(.2–1.8)													
Hay and Goodrowe[15]	74						(77.9–127)						(66–79)					32–51		
Sperm Recovered from Male's Postejaculation urine																				
Dooley et al.[11]	8	8							38.65		22.06									
Dooley et al.[11]	5	5							20.12		46.82									
Sperm Recovered from Vaginal Lavage Fluid after Mating (1.0 ml buffered saline)																				
Dooley et al.[11]	5	5							2.72		4.37									

* Epididymis and 20-mm vas deferens minced in 300 μl extender.
N = normal; 1° = primary abnormalities; 2° = secondary abnormalities; Abn = total abnormal

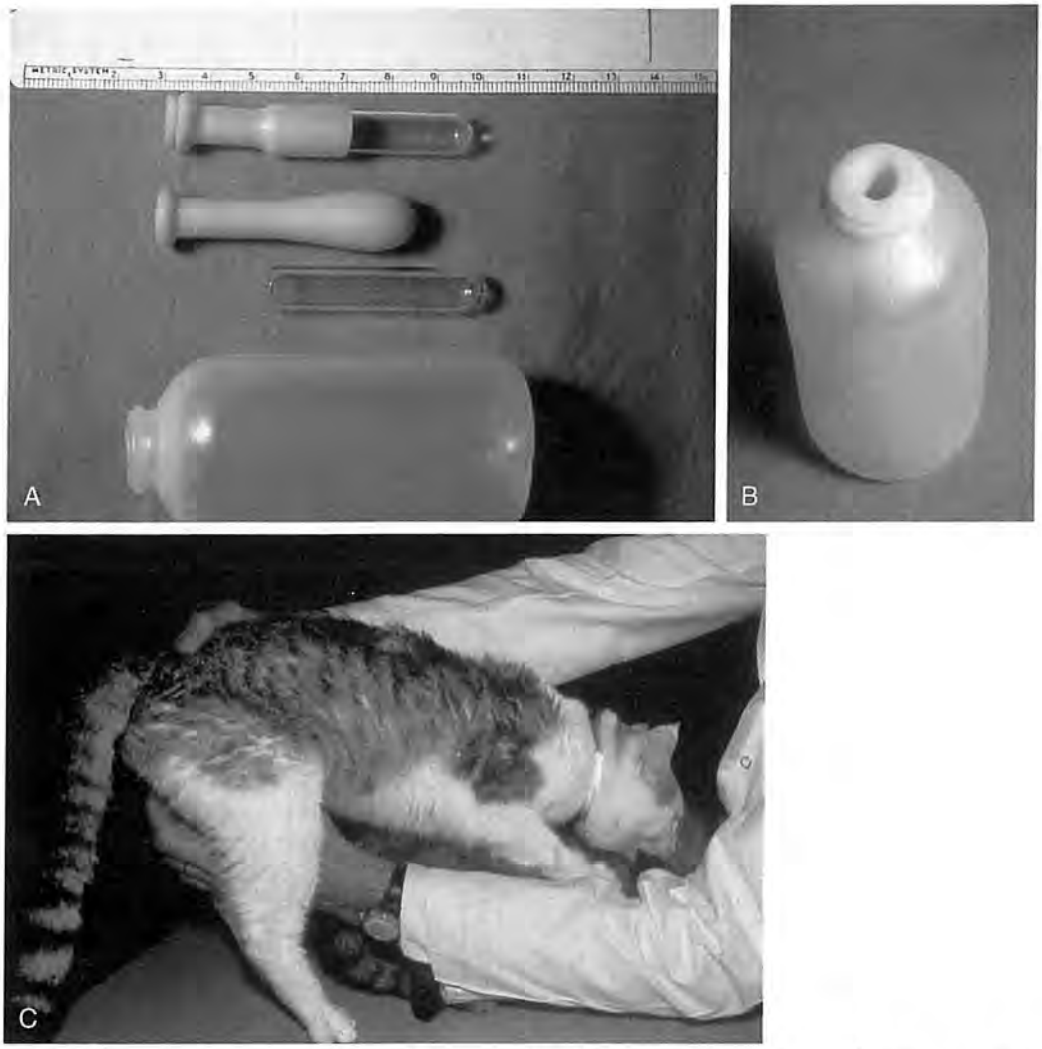

Figure 37–1. Components for an artificial vagina for tomcats (**A** and **B**), and collection of semen from a trained male (**C**). Components include an assembled collection tube, a Pasteur pipette bulb, the 6 × 50-mm glass test tube, and a 100-ml polyethylene bottle, which serves as a warm water jacket (44° to 46°C) to control temperature. The collection apparatus is out of sight between the male's hind legs; the male is holding the teaser queen by the neck. (From Sojka NJ: Management of artificial breeding in cats. *In* Morrow DA [ed]: Current Therapy in Theriogenology: Diagnosis, Treatment, and Prevention of Reproductive Diseases in Small and Large Animals, 2nd ed. Philadelphia, WB Saunders, 1986, pp 805–808, with permission.)

the high degree of variation in sperm number observed in this species. Electroejaculation of semen also has been reported in male cats anesthetized with a mixture of xylazine and ketamine given intravenous (IV) or with sodium thiopentone and atropine given intramuscularly (IM).[4] Anesthetic and voltage protocols for electroejaculation of the tom cat are listed in Table 37–2.

Vaginal lavage of the postcoital queen, or collection of a vaginal cytology specimen following copulation, may reveal presence of spermatozoa (Fig. 37–3). When vaginal lavage with 1 ml saline was performed immediately after mating between five normal males and five normal females, 40,000 to 10,240,000 sperm were recovered.[11]

The tom cat is reported to ejaculate from 15 to 90 per cent (mean = 46.8 per cent) of the ejaculate retrograde into the urinary bladder during ejaculation (Table 37–3).[6,11,21] Collection of urine by cystocentesis from the tom following ejaculation with examination of the urine sediment for sperm is a useful procedure in small animal practice to determine whether a cat is producing sperm.

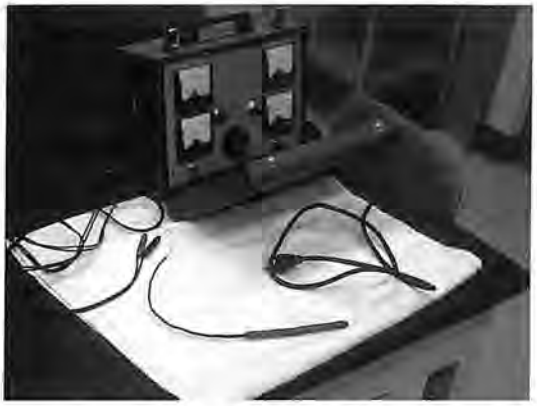

Figure 37–2. Electric stimulator and Teflon rectal probe for electroejaculation of the tom cat. The probe is inserted 9 cm into the rectum of the anesthetized cat, and variable numbers of 2- to 7-V stimuli are applied (see Table 37–2). (Courtesy of P-T Electronics, Boring, OR.)

Semen Evaluation

Semen Volume

Evaluation of cat semen has been superficial, due to the difficulty with which this fluid is obtained as well as its small volume. Ejaculate volumes collected by artificial vagina have been reported to range from 0.01 to 0.12 ml, and those collected by electroejaculation from 0.001 to 0.738 ml; these volumes have contained 3 to 143 million (AV), and 0.05 to 153 million (electroejaculation) sperm per ejaculate (Table 37–1). Semen volume is determined using a variable volume or 100-μl calibrated pipette.[2]

Sperm Cell Numbers

Sperm concentration usually is determined using a standard hemacytometer counting procedure, with undiluted semen, or at dilutions of 1 : 10, 1 : 25, 1 : 50, or 1 : 100 with distilled water.[5] Sperm counted in the 1-mm^2 area of the Neubauer hemacytometer and multiplied by 10^6 comprise the sperm concentration per milliliter in samples diluted 1 : 100. One must be cautious in comparing volume or sperm number values obtained by different investigators because of observations that electroejaculation technique alters semen volume and sperm number.[5,7] For example, Johnstone reported great variation in quality between collections from the same cat, and smaller total numbers than reported by others; in Johnstone's electroejaculation protocol, however, maximum applied voltage was usually 2 or 3 V, whereas Pineda and Dooley reported that 4- or 8-V pulses resulted in greater numbers of sperm per ejaculate than 1- or 2-V pulses.[4,7] Also, influence of magnitude of voltage application on magnitude of retrograde ejaculation has not been reported.[6]

Sperm counts in ejaculates collected by electroejaculation from 10 adult male cats undergoing bilateral occlusion of the ductus deferens were zero in 3 of 10 at 2 days following occlusion; 3 of 10 and 4 of 10 produced spermatozoa until electroejaculations on days 5 and 10, respectively, after ductus occlusion. All cats were azoospermic by day 10.[22]

Progressive Motility of Spermatozoa

Percentage of progressively motile sperm usually is estimated by examining a drop of undiluted semen at 400× magnification immediately after semen collection; mean progressive motilities of 60 to 90 per cent have been reported as normal in this species.

Sperm Cell Morphology

Feline sperm are approximately 26 μm in length, compared to dog spermatozoa, which are approximately 36 μm long.[23] Percentage of morphologically abnormal sperm in the ejaculate is determined by examining 200 sperm using phase-contrast microscopy or by routine light microscopy following staining with Diff-Quik* or eosin-nigrosin stain.†

Normal feline sperm examined by the light and scanning electron microscopes are depicted in Figure 37–4. Mean percentages of morphologically normal sperm have been reported at about 70 per cent in the cat (Table 37–1). Morphologic abnormalities of the feline sperm that have been reported include macrocephaly, microcephaly, double head, double tail, proximally coiled tail (Fig. 37–5A), bent midpiece, proximal and distal retained cytoplasmic droplet (Fig. 37–5B,C), detached head, and bent tail (Fig. 37–5D).[10]

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* Diff Quik Stain Set; Harleco, Gibbstown, NJ. Prepare a smear of the semen on a clean glass slide, and allow to air-dry. Immerse slide for 5 minutes in each of the three (fixative, eosin stain, hematoxylin stain) fluids provided in the stain set, then rinse gently with distilled water and allow to air-dry. Examine under oil immersion (1000×) without a coverslip.

† Eosin-Nigrosin Morphology Stain; Society for Theriogenology, Hastings, NE. Mix one drop of semen with one drop of stain and draw, with a spreader slide, over a microscope slide. Allow to air-dry, and examine under oil immersion (1000×) without a coverslip.

Table 37–2. Protocols for Electroejaculation in the Cat

Reference	Anesthesia	Rectal Probe¶	Voltage Applications
2	Ketamine HCl* (33 mg/kg) IM; *or* CI 744† (5.5 mg/kg) IM	12 × 1-cm diameter, Teflon 3 1.5 × 5-cm stainless steel electrodes, mounted ventrally Probe inserted 9 cm into rectum	Three sets of 60 stimuli; 5 minute rest between sets Each stimulus = 2 seconds up; 3 seconds on; 2 seconds off Set 1 = 15 stimuli each at 2, 3, 4, 5 V Set 2 = 5, 10, 15, 15, 15 stimuli at 2, 3, 4, 5, 6 V Set 3 = 5, 5, 5, 15, 15, 15 stimuli at 2, 3, 4, 5, 6, 7 V
4	Xylazine‡ (1 mg/kg) IM, and ketamine HCl (10 mg/kg) IM; *or* Na thiopentone§ (2.5%) and atropine‖ (0.65 mg/ml) IV to effect	14 × 1-cm diameter, plastic Four strip electrodes 35 × 3 mm every 90 degrees Probe inserted 9 cm into rectum	10 stimuli at low voltage, then one "maximum" stimulus; 10-minute rest, then repeat 4–9 times Each stimulus = 3 seconds up; 3 seconds on; 3 seconds off Each stimulus at low voltage = 2 or 3 V (to muscle contraction)
5	Ketamine HCl (30 mg/kg) IM	15 × 1-cm diameter Bipolar with three longitudinally directed nickel-silver electrodes Probe inserted 6 cm into rectum	Four sets of 60 stimuli; 5-minute rest between sets Each stimulus = 2 seconds up; 2 seconds rest Each set = 40 stimuli at 2 V and 20 stimuli at 3 V
10	Ketamine HCl (25 mg/kg) IM	13 × 1-cm diameter, Teflon Three longitudinal stainless steel electrodes, 2.6 mm in width and 3.75 cm in length Probe inserted 7–9 cm into rectum	Three sets of stimuli (30, 30, and 20); 2- to 3-minute rest between sets Each stimulus = 1 second up; 2–3 seconds on; abrupt return to 0 for 3 seconds Set 1 = 10 stimuli each at 2, 3, and 4 V Set 2 = 10 stimuli each at 3, 4, and 5 V Set 3 = 10 stimuli each at 4 and 5 V

* Ketaset; Bristol Laboratories, Syracuse, NY.
† Parke Davis & Co, Detroit, MI.
‡ Bayer Aust Ltd, Botany, New South Wales.
§ Ceva Chemicals; Aust Pty Ltd, Hornsby, New South Wales.
‖ Parnell Laboratories Pty Ltd, Kirrawee, New South Wales.
¶ Probes available from P-T Electronics, Boring, OR; Beltron Instruments, Bryan TX; description of probe/ejaculator construction in Dooley et al.[2]

Figure 37–3. Vaginal cytology specimen from an estrous queen showing presence of a sperm head (arrow) with a cornified epithelial cell. Hematoxylin and eosin.

Table 37–3. Volume of and Total Number of Spermatozoa in the Ejaculate; Volume of Urine, Spermatozoal Concentration, Motility, and Total Number of Spermatozoa in the Postejaculation Urine; and Percentage of Retrograde Flow of Spermatozoa into the Urinary Bladder of Cats During Semen Collection with an Artificial Vagina

	Ejaculate		**Postejaculation Urine**				
				Spermatozoal			
Cat No.	Volume (ml)	Total No. of Spermatozoa (10^6)	Volume (ml)	*Concentration* (10^6/ml)	*Motility*	Total No. Spermatozoa (10^6)	Retrograde Flow (%)
2	0.02	65.20	8.0	1.700	M	13.60	17.26
3	0.05	54.34	10.4	0.890	M	9.26	14.56
6	0.01	16.80	22.0	0.815	M	17.93	51.63
7	0.02	9.57	2.0	7.275	M	14.55	60.32
8	0.01	4.85	6.2	7.300	NM	45.26	90.32
Mean	0.02	30.15	9.72	3.596	—	20.12	46.82
SD	0.02	27.64	7.52	3.388	—	14.39	31.67

M, motile spermatozoa; NM, nonmotile spermatozoa.
From Dooley MP, Pineda MH, Hopper JG, Hsu WH: Retrograde flow of spermatozoa into the urinary bladder of cats during electroejaculation, collection of semen with an artificial vagina and mating. Am J Vet Res 52:687–691, 1991, with permission.)

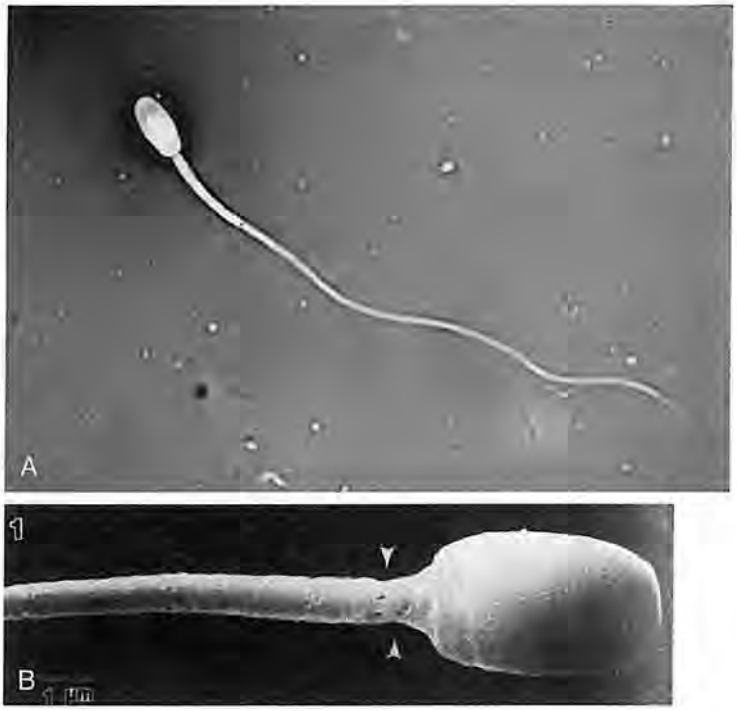

Figure 37–4. Normal feline spermatozoan examined by light microscopy using eosin-nigrosin stain (1000×) (**A**) or scanning electron microscopy (**B**). In the scanning electron micrograph, the midpiece tapers inward (arrows) in the neck area. (From Schmehl ML, Graham EF: Ultrastructure of the domestic tom cat [*Felis domestica*] and tiger [*Panthera tigris altaica*] spermatozoa. Theriogenology 31:861–874, 1989, with permission.)

Presence of intact acrosomes in sperm that have not undergone the acrosome reaction may be determined in cat semen samples stained with a solution of 1% fast green FCF (Eastman Kodak Co, Rochester, NY 14650), 1% rose bengal, and 40% ethyl alcohol in 0.1 M citric acid–0.2 M disodium phosphate buffer.[13] Semen is diluted with 2.9% sodium citrate, and an equal volume of staining solution is added and incubated for 70 seconds at room temperature. A drop of this mixture is then smeared onto a slide and air dried at 37°C. Acrosome status is evaluated at 1000× magnification; the intact acrosome stains purplish blue over the anterior portion of the sperm head. If acrosomal loss has occurred, the anterior portion of the head is colorless to light pink.[13]

Feline Sperm Cell Ultrastructure

Tom cat sperm examined by transmission and scanning electron microscopy have elongated, oval shaped heads with two planes of symmetry (longitudinal and lateral) (Fig. 37–4).[24] The neck tapers inwardly between the head and midpiece.[24,25] Longitudinal section of the head and neck (Fig. 37–6A) shows a dense acrosomal matrix, with electron-dense material surrounding the microtubules of the proximal centriole. Cross sections through the midpiece of the tom cat sperm (Fig. 37–6B and C) show the central pair of microtubules surrounded by nine microtubule doublets and nine dense rods.

Seminal Plasma Chemistries

Seminal plasma pH and other chemistries in feline seminal plasma are listed in Tables 37–1 and 37–4. Specific gravity, osmolality, and anion gap of feline seminal plasma are similar to those of blood serum. Significantly higher alkaline phosphatase is present in seminal plasma originating at the level of the testes and/or epididymides, suggesting that, in the cat, as in the dog, this enzyme may serve as an epididymal marker (Table 37–4).[26]

Seminal Plasma Microbiology

Aerobic bacterial flora in ejaculated semen from normal cats include *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxytoca, Streptococcus* and *Staphylococcus* (Table 37–5).[27] These are probably normal urethral flora flushed out in the (low-volume) feline ejaculate, as they are the same as the organisms collected at preputial culture.[27,28] In 29 semen samples from six young fertile males (with negative urine cultures collected by cystocentesis), 28 had positive bacterial growth, ranging from one isolate ($n = 8$ of 28) to four isolates

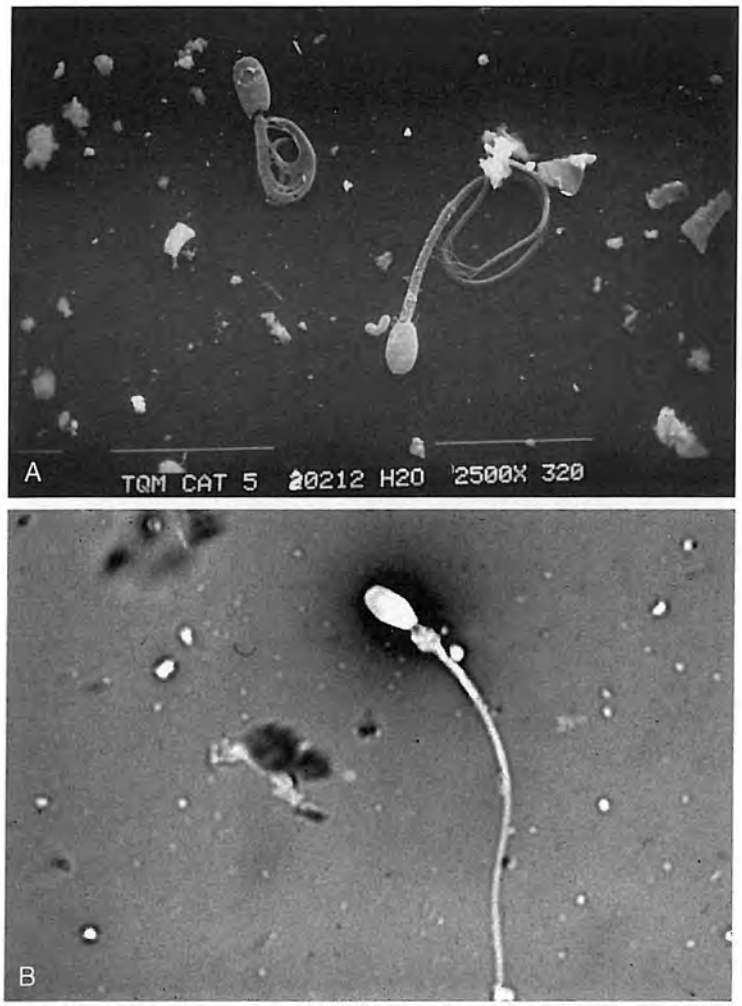

Figure 37–5. Abnormal feline spermatozoa examined by scanning electron microscopy (**A**) or light microscopy using eosin-nigrosin stain (1000×) (**B** through **D**). Abnormalities include proximally coiled tail (a), proximal retained cytoplasmic droplet (b), distal retained cytoplasmic droplet (c), and bent tail (d).

Illustration continued on following page

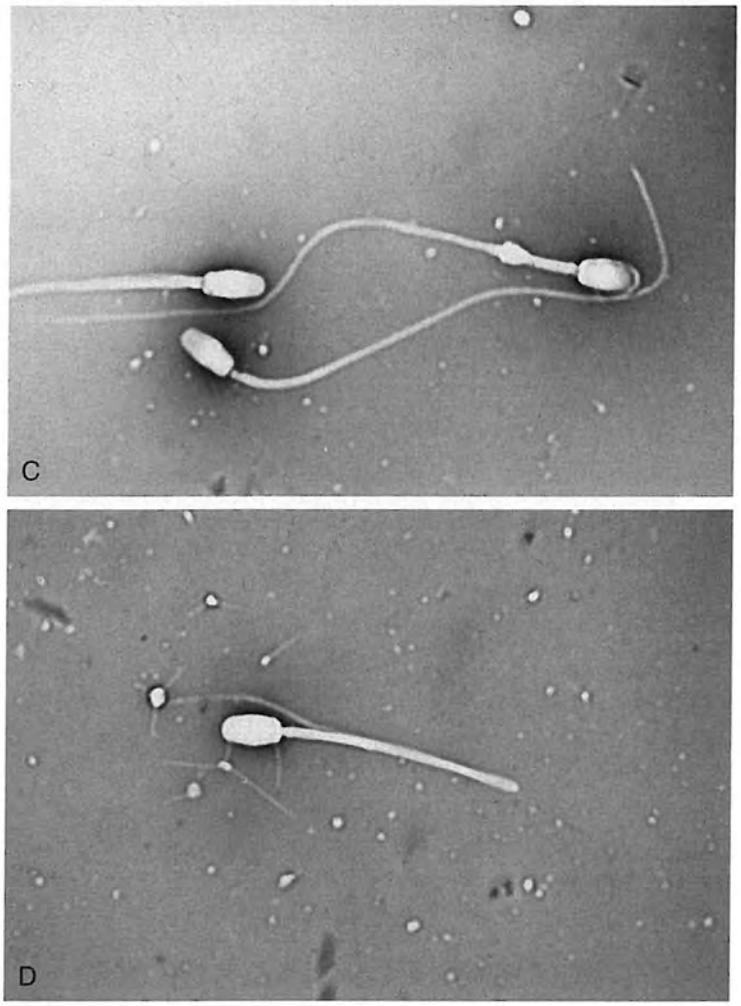

Figure 37–5. *Continued*

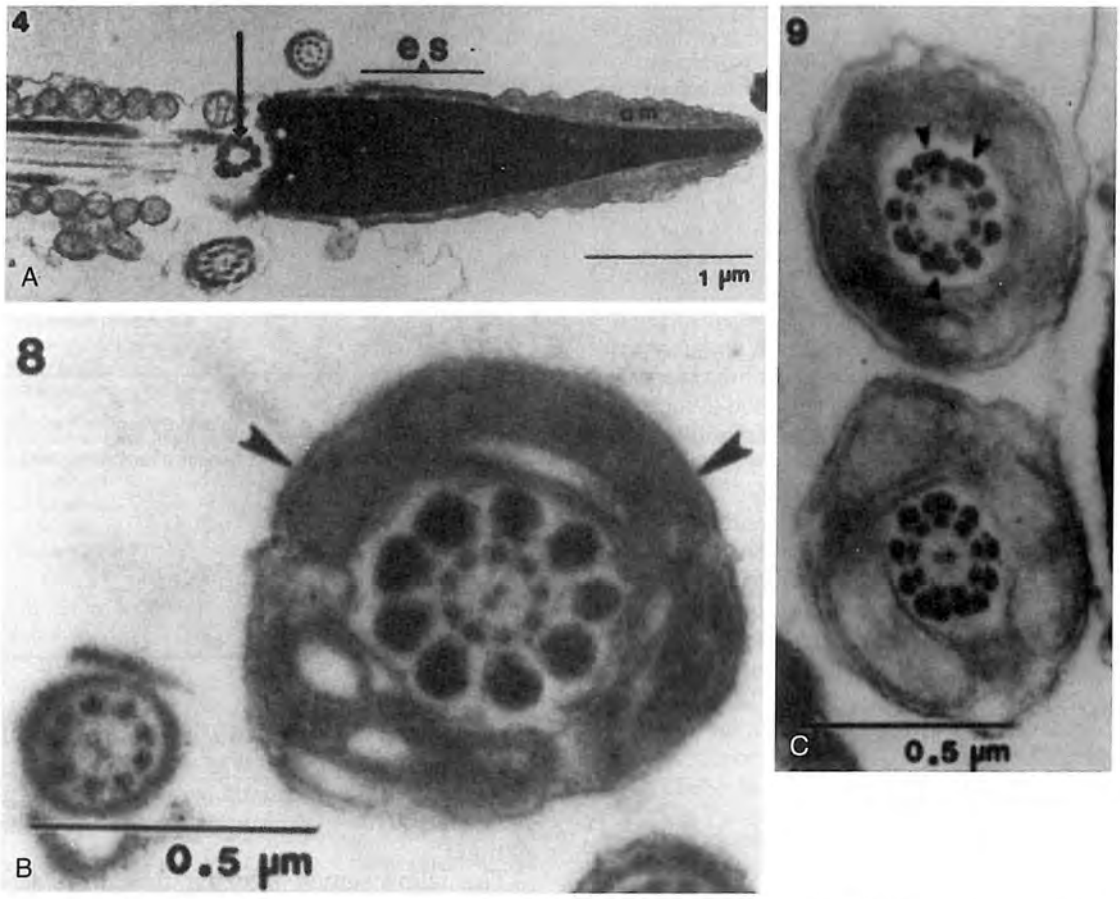

Figure 37–6. Ultrastructure of feline sperm examined by transmission electron microscopy. Longitudinal section of the head and neck (**A**) show a distinct equatorial segment (es) and dense material (arrows), which surrounds the microtubules in the proximal centriole. Cross sections through the midpiece (**B** and **C**) show the central pair of microtubules surrounded by nine microtubule doublets and nine dense rods. The plasma membrane (arrows, **B**) covers the ring of mitochondria that encircles the microtubules. A section farther caudal (**C**) shows less dense, and occasionally bilobed (arrows) rods. (From Schmehl ML, Graham EF: Ultrastructure of the domestic tom cat [*Felis domestica*] and tiger [*Panthera tigris altaica*] spermatozoa. Theriogenology 31:861–874, 1989, with permission.)

Table 37–4. Feline Seminal Plasma Characteristics and Chemistries in Samples Collected by Electroejaculation from Six Male Cats Before and after Prescrotal Vasectomy or Prescrotal Vasectomy and Bulbourethral Gland Excision*

Parameter	Seminal Plasma (S)	Prostatic and Bulbourethral Fluid (P+B)	Prostatic Fluid (P)
Volume/ejaculate, ml	0.125* (0.011)†	0.165* (.019)†	0.04* (0.006)†
pH	6.6 (0.1)	6.7 (0.1)	7.8 (0.6)
Specific gravity	1.007 (0)	1.006 (0)	1.007 (0)
Osmolality, mOsm/L	323 (3)	327 (2)	331 (3)
Anion gap	−6.5 (1.4)	−6.7 (2.5)	−1.4 (3.6)
Albumin, g/dl	0.155 (0.008)	0.115 (0.024)	0.115 (0.009)
Acid phosphatase, U/L	23.6 (2.8)	3.7 (1.1)	7.0 (1.8)
Alanine transaminase, U/L	18.0 (5.6)	41.2 (5.3)	37.5 (9.4)
Alkaline phosphatase, U/L	160,355 (15,558)	445 (170)	281 (164)
Aspartate transaminase, U/L	16 (2)	13 (2)	22 (4)
Calcium, mg/dl	<4	<4	<4
Chloride, mEq/L	161 (1.4)	163 (3)	159 (0)
CO_2, total, mEq/L	11.4 (0.5)	12.3 (0.7)	12.4 (1.6)
Glucose, mg/dl	1.0 (0.5)	0 (0)	1.5 (0.5)
Phosphorus, mg/dl	1.18 (0.17)	0.20 (0.04)	0.75 (0.15)
Potassium, mEq/L	14.1 (0.5)	16.0 (0.6)	15.5 (0.5)
Protein, total, g/dl	0.31 (0.04)	0.17 (0.02)	0.19 (0.04)
Sodium, mEq/L	166 (2)	168 (1)	151 (17)
Urea nitrogen, mg/dl	16.2 (0.5)	14.0 (0.7)	15.5 (2.5)

* Means.
† Standard errors.
From Johnston SD, Osborne CA, Lipowitz AJ: Characterization of seminal plasma, prostatic fluid, and bulbourethral gland secretions in the domestic cat. *In* Proceedings of the 11th International Congress on Animal Reproduction and Artificial Insemination, Dublin, Ireland, 1988, Vol IV, p 560, with permission.

(n = 3 of 28) per sample. Twenty of the 60 isolates contained more than 100,000 bacteria per milliliter of semen, 21 had 10,000 to 95,000 bacteria per milliliter of semen, and 19 had less than 10,000.

Sperm Penetration Assays

Penetration of zona-free hamster oocytes and zona-intact domestic cat oocytes by capacitated feline sperm has been reported.[14,29–32] Percentage penetration of zona-free hamster ova by normospermic cat spermatozoa was reported as 10.5 per cent (n = 1065); percentage penetration of zona-intact cat ova by normospermic cat spermatozoa was reported as 62.6 per cent (n = 222).[30,31] Percentage penetration by teratospermic (<40 per cent morphologically normal) cat spermatozoa was 2.8 per cent (n = 1076) and 14.6 per cent (n = 164), respectively. Hamster ova penetration percentage of 5 per cent (12 of 238) and 28 per cent (67 of 240) have been reported using fresh and overnight chilled feline epididymal spermatozoa, respectively.[32] Feline sperm from the ductus deferens can fertilize feline ova in vitro without in vivo capacitation.[29]

Semen Extension and Preservation

Frozen Semen

Pregnancy in domestic cats inseminated with frozen thawed semen was first reported in 1976, with description of a one-kitten pregnancy at 49 days following vaginal insemination.[19]

The feline semen freezing procedure first described involves mixing the ejaculate with 200 μl sterile 0.9 % NaCl solution (to increase total volume of ejaculate) and 200 μl diluent at room temperature (22° to 23°C).[1] The diluent is composed of 20% (v/v) egg yolk, 11% (w/v) lactose, and 4% (v/v) glycerin in deionized water, to which 1000 μg streptomycin sulfate per milliliter, and 1000 IU penicillin G potassium per milliliter are added. The sample is then measured and 400 μl subtracted from the total to determine initial ejaculate volume; if initial ejaculate volume exceeds 200 μl, a further volume of diluent is added to ensure a 1:1 semen: diluent (v/v) ratio. The sample is then equilibrated at 5°C for 20 minutes after which time a volume of 200 μl plus initial ejaculate volume of 5°C diluent is added and

Table 37-5. Aerobic Bacteria in Feline Semen Collected by Electroejaculation and on Preputial Mucosa from Young Adult Male Cats with Normal Semen Quality*

	Preputial Mucosa	Semen
Bacteria identified:		
Escherichia coli, β-Hemolytic	15	14
Pseudomonas aeruginosa	11	14
Proteus mirabilis	10	10
Klebsiella oxytoca	7	10
Streptococcus sp.	8	5
Escherichia coli, Nonhemolytic	4	1
Enterococcus sp.	3	
Bacillus sp.	2	1
Serratia odorifera	2	
Streptococcus enterococcus	2	3
Staphylococcus sp.	2	1
Yersinia intermedia	1	
Acinetobacter sp.		1
No growth	3	1
Total	70	61
Number of Bacterial Isolates		
4 isolates	5 samples	3 samples
3 isolates	9 samples	6 samples
2 isolates	8 samples	11 samples
1 isolate	4 samples	8 samples
No growth	3 samples	1 sample
Number of Colony-Forming Units per ml Sample Per Isolate:		
>100,000	10 isolates	20 isolates
10,000 to 95,000	1 isolate	21 isolates
<10,000	56 isolates	19 isolates
No growth	3 samples	1 sample

* Preputial cultures were collected by rolling a sterile, cotton-tipped swab around the circumference of the penis and transporting the swab to the laboratory in 3 ml lactated Ringer's solution. $n = 29$.

From Johnston SD, Root MV, Olson PNS: Ovarian and testicular function in the domestic cat: Clinical management of spontaneous reproductive disease. Anim Reprod Sci 42:261–274, 1996, with permission.

the sample allowed to equilibrate at 5°C for an additional 10 minutes. The sample is frozen by dispensing single drops of diluted semen with a Pasteur pipette into 3 × 4-mm indentations in a block of solid CO_2 (dry ice). Frozen pellets subsequently are deposited into a bath of liquid nitrogen, then transferred to labeled 5- or 8-ml Nalgene vials for liquid nitrogen storage.[1]

Feline semen diluted 1:3 with glycerol-lactose–egg yolk media at 5°C was frozen for 10 minutes in drop-sized pellets on dry ice, after which the pellets were stored in liquid nitrogen at −196°C. Semen stored 49 and 81 days and then thawed in equal volumes of 3.2% Na citrate resulted in 13 and 17 per cent fertilization rates, respectively, after being inseminated vaginally into estrous queens.[33]

Feline semen also has been frozen in straws, using a diluent composed of tes-tris (Test) egg yolk (20%) buffer (diluent volume not specified).[34] Samples were cooled to 4°C over 2 to 3 hours; 4°C glycerol at a final concentration of 5% was added after the semen sample reached 4°C. Diluted samples were then loaded into 0.25-ml straws and cooled at 10°C/min to −80°C, after which straws were plunged into liquid nitrogen (−196°C). Better post-thaw motility, percentage of live sperm, and acrosome integrity were reported for freezing in straws compared to pellets.[34]

Chilled Extended Semen

Conceptions with feline semen stored at 4°C for up to 3 days following dilution with equal volumes of 37°C 3% buffered citrate–egg yolk media have been reported. The diluted sample is submerged in an 800-ml beaker of 37°C water and placed in a 4°C refrigerator to equilibrate to 4°C over 4 to 5 hours; samples stored for 0, 1, 2, and 3 days resulted in percentages of fertilized ova in queens of 100, 67, 30, and 23 per cent, respectively.[12,35]

Short-term storage of feline spermatozoa at 4°C was reported for samples diluted in Test-yolk buffer as described for semen freezing, and held at 4°C for 24 hours.[36] Motile spermatozoa have been recovered from feline semen samples stored for up to 1 week in 325 mM *N*-tris (TES) buffer with 2 to 20% (v/v) egg yolk; the yolk, however, significantly decreased the percentage of progressively motile cells, leading to the conclusion that cat sperm may be less susceptible to cold shock than are sperm of other species.[37,38]

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Chapter 38

Prevention of Fertility in the Tom Cat

Indications for preventing fertility in the male cat include attempts to decrease the pet overpopulation problem (see Chapter 30), to control feral cat colonies, and to exert desirable effects on reproductive and urine marking behaviors afforded by castration in this species. Methods of preventing reproduction in the male cat include castration, vasectomy, injection of sclerosing agents into the epididymides (chemical vasectomy), and administration of progestogens.

Castration

Castration is the most widely used method of preventing reproduction of the male cat, because it is easy, effective, irreversible, and it eliminates most objectionable reproductive behaviors of aggression, roaming, and urine marking.

Castration is performed in male kittens or cats older than 6 weeks of age under general anesthesia. Kittens (6 to 14 weeks of age) should be fasted for no more than 4 hours preoperatively, maintained on a warm water blanket during surgery and recovery, and given a small meal within 1 hour of standing so as to prevent hypothermia and hypoglycemia, which are of special concern in pediatric patients.[1-3] Standard open technique of feline orchiectomy includes incision of the scrotum, subcutaneous tissue, and parietal vaginal tunic over each testis. The parietal tunic is grasped, separated from the testis, and excised. The spermatic cord is occluded by ligation, by a knot on itself, or by tying the ductus deferens to the spermatic vessels.[4] Ligation of the cord with suture is preferred in kittens (6 to 14 weeks of age) because of the fragility of the spermatic cord blood vessels at this age. The spermatic cord is transected distal to the ligature/knot, and the testis is removed. The scrotal incision is not sutured.[4]

Castration eliminates sexual behavior immediately in most toms, although the occasional experienced male may achieve erection and copulate with estrous queens after castration.[5] In one report, none of six cats without previous sexual experience exhibited mating behavior by 1 week after castration, while 4 of 12 experienced males continued some neck grip, mounting, or pelvic thrusting behavior for as long as 5 weeks; one of the four continued to copulate with estrous queens for up to 1.5 years.[6,7] Prepuberal castration is most effective in eliminating or preventing undesirable sexual behavior.[6,8-10] Vasectomy has been associated with presence of spermatozoa in the feline ejaculate for as many as 49 days after vasectomy, suggesting that castrated cats should not be presumed sterile until at least this long following surgery.[11] Flushing the vasa deferentia with 10 to 12 ml of a 0.007% solution of trypan blue dye in 0.9% isotonic saline solution immediately following vasectomy was reported to decrease the number of intact spermatozoa in the ejaculate to zero by day 7.[12] Male cats undergoing prepuberal castration do not develop the thick cervical dermis and stocky neck that is typical of the male cat phenotype. Castration causes immediate decrease in feline serum testosterone concentrations to nondetectable (<0.05 ng/ml) concentrations.[13,14]

Desirable Effects of Feline Castration

Desirable effects of castration of the tom include decreased aggressive behavior, mount-

ing, roaming, urine marking, and urine odor.[9,13,15] Decline in fighting (88 per cent of cats), roaming (94 per cent of cats), and urine spraying (84 per cent of cats), were reported in 42 male cats castrated in adulthood.[9,13]

Prepuberal castration usually prevents urine spraying.[9,14,17] Approximately 10 per cent of 134 prepuberally gonadectomized male and 5 per cent of 152 prepuberally gonadectomized female cats were reported to show urine spraying behavior as adults; the male cats were more likely to spray if housed with female cats than with other male cats.[17] Age of prepuberal castration (6 to 10 months of age) had no influence on spraying later in life. Male cats castrated in adulthood usually show postoperative decline in urine marking. A rapid decline in urine spraying occurred in 78 per cent of 42 male cats castrated as adults, and another 9 per cent showed gradual decline in spraying.[18] Age at castration was not related to the rate of decline.

Undesirable Effects of Feline Castration

Undesirable effects of castration include adhesion of the prepuce to the penis and delayed long bone physeal closure in cats castrated prior to 7 months of age, decreased nutritional caloric needs that may predispose to obesity, and surgical complications that may develop intra- or postoperatively.

Breakdown of penile/preputial adhesion is an androgen-dependent event that occurs in most mammals postpartum.[19,20] Male kittens $n = 6$) castrated at 7 weeks of age all showed persistence of such adhesion with inability to protrude the penis at 22 months of age; three of five castrated at 7 months of age showed such adhesion.[1] Four of 10 male kittens castrated at 5 months of age showed variable adhesions of the prepuce to the penis; some adhesions could be separated partially by manipulation, while in other areas a common stratified squamous epithelium joined the prepuce to the penis.[19,20] None of the 10 male kittens castrated at 5 months of age and given replacement therapy with testosterone or 10 male kittens left intact as controls showed these adhesions. In theory, these adhesions may be associated with buildup of debris and urine, and predispose to local irritation, inflammation, or ascending urinary tract infection, although observation of such adverse sequelae has not been reported.[20]

Physeal closure is delayed 5 to 7 months in male cats gonadectomized at 7 weeks or 7 months of age when compared to intact littermates; this results in long bone length that is about 10 per cent longer than that of sexually intact littermates (see Fig. 30–1).[1,21,22] In 24 male cats referred for repair of physeal fractures over a 4-year period, there were 12 fractures of unexpectedly open physes in neutered males 12 to 16 months of age; fractures in these cats involved open physes of the proximal humerus, distal radius, distal femur, or distal tibia.[21] No conclusions were drawn from the sample size as to whether the open physes predisposed to physeal fracture.

Heat production, a measure of metabolic rate, was decreased an average of 28 per cent in cats castrated at 7 weeks or 7 months when compared to intact toms.[23,24] This suggests that gonadectomized male cats may require intake of 28 per cent fewer calories than do sexually intact male cats in order to avoid signs of obesity. Heat production was not different between male cats castrated at 7 weeks or 7 months of age.

Undesirable surgical complications of castration in male cats include hemorrhage, scrotal bruising and swelling, and infection at the incision site.[4] Priapism due to a thrombotic remnant at the base of the spermatic cord has been reported; the cat required amputation of the penis.[25]

Proposed undesirable effects of feline castration that have been disproven in controlled studies include proposed decrease in urethral diameter and proposed increase in lower urinary tract diseases in castrated, compared to intact, male cats. Studies have documented that there is no difference in urethral diameter of male cats left sexually intact, castrated pre- or postpuberally, or castrated prepuberally and given testosterone replacement therapy (Fig. 38–1).[1,10,19] Cats fed calculi-inducing diets showed no effect of castration on development or severity of urethral obstruction or lower urinary tract disorder signs.[26]

If orchiectomy is incomplete, or if intra-abdominal testicular tissue remains as a cryptorchid testis, reproductive behaviors (i.e., mounting, intromission), urine marking, and the thick cervical dermis of the male persist. Presumptive diagnosis of retained testicular tissue is based on persistence of the androgen-dependent penile spines (see Chapter 36), or by measuring serum testosterone concentrations greater than 1 ng/ml 1 hour after administration of gonadotropin-releasing hormone (GnRH) (25 μg intramuscularly [IM]) or 4 hours after administration of human chorionic gonadotropin (hCG) (250 IU IM).[27]

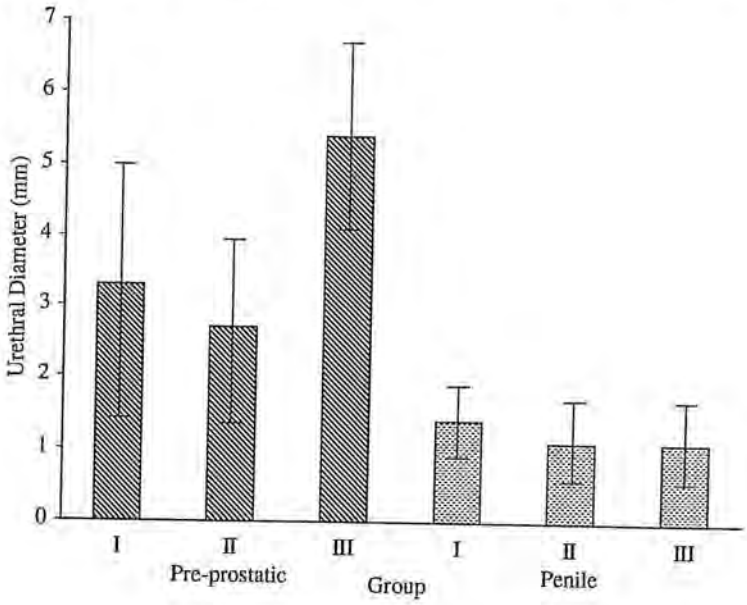

Figure 38–1. Mean diameter (±SD) in millimeters of the preprostatic and penile urethra in male cats gonadectomized at 7 weeks of age (group I) or 7 months of age (group II) or left intact (group III). Diameter is not significantly different by group. (From Root MV: The effect of prepuberal and postpuberal gonadectomy on the general health and development of obesity in the male and female domestic cat. PhD Thesis, University of Minnesota, 1995, with permission.)

Vasectomy

Vasectomy is performed in the anesthetized cat by double-ligating and surgically removing a section of each ductus deferens through a 1.5- to 2.0-cm ventral midline incision just cranial to the scrotum.[11,28–30] Gentle caudal traction on the testes aids in identifying the spermatic cords after the skin incision is made, and the vasa deferentia are isolated by blunt dissection. Alternatively, the ductus deferens may be identified intra-abdominally using a laparoscope, and 1 to 2 cm of the ductus is occluded using ancillary bipolar forceps and electrocoagulation.[31] Live spermatozoa were present in ejaculates from vasectomized cats for up to 49 days after prescrotal vasectomy, or 120 hours after intra-abdominal electrocoagulation of the ductus deferens.[11,31] Vasectomy does not alter libido or copulatory ability in adult male cats. Spermatogenesis was active in cats examined histologically 7 and 11 months following laparoscopic vasectomy by electrocoagulation, although spermatoceles of the ductus deferens and epididymis were presented in some animals.[31]

Injection of Epididymal Sclerosing Agents

Sclerosis of the epididymis, or chemical vasectomy, may be induced by injection of 0.05 or 0.10 ml 4.5% chlorhexidine digluconate into the cauda of the feline epididymis.[11] This sclerosing agent is injected through a 27- or 30-gauge, 12.7-mm needle into the most prominent area of the cauda epididymis of the anesthetized cat while the testis is fixed against the scrotum. Of eight cats so injected (four with 0.05 and four with 0.10 ml per epididymis) long-lasting oligospermia (three) or azoospermia (four) was induced; four of the eight had some sperm (0.010 to 12.788 million) in the ejaculate 231 to 252 days after treatment, but only one of these had normal sperm numbers.[11]

Progestational Drugs

Progestins have been used to alter feline sexual behavior and urine marking behavior, but their effect on semen quality is unknown.[13,32–34] Delmadinone acetate (DMA) at doses of 0.25 to 1.0 mg/kg orally daily for 7 to 14 days or 10 to 20 mg/kg subcutaneously (SC) once caused termination of roaming and in-house urine marking within 3 to 7 days; these behaviors returned in 3 to 12 months, requiring repeated treatment.[32] Complete control of sexual interest was reported in two toms given single oral doses of 5 mg of megestrol acetate, chlormadinone acetate, or 2.5 mg DMA once weekly.[34] Full drug effects were reported to occur in less than 36 hours, and to be lost by 8 to 10 days following treatment. Medroxyprogesterone acetate, at a dose of 2.2 to 11 mg/kg SC or IM, also has been used to alter male sexual behavior in this species, and is reported to affect spermatogenesis adversely, but quantitative studies on sperm numbers after treat-

ment are not available.[13,33] Chronic progestin administration in the male cat may cause undesirable side effects (e.g., diabetes mellitus, mammary hypertrophy, possibly mammary neoplasia), so it should not be used casually or for prolonged periods.[13,35]

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Chapter 39

Disorders of the Feline Testes and Epididymides

Disorders of the Testes

Congenital Anomalies

TESTICULAR APLASIA (MONORCHIA)

Absence of one testis (monorchia) has been reported occasionally in the cat. In most cases, diagnosis was made at laparotomy to search for an abdominal cryptorchid testis, when the spermatic cord and testicular blood vessels were traced to a blunt terminus. The epididymis was present in the abdomen on the side of the missing testis in a case reported by McEntee.[1] Two mixed-breed cats with monorchidism, one with left side affected and one with right side affected, were detected in a population of 1345 cats presenting for orchiectomy over a 10-year period; 23 cats in this group were cryptorchid.[2] Testicular aplasia was diagnosed in an Abyssinian and a Burmese cat in a retrospective study of 50 cryptorchid cats castrated at the Animal Medical Center.[3] Because the cryptorchid testis may be difficult to find at exploratory surgery, diagnosis of monorchia should be confirmed using serum testosterone concentrations following excision of contralateral, scrotal testis (see Cryptorchidism below).

ABNORMALITIES OF SEXUAL DIFFERENTIATION

In the normal male cat, sex is determined at time of conception, when a Y-chromosome-bearing sperm penetrates the X-chromosome-bearing ovum and initiates fertilization. When a region on the Y chromosome called the sry (sex-determining region of the Y chromosome) is present, undifferentiated gonadal ridge tissue begins to differentiate as testis. The testis then induces persistence and differentiation of the male tubular tract (the mesonephric or wolffian duct system) and external genitalia by secreting testosterone, and induces regression of the internal female tubular tract (the paramesonephric or müllerian duct system) by secreting müllerian inhibiting substance (MIS). Major categories of abnormalities of sexual differentiation include numerical abnormalities of chromosomal sex (such as 39,XXY trisomy or 38,XX/38,XY chimeras or mosaics), abnormalities of gonadal sex (such as presence of ovotestes in true hermaphrodites), and abnormalities of phenotypic sex (such as male pseudohermaphrodites, or XY males with testes and a persistent uterus masculinus). Accurate diagnosis of abnormality of sexual differentiation relies on knowledge of the karyotype, gonadal histology, wolffian and/or müllerian duct remnants in the abdomen, and external genitalia in the affected individual.

Abnormalities of Chromosomal Sex: Testicular Hypoplasia in the Male Tortoiseshell or Calico Cat. The male tortoiseshell (black and orange) or calico (black, orange, and white) (T-C) cat has been known since the early 1900s to be exceedingly uncommon, and, when found, to have a high incidence of sterility.[4–12] Incidence of male T-C cats is estimated at 1 in 3000 T-C cats.

First drawings of testicular histology of the male T-C cat were published in 1915; these showed presence of small seminiferous tubules lined by Sertoli cells and absence of spermatogonia and spermatogenesis.[13] In a 1931 report of 14 adult male T-C cats on record, 5 (36 per cent) were known sterile, 3 (21 per cent) were known fertile, and 6 were of unknown fertility.[14] Testes from six male T-C cats included four with absence of spermatogenesis

and two with normal spermatogenesis when compared to testes of normal, non–T-C male cats.[15] One male tortoiseshell cat was reported to have sired 65 kittens in 2 years; and 11 tortoiseshell males (none of which were reported fertile or infertile) were among the 43 sexed offspring.[16]

In early work first from England, and then the United States and Japan, careful descriptions of results of matings of orange, black, and T-C cats provided evidence that orange and black coat colors are sex-limited traits in the cat, and that black and orange may occur together in the female, but usually do not occur together in the male.[17–30] Evidence indicated that orange coat color in this species is determined by a single gene located on the X chromosome; black coat color may be a codominant allele at the same locus or an autosomal trait masked by the orange, sex-linked allele and unmasked by the nonorange allele (epistasis). Females homozygous for the orange trait are orange; those homozygous for black or nonorange are black, and those heterozygous for orange and nonorange contain scattered patches of both colors, and are tortoiseshell. Presence of (autosomal) white spotting in a tortoiseshell animal causes the calico appearance, and dilution genes may alter appearance of the black to blue or orange to cream colors. Male cats, bearing only one X chromosome, should therefore be able to show orange or black coat color, but not both. Females heterozygous for orange and black coat colors may show both colors, in a brindle or scattered patch fashion over the skin. The female pattern is due to random X-chromosome inactivation, which occurs in all female mammals early in embryonic development; the inactivated X chromosome in any given cell (which may be of maternal or paternal origin in different cells of the same animal) may be visible in some cells (e.g., buccal mucosa epithelium, polymorphonuclear leukocytes) as sex chromatin or the Barr body.[31–33]

Many theories were advanced to explain the coexistence of unusual coat color and sterility in the male T-C cat: somatic point mutation at the color locus, failure of sex linkage, sex reversal, meiotic nondisjunction, partial chromosome nondisjunction, crossing over of homologous regions of the X and Y chromosomes, and prenatal vascular anastomosis leading to freemartin-like chimerism. In 1961, however, just 5 years after the cytogenetic breakthrough technique for counting and analyzing human chromosomes was first reported, and 2 years after it was discovered that the sex-chromatin–positive Klinefelter's syndrome men have an extra X chromosome (47,XXY), Thuline and Norby reported the presence of 39 chromosomes (instead of the normal 38) in two phenotypically male T-C cats.[34] Both of these cats contained a female-type buccal mucosal smear (sex chromatin present), and absence of spermatogenesis on testicular histology was observed in one. Their report appeared slightly before one by Frota-Pessoa hypothesizing that male T-C cats might be XXY aneuploids like human Klinefelter's syndrome patients.[35] Chu, Thuline, and Norby reported detecting a mixture of diploid (38,XX) and triploid (57,XXX) cells from cultured somatic cells of a sex-chromatin–positive male T-C cat with seminiferous tubules lined only by Sertoli cells.[36] Their work was the first to document presence of more than one X chromosome and/or chimerism in the male T-C cat. At the time their paper appeared, the correct chromosome number of the cat (38,XX or 38,XY) had been established by a number of techniques and investigators.[37] Figure 39–1 depicts the karyotype and testicular histology of a male calico cat.[34]

Between 1956 and 1984, 38 cases of male T-C cats were published that included karyotype and information on fertility.[12,15,36–55] Among these 38 animals were cats with one of eight different chromosome constitutions (Table 39–1); 10 were known fertile, and 25 were known sterile. All of the 11 39,XXY cats were sterile, as were 2 chimeric-mosaics with multiple cell populations. Most of the 38,XY cats (assumed to be 38,XY/38,XY mosaics or chimeras) were fertile.

Data presented in Table 39–1 must, however, be interpreted with caution, because of the limitations and laboratory variability with the karyotype technique. First, in many reports blood lymphocyte culture is used to determine karyotype when, in fact, karyotype of the gonad or skin may differ from that of blood cells. Second, culture technique may alter results; Chu et al. observed that triploid cells increased from 70 to 92 per cent of the total after 3 to 9 weeks in culture; cell culture duration prior to karyotype usually is not specified in reports on these cats.[36] Third, the decision to call a modal number of chromosomes (e.g., 39,XXY) the karyotype when a small percentage of cells contain 38 chromosomes often has been made subjectively, based on observation of occasional artifactual chromosome loss from cells of normal individuals; this may mean that some chimeric cats are incorrectly categorized

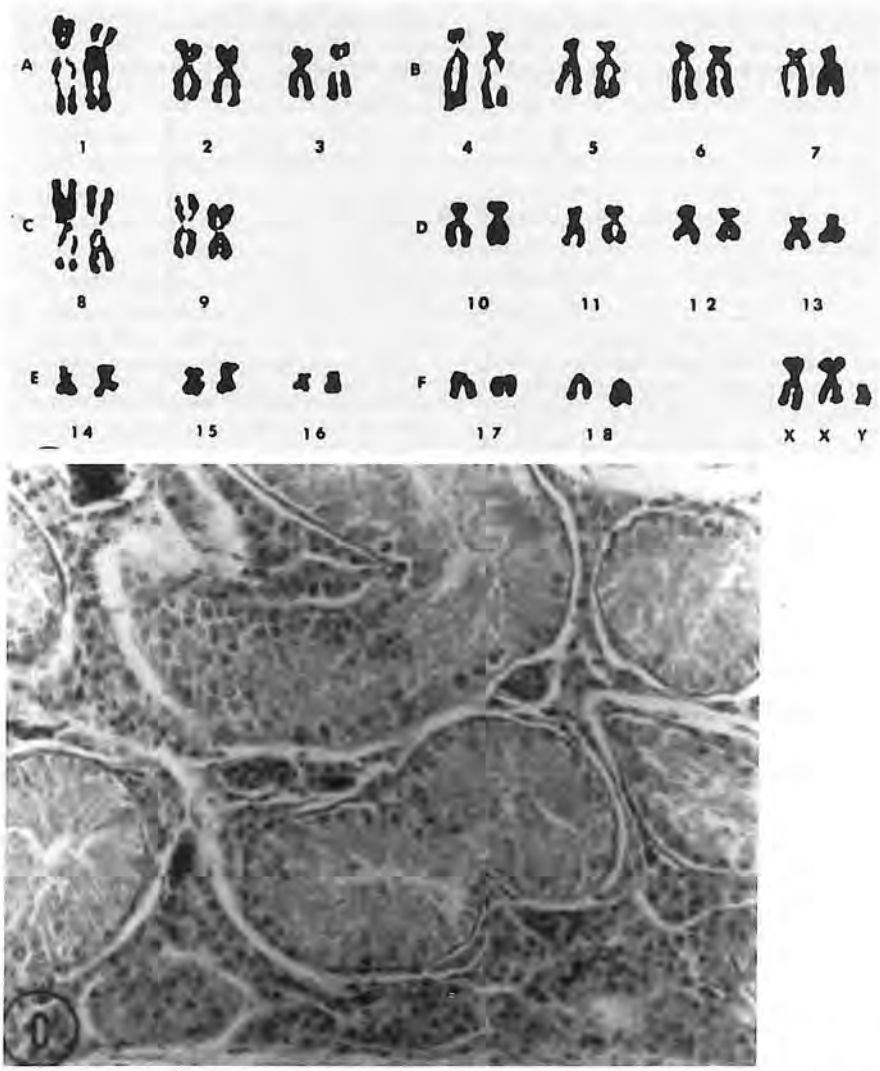

Figure 39–1. Karyotype (top) and testicular histology (low power, 160×) of a 2-year-old longhaired male cat, colored gray, cream, and white. The karyotype depicts 39,XXY from each of 45 cells counted at two separate laboratories from cultured skin fibroblasts. The testicular histology shows well-developed seminiferous tubules lined by Sertoli cells only. Evidence of spermatogonia is not seen. Tubules are surrounded by increased numbers of interstitial cells. (From Centerwall WR, Benirschke K: An animal model for the XXY Klinefelter's syndrome in man: Tortoiseshell and calico male cats. Am J Vet Res 36:1275–1280, 1975, with permission.)

as 39,XXY aneuploids. These technique problems also make difficult the task of assessing etiopathogenesis of the chromosome abnormalities and their mechanism of adversely affecting fertility, because hypotheses may be based on incorrect karyotypes. Still, all of the fertile male T-C cats have had a detectable 38,XY cell line, suggesting that normal 38,XY cells may exist in the gonads of these individuals as well as in tissues cultured (Table 39–1).[53] In addition, Malouf et al. detected two histologically different populations of seminiferous tubules in a 38,XX/38,XY male calico cat; some showed normal spermatogenesis and some were completely devoid of germ cells, a condition observed by us in a 38,XY male tortoiseshell cat (Fig. 39–2).[47]

The XXY or chimeric chromosome status in the phenotypic male has been detected in a Himalayan cat with tortoiseshell points, in a Siamese crossbreed with calico points, in a blue-cream Burmese, and in three brown tortoiseshell Burmese cats as well as in the T-C domestic shorthair and longhair breeds.[45,50–52] Investigators have karyotyped these cats because of their unusual coat color, which is a marker of the XXY or chimeric state; however, other male cats may carry abnormal genotypes

Table 39–1. Chromosome Complements and Fertility in Male Tortoiseshell-Calico (T-C) Cats

Chromosome Complement	No. of Cats	No. Fertile	No. Sterile	No. Unknown
39,XXY	11	0	11	0
38,XX/38,XY	7	3	3	1
38,XY*	6	5	1	0
38,XY/39,XXY	6	1	4	1
38,XX/57,XXY	4	0	4	0
38,XY/57,XXY	2	1	0	1
38,XY/39,XXY/40,XXYY	1	0	1	0
38,XX/38,XY/39,XXY/40,XXYY	1	0	1	0
Totals	38	10	25	3

* 38,XY cats assumed to be 38,XY/38,XY chimeras.
Data from Jones,[12] Ishihara,[15] Chu et al.,[36] San Juan Conference on Karyotype of Felidae,[37] Biggers and McFeely,[38] Centerwall and Benirschke,[39] Gregson and Ishmael,[40] Hageltorn and Gustavsson,[41] Jones,[42] Konig et al.,[43] Long et al.,[44] Loughman and Frye,[45] Loughman et al.,[46] Malouf et al.,[47] Matano,[48] McFeely et al.,[49] Moran et al.,[50] Nicholas et al.,[51] Pyle et al.,[52] Ramberg et al.,[53] Thuline,[54] and Thuline and Norby.[55]

with normal coat color (e.g., black or orange). Any male cat known to be sterile since birth, of any coat color, should be examined for karyotype in order to rule out this abnormality. The approximate 2:1 sterility:fertility ratio of male T-C cats (Table 39–1) may be biased because of selection of males for fertility.[13–15,39]

Because of the various genotypes (Table 39–1) and testicular histologies (i.e., normal spermatogenesis; some seminiferous tubules normal and some without spermatogonia; all tubules with Sertoli cells only) present in male T-C cats, it is difficult to advance a single genetic explanation for this phenomenon. Mechanisms that may be involved include disjunction of the sex chromosomes during meiosis (leading to a 20,XX or 20,XY gamete), fertilization of one ovum by two sperm (leading to a 57,XXY cell), mosaicism (i.e., an individual with cell populations of more than one genotype derived from a single zygote through events such as somatic mutation, somatic crossing-over, mitotic nondisjunction) or chimerism (i.e., an individual with cell populations of more than one genotype arising from a mixture of different zygotes such as transplantation, chorionic vascular anastomosis, double fertilization, or participation of both zygotes into one developing embryo).[56] Gene instability or mutation at the orange locus is a possible explanation for the fertile 38,XY male T-C cat; data from offspring of fertile male 38,XY cats support such an explanation.[50,57]

Abnormalities of Gonadal Sex: True Hermaphroditism. Abnormality of gonadal sex occurs when gonadal sex is not consistent with diploid chromosomal sex, or when an ovotestes or ovary and testes are present in a single individual. Because of the small number of feline karyotyping services available, abnormalities of gonadal sex have not been widely reported in this species. A right abdominal

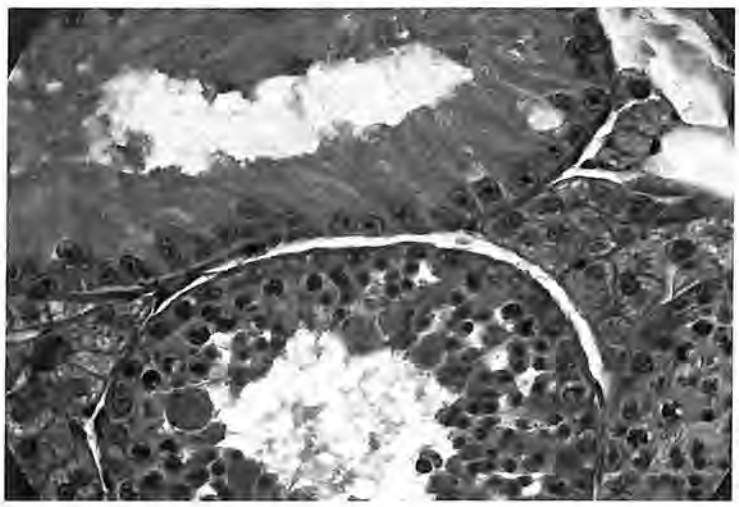

Figure 39–2. Histologic appearance of the seminiferous tubules of a male calico cat with 38,XY chromosome complement. One tubule appears normal, and the other is lined by Sertoli cells with no evidence of spermatogenesis. Hematoxylin and eosin stain.

ovotestis was present in a feline cadaver that also contained a left scrotal testis that was histologically normal; a right uterine tube and uterine horn, which entered the urethra through the region of the right side of the prostate gland, also were present.[58] Bilateral ovotestes were detected in a 4-year-old American shorthair cat examined for urinary incontinence of 7 months' and hematuria of 1 month's duration.[59] On retrograde urethrocystography, a bicornuate structure was identified dorsal to the urinary bladder which, at exploratory surgery, was found to be a thin-walled uterus. Urine dribbling ceased following surgical removal of the uterus and gonads. Ovaries and uterus were present in a 6-month-old phenotypically male Siamese cat examined for showing signs of estrus; the gonads appeared to be ovaries grossly, but may, in fact, have been ovotestes due to the unusual finding of the penis (which should differentiate only under androgenic stimulation).[60,61] (Alternatively, the cat's mother could have received or secreted virilizing hormones during pregnancy, in which case the kitten would be a female pseudohermaphrodite.)

Abnormalities of Phenotypic Sex: Pseudohermaphroditism. Pseudohermaphrodites are individuals with external genitalia or excurrent reproductive ducts of the gender opposite that represented by their gonads. Pseudohermaphrodites are named by gonadal sex, so a male pseudohermaphrodite is an XY individual with testes and the phenotype or tubular tract of the female. Insufficient or ineffective androgen or androgen receptors or 5α-reductase (i.e., the enzyme that converts testosterone to dihydrotestosterone, which induces closure of the urethral and labioscrotal folds in the male) results in female external genitalia. Inadequate or ineffective MIS or MIS receptors results in persistence of the müllerian duct system (uterus masculinus) internally. Male pseudohermaphroditism has been described in the cat as it has in other species, and the uterus masculinus has been observed in phenotypic males with testes as well as in the true hermaphrodites just described. Signs of sexual excitement were described in a 3½-month-old kitten with one scrotal testis and a rudimentary uterus.[62] Abdominal testes and uterus were present in a 1-year-old blue tabby cat with a vulva that was slightly less elongated than normal and that contained a small penis-like structure that could be protruded from the vulvar cleft.[63] Barr bodies (inactivated X chromosomes) were not observed during examination of 400 cells collected from the buccal mucosa, suggesting that the cat was a genetic male. A uterus masculinus was removed surgically from a 38,XY 6-year-old male castrate domestic longhair cat referred to the University of Minnesota for persistent dysuria following perineal urethrostomy 2 weeks earlier; the uterus compressed the urethral lumen at the region of the trigone (Fig. 39–3).[64] Pyometra of the uterus masculinus was detected in a 10-month-old neutered male cat with a history of intermittent antibiotic-responsive fever, lethargy, and stranguria; signs resolved following hysterectomy.[65]

Testicular feminization is a syndrome associated with normal male karyotype and func-

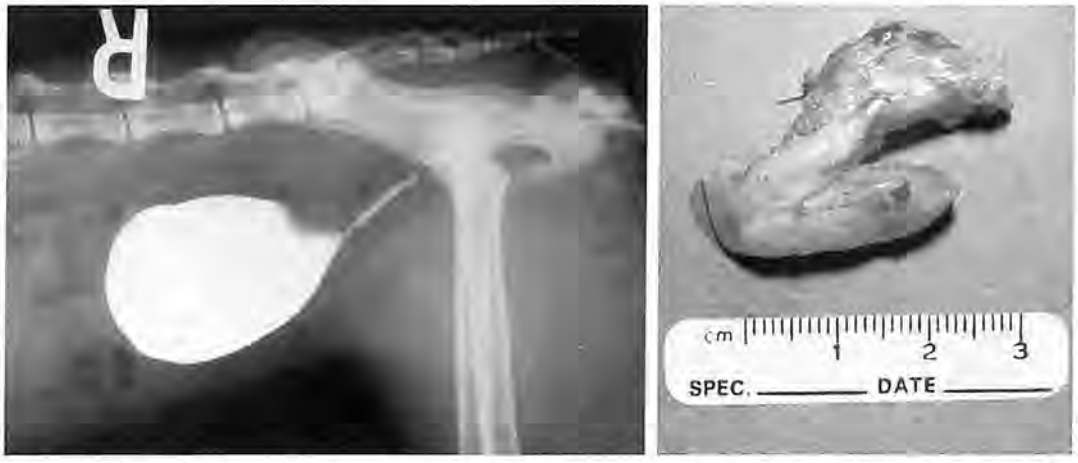

Figure 39–3. Radiographic (retrograde cystourethrography) and gross appearance of a uterus masculinus removed surgically from a 38,XY 6-year-old male castrate domestic longhair cat with persistent dysuria and compression of the urethral lumen at the region of the trigone. (From Osborne CA, Johnston GR, Polzin DJ, et al: Redefinition of the feline urologic syndrome: Feline lower urinary tract disease with heterogeneous causes. Vet Clin North Am 14:409–438, 1984, with permission.)

tional testes, but nonfunctional androgen receptors throughout the body; affected individuals have female external genitalia, and lack internal wolffian (epididymis) and müllerian (uterus) duct derivatives.[66] Testicular feminization is sometimes called complete androgen resistance. This syndrome was reported in a 6-month-old tabby cat admitted for routine ovariohysterectomy; the external genitalia consisted of a vulva and clitoris of normal shape and size, and two abdominal testes were removed that had histologic features similar to those of undescended testes in other animals.[66] Serum testosterone concentrations were not measured prior to castration. No histologic evidence of epididymides was present, nor were androgen receptors present in fibroblasts cultured from genital skin. Testes usually are removed from human patients with this disorder because of the finding that gonadal neoplasia may occur; similar recommendation is indicated in the cat in order to confirm diagnosis.[66]

CYSTIC RETE TESTIS

Cystic rete testis was reported in the right testis of an 8-month-old cat presenting for routine castration (Fig. 39–4).[67] Approximately 50 per cent of the right testis was occupied by an irregular shaped cyst that communicated with a 9 × 16-mm cyst in the head of the right epididymis. The right epididymis contained proteinaceous material but no sperm. The seminiferous epithelium of the affected testis contained focal areas of degeneration as well as active spermatogenesis.

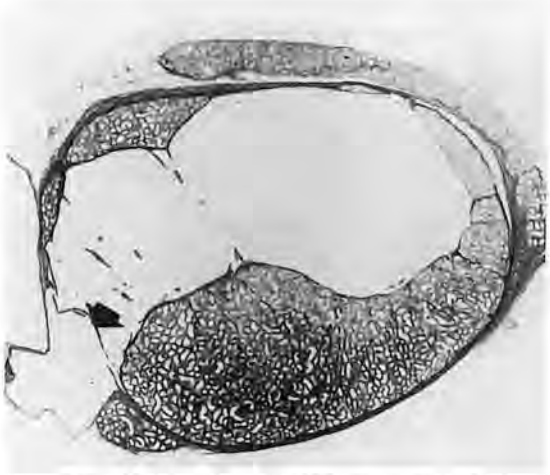

Figure 39–4. Midsagittal section of feline testis and epididymis from a cat with cystic rete testis. Central cystic cavity is surrounded by testicular parenchyma. Arrow points to portion of extratesticular cystic rete testis. (From Gelberg HB, McEntee K: Cystic rete testis in a cat and fox. Vet Pathol 20:634–636, 1983, with permission.)

Cryptorchidism

The normal feline testis descends into the scrotum prior to birth; testes may, however, move freely up and down in the inguinal canal prior to puberty, so that definitive diagnosis of cryptorchidism should not be made in cats less than 7 to 8 months of age.[68,69] One author has reported that the "vast majority" of cryptorchid male kittens presenting for castration at less than 6 months of age have normal scrotal testes when re-examined 6 weeks later with no treatment.[70]

Incidence of cryptorchidism in the cat has been reported as 0.37 per cent (23 of 6290 male cats presenting for castration at London clinics in 1 year) and 1.7 per cent (23 of 1345 cats admitted for castration).[2,70] Cryptorchidism was the most common congenital defect of the urogenital systems in 19,646 feline patients reported by Priester et al. (*n* affected = 14).[71] Feline cryptorchidism is more commonly unilateral than bilateral; only 1 of 10 (10 per cent), 5 of 23 (22 per cent), and 5 of 50 (10 per cent) affected cats were bilateral cryptorchids.[2,3,70] Location of the testes in 10 bilateral cryptorchid cats was bilateral abdominal (7), bilateral inguinal ring (1), and right testis inguinal/left testis abdominal (2).[2,3] In 63 unilateral cryptorchid cats, left and right testes were equally affected.[2,3] Locations of 43 unilateral cryptorchid feline testes were abdominal (28 per cent), inguinal ring (14 per cent), and inguinal (58 per cent).[3]

There are no data at present that demonstrate that cryptorchidism is a hereditary defect in the cat.[72] Some authors suggest that it should be considered hereditary because of its heritable nature in other species and because Persian cats are over-represented in surveys of affected cats.[2,3]

Cryptorchid testes produce testosterone, so male phenotype (thick cervical dermis), libido, aggressive behavior, urine marking, and "tomcat" urine odor usually are present in affected males. Cats with unilateral abdominal cryptorchidism have been reported where male behavior or yowling to get outdoors persisted after unilateral castration of the scrotal testis; this behavior ceased following laparotomy and removal of the abdominal testis.[73–76] Bilateral cryptorchid cats are sterile, because of the higher temperature in the abdomen or at the inguinal ring; unilateral cryptorchidism may

occur in a fertile male.[72] Affected testes usually lack spermatogonia and products of their meiosis (i.e., spermatocytes, spermatids, spermatozoa), but contain normal complements of interstitial and sustentacular (Sertoli) cells. Affected toms usually are otherwise clinically normal, although a few have shown other congenital defects. These include bilateral medial patellar luxation, congenitally shortened tail, kinked tail, tetralogy of Fallot, tarsal deformity, microphthalmia, and upper eyelid agenesis. In dogs, there is an association between cryptorchidism and patellar luxation in breeds not at risk for the latter; the other defects in the cat have not been reported in association with cryptorchidism in other species.[3]

Diagnosis of cryptorchidism is based on inspection of the scrotum of male cats at or after 5 months of age; feline testes move up and down in the inguinal canal prior to puberty, so that inspection prior to that time may falsely suggest a diagnosis of cryptorchidism.[69] When male cats of unknown history are presented with no scrotal testes, two diagnostic tests may be used as presumptive evidence of an occult, retained testis. The first of these is examination of the penis for presence of androgen-dependent penile spines, because penile spines atrophy by 6 weeks following complete castration (see Chapter 36 and Fig. 36–5). Second, serum testosterone concentration following stimulation with human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (GnRH) may support a diagnosis of cryptorchidism. Table 39–2 contains serum testosterone concentrations in normal intact and cryptorchid male cats before and after stimulation with hCG or GnRH. Note that, because testosterone is secreted in pulsatile bursts, intact male cats may not have detectable serum concentrations at a single bleeding; therefore, provocative stimulation is required for diagnosis. Diagnosis of cryptorchidism is confirmed by finding the retained testes at surgical exploration.

Medical therapy with anterior pituitary or chorionic gonadotropin hormones is not effective in inducing descent of cryptorchid feline testes.[72,79] The veterinarian's decision, therefore, is to do nothing or to recommend surgical removal of the retained testis or testes. Surgical removal is the treatment of choice in other species, where testicular neoplasia and/or torsion of the spermatic cord are well-documented sequelae to cryptorchidism, and where the hereditary nature of the defect is an indication for bilateral castration of potentially fertile unilateral cryptorchid animals. In the cat, the heritable nature of this defect is not known, but is suspected. Spermatic cord torsion has not been documented in this species; and only a small number of feline testicular tumors have been reported, with only one affected animal also being a (bilateral) cryptorchid.[80] Neoplasia was not present in cryptorchid testes removed from 14 cats, although all of these castrations were in young cats, and neoplasia of the cryptorchid testis usually occurs in older individuals in other species.[69,73,74,81]

Both midline abdominal and inguinal ring surgical approaches have been described to find and remove cryptorchid testes in the cat.[82–84] The retained testis may be located anywhere between the caudal pole of the kidney and the scrotum; use of a spay hook to retrieve the vas deferens via an abdominal approach has been advocated by some authors, but is not recommended because of the risk of damaging the ureters. One author reported avulsion of a ureter from the trigone of the bladder when

Table 39–2. Serum Concentrations of Testosterone in Normal Intact and Cryptorchid Male Cats Before and After Administration of Human Chorionic Gonadotropin (hCG) or Gonadotropin-Releasing Hormone (GnRH)

Animals	No. of Cats	Serum Testosterone Concentration (ng/ml)		
		Resting	Post-hCG*	Post-GnRH†
Normal intact male cats	6	ND‡–3.0	3.1–9.0	5.0–12.0
DSH cat with two deep inguinal testes	1	1.3		2.7
DLH cat with one testis in subcutis overlying the pubis[76]	1	0.68	10.5§	

* Post-hCG samples drawn 4 hours after 250 IU hCG IM.
† Post-GnRH samples drawn 1 hour after 25 μg GnRH IM.
‡ ND = nondetectable, <0.05 ng/ml.
§ Post-hCG sample drawn 2 hours after 500 IU hCG IV.
Data from Memon et al.,[76] Johnston,[77] and Johnston et al.[78]

a spay hook was used in an attempt to exteriorize a retained testis.[3] Because the retained testis usually is not palpable per abdomen, both inguinal and abdominal approaches have been advocated. In the former, a ventral midline incision just cranial to the pubis permits blunt dissection and lifting of the inguinal fat pads, and visualization of the external inguinal rings.[83] Alternatively, a ventral midline abdominal incision starting 4 cm caudal to the umbilicus and extending 4 cm caudally permits examination of the bladder, prostrate and internal inguinal ring; if the testis is not identified, the incision can be extended cranially to examine the caudal pole of the kidney.[3] Memon et al. describe finding a cryptorchid testis that could not be located at two previous laparotomies by following the left ductus deferens from the prostate through the inguinal ring caudally into subcutaneous tissue and a fascial plane lateral to the symphysis pubis.[76] Gentle traction may be applied to the spermatic cord or gubernaculum at the inguinal ring while dissecting bluntly to expose the testis.

Injury

Injury and displacement of the testes are unusual in cats because of the firm apposition of these organs to the body, but occasionally injury occurs following a cat being hit by a car or following bite wounds from a cat fight. Broad-spectrum antibiotic therapy, establishment of drainage in presence of infection, and application of cold packs to decrease inflammation are indicated in these cases.[79]

Orchitis

Orchitis is rare in the cat, but has been observed following testicular infection with tuberculosis, *Brucella* spp., the feline infectious peritonitis (FIP) virus, or aerobic bacteria introduced by bite wounds; noninfectious lymphocytic orchitis also has been observed.

Feline genital tuberculosis has been reported outside of the United States.[85–88] One or both testes may be involved; bilateral testicular enlargement to five times normal size with spermatic cord and epididymal enlargement occurred in a 2-year-old Siamese cat with disseminated tuberculosis and probable hematogenous spread.[87]

Feline orchitis due to brucellosis has been reported, although cats are considered resistant to natural infection with *Brucella* spp.[79,89]

Bacterial orchitis in the cat, as in other species, is associated with scrotal swelling, pain, and redness. Appropriate treatment is aggressive therapy with a broad-spectrum antibiotic for 2 to 3 weeks.

Scrotal distention due to FIP inflammation of the testicular tunics was reported by Stein and observed by the author in a 9-week-old kitten with bilaterally swollen testicles, fever (104°F), lethargy, and a negative titer to FIP (Fig. 39–5).[79] Observed cases have not shown antemortem evidence of severe ascites, but fibrinous fluid exudate was present in the peritoneal cavity at necropsy. The vaginal cavity surrounding each testicle is continuous with the peritoneal cavity, and extension of a peritoneal inflammatory process, therefore, may occur in the male.

Reactive, lymphocytic aggregates (i.e., noninfectious orchitis) have been observed in the testes of two cats, ages 8 and 9 years, and two testicular sperm granulomas (dilated tubules with no observable germinal epithelium or Sertoli cells but tightly packed luminal spermatozoa) were present in the oldest of these cats.[90] Spermatozoa are antigenically different than tissues of the male that produced them, and normally are protected from encountering immunocompetent cells by the blood-testis barrier. Should a rent in this barrier occur (i.e., in the testis, epididymis, or vas deferens), a sperm granuloma may occur, with regional monocyte infiltration and inflammatory response. Sperm granulomas generally are not associated with abnormal clinical signs except for infertility, and are diagnosed by histology of affected testes. Their importance in congenital or acquired infertility in the cat is unknown.

Testicular Degeneration/Atrophy

Atrophy is a nonspecific lesion of mammalian testes that may occur following nutritional, thermal, radiation, endocrine, inflammatory, metabolic, or immune-mediated testicular insult.

Cats fed diets deficient in riboflavin, vitamin A, or essential fatty acids (specifically linoleate, which the cat testis can convert to arachidonate) have been shown to undergo testicular degeneration and atrophy.[91–94] Male cats fed a riboflavin-deficient diet for 6 months showed complete aspermia, with decreased numbers of spermatids and spermatocytes, and an occasional increase in number of Sertoli cells.[91] Four male kittens fed a raw-meat diet devoid of vitamin A from 12 weeks of age failed to show

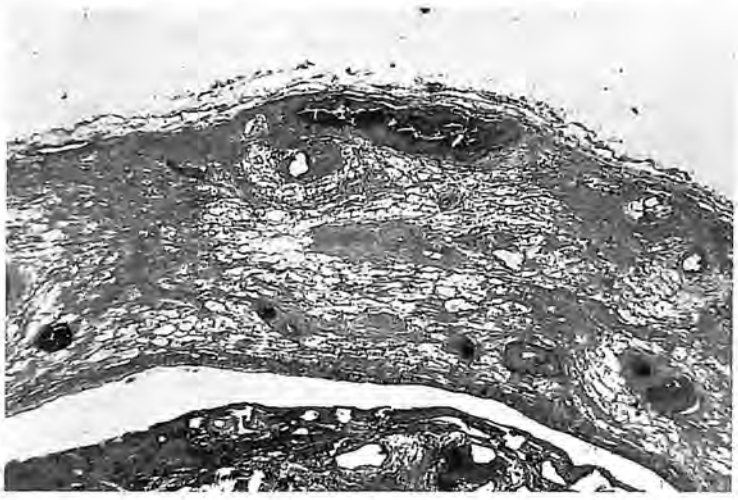

Figure 39–5. Histologic appearance of the testis from a 9-week-old kitten with bilaterally swollen testes due to feline infectious peritonitis. The tunica albuginea and tunica vaginalis have multiple foci of necrotizing inflammation, with fibrinoid necrosis of vessel walls and fibrin thrombi. The foci are composed of plasma cells, neutrophils and fibrin. Hematoxylin and eosin stain.

puberal testicular development.[92] Three of four adult male cats fed a linoleate-deficient diet exhibited extensive testicular degeneration; very few sperm and some intraepithelial cysts were present in the epididymides.[93] Excess dietary vitamin A was reported to induce testicular degeneration in cats after 12 to 15 months of a diet of liver.[95]

Thermal effect on testicular function of the cat is best demonstrated by the histologic appearance of abdominal cryptorchid testicles. Such testes generally show complete absence of spermatogenesis.[75] Testicular atrophy also may occur following administration of sex steroids.[72,96]

Degeneration of seminiferous tubules and thickening of the seminiferous tubular basement membrane were reported to increase with age in a study of 42 pairs of feline testes and epididymides removed at routine castration.[90] In testes from one 9-year-old cat included in the study, severe seminiferous tubular degeneration and absence of the germ cell line were observed with lymphocytic aggregates containing plasma cells, macrophages, and mitotic figures.

Diagnosis of testicular atrophy is based on histologic examination of the testis or a biopsy sample of testis. Supporting diagnostics include physical examination findings of small soft testes, azoospermia, and/or detection of insufficient (if suppressed by sex steroids) or elevated (with reduced testicular function) serum gonadotropins (i.e., luteinizing hormone [LH] and follicle-stimulating hormone [FSH]).

Testicular Neoplasia

The incidence of testicular tumors in cats is low; no testicular tumors were present in 56 tumors in 11,909 cats, in 621 feline tumor cases from both sexes, or in surveys of 328 and 571 feline neoplasms.[97–100]

Occasional case reports have documented Sertoli cell tumors (SCTs), interstitial cell tumors, a seminoma, undifferentiated carcinomas, and one testicular teratoma in aged male cats.[1,80,99,101–103] One report documents an SCT in the right testis and an adenoma and undifferentiated carcinoma in the left testis of a bilaterally cryptorchid aged domestic shorthair cat.[80] An interstitial cell tumor measuring 5 × 9 mm at midsagittal section in a 11 × 13-mm testis section was an incidental finding at necropsy in a 13-year-old cat.[1] A functional (testosterone-secreting) ectopic interstitial cell tumor was detected attached to an atrophic remnant of a spermatic cord in the subcutis near the scrotum in a 12-year-old male cat castrated at 6 months of age that had been exhibiting urine marking for the past 2 years.[103]

Metastasis of a feline Sertoli cell tumor to the liver and spleen and of a malignant seminoma to the lymph nodes and soft tissues of the sublumbar region have been reported.[80,102] Lymphosarcoma metastasizes to the testes occasionally, but always as part of more generalized disease.[102]

Testicular neoplasia is diagnosed by palpation of a testicular mass in the scrotal or ectopic testis, followed by excision biopsy and histopathology. Paraneoplastic syndromes such as feminization or bone marrow suppression by estrogen secretion of SCTs have not been associated with testicular neoplasia in the cat. These tumors may occur unilaterally or bilaterally.[89]

Treatment of feline testicular neoplasia is orchiectomy of the affected testes. Hemicastra-

tion may be considered in valuable breeding animals that are not cryptorchid, but bilateral orchiectomy is preferred because of the occurrence of bilateral testicular tumors and because atrophy of the contralateral testis often occurs, reducing fertility.

Prognosis for life of the animal is good in absence of metastasis.

Disorders of the Epididymides, Spermatic Cord, and Vas Deferens

Anomalies

Congenital absence of the right vas deferens, kidney, and ureter has been reported in a cat.[104] Spermatogenesis was present in both the right and left testes, but the left testis (vas deferens present) contained germ cells in all stages of development, whereas the right testis showed few cells that had progressed to the spermatid stage.[104] Aplasia of the left epididymis and vas deferens occurred in a 39,XXY male tricolor cat.[43] A 9 × 16-mm cyst in the head of the right epididymis was detected and removed at routine castration from an 8-month-old cat (Fig. 39–4); the epididymal cyst communicated with a cyst occupying approximately 50 per cent of the right testis.[67] The epididymis contained proteinaceous material but no sperm, and it appeared that the efferent ductules of the right testes were distended with sperm that could not reach the epididymis. The left testis and epididymis were normal.

Spermatoceles

Spermatoceles are cystic dilations of the epididymal duct with accumulation of sperm in the cyst; they occur following congenital or acquired occlusion of the duct or a break in the epididymal epithelium leading to formation of a sperm granuloma.[89] Spermatoceles have been described in pathologic surveys of testes obtained from clinically normal cats at routine castration, and in cats following electrocautery vasectomy or intraepididymal injection of sclerosing agents to induce sterility.[90,105,106] Although cystic dilations of the epididymis occasionally are palpable through the scrotum, there may, as often, be no palpable mass. Diagnosis is confirmed by histologic examination.

Inflammation

Funiculitis, inflammation of the spermatic cord, may occur following infection acquired at castration. Chronic suppurative funiculitis (scirrhous cord) is sometimes seen in cats, and the severed end of the spermatic cord may be adherent to the scrotum or scrotal wound.[107,108] Abscessation may be present in these cases, and affected animals usually are febrile, lethargic, and anorexic. Testicular tuberculosis also may be associated with tubercular inflammation of the spermatic cord.[109]

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Chapter 40

Disorders of the Feline Prostate and Bulbourethral Glands

Disorders of the Prostate

With the exception of a small number of prostatic adenocarcinomas, prostate disease is almost unknown in the cat. The normal feline prostate is not easily palpable, feline prostatic fluid is very difficult to collect, and the prostate atrophies after castration.

Congenital anomaly of the feline prostate has not been reported.[1] Prostatic atrophy occurs after castration or in androgen-insufficiency states, which may occur as abnormalities of sexual differentiation. Squamous metaplasia and prostatic enlargement occur following experimental administration of estrogen.[2-4] Benign prostatic enlargement has been reported only a single time, in a 6-year-old intact male with clinical signs of straining to defecate and an enlarged prostate discovered at rectal palpation under anesthesia. When castration and stilbestrol therapy did not lead to prostate involution or remission of signs, the cat was euthanized; at autopsy a walnut-sized prostate was found to consist of a 0.5-inch central cyst and undifferentiated fibrous connective tissue with much collagen and scattered remnants of ducts.[5] Although Bloom reports that prostatitis does not occur in the cat, a 2-cm-diameter abscessed prostate was detected at necropsy of an aged cat at Angell Memorial Hospital (and J. Holzworth, personal communication, 1982).[2]

A few cases of prostatic adenocarcinoma have been diagnosed in cats, with variable description of clinical signs and presence of metastasis (Fig. 40–1)[1,6-8] (C. A. Osborne, personal communication, 1983). Four cases of prostatic adenocarcinoma were described in castrated male cats of ages 10, 15, 17, and 22 years[6,9] (C. A. Osborne, personal communication, 1983). Clinical signs in affected cats included hematuria, dysuria, pollakiuria, and outflow obstruction of the urinary tract. A hard mass at the pelvic brim was palpated rectally in two of these cats (C. A. Osborne, personal communication, 1983). Irregularity and filling defects of the prostatic urethra were detected by retrograde urethrography in one cat with prostatic adenocarcinoma and tumor metastasis to the lungs (Fig. 40–2).[6] Since most prostatic adenocarcinomas have been detected at necropsy in this species, there is little information about their biologic behavior or response to treatment. A prostatic fibroadenoma was reported in a 13-year-old male castrate cat.[9]

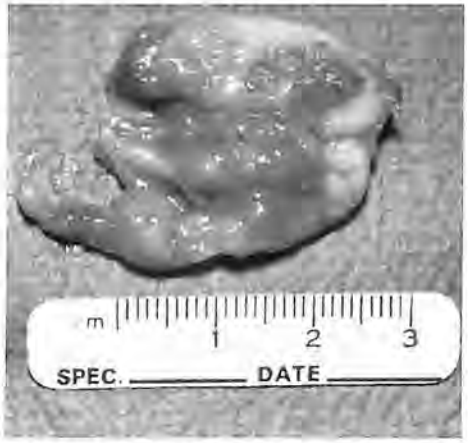

Figure 40–1. Gross appearance of a prostatic adenocarcinoma from a cat. (Courtesy of Dr. Carl Osborne, University of Minnesota, College of Veterinary Medicine.)

Disorders of the Bulbourethral Glands

Bloom reports that lesions of these accessory sex organs of the cat are infrequent and are of

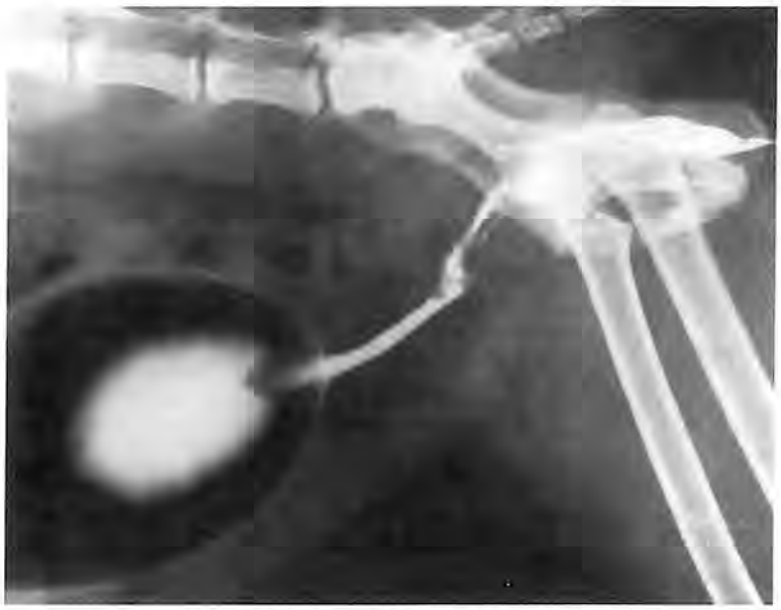

Figure 40–2. Double-contrast cystogram showing filling defects in the prostatic urethra of a 10-year-old neutered male cat with hematuria, dysuria, and pollakiuria; necropsy revealed presence of prostatic adenocarcinoma with metastasis to the lungs. (From Hawe RS: What is your diagnosis? J Am Vet Med Assoc 182:1257–1258, 1983, with permission.)

little clinical significance.[2] Atrophy (i.e., slight reduction in gland size, decreased number of acini, decreased size of glandular lumens, flattened to cuboidal epithelium, and increased stroma) occurs some time following castration. Inflammation (i.e., large, focal or diffuse infiltration of lymphocytes, catarrhal changes with desquamation of lining cells into the glandular lumens) may be observed histologically in cats with cystitis or urethritis.[2] Finally, focal cystic changes of bulbourethral gland acini may occur in old, intact toms.[2] Bulbourethral gland lesions in cats have been detected at routine necropsy, and are not associated with known clinical signs.

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Chapter 41

Disorders of the Feline Penis and Prepuce

Congenital Anomalies

Congenital defects of the penis are rare in cats; none were reported in 26 cats with single or multiple congenital defects of the urogenital systems from a patient population of 19,646 cats seen at 10 North American veterinary college clinics over approximately 5 years.[1] Congenital defects of the penis that have been observed in cats include underdeveloped penis and hypospadias.[2] A small penis was reported in a tortoiseshell and white cat with one hypoplastic testis, a female internal genital tract and a chromosome constitution in some cells of $2n$ = 38,XX chromosomes, and in some cells of $3n$ = 57,XXY chromosomes (38XX/57,XXY).[3] A small (3-mm) penis was observed in the vagina of a 3-year-old domestic shorthair cat assumed to be a male pseudohermaphrodite.[4]

Phimosis/Paraphimosis

Phimosis is constriction of the preputial orifice that prevents protrusion of the penis. Congenital phimosis was reported in an adult intact male domestic shorthair cat with signs of dysuria; it also has been observed in association with signs of urethral obstruction in young or young adult cats; signs include dysuria, hematuria, and distended bladder.[5] Diagnosis is based on inspection of the prepuce and inability to protrude the penis. Differential diagnoses include presence of hair rings that may encircle the penis and compress the lumen of the penile urethral (see Injury below) and preputial adhesion following prepuberal castration (see Chapter 38). Treatment is surgical enlargement of the preputial orifice, generally by the removal of a broad V-shaped piece of tissue from the preputial orifice.

Paraphimosis is presence of a constricting preputial orifice with a protruded penis causing painful swelling of the penis and inability of penile retraction into the prepuce. If not corrected, paraphimosis leads to drying of the glans penis, and may result in penile gangrene. Paraphimosis is less common than phimosis in the cat, but may occur.[5] Paraphimosis is diagnosed by inspection at physical examination, and treatment is replacement of the penis after lubrication or surgical enlargement of the preputial orifice.

Injury

Penile injury may occur in cats due to cat fight trauma, pelvic/perineal injury if hit by a car, or presence of hair rings encircling the glans penis, which may obstruct urethral urine outflow; penile hematoma may occur following urethral obstruction with crystals or discrete calculi, or following catheterization attempts to relieve urethral obstruction.

Wooldridge described urethral obstruction in a 4-month-old intact male Persian cat presenting with abdominal pain and a distended bladder.[6] Hair was observed protruding from the preputial orifice, and gentle traction on that hair caused extrusion of the penis, around which were seen several coils of hair. The penis was lacerated, suggesting a stricture. Removal of the hair ring resulted in good urethral patency within several days time. Hart and Peterson reported on five adult male cats in which a hair ring around the base of the glans penis prevented intromission and successful mating.[7] Behavior of the affected toms in-

cluded persistent mounting with intense or prolonged pelvic thrusting. All of the rings could be removed easily by sliding them off of the shaft of the penis, and normal mating behavior was possible immediately thereafter.[7]

Penile hematoma is a common sequel to urethral obstruction, and traumatic lesions of this organ may progress to gangrene of the distal portion of the penis.[8] Diagnosis is based on history and inspection, and treatment includes antibacterial and anti-inflammatory drugs as well as use of gentle manipulation in attempting to relieve urethral obstruction.

Priapism

Priapism is persistent abnormal erection of the penis (Fig. 41–1). Priapism in mammals may be caused by spinal cord injury, thromboembolism of the corpus cavernosus penis including that caused by trauma, amyloidosis and tumor metastasis, local inflammation, and drugs such as amphetamines. Priapism is a very rare disorder in the cat; it has been reported in nine cases.[9–11]

Six of seven affected cats in one report were Siamese, and four of these were neutered males.[11] History in three neutered males and one intact male included trying to mate with an estrous queen. Presenting signs included priapism and slow, difficult urination. Five of the cats were treated with penile amputation and perineal urethrostomy (PU) and four recovered well; one cat died 3 days following PU, and postmortem was not allowed. Similar microscopic changes were observed in the penises of six affected cats undergoing penile amputation; these included thrombosis of the corpus cavernosum penis and superficial erosion or ulceration of the external surface of the penis (Fig. 41–2).[11]

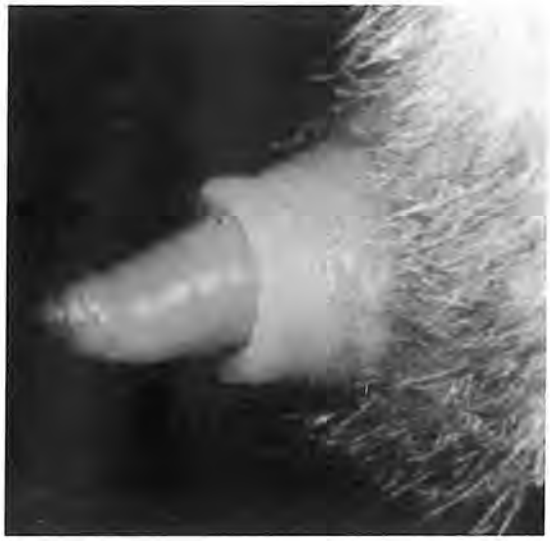

Figure 41–1. Priapism in a neutered male cat. Note the dry penile tip, which is devoid of androgen-dependent penile spines because the cat is neutered. (From Gunn-Moore DA, Brown PJ, Holt PE, Gruffydd-Jones TJ: Priapism in seven cats. J Small Anim Pract 36:262–266, 1995, with permission.)

Penile amputation and PU are recommended for affected cats because of the danger

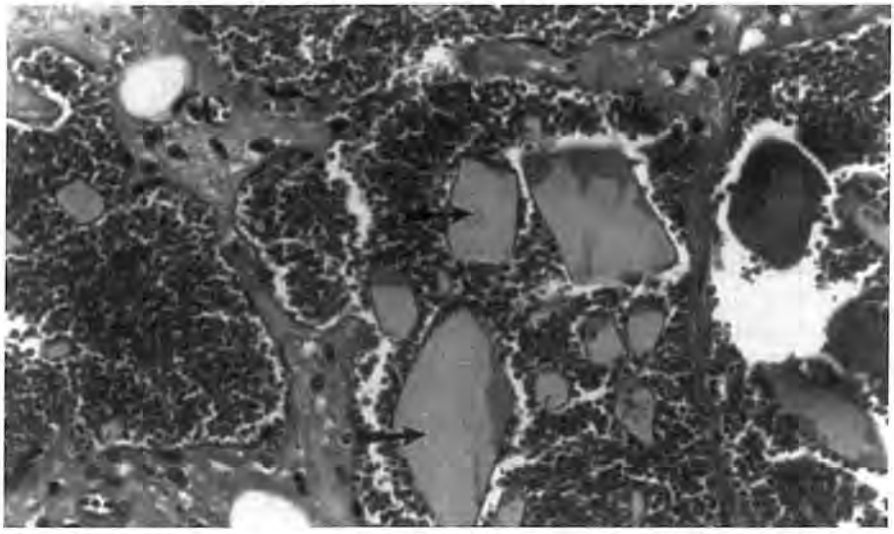

Figure 41–2. A section through the corpus cavernosum of the penis of a cat with thrombosis and priapism, showing congestion and large haemoglobin crystals (arrows). Haematoxylin and eosin; 175X. (From Gunn-Moore DA, Brown PJ, Holt PE, Gruffydd-Jones TJ: Priapism in seven cats. J Small Anim Pract 36:262–266, 1995, with permission.)

of gangrene and/or urethral obstruction secondary to priapism in this species.

Neoplasia

Tumors of the penis and prepuce have not been reported in the cat, except for single cases of penile carcinoma[12] and sarcoma.[13]

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Chapter 42

Disorders of the Mammary Gland in the Male Cat

Mammary Hyperplasia

Mammary hyperplasia may occur in male cats (intact or neutered) receiving long-term therapy with progestrogens such as megestrol acetate (MGA) (Ovaban; Schering Corp., Kenilworth, NJ). Usually a hyperplastic nodule occurs in a single gland in affected male cats, but multiple nodules also have been reported. Eight neutered male cats with mammary hyperplasia (Table 42–1) have been reported with descriptions of 62 intact or neutered female cats.[1-5] Progestogen-induced mammary nodules in male cats range in size from 1.0 cm^3 to 11 × 8 × 5 cm.

Both diffuse fibroepithelial hyperplasia and intraductular papillary fibroepithelial hyperplasia have been described in affected males.[1,4] Ultrastructure of hyperplastic mammary tissue from progestin-treated male and female cats is similar, and neither light microscopic nor electron microscopic examination of tissue permits distinction between mammary hyperplasia in young intact females from lesions in older male and female cats given progestins.[3]

Because mammary cancer has been reported in the male cat treated with progestogens, diagnosis should be based both on history of drug therapy and on excision biopsy histology.[5] Treatment is surgical excision of the mammary nodule and cessation of progestogen therapy.

Neoplasia

Although mammary neoplasia is very common in the queen, incidence of this disorder in male cats is low. Seven reports of malignant mammary tumors in cats describe 438 females and 7 (1.6 per cent) neutered males.[5-11] The cases of mammary neoplasia in male cats oc-

Table 42–1. Clinical Features of Hyperplastic Mammary Nodules in Progestogen-Treated Male Cats

Age (Years)	Sex	Breed	Mass	Previous Progestogens		Reference
				Total Dose	Months	
4	MC	Unkown	11 × 8 × 5 cm, gland 4	320 mg MGA	14	Hinton and Gaskell[1]
5	MC	Unkown	3.5 × 3 × 1 cm, cystic	600 mg MGA	48	Hinton and Gaskell[1]
4.5	MC	Domestic	2 × 1.5 cm, gland 3	100 mg MDA	4	Hayden et al.[2]
2	MC	Domestic	1.5 cm	150 mg MGA	2	Hayden et al.[2]
10	MC	DLH	4 cm diameter, gland L 4	260 mg MGA	6	Dorn et al.[4]
10	MC	DSH	2 cm fluid filled space w/0.8-cm mass, gland L 3	140 mg MGA	3	Hayden et al.[5]
3	MC	DSH	4 × 3.5 × 3 cm	92.5 mg MGA	4	Hayden et al.[5]
3	MC	Domestic	1-cm^3 masses, glands L 3 R 3, and L 4	180 mg MGA	3	Hayden et al.[5]

MGA, megestrol acetate, given per os; MDA, medroxyprogesterone acetate, given IM; MC, male castrate; DLH, domestic longhair; DSH, domestic shorthair; L, left; R, right.

curred in castrated males; information on age at diagnosis, tumor type, clinical signs, metastasis, and survival time was not reported in most cases. A tubulopapillary carcinoma was observed in the third mammary gland (side not specified) of an adult male castrate domestic shorthair cat previously treated periodically with oral megestrol acetate.[5] Two other males were reported with mammary carcinomas.[10] Diagnosis is based on palpation of mammary masses and tumor histology. Treatment is surgical excision of affected glands (simple mastectomy). Until more cases are reported, prognosis should be based on tumor histology and criteria developed for females.

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Chapter 43

Clinical Approach to the Complaint of Infertility in the Male Cat

Infertility in the male cat has not been well characterized because of the difficulty in obtaining semen in this species. There are, however, diagnostic approaches to an infertility complaint that are possible for most small animal veterinarians, and which are described here in a case work-up approach. Thorough approach to male infertility also relies on complete evaluation of the queen(s) in question (see Chapter 35). The four steps in the clinical approach to male infertility in this species are acquisition of a complete minimum data base, definition of the problem, diagnostic plans to rule out known causes of infertility, and therapeutic plan.

Acquisition of the Minimum Data Base

History

The goals of the history are to characterize the male's reproductive performance and to identify factors that may cause or contribute to infertility/sterility. Reproductive history includes information on whether the male has shown interest in mounting and copulating with estrous females, whether prolonged mounting and pelvic thrusting have been observed, and whether apparently normal copulation resulting in a female "after-reaction" (signal of intromission and vaginal stimulation) has occurred. The after-reaction is characterized by the female's crying out, striking out at the tom, and frantic rolling with vulvar licking (see Chapter 26). Number of females bred, numbers of breedings per queen, numbers and dates of litters sired, litter size, litter mortality, and interestrous intervals of queens that did not conceive following breeding should be recorded.

Nonreproductive history that is of interest includes diet, vaccination history, presence of viral disease in the affected tom and in the cattery, presence of fever or other systemic illness, and previous medical treatment, if any. All-meat diets or noncommercial diets may cause aspermia due to vitamin A deficiency or other nutritional causes of testicular degeneration (see Chapter 39). Medical therapy with steroids, including glucocorticoids, progestogens, or androgens, may suppress gonadotropin secretion and adversely affect spermatogenesis. Degree of inbreeding, if known, also may be of interest, as this practice in some species is associated with decreased fertility.

Physical Examination

A complete physical examination should be performed. Problem-specific physical examination includes assessment of testicular size and consistency; small soft testes support a diagnosis of testicular hypoplasia or atrophy, while testicular enlargement supports an inflammatory or neoplastic disorder. Coat color (looking for orange or cream together with black or smoke colors in the XXY or chimeric male), coat quality (an indicator of endocrine or nutritional imbalance), and presence of the thick cervical dermis of the male phenotype (which occurs only in the presence of androgens) should be noted. The penis should be protruded in order to rule out congenital phimosis or penile hair rings; and penile size and presence of penile spines (indicators of adequacy of gonadotropin and testosterone secretion) should be noted.

Semen Evaluation

Confirmation that sperm are present in the ejaculate and that sperm numbers, progressive motility, and morphology are normal is the next step in the infertility work-up. At best, semen should be obtained by electroejaculation or by collection into an artificial vagina (see Chapter 37) if one of these procedures is available, even as a referral service.

If electroejaculation is not possible, the male should be allowed to copulate with an estrous queen. Following such copulation, a vaginal cytology specimen can be collected from the queen, a urine sample collected by cystocentesis from the male, and both specimens examined for presence of sperm. Normal sperm may enter the uterus quickly, leaving abnormal sperm in the vagina. Sperm recovered from vaginal cytology samples may show a high percentage of morphologic defects, and their relation to original semen quality is unknown. These samples may, however, help distinguish cats with azoospermia from those with sperm in the ejaculate. Feline sperm may be recovered from the urinary bladder of the male by cystocentesis following ejaculation, because cats deposit some semen in the urinary bladder every time they ejaculate (see Chapter 37).[1] As with the vaginal cytology sample, quality of sperm or seminal plasma obtained by this technique may not be a good indicator of total ejaculate quality, but sperm presence may enable the clinician to rule out azoospermia.

Definition of the Problem

Failure to Attempt Copulation: Poor Libido

Poor libido is diagnosed based on owner observation of the tom's behavior in the presence of an estrous queen. Causes of this problem include congenital and acquired (penile hair ring) phimosis, inadequate testosterone, and mate preference.[2–4]

Failure to Achieve Complete Intromission

Failure to achieve complete intromission is diagnosed based on prolonged mounting, neck biting, and treading along the queen's flanks with pelvic thrusting, not accompanied by rapid dismount and the queen's after-reaction. In the absence of careful observation of breeding behavior, failure of the estrous queen to have been induced to ovulate (serum progesterone concentrations should exceed 1 ng/ml for about 40 days after breeding if ovulation occurred) supports this diagnosis. Causes of this problem are the same as those associated with failure to attempt copulation, as well as congenital vulvovestibulovaginal anomaly of the queen.

Failure to Induce Ovulation in the Queen

Failure of the tom to induce ovulation in the queen is suspected in queens that exhibit interestrous intervals less than 40 days after breeding, and is confirmed by demonstrating low (<1 ng/ml) serum progesterone concentrations in bred queens during the 40 days after breeding.[5] Causes of ovulation failure include inadequate number of copulations (usually only one), inadequate vaginal stimulation at mating, congenital vaginal anomalies of the queen, and possibly inadequate secretion of luteinizing hormone (LH) by the queen.[6]

Abnormal Semen Quality

Abnormal semen quality is characterized as aspermia (no ejaculate), azoospermia (no sperm in a clear fluid ejaculate), oligospermia (low sperm numbers), asthenozoospermia (<25 percent progressively motile sperm), and teratozoospermia (abnormal sperm morphology). The most commonly reported cause of abnormal semen quality in this species is azoospermia caused by abnormal sexual differentiation associated with abnormal karyotype (see Chapter 39).[7,8] Twenty-five of 38 male calico/tortoiseshell cats, some with 39,XXY and some with chimeric chromosome complements, were reported sterile (see Table 39–1). Some chimeric cats have more than one population of seminiferous tubules, which may or may not produce sperm (see Fig. 39–2).

Other possible causes of abnormal semen quality or absence of ejaculate in the tom include testicular causes (e.g., congenital cysts, fever, infection, steroid administration, hypoandrogenism, radiation, thermal insult, trauma, systemic illness, degeneration, neoplasia) and posttesticular causes (e.g., epididymal cysts, epididymitis, segmental aplasia of the wolffian duct system, congenital or acquired impatency of the colliculus seminalis, inflammation of the prostate or bulbourethral glands). Most of these potential causes have

not been observed to cause infertility in the tom, in part because of the difficulty in collecting semen in this species and characterizing its quality under various conditions.

Diagnostic Procedures to Rule Out Known Causes of Infertility

Failure to Attempt Copulation: Poor Libido

Diagnostic approach to poor libido is to confirm observation of this behavior in the presence of an estrous queen; to protrude and examine the penis for evidence of phimosis, penile hair ring, and penile spines; to perform a karyotype on lymphocytes from peripheral blood; and to attempt to breed the male to different estrous queens. Serum testosterone concentration may be measured following stimulation (described below) with human chorionic gonadotropin (hCG), which has LH-like activity, or gonadotropin-releasing hormone (GnRH), which induces release of endogenous LH. If reproductive disease is ruled out, one may observe response to treatment, which follows.

Karyotype laboratory service is available at several veterinary colleges in North America. In general, the sample requirement is 7 to 10 ml heparinized blood that must be transported to the laboratory at room temperature in less than 24 hours from time of sample collection; this requires use of an overnight mail or courier service and coordination with the laboratory regarding day of receipt. While circulating blood lymphocytes usually have the same karyotype as the diploid spermatogonia of the testes, it is possible in chimeras that they may not, and that a normal lymphocyte karyotype may be present in an animal with an abnormal karyotype of testicular cells. Therefore, karyotype of testicular parenchyma itself or of fibroblasts from skin tissue obtained by punch biopsy may be of value.

Resting serum testosterone in six normal cats ranged from nondetectable (<0.05 ng/ml) to 3.0 ng/ml (see Table 39–2).[9,10] Serum testosterone 1 hour after administration of 25 μg GnRH intramuscularly (IM) ranged from 5 to 12 ng/ml. Serum testosterone 4 hours after 250 IU hCG IM ranged from 3.1 to 9.0 ng/ml. Serum gonadotropin (LH, follicle-stimulating hormone [FSH]) assay to detect insufficiency states (which might be indicators of therapeutic direction) or elevated concentrations of these hormones (which occur after castration or irreversible testicular atrophy) is not available commercially at present for this species.

Failure to Achieve Complete Intromission

Diagnostic approach to the problem of incomplete intromission is the same as for poor libido. Low (<1 ng/ml) serum progesterone in the queen following the mating attempt indicates she did not ovulate; this may support the diagnosis of intromission failure, although some queens ovulate spontaneously in the absence of vaginal stimulation.[11] In addition, about half of the queens undergoing a single normal intromission and insemination do not ovulate, so low progesterone may not distinguish intromission failure from ovulation failure.[6] Contrast radiography of the vestibule and vagina of the estrous queen is indicated to look for congenital anomalies of the queen that prevent intromission.[12,13]

Failure to Induce Ovulation in the Queen

Ovulation failure is diagnosed by demonstrating low (<1 ng/ml) serum progesterone concentrations during the 40 days following intromission.[5] If intromission, insemination, and semen quality are normal, ovulation failure may be confirmed by observing response to medical ovulation induction and repeat breeding (see Therapeutic Plan below).

Abnormal Semen Quality

Diagnostic tools for approach to abnormal semen quality in the tom include collection of semen by electroejaculation, karyotype, measurement of alkaline phosphatase in urine following ejaculation in the azoospermic cat (see Chapter 37), seminal plasma culture, testicular and epididymal ultrasonography, and testicular biopsy. If the semen contains blood or evidence of infection, a retrograde urethrogram may be of value in localizing the site of origin or extent of the disease process.[12,13] See Chapter 39 for description of testicular disorders in the male cat. The diagnostic approach should be tailored to the signalment, history, and physical examination findings of the male cat.

Testicular biopsy is a valuable prognostic test in infertile male cats with a carefully documented reproductive history and evidence of

azoospermia; biopsy is, however, invasive, expensive, and there is danger of subsequent testicular inflammation and immune-mediated reaction against sperm antigens, which normally do not contact immunocompetent cells. Testicular biopsy should be performed with the patient under general anesthesia. Blunt dissection is made to the testis through a prescrotal incision; a 0.5-cm incision is made through the fascia spermatica interna into the vaginal cavity, and then a pointed blade is used to make a stab incision into the testicular parenchyma, resulting in protrusion of a small portion of seminiferous tubular tissue that can be sliced off for histology.[14] Alternatively, a small wedge of tissue may be excised from the testicular parenchyma. Use of Bouin's fixative is advocated in preference to formalin in order to preserve testicular architecture.[14]

Therapeutic Plan

Failure to Attempt Copulation: Poor Libido

Poor libido caused by congenital and acquired (penile hair ring) phimosis may be treated by removal of the hair ring or surgical correction of congenital phimosis. If the male has normal testes on physical examination and normal karyotype, libido may be improved by administration of 25 μg GnRH IM about 1 hour prior to attempted breeding.

Failure to Achieve Complete Intromission

Therapeutic plan for failure to achieve complete intromission is the same as that for poor libido. In addition, if abnormality of the female vagina or vestibule are diagnosed, surgical correction or artificial insemination (see Chapter 26) may be attempted.

Failure to Induce Ovulation in the Queen

Ovulation failure of the queen is treated by administering 25 μg GnRH or 250 IU hCG IM to the queen at time of mating.[15,16] Alternatively, mating may be attempted multiple times over 1 to 3 days during estrus.

Abnormal Semen Quality

Abnormal semen quality of the tom due to abnormal karyotype, most other testicular causes (e.g., congenital cysts, radiation, trauma, systemic illness, testicular degeneration or neoplasia) or post-testicular causes (e.g., epididymal cysts, epididymitis, segmental aplasia of the wolffian duct system, congenital or acquired impatency of the colliculus seminalis, inflammation of the prostate or bulbourethral glands) usually is not reversible. Sexual rest (3 to 4 months) may result in spontaneous recovery of infertile males with prior fever, infection, steroid administration, or thermal insult. Administration of antibiotics for a 3- to 4-week course may be effective in treating bacterial infection of the male reproductive tract, remembering that such infection is extremely rare, and that cat semen normally contains high numbers of bacteria originating from the penile urethra at time of ejaculation.[10] In dogs with testicular neoplasia, unilateral orchiectomy has been associated with subsequent fertility from the contralateral testes, and the same may be possible in a similarly affected tom.

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Appendix

Guide to Congenital Defects of Dogs

* Reprinted from Hoskins JD, Taboada J: Congenital defects of the dog. Compend Contin Educ Small Anim 14:873–897, 1992.

Guide to Congenital Defects of Dogs

Condition	Breeds Affected	Remarks
BODY WALL		
Hernia	Weimaraner, German shepherd	Peritoneopericardial hernias are more common than pleuroperitoneal hernias; presenting signs depend on amount of displaced tissue contained in the hernia
Diaphragmatic		
Peritoneopericardial and pleuroperitoneal		
Hiatal	Brachycephalic breeds and Chinese shar pei	Defect of the phrenoesophageal ligament that allows displacement of the gastroesophageal junction forward into the thoracic cavity
Inguinal	Basset hound, cairn terrier, basenji, Pekingese, West Highland white terrier	Defect in formation of the aponeuroses of the inguinal ring and linea alba
Umbilical	Airedale terrier, basenji, Pekingese, pointer, weimaraner	Failure of normal closure of the umbilical ring; increasing abdominal pressure with advancing age forces the omentum or occasionally the intestines into the defect
Pectus excavatum	Many breeds	Intrusion of the sternum into the thorax; the ventral ends of the ribs turn medially to join dorsally the displaced sternebrae
BONES AND JOINTS		
Achondroplasia		
Appendicular	Basset hound, dachshund, miniature poodle, Scottish terrier	Cartilage of epiphyseal plate grows in irregular directions and is scant
Axial	Poodle, Scottish terrier	Chondrodystrophia fetalis
Anury	Cocker spaniel	Absence of one to all caudal vertebrae
Brachydactyly	Many breeds	Reduced size and function of outer toes
Brachyury (short tail)	Beagle, cocker spaniel, English bulldog, toy griffon	Condition that occurs in normally longer-tailed breeds
Carpal subluxation	Labrador retriever, Irish setter	Condition that occurs bilaterally and is limited to the carporadial joints; appears when puppies begin to walk at about three weeks of age
Cartilaginous exostosis	German shepherd, Alaskan malamute, Yorkshire terrier	Radiographically visualized as localized osteochondromatous outgrowths protruding from long bones, scapula, ilium, cervical and thoracic vertebrae, metatarsus, and phalanges
Cervical calcinosis circumscripta	Great Dane	Calcinosis masses typically attach to tendons inserting on lateral processes of C_4 and C_5 just below muscle; histopathologic examination reveals dense collagen, granulomatous reaction, and islands of trabecular bone and marrow
Cervical vertebral instability (wobbler syndrome)	Basset hound, Doberman pinscher, English sheepdog, fox terrier, Great Dane, Irish setter, Rhodesian ridgeback, Saint Bernard	Condition that exists when the ventral spinal canal is narrower dorsoventrally than the dorsal canal between C_3 and C_7; deformed vertebral bodies result in spinal neurosis; in basset hounds, a malformed C_{2-3} may be involved
Chondrodysplasia (dwarfism)	Alaskan malamute, Shetland sheepdog, Labrador retriever	Stunted forelegs, lateral deviation of paws, and sloping top line attributable to impaired endochondral bone growth
Cranioschisis	Cocker spaniel	Soft spots in the cranium (skull fissures); defects apparently are calvarium developmental abnormalities or persistent fontanels
Ectromelia	Pointer	Condition in which the scapula is the only bone of the forelimb present
Elbow dysplasia (see Ununited anconeal process)		
Epiphyseal dysplasia	Beagle, poodle	Characterized by stippling defects of the epiphyseal sites; abnormal movement of the hindlimbs and a swaying gait result

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Foramen magnum dysplasia	Chihuahua, cocker spaniel, Skye terrier	Characterized by malformation of the occipital bone with enlargement of the foramen magnum and exposure of the cerebellum and brain stem; hydrocephalus may be present
Hip dysplasia	Primarily large and giant breeds; also cocker spaniel and Shetland sheepdog	Deformity of coxofemoral joint attributed to disparity between development of the primary muscle mass and the skeleton; onset most frequent at five months of age
Legg-Calvé-Perthes disease	Small breeds, including Manchester terrier, Pekingese, poodle, Chinese pug, schnauzer, wirehaired fox terrier	Characterized by increase in trabular bone of the femoral head followed by aseptic necrosis secondary to ischemia; revascularization of the bone is followed by demineralization
Lumbosacral malarticulation	German shepherd	Condition in which compression is created on the cauda equina; attributable to subluxation, stenosis, or spondylosis of the lumbosacral articulation
Odontoid process dysplasia (nonunion with C_2)	Chihuahua, Pekingese, Pomeranian, poodle, Yorkshire terrier	Condition that results in atlantoaxial subluxation; signs vary from neck pain to quadriplegia
Osteoporosis	Dachshund	Condition caused by abnormal bone resorption but radiographically uniformly dense bones; puppies usually present as swimmers
Panosteitis (enostosis)	German shepherd, Basset hound, and other breeds	Excessive formation of bone in various states of maturity of the ulna, humerus, radius, femur, and tibia and excessive osteoblastic activity with formation of new bone by fibrous metaplasia; radiographically evident as opacity in the area of the nutrient foramen; intermittent leg lameness at 6 to 12 months of age
Patellar luxation	Toy breeds	Condition resulting from alteration of structures that maintain the normal position of patella; usually medial, being unilateral or bilateral; onset usually evident at four to six months of age
Polydactyly	Many breeds	Reappearance of first digit on the hindlimbs
Radial agenesis	Many breeds	Complete lack of development of the radius evident as a unilateral or bilateral problem at an early age; lack of radial support for the carpus medially results in medial deviation of the paw
Radius, premature closure of	Many breeds	When the distal radial physis prematurely closes, one side of the radius usually continues to grow while the other side closes; resultant deformity is angulation of the carpus and metacarpal bones toward the closed side; closure of the proximal radial physis results in progressive separation of the radial head from the distal humerus
Short spine	Greyhound, Shiba Ina	Abnormal development produces short vertebral column, kyphosis, and scoliosis; high shoulders and back sloping sharply to the tail are seen
Shoulder luxation	Chihuahua, griffon, King Charles spaniel, miniature pinscher, miniature poodle, Pomeranian, wirehaired fox terrier	First occurs at three to four months of age; in severely affected individuals, may lead to medial shoulder luxation at early age; radiographs will confirm shoulder luxation if a flexed and rotated view of the shoulder region is taken
Spina bifida	Beagle, English bulldog	Condition resulting from defective fusion of vertebral arches

Table continued on following page

Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Ulna, premature closure of	Many breeds	When the distal ulnar physis prematurely closes, majority of ulnar lengthening stops while the radius grows unabated; the resultant deformity is progressive bowing and twisting of the radius, as the ulna acts as a bowstring
Ununited anconeal process (elbow dysplasia)	German shepherd, Labrador retriever, basset hound, French bulldog, Great Dane, bull mastiff, Great Pyrenees, Irish wolfhound, weimaraner, Newfoundland	Failure of normal fusion of anconeal process to diaphysis of ulna; may be unilateral or bilateral; stunted and usually loose process contributes to elbow joint laxity and synovitis that produce progressive arthritic changes; frequently inapparent until secondary arthritis has occurred
Vertebral anomalies	Brachycephalic and screwtail breeds: Boston terrier, English and French bulldogs, Pomeranian, Chinese pug	Hemivertebrae are shortened or misshapen vertebrae that occur when the right and left halves of the vertebral body develop asymmetrically or fail to fuse (most common site is T_{7-9} area); block or fused vertebrae occur when there is incomplete segmentation of two or more adjacent vertebrae; butterfly vertebrae result because of persistence or sagittal cleavage of the notochord, leading to sagittal cleft in the vertebral body; spinal cord compression often occurs with these vertebral anomalies
CARDIOVASCULAR SYSTEM		
Aortic stenosis	Newfoundland, boxer, German shepherd, German shorthaired pointer, golden retriever	Subvalvular most common form; a ridge of fibrocartilaginous tissue is located below the aortic valve; valvular and supravalvular less common
Atrial anomalies	Boxer and other breeds	Seldom recognized as individual anomalies and are usually with other cardiac defects; another atrial defect is cor triatriatum, which results from persistence of the embryonic eustachian valves within the right atrium
Endocardial fibroelastosis	Many breeds	Characterized by proliferation of elastic and collagenous fibers within the endocardium; left atrium and ventricle usually exclusively involved
Mitral valve malformation	Great Dane, German shepherd, English bulldog, Chihuahua	Dilatation of the mitral annulus, anomalies of valve leaflets, and altered chorda tendineae and papillary muscles
Patent ductus arteriosus	Poodle, Pomeranian, collie, German shepherd, Shetland sheepdog	Condition occurring when the normal fetal vessel (ductus arteriosus) that shunts blood past the nonfunctional fetal lungs into the aorta fails to close within first two to three days of life
Persistent atrial standstill	English springer spaniel	Bradycardia of persistent atrial standstill does not respond to atropine; symptomatic dogs must be treated by permanent pacemaker implantation
Persistent right aortic arch	German shepherd, Irish setter	Aorta originates from right fourth aortic arch rather than left; communication with ligamentum arteriosus results in esophageal blockage
Pulmonic stenosis	Beagle, English bulldog, Chihuahua, fox terrier, Samoyed, miniature schnauzer	Narrowing or obstruction between the right ventricle and pulmonary trunk; although stenosis may occur in supravalvular, valvular, or subvalvular area, pulmonary stenosis attributable to valvular dysplasia is most common
Stenosis of atrioventricular bundle (bundle of His)	Chinese pug	Individuals with stenosis of atrioventricular bundle have syncopal attacks that begin during the first several months of life

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Tetralogy of Fallot	Keeshond	Tetralogy of Fallot includes ventricular septal defect, right ventricular outflow obstruction, hypertrophy of the right ventricle, and dextropositioned aorta that accept blood from both ventricles
Tricuspid valve dysplasia	Great Dane, weimaraner	A spectrum of abnormalities, including anomalies of valve cusps, chorda tendineae, papillary muscles, and valvular tissues; enlargement of right atrium and ventricle occurs secondary to valvular incompetence
Ventricular preexcitation syndrome	Many breeds	An isolated abnormality that occurs in association with anatomic congenital defects
Ventricular septal defect	English bulldog	Usually a single defect located high in the septum just below the tricuspid and aortic valves
DIGESTIVE SYSTEM		
Anorectal defects	Many breeds	Imperforate anus, segmental aplasia, rectovaginal fistula, rectovestibular fistula, anovaginal cleft, and rectal urethral fistula; of these defects, imperforate anus is the most common
Brachygnathia	Many breeds	Upper jaw is longer than the lower jaw
Cleft palate/cleft lip complex	Brachycephalic breeds, beagle, cocker spaniel, dachshund, Shetland sheepdog, schnauzer, Labrador retriever, German shepherd	Cleft lip usually occurs as unilateral defect in the lip or in the floor of the nostril; cleft palate may be identified as onset palatal rugae on the roof of the oral cavity, incomplete fusion of the soft palate, or oronasal fistula through a cleft palate
Cricopharyngeal achalasia	Toy breeds	Failure of cricopharyngeal muscle and part of the thyropharyngeal muscle to relax and thus permit a food bolus to move from the pharynx into the cranial esophagus
Dentition, abnormal	Many breeds	Disorders affecting dentition include anodontia (absence of one or more teeth), retained deciduous teeth, supernumerary teeth, dens in dente, and shape abnormalities
Esophageal diverticula	Many breeds	Segments of the esophagus typically involved with diverticula are areas just cranial to the thoracic inlet and diaphragm; periodic diverticularization of the esophagus at the thoracic inlet is considered to be a normal finding for most young English bulldogs
Lymphangiectasia, intenstinal	Many breeds	Malformation of the lymphatic system that contributes to clinical signs reflective of protein-losing enteropathy
Intestinal anomalies	Many breeds	Congenital defects of intestinal tract include atresia or duplication of an intestinal segment; usually incompatible with life unless surgically corrected
Meckel's diverticulum	Many breeds	Sacculation or appendage of the ileum derived from an unobliterated yolk stalk.
Megaesophagus, idiopathic	Great Dane, German shepherd, Irish setter, dachshund, miniature schnauzer	Characterized by primary motor system disturbances of the esophagus that result in abnormal or unsuccessful transport of ingesta between the pharynx and stomach
Microcheilia	Schnauzer	Reduced oral fissure
Parotid salivary gland, enlargement of	Dachshund	Affected individuals present with enlarged parotid salivary glands and hypersalivation (profuse drooling)
Prognathism	Brachycephalic breeds	Upper jaw is shorter than lower jaw
Pyloric stenosis	Boxer, Boston terrier	Probably caused by excessive secretion of the gastrointestinal hormone, gastrin, which is produced by the G cells in the stomach wall and has a potent trophic effect on pyloric circular smooth muscle as well as the mucosa

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■ ■ ■ Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
EAR		
Deafness	Akita, Australian heeler and shepherd, border collie, Boston terrier, collie, bullterrier, cocker spaniel, dalmatian, dachshund, Doberman pinscher, English setter and bulldog, foxhound, fox terrier, Great Dane, Old English sheepdog, Scottish and Sealyham terriers, Norwegian dunker hound, Shetland sheepdog	Lack or loss of the sense of hearing; congenital deafness is most common type; may be partial or complete and affect only one ear or both ears; unilateral deafness is most common form in dogs; of the electrodiagnostic techniques used for determining extent of deafness, recording brain stem auditory-evoked potentials and impedance audiometry are most frequently used; hearing loss occurs secondary to degeneration, hypoplasia, or aplasia of the spiral organ of the inner ear
External ear canal, malformations of	Brachycephalic breeds	Incomplete development of canals or may be shorter, more tortuous than normal, or atretic
Pinna, malformations of	German shepherd, wirehaired terrier, collie, Irish setter	Deviations in size and shape typical for breed, such as gross variations in size of the pinna (macrotia, microtia) or its complete absence (anotia)
ENDOCRINE AND METABOLIC SYSTEMS		
Adrenal hyperplasia	Great Dane	Deficiency in one of the enzymes (17-hydroxylase) necessary for cortisol synthesis; signs include poor growth and those of glucocorticoid deficiency
Diabetes insipidus	Miniature poodle, German shepherd, Boston terrier, Norwegian elkhound, schnauzer, German shorthaired pointer	Can be hypothalamic-neurohypophyseal or nephrogenic in origin; hypothalamic-neurohypophyseal is most common; signs in puppies usually restricted to polyuria and polydipsia
Diabetes mellitus	Keeshond, golden retriever, whippet, West Highland white terrier, Alaskan malamute, standard poodle, Old English sheepdog, Doberman pinscher, miniature schnauzer and pinscher, schipperke, German shepherd, Labrador retriever, Finnish spitz, Manchester terrier, English springer spaniel, chow chow, mixed breed	May become evident as early as two to six months of age; pancreatic lesions often include atrophy of B cells and associated noninflammatory atrophy of a few acinar cells; affected individuals usually exhibit decreased rate of growth in addition to polyphagia, polyuria, and soft or diarrheic stools
Dysbetalipoproteinemia	Miniature schnauzer	Defective synthesis of APO lipoprotein; signs include abdominal distress and seizures
Glycogen storage disease	German shepherd	Results from enzyme deficiency, amylo-1, 6-glucosidase; signs reflect severity of hypoglycemia
Hyperchylomicronemia	Miniature schnauzer	Results from enzyme deficiency, lipoprotein lipase; signs include abdominal distress and seizures with onset
Hypoadrenocorticism	Many breeds	Signs caused by deficiency of glucocorticoids or mineralocorticoids and glucocorticoids; congenital hypoplasia of adrenal glands probably causes early puppy death and thus goes unrecognized
Hypothyroidism	Giant schnauzer, Scottish deerhound	Results from thyroid dysgenesis, circulating thyroid hormone transport abnormalities, dyshormonogenesis, congenital thyroid-stimulating hormone deficiency, and severe iodine deficiency; congenital defects probably cause early puppy death and thus goes unrecognized

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Neonatal hypoglycemia	Toy breeds	Occurs during the nursing period because of inadequate glycogen or protein substrate stores or immature liver enzyme systems; also occurs from failure to adapt to fasting during postweaning period; signs are related to severity
Pituitary dwarfism	German shepherd, toy pinscher, weimaraner, spitz, carnelian beardog, giant schnauzer	Caused by deficiencies of growth hormone and sometimes other adenohypophyseal hormones; signs include proportional limb-to-trunk dwarfism, prognathism, altered mentation, delayed eruption of permanent teeth, retained puppy coat leading to eventual alopecia, and suppressed immune responses
Primary parathyroid hyperplasia	German shepherd	Affected individuals develop signs at two weeks of age; including stunted growth, muscular weakness, polyuria, and polydipsia
Urea cycle defect (citrullinemia)	Golden retriever, beagle	Results from urea cycle enzyme deficiency, argininosuccinate synthetase; signs include vomiting; seizures, and altered mentation
EYE		
Aberrant canthal dermis	Many breeds	Encroachment of dermis at the nasal canthus on bulbar and palpebral conjunctiva
Agenesis of the eyelid	Many breeds	Absence of varying segments of the eyelid margin, usually temporal one third of the upper eyelid
Anophthalmos	Many breeds	Complete absence of the globe
Aphakia	Saint Bernard	Congenital absence of the lens; usually occurs with other ocular defects
Blepharophimosis	Many breeds	Abnormal narrowing of the space between the eyelids
Cataracts	Afghan hound, cocker spaniel beagle, Boston terrier, Australian shepherd, Chesapeake Bay retriever, German shepherd, Bedlington and Sealyham terriers, golden retriever, Old English sheepdog, miniature schnauzer, Siberian husky, Staffordshire terrier, standard poodle, Welsh springer spaniel	In animals younger than six months of age, are classified as either congenital or juvenile; congenital cataracts are present at birth, although they may not be noticed until six to eight weeks of age; and may be inherited or secondary to in utero influences, so it is essential to question the owner regarding presence of cataracts in the sire, dam, previous litters, or pedigrees; juvenile cataracts develop from newborn period until six years of age; heredity is major factor, although other causes may contribute to juvenile cataracts formation; course of juvenile cataracts usually progressive, but rate at which progression occurs varies; complete opacification of the lens may occur in less than one year following diagnosis; if functional vision is present, congenital or juvenile cataracts in puppies usually undergo spontaneous resorption within the first year
Collie eye anomaly	Smooth- and rough-coated collies, Shetland sheepdog	Characterized by an array of posterior segment abnormalities; included in order of increasing severity are choroidal hypoplasia, optic nerve and scleral colobomas, and retinal detachment
Dermoid	Saint Bernard, German shepherd, dachshund, dalmatian	First noticed as skinlike appendage soon after eyelids open; usually involves temporal cornea and conjunctiva and affects one eye or both

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Distichiasis	English bulldog, toy and miniature poodle, cocker spaniel, Pekingese, boxer, alsatian, Shetland sheepdog, Bedlington and Yorkshire terriers, Shih Tzu, Chinese pug, Saint Bernard	Extra row of eyelashes that protrude from orifices of Meibomian glands within the intermarginal space of the eyelid; upper, lower, or both eyelids may be involved
Divergent strabismus	Brachycephalic breeds	First noted when eyelids open; normal ocular alignment develops during the second postnatal month
Ectropion	Cocker spaniel, Saint Bernard, bloodhound, basset hound	Everted eyelids, allowing exposure of underlying bulbar conjunctiva; animals affected tend to display mucopurulent exudation and hyperemia of exposed conjunctiva and diminished Schirmer tear test values
Enophthalmos	Saint Bernard, Great Dane, Doberman pinscher, golden retriever, Irish setter	Recession of the globe within the orbit; most often associated with microphthalmos
Entropion	Many breeds	Inward deviation of the eyelid; lower eyelid is more commonly affected because of poorly formed tarsal plate
Glaucoma	Beagle, basset hound, cocker and English springer spaniels, poodle, Samoyed, wirehaired terrier	Pressure increases occurring within the first year of life are rare, despite existence of congenital iridocorneal abnormalities
Hemeralopia (day blindness)	Alaskan malamute	Affected individuals are visually impaired in daylight, but function well at night and on overcast days; ocular fundus appears normal
Heterochromia	Merled collie, Shetland sheepdog, Australian shepherd harlequin Great Dane, Siberian husky, Alaskan malamute, dalmatian, American foxhound, Norwegian dunker hound	Variation in iris color, occurs commonly in subalbinotic animals; variations in tapetal development and degree of pigmentation in retinal pigment epithelium and choroid may occur simultaneously
Iridocorneal abnormalities	Many breeds	Congenital mesodermal remnants occur in corneal angle in basset hounds
Microcornea	Basenji, collie, Saint Bernard, miniature schnauzer, Australian shepherd, poodle	Affected eyes have more bulbar conjunctiva exposed medially and laterally but no apparent visual problems
Microphakia	Saint Bernard, beagle	Margin of abnormally small lens along with elongated ciliary processes in microphakia may be seen following pupillary dilatation
Microphthalmos with colobomas	Australian shepherd, merled Shetland sheepdog, harlequin Great Dane	Large equatorial staphylomas, up to 20 diopters deep, are evident
Microphthalmos	Australian shepherd, Great Dane, collie, Shetland sheepdog, dachshund, miniature schnauzer, Old English sheepdog, akita, Cavalier King Charles spaniel, Bedlington and Sealyham terriers, Labrador retriever, Doberman pinscher	Failure of the globe to develop to normal size; characterized by varying degrees of enophthalmos with or without other ocular defects; commonly associated with other ocular defects, including colobomas, persistent pupillary membranes, cataract, equatorial staphylomas, choroidal hypoplasia, retinal dysplasia and detachment, and otic nerve hypoplasia; vision often impaired
Optic nerve colobomas	Collie, Shetland sheepdog, Australian shepherd, basenji	Pits or excavations in the optic disk that occur as part of the collie or shelty eye anomaly or as single lesions

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Condition	Breeds Affected	Remarks
Optic nerve hypoplasia	Beagle, dachshund, collie, Russian wolfhound, German shepherd, Great Pyrenees, Saint Bernard, miniature poodle	Poor vision is common in bilaterally affected animals, although owners usually fail to recognize the vision deficit while the animal is with its siblings; unilateral lesions often incidental findings, as the puppy compensates with the unaffected eye; affected eyes exhibit sluggish to absent direct pupillary light reflexes; resting pupil size may be larger than normal; affected optic disk often less than half its normal size, its center is depressed, and periphery is pigmented
Persistent hyperplastic primary vitreous	Doberman pinscher, Staffordshire terrier, standard schnauzer	Characterized by presence of fibrovascular membrane on posterior lens surface; results from persistence of hyaloid vasculature coupled with proliferation of mesoderm within the arborizing vascular tunic surrounding the lens
Persistent pupillary membranes	Basenji and other breeds	Arise from anterior iris surface and represent remnants of embryonic vascular system
Progressive retinal atrophy	Collie, Irish setter, Norwegian elkhound, miniature schnauzer, Cardigan Welsh corgi	Disorder affecting retinal photoreceptor layer; animals first demonstrate visual deficits in dim lighting; progressive loss of day vision then occurs, followed by total blindness and dilated pupils; retinal appearance varies with stage of disorder
Progressive retinal degeneration	Borzoi	First occurs at six months of age; initial retinal lesions appear as focal areas of hyperreflectivity in extreme peripheral tapetum; later, lesions coalesce into diffuse retinal degeneration
Pupillary anomalies	Many breeds	Ventronasal, notchlike defect (coloboma) in pupillary border results in keyhole-shaped pupil; eccentric pupil (corectopia) may accompany multiple ocular defects in Australian shepherd
Retinal dysplasia	English springer spaniel, Labrador retriever, Bedlington terrier, cocker spaniel, beagle, akita, Australian shepherd, Doberman pinscher, Old English sheepdog, rottweiler, Yorkshire terrier	Characterized by folds in outer retinal layers and by retinal rosettes, in which variably differentiated retinal cells are arranged around central lumen; more severe forms may demonstrate retinal detachment attributable to subretinal fluid accumulation; may occur alone or in association with other congenital ocular defects
Retinal folds	Collie and other breeds	Usually appear in nontapetal portion of the fundus; believed to be caused by transient growth differential between inner and outer layers of optic cup and usually disappear when dogs are about six months of age
Stationary night blindness	Tibetan terrier, briard	Night blindness first evident by six weeks of age; fundus of the briard appears normal, while low-level illumination demonstrates increased tapetal granularity in the Tibetan terrier; Tibetan terrier may subsequently develop progressive retinal atrophy
Tapetal hypoplasia	Beagle	Lack of visible tapetum and uniform reddish brown fundus reflex evident
Trichiasis	Many breeds	Condition in which otherwise normal eyelashes are deviated from their normal position, allowing contact between the deviated eyelash and the cornea
HEMATOPOIETIC AND LYMPHATIC SYSTEMS		
Anasarca	English bulldog	Affected individuals have generalized subcutaneous edema and fluid accumulation

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Coagulation protein disorders		
Factor XII Factor XI	Standard and miniature poodles, German short-haired pointer English springer spaniel, Great Pyreness, weimaraner, Kerry blue terrier	Not associated with bleeding diathesis; affected individuals may be predisposed to infection and/or thrombosis Severe Factor XI deficiency characteristically a minor bleeding diathesis that becomes major after trauma or surgery
Factor X	American cocker spaniel	Individuals homozygous for gene usually stillborn or die within first weeks of life from massive pulmonary and/or abdominal hemorrhage; heterozygotes have mild to severe bleeding tendency
Factor IX (hemophilia B)	Many breeds	Sex-linked hemorrhagic disorder; excessive bleeding from umbilical cord or tail or feet at time of tail-docking and dew-claw removal are common signs; hemarthrosis, gingival bleeding during tooth eruption, and spontaneous hematoma formation are other typical manifestations
Factor VIII (hemophilia A)	Many breeds	One of the most common inherited hemorrhagic disorders; bleeding tendencies are same as for Factor IX deficiency
Factor VIII (von Willebrand's disease)	Many breeds	Attributable to defective or deficient Factor VIII-related antigen (von Willebrand factor) and is most common inherited bleeding disorder; bleeding tendencies are same as for Factor IX deficiency
Factor VII	Beagle, miniature schnauzer, Alaskan malamute, boxer, bulldog	Usually not accompanied by detectable bleeding, although affected individuals may experience bruising or prolonged bleeding after surgery
Factor II	English cocker spaniel, boxer	Disorders of prothrombin including detectable bleeding tendencies, usually epistaxis and gingival bleeding
Factor I	Saint Bernard, vizsla, Russian wolfhound	Affected individuals with dysfibrinogenemia or hypofibrinogenemia experience mild bleeding manifested by lameness and epistaxis; challenge with surgery or trauma results in life-threatening bleeding
Erythrocyte defects		
Pyruvate kinase deficiency	Basenji, beagle, West Highland white terrier	Premature red blood cell destruction and moderate to severe anemia with evidence of red blood cell regeneration
Stomatocytosis	Alaskan malamute, miniature schnauzer	Stomatocytes and polychromasia in association with autosomal recessive-transmitted chondrodysplasia in malamutes
Familial nonspherocytic anemia	Miniature poodle	Markedly regenerative red blood cell response, hepatosplenomegaly, and bone marrow myelofibrosis and osteosclerosis
Nonspherocytic hemolytic disorders	Beagle	Mild anemia and polychromasia
Phosphofructokinase deficiency	English springer spaniel	Results in primary hemolytic disorder with appropriate bone marrow response (reticulocytosis)
Glucose-6-phosphate dehydrogenase deficiency	Weimaraner	Generally no anemia or polychromasia
Cytochrome b_5 reductase deficiency	Many breeds	May have no anemia, cyanosis, and exercise intolerance
Elliptocytosis	Mixed breed	Attributable to decrease in red blood cell membrane protein, a protein band 4.1 deficiency; affected red blood cells are mechanically unstable, resulting in mild to moderate regenerative hemolytic anemia

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Condition	Breeds Affected	Remarks
Increased osmotic fragility	English springer spaniel	May have no anemia, polychromasia, poikilocytosis, and exercise-induced hyperthermia
High-potassium erythrocytes	Akita, Japanese mongrel	No anemia but increased erythrocyte and serum potassium (pseudohyperkalemia)
Familial microcytosis	Akita	No anemia but prominent microcytosis
Cyclic hematopoiesis	Gray collie	Intermittent cytopenias usually present
Selective cobalamin malabsorption	Giant schnauzer	Usually have moderate anemia, nonregenerative megaloblasts, hypersegmented neutrophils, cachexia, and dementia
Familial macrocytosis and dyshematopoiesis	Miniature and toy poodles	No anemia, macrocytosis, hypersegmented neutrophils, and normal osmotic fragility
Lymphoedema	English bulldog, Old English sheepdog, German shepherd, borzoi, Labrador retriever, Great Dane, poodle, Belgian and Tervuren shepherds	Primary lymphoedema attributable to developmental abnormalities within lymphatic system: lymphatic channels may be aplastic, hypoplastic, or hyperplastic; lymph nodes may be normal, hypocellular, or absent; characterized by soft, pitting, nonpainful edema of one or more extremities, usually involving hindlimbs; rarely, abdominal or pleural effusions may develop
Methemoglobinemia	Borzoi, English setter	Results from enzyme deficiency, NADH-methemoglobin reductase; affected animals have evidence of cyanosis, brownish mucous membranes and dark brownish blood that does not turn red on exposure to oxygen, and exercise intolerance.
Thrombasthenic thrombopathia	Otterhound	Intrinsic platelet disorder, defect distinguished by presence of bizarre, giant platelets and reductions in membrane glycoproteins II and III; platelets fail to support normal clot retraction, have reduced retention on glass bead surfaces, and fail to aggregate normally in response to adenosine diphosphate, collagen, and thrombin
Thrombopathia	Basset hound	Intrinsic platelet disorder; affected animals exhibit signs typical of quantitative and qualitative platelet defects, including epistaxis, gingival bleeding, and petechiation; characterized by abnormal fibrinogen receptor exposure and impaired dense granule release
Thymic branchial cyst	Many breeds	Arises from remnants of branchial pouch epithelium, the embryonic precursor of thymic tissue; occurs in thymus or subcutis of the neck
IMMUNE SYSTEM		
Combined immunodeficiency	Basset hound	Affected individuals develop severe bacterial skin infections, stomatitis, and otitis within first few weeks of life and have lymphopenia, depressed T lymphocyte function with low serum IgA and IgG and variable IgM concentrations
Complement deficiency	Brittany spaniel	Absence of complement 3 and impaired function of phagocytes
Cyclic hematopoiesis	Collie, cocker spaniel, Pomeranian	Basis for cyclic hematopoiesis is bone marrow stem cell defect resulting in cyclic fluctuation in neutrophils, reticulocytes, and thrombocytes from the bone marrow; additional defects in lysosomal function result in decreased bactericidal capacity of neutrophils; respiratory, umbilical, and septicemic infections are common

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Condition	Breeds Affected	Remarks
Granulocytopathy	Irish setter, Doberman pinscher, weimaraner	Defect in neutrophilic bactericidal capacity; affected individuals are stunted, have recurrent bacterial infections, and require continual antibiotic therapy
Pelger-Huët anomaly	American foxhound, basenji	Decreased lobulation of granulocytic cells; abnormal nuclear shape may contribute to reduced cell mobility and abnormal chemotaxis; not all affected animals have chemotactic defects, and none has increased risk of infection
Pneumocystosis	Dachshund	Most cases occur in animals younger than six months of age with suspected congenital immunodeficiency
Selective IgA deficiency	German shepherd, beagle, Chinese shar pei, Airedale terrier	Affected animals have low or undetectable serum or secretory IgA concentrations or both and experience chronic recurrent upper and lower respiratory infections, otitis externa, and dermatitis; despite selective low IgA levels in many related animals, some animals will not be symptomatic
Thymic atrophy	Weimaraner	Affected individuals with thymic atrophy can be detected within one to three months of age; signs include stunted growth, chronic washing, and suppurative pneumonia; additional defects noted include decreased growth hormone concentration and T cell function
LIVER AND PANCREAS		
Biliary atresia		Failure of biliary tract to develop creates incomplete functional connection between liver and duodenum
Copper-associated hepatopathy	Bedlington and West Highland white terriers, Doberman pinscher	Age-related accumulation of copper in hepatic lysosomes associated with chronic active hepatitis; in the Bedlington terrier, only dogs homozygous for trait accumulate copper in the liver; in other breeds, copper accumulation probably related to degree of active liver disease
Gallbladder anomalies	Many breeds	Congenital malformations include trilobed or bilobed gallbladders; development of two separated gallbladders with cystic ducts united in a common duct; ductular bladders developing as supernumerary vesicles derived from hepatic, cystic, or common bile ducts; and trabecular bladders derived from vesicular outgrowths of liver trabeculae
Hepatic cyst	Cairn terrier and other breeds	May be parenchymal or ductal in origin; most are of ductular origin, arising from one or more primitive bile ducts lacking connection with biliary tract and subsequently developing into retention cysts
Intrahepatic arterioportal fistulae	Many breeds	Result from failure of the common embryologic anlage to differentiate; contribute to portal hypertension and shunting through multiple portosystemic collaterals and ascites
Pancreatic hypoplasia	German shepherd, Doberman pinscher, Irish setter, beagle, Labrador retriever, Saint Bernard	Associated with generalized reduction in pancreatic exocrine (acinar) cells, but islets of Langerhans remain intact
Portosystemic venous shunts		
Intrahepatic	Doberman pinscher, golden and Labrador retrievers, Irish setter, Samoyed, Irish wolfhound	Most often remnant of fetal ductus venosus that remains patent; other large intrahepatic venous communications may be present; ductus venosus is embryonic venous channel originating from umbilical vein that traverses liver and drains into left hepatic vein and then into caudal vena cava

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Condition	Breeds Affected	Remarks
Extrahepatic	Miniature schnauzer and poodle, Yorkshire terrier, dachshund	Shunt between portal vein and postcava or between portal vein and azygous vein; complete absence of portal vein entry into liver is unusual
Urea cycle enzyme deficiency	Golden retriever, beagle	Significant reductions in urea cycle enzyme argininosuccinate synthetase lead to inability to handle endogenous ammonia and signs of hepatic encephalopathy
NERVOUS SYSTEM		
Afghan myelomalacia	Afghan hound	Demyelination with accompanying myelomalacia predominantly occurs in dorsal funiculi of caudal cervical spinal cord, all funiculi of thoracic segments, and ventral funiculi of the lumbar area
Ataxia of fox terriers	Smooth-haired and Jack Russell fox terriers	Progressive demyelination of spinal cord segments, especially spinal cord segments of the pelvic limbs
Cranial dysraphism	Many breeds	Defects occurring because of faulty closure of neural tube; conditions that may be seen in association with cranial dysraphism include anencephaly (brain is absent at birth or more commonly only the basal nuclei and cerebellum are well developed), exencephaly (brain is exposed as result of congenital cleft in skull), cranium bifida (open cleft in skull), encephalocele and meningocele (brain or meninges, respectively, protrude through congenital cleft in skull), and cyclopia (developmental anomaly characterized by single orbital fossa with either complete or partial agenesis of the globe)
Cerebellar abiotrophies	Kerry blue terrier, Gordon setter, and other breeds	Loss of vital, nutritional substances; cerebellum is normal in gross appearance, but marked depopulation involving principally Purkinje cells is present; other areas of brain may be affected
Cerebellar hypoplasia	Chow chow, Irish setter, wirehaired fox terrier	Uniform forms of cerebellar hypoplasia in which clinical signs of cerebellar dysfunction are present at birth and do not progress occur
Cerebellar vermis hypoplasia	Boston terrier and bullterrier	Caudal cerebellar vermis is preferentially hypoplastic, although other portions of cerebellum often are hypoplastic to lesser degree and some have concomitant hydrocephalus
Degenerative changes in cerebellum	Airedale terrier, Finnish harrier, Bernese mountain dog, bull mastiff, rough-coated collie, Irish setter, miniature poodle, beagle	Other breed-specific syndromes in which there are degenerative changes in the cerebellum alone or together with other areas of the central nervous system occur; clinical signs in some cases are progressive, whereas in others they are apparently static
Demyelination of miniature poodles	Miniature poodle	Progressive demyelination involving principally the spinal cord leads to paraparesis at two to four months of age and subsequently tetraplegia
Epilepsy	Beagle, Belgian and Tervuren shepherds, keeshond, collie, dachshund, poodle, German shepherd, setters, retrievers, spaniels	Recurrence of seizures; genetic predisposition suspected in several dog breeds, but potential heritable basis for epilepsy include beagle, Belgian and Tervüren shepherds, German shepherd, and keeshond

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Condition	Breeds Affected	Remarks
Hydrocephalus	Maltese, Yorkshire terrier, English bulldog, Chihuahua, Lhasa apso, Chinese pug, toy poodle, Pomeranian, cairn and Boston terriers, Pekingese	Excessive accumulation of cerebrospinal fluid within skull; the terms *internal* and *external* denote excess fluid within or outside the ventricular system, respectively; congenital forms may occur because of structural defects that either obstruct cerebrospinal fluid outflow at the mesencephalic aqueduct or impede cerebrospinal fluid absorption
Hypertrophic neuropathy	Tibetan mastiff	Affected individuals have prominent concomitant demyelination and remyelination that occur at eight weeks of age and progresses to tetraparesis
Hypomyelination, dysmyelination	Chow chow, springer spaniel, Samoyed, weimaraner, Bernese mountain dog	Reduced (hypomyelination) and abnormal (dysmyelination) myelination of the central nervous system occur, suggesting the responsible lesion may involve either failed or delayed differentiation of oligodendrocytes
Lissencephaly	Lhasa apso, Irish setter, wirehaired fox terrier	Marked reduction or absence of cerebral gyri; may occur as single entity or concurrently with cerebellar hypoplasia, cyclopia, and hydrocephalus
Lysosomal storage diseases		
Ceroid lipofuscinosis	English setter, cocker spaniel, Chihuahua, dachshund, Saluki, border collie	Results from unknown, perhaps *p*-phenylene diamine, enzyme deficiency
Fucosidosis	English springer spaniel	Results from enzyme deficiency, α-L-fucosidase
GM_1 gangliosidosis	Beagle, English springer spaniel, Portuguese water dog	Results from enzyme deficiency, β-galactosidase
GM_2 gangliosidosis	German shorthaired pointer, Japanese spaniel	Results from enzyme deficiency, β-hexoseaminidase
Globoid cell leukodystrophy	Cairn and West Highland white terriers, poodle, bluetick hound, beagle, basset hound, Pomeranian	Results from enzyme deficiency, β-galactocerebrosidase
Glucocerebrosidosis	Silky terrier	Results from enzyme deficiency, β-glucosidase
Glycogenosis	German shepherd	Results from enzyme deficiency, α-glucosidase
Sphingomyelinosis	Poodle	Results from enzyme deficiency, sphingomyelinase
Motor neuronopathies	Brittany spaniel, collie, Swedish Lapland, rottweiler, pointer, Great Dane	Degeneration of previously differentiated ventral horn cells of the spinal cord (spinal abiotrophies)
Narcolepsy-cataplexy	Doberman pinscher, Labrador retriever, miniature poodle, dachshund, beagle, Saint Bernard	Excessive daytime sleep (narcolepsy); periods of acute muscular hypotonia often seen in association with narcolepsy (cataplexy)
Neuroaxonal dystrophy	Shetland sheepdog, rottweiler, Chihuahua	Marked axonal distention (axonal spheroids) in central nervous system; signs manifested reflect predominant location of axonal spheroids within central nervous system
Neuronal degeneration	Cocker spaniel	Occurs in young cocker spaniels; causes ataxia, tremors, abnormal behavior, and seizures by several months of age
Peripheral vestibular disorders	German shepherd, English cocker spaniel, Doberman pinscher, Shetland sheepdog	Absence of signs of central vestibular disease in affected animals suggests that developmental lesions affecting peripheral labyrinth are involved; affected animals have signs of peripheral vestibular dysfunction, including head tilt, circling, and rolling at birth or within a few weeks
Progressive axonopathy	Boxer	Affected individuals have axons that are markedly enlarged in both peripheral and central nervous systems; pelvic limb ataxia typically begins at two months of age; other neurologic dysfunctions are noted as disease progresses

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Condition	Breeds Affected	Remarks
Pug encephalitis	Chinese pug	Affected individuals have signs principally of forebrain dysfunction, including seizures, attitude change, and circling; marked, predominantly mononuclear pleocytosis noted in affected animals
Sensory neuronopathies and neuropathies	Dachshund, shorthaired and English pointers, border collie, Siberian husky	Loss of sensory nerve fibers, neuronal cell bodies, or both results in pelvic limb ataxia and/or hyporeflexia, urinary incontinence, gastrointestinal dysfunction, loss of conscious proprioception, depressed pain sensation, and self-mutilation of extremities
Spinal dysraphism	Weimaraner	Refers collectively to anomalies involving spinal cord, vertebral column, and skin subsequent to faulty closure of neural tube; meninges (meningocele), spinal cord or roots (myelocele), or both (meningomyelocele) may protrude through defective fusion of vertebral arch (spina bifida); neural lesions may be noted; spinal clefts (myeloschisis) may communicate with dilated central canal (hydromyelia) or cystic spaces within spinal parenchyma (syringomyelia)
Spongiform encephalopathies	Labrador retriever, Samoyed, Silky terrier, dalmatian	Manifested by multifocal neurologic dysfunction and marked vacuolation of central nervous system white matter
NEUROMUSCULAR SYSTEM AND MUSCLES		
Dermatomyositis	Collie, Shetland sheepdog	Idiopathic inflammation of skin and muscles; family history of syndrome exists; almost all individuals with skin lesions have some degree of muscle involvement; signs range from mild symmetric temporalis muscle atrophy to generalized muscle atrophy and weakness; megaesophagus and trismus can develop in severely affected individuals
Familial myoclonus	Labrador retriever	Episodes of marked muscular hypertonicity that begins at three weeks of age; extensor rigidity and opisthotonos become more pronounced on stimulation
Labrador retriever myopathy	Labrador retriever	Progressive degenerative myopathy first noted at three to four months of age as stiffness of gait and simultaneous advancement of pelvic limbs (bunny hop); signs do not progress significantly in some dogs beyond six to eight months of age
Myasthenia gravis	Jack Russell and smooth-coated fox terriers, springer spaniel	Leads to failure of neuromuscular transmission because of congenital deficiency of acetylcholine receptors in postsynaptic membrane; may be first noted at six to nine weeks of age and older
Myotonia	Chow chow, Great Dane, Staffordshire terrier, Rhodesian ridgeback	Persistent muscle contraction subsequent to either voluntary contraction or stimulation; prominent stiffness of gait noted when affected animals first become ambulatory and lessens with further exercise, being worse in pelvic limbs
Scotty cramp	Scottish terrier, dalmatian	Characterized by paroxysms of muscular hypertonicity; episodes usually begin at six to eight weeks of age and are generally precipitated by fear or excitement
X-linked muscular dystrophy	Irish terrier, golden retriever	Occurs homologous with Duchenne muscular dystrophy in humans; only affected males and homozygous females first show stilted gait and simultaneous advancement of pelvic limbs (bunny hop) at 8 to 10 weeks of age

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Condition	Breeds Affected	Remarks
REPRODUCTIVE SYSTEM		
Aplasia of the duct system	Many breeds	Failure of any part of testicular duct system to develop results in impaired transportation of spermatozoa to urethra, accumulation of sperm proximal to obstruction, and potential development of sperm granuloma and testicular degeneration
Chimeras		
True hermaphrodite	Many breeds	True hermaphrodites have both ovarian and testicular tissue present in same individual; true hermaphrodite chimeras have either XX/XY or XX/XXY chromosome constitutions, enlarged clitoris, little testicular tissue, and external female appearance
XX/XY chimeras with testes	Old English sheepdog	External genital opening with cranially displaced vulvalike structure, hypoplastic penis contained in vulvalike structure, no external scrotum or testes (testes are located near caudal pole of kidneys), and bicornuate uterus
Chromosomal number abnormalities		
XXY syndrome		Not recognized as readily in dogs as in tortoise shell cats because the haircoat color paradox does not signal its presence; normal male external phenotype with 79,XXY chromosome constitution has small testes with seminiferous tubular dysgenesis and no evidence of spermatogenesis
XO syndrome	Doberman pinscher	Occurs in females that have normal phenotype and not cycled by 24 months of age
Triple-X syndrome	Airedale terrier	Occurs in females that have normal phenotype, underdeveloped genitalia, and not cycled by 24 months of age
Chromosomal sex abnormalities	Many breeds	Dogs have 78 chromosomes, including the X and Y chromosomes; affected phenotypic males and females with abnormal sex chromosome constitutions, except chimeras and mosaics, have underdeveloped genitalia; with few exceptions, individuals are sterile
Cryptorchidism	Toy and miniature poodles, Pomeranian, Yorkshire and cairn terriers, dachshund, Chihuahua, Maltese, boxer, Pekingese, English bulldog, miniature schnauzer, Shetland sheepdog	Normally, canine testes descend to scrotum by 10 days after birth; if both testes are not within the scrotum by eight weeks of age, diagnosis of cryptorchidism is warranted; both males and females may carry gene for cryptorchidism and pass to offspring; heterozygous males, heterozygous females, and homozygous females are phenotypically normal carriers; only homozygous males are cryptorchid
Hypospadias	Boston terrier and other breeds	Abnormality in location of urinary orifice, being ventral and proximal to normal site in glans penis; urinary orifice may be located in glans penis (mild hypospadias), penile shaft (moderate hypospadias), or penoscrotal junction, scrotum, or perineum (severe hypospadias); may be accompanied by cryptorchidism, scrotal abnormalities, persistent Müllerian structures, and intersexuality
Os penis deformity	Many breeds	May result in deviation of penis and, depending on severity, inability to retract penis fully into preputial sheath; persistent exposure of portion of glans penis results in desiccation, trauma, or necrosis

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■ ■ ■ Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Persistent penile frenulum	Many breeds	Persistence of band of connective tissue extending from ventral tip of glans penis to either prepuce or ventral surface of penis
Prepuce anomaly	Many breeds	Abnormal shortening of prepuce results in persistent exposure of glans penis; may result in desiccation, trauma, or necrosis
Pseudohermaphroditism		
Female	Many breeds	Female hermaphrodite has XX chromosome constitution and ovaries but internal or external genitalia are masculinized
Male	Miniature schnauzer, poodle, Pekingese	Male hermaphrodite has XY chromosome constitution and testes but internal or external genitalia are to some degree those of female
Testicular hypoplasia	Many breeds	Abnormal development of the seminiferous tubular germinal epithelium; results in oligospermia or azoospermia and sterility; may be unilateral or bilateral and usually first noticed soon after puberty
Vaginal prolapse	Large breeds	Protrusion of edematous vaginal tissue into vaginal lumen, often through vulva of intact female during time of estrogen stimulation
XX sex reversal	Cocker spaniel, beagle, Chinese pug, Kerry blue terrier, weimaraner, German shorthaired pointer	Animals in which chromosomal and gonadal sex do not agree are called *sex reversed;* dogs with XX sex reversal have 78,XX chromosome constitution and varying amounts of testicular tissue in gonad; individuals are true XX hermaphrodites or XX males and have mild to severe gonadal masculinization
RESPIRATORY SYSTEM		
Bronchial cartilage hypoplasia	Pekingese	Seen during first several months of life, usually as severe respiratory distress
Bronchoesophageal fistula	Many breeds	Connection between esophagus and airways that may allow saliva and ingested material to enter lungs
Laryngeal hypoplasia	Skye terrier	Incompletely developed larynx; signs, when present, vary with degree of laryngeal narrowing
Laryngeal paralysis	Bouvier des Flandres, Siberian husky	Failure of larynx to abduct during inspiration produces muted bark and soft, moist cough; later, roaring sound of inspiratory dyspnea becomes dominant sign
Pulmonary emphysema	Many breeds	Abnormally large air spaces occur distal to terminal bronchi; affected individuals may show signs of respiratory distress as early as six weeks of age
Primary ciliary dyskinesia	English pointer, English springer spaniel	Abnormal functioning cilia of respiratory epithelium, resulting in reduced mucociliary clearance of respiratory secretions, inhaled particles, and infectious agents
Stenotic nares	Brachycephalic breeds	Predisposes to laryngeal collapse by causing formation of a partial vacuum with inspiration; dyspnea, mouth breathing, and snoring sounds are common
Tracheal collapse	Brachycephalic and miniature breeds, especially Chihuahua, poodle, Pomeranian	Occurs because malformations of tracheal rings cause dorsoventral flattening of trachea
Tracheal hypoplasia	Brachycephalic breeds and Chinese shar pei	Inadequate growth of tracheal rings; commonly associated with secondary respiratory tract infections

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
SKIN		
Acanthosis nigricans	Dachshund	Cutaneous reaction pattern characterized by bilateral axillary hyperpigmentation, lichenification, and alopecia
Acral mutilation syndrome	German shorthaired and English pointers	Sensory neuropathy that results in progressive mutilation of distal extremities; begins as biting and licking at paw(s) with hindlimbs being most severely involved
Acrodermatitis	American bullterrier	At birth, affected individuals have skin pigmentation lighter than normal, are physically weak; cannot chew or swallow well, and retarded growth; by six weeks of age, skin lesions appear on foot pads, ears and muzzle, and around all body orifices
Alopecia universalis	American hairless terrier, beagle	Generalized lack of hair coverage; adnexal abnormalities concurrently not present
Aplasia cutis (epitheliogenesis imperfecta)	Many breeds	Discontinuity of squamous epithelium; present at birth as glistening red, well-demarcated defect in skin; defect is covered with one to three layers of flat to cuboidal epithelium and a stroma devoid of all adnexae
Black hair follicular dysplasia	Black and white mixed breeds, bearded collie, basset hound, papillon, schipperke, dachshund	Defective haircoat found only in black haircoat regions; includes hypotrichosis, fractured stubby hairs lacking normal sheen, and periodic scaliness of skin
Collagen disorder of the foot pads	German shepherd	All foot pads are softer than normal, often tender; discrete ulcers may develop on one or more pads, especially the carpal and tarsal pads; lesions contain multifocal areas of collagenolysis and neutrophilic inflammation
Color mutant alopecia	Doberman pinscher, Irish setter, chow chow, dachshund, standard poodle, Great Dane, greyhound, whippet, basset hound, Boston terrier, Chihuahua	Ectodermal defect of color mutants characterized by partial alopecia, dry lusterless haircoat, scaliness, and papules; defects in melanization and cortical structure of affected hairs also occur
Cutaneous asthenia (Ehlers-Danlos syndrome, dominant collagen dysplasia, dermal fragility syndrome, dermatosparaxis)	Beagle, dachshund, boxer, Saint Bernard, German shepherd, English springer spaniel, greyhound	Connective tissue disease characterized by loose, hyperextensive, abnormally fragile skin easily torn by minor trauma
Cutaneous mucinosis	Chinese shar pei	Produces peculiar puffed face appearance favored in some breed lines and contributes to thickness of multiple skinfolds
Dermatomyositis	Collie, Shetland sheepdog	Idiopathic inflammation of skin and muscles; family history of syndrome exists; early skin lesions favor locations over bony prominences that are especially exposed to trauma; almost all individuals with skin lesions have some degree of muscle involvement
Dermoid sinus	Rhodesian ridgeback, Shih Tzu, boxer	Neural tube defect resulting from incomplete separation of skin and neural tube during embryonic development; sinus is tubular indentation of skin extending from dorsal midline as blind sac ending in subcutaneous tissue or extending through spinal canal to dura mater
Digital hyperkeratosis	Irish terrier	Hyperkeratosis of the foot pads of all four paws develops at an early age; affected pads tend to fissure, become secondarily infected, and painful

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Ectodermal defect	Miniature poodle, whippet, cocker spaniel, Belgian shepherd, Lhasa apso, Yorkshire terrier, miniature poodle	Affected individuals are born with two thirds of normally haired parts of body exhibiting hairlessness; hairless skin is extremely thin and contains no cutaneous appendages
Epidermal dysplasia	West Highland white terrier	Familial defect in keratinization that first presents as erythema and pruritus of extremities and ventrum, progressing to severe hyperpigmentation and seborrhea
Epidermolysis bullosa	Collie, Shetland sheepdog	Probably a mild form of canine familial dermatomyositis in which muscle lesions are inapparent
Hypotrichosis	Beagle, Yorkshire terrier, Labrador retriever, Lhasa apso, Irish water spaniel, toy poodle	Incomplete ectodermal defect in that affected individuals have remnants of hair follicles and other epidermal appendages in skin; in some cases, may be confined to certain hair color pattern; hypotrichosis may also develop after birth as delayed-onset trait
Ichthyosis	West Highland white terrier, American pit bull terrier, Boston terrier, Doberman pinscher	Extreme hyperkeratosis on all or part of skin and exaggerated thickening of digital, carpal, and tarsal pads; present at birth and becomes progressively more severe with age
Lichenoid-psoriasiform dermatosis	Springer spaniel	Asymptomatic, generally symmetric, erythematous, lichenoid papules and plaques initially noted on pinnae and in external ear canal and inguinal region; with time, lesions become more hyperkeratotic and spread to face, ventral trunk, and perineal area
Nevi	German shepherd, miniature poodle and schnauzer, Shetland sheepdog	Circumscribed developmental defect in skin; when nevus forms a hyperplastic mass, it is referred to as hamartoma; various other types include sebaceous, hyperpigmented epidermal, and mucocutaneous angiomatous nevi
Partial alopecia	Chinese crested dog, Mexican hairless dog, Chihuahua, Abyssinian sand dog, Turkish naked dog, Peruvian hairless dog, Xoloitzcuintli	These breeds are bred specificially for varying degrees of alopecia and as such become accepted standard
Seborrhea, congenital	English springer spaniel	Affected individuals born with dry skin and discolored hair; patches of hyperkeratosis and scale then develop, and adherent scale and debris accumulate on hair shafts
Tyrosinase deficiency	Chow chow	Changes in color of tongue, buccal mucosa, and portions of hair shaft are result of deficiency of tyrosinase, the enzyme necessary in chemical reactions that produce melanin
Tyrosinemia	German shepherd	Early-age onset of eye and skin lesions with mental retardation; serum tyrosine levels are elevated because of deficiency of cytosolic hepatic tyrosine aminotransferase; inflammatory response to tyrosine crystals deposited in tissue results in eye and possibly skin lesions
Vitiligo	Doberman pinscher, rottweiler, Belgian shepherd, Tervuren and German shepherds, Old English sheepdog, dachshund	Loss of skin pigment, especially around nose, lips, buccal mucosa, and facial skin; foot pads and nails as well as haircoat may be affected
URINARY SYSTEM		
Ectopic ureter	Siberian husky	May occur unilaterally or bilaterally and may be associated with other urinary tract anomalies; affected individuals, mostly females, have history of incontinence since birth or weaning

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Guide to Congenital Defects of Dogs *Continued*

Condition	Breeds Affected	Remarks
Malposition of urinary bladder (pelvic bladder)	Doberman pinscher	Caudal malposition of urinary bladder; may be cause urinary incontinence and is associated with other urinary tract abnormalities
Renal defects		
Agenesis or absence of kidneys	Many breeds	Can be unilateral or bilateral and is usually accompanied by associated ureteral aplasia; is fatal and a recognized cause of fading puppy syndrome
Amyloidosis	Chinese shar pei	Renal function in affected individuals varies depending on degree and duration of renal involvement; advanced signs of renal failure eventually occur and typically are associated with severe proteinuria and hypoproteinemia
Cystinuria	Dachshund, basset hound, bulldog, Chihuahua, Yorkshire and Irish terriers, mixed breed	Caused by specific defect in renal tubules; results in defective resorption of certain amino acids including cystine
Familial renal disease	Basenji, cocker spaniel, Doberman pinscher, Lhasa apso, Shih Tzu, Norwegian elkhound, Samoyed, soft-coated wheaten terrier, chow chow, standard poodle	Renal function in affected individuals varies depending on degree and duration of renal involvement; polyuria and polydipsia, anorexia, lethargy, weight loss or inability to gain weight, and eventually nonregenerative anemia, azotemia, skeletal changes, gastrointestinal signs occur in most of affected individuals
Fanconi's syndrome	Basenji	Caused by resorptive defect in proximal nephron leading to glycosuria, aminoaciduria, proteinuria, phosphaturia, renal tubular acidosis, and resorptive abnormalities of sodium, potassium, and urate
Fusion (horseshoe) kidney	Many breeds	Fusion of embryonic kidneys; usually incidental finding and not associated with clinical signs
Polycystic kidneys	Cairn terrier	Characterized by variable number of fluid-filled cysts in renal parenchyma, ranging from extremely small to massive; affected individuals may be asymptomatic or show evidence of rapidly progressive renal failure
Renal duplication	English bulldog	Usually an incidental finding and not associated with clinical signs of altered renal function
Renal ectopia	Many breeds	Arrest in normal embryonic ascent of kidney; kidney appears as caudal abdominal mass within pelvis or in sublumbar area; usually incidental finding and not associated with clinical signs
Renal glucosuria	Norwegian elkhound	Isolated tubular defect for resorption of glucose; may predispose affected individuals to urinary tract infection
Renal hematuria	Many breeds	For unknown reasons, may cause significant blood loss via urine; renal function may be not compromised even though there is persistent renal hematuria
Urate defect	Dalmatian	Urate metabolism and transport defect affect cell membranes, particularly hepatocytes, and renal tubular epithelium, which allows excessive secretory flux of urate into urine; in dalmatian, not a clinical problem unless there is an occurrence of urate uroliths
Urachal anomalies	Many breeds	Vary from complete persistence of urachus with communication between urinary bladder and umbilicus to blind cysts to cranioventral urachal diverticulum
Ureterocele	Many breeds	Dilatation of submucosal segment of intravesical ureter resulting in bulging of dilated segment into urinary bladder

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Condition	Breeds Affected	Remarks
Urethral anomalies	Many breeds	Include hypospadias (urethral opening on underside of penis or on perineum), imperforate urethra, ectopic urethra, urethral aplasia (associated with penile aplasia), duplicate urethra, and urethrorectal fistula
Urinary bladder anomalies	English bulldog, Doberman pinscher	Include exstrophy of bladder (absence of ventral abdominal body wall and ventral bladder wall), duplication of urinary bladder, and agenesis of bladder

* Reprinted from Hoskins JD, Taboada J: Congenital defects of the dog. Compend Contin Educ Small Anim 14:873–897, 1992.

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