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Series Preface

The Practical Veterinarian series was developed to help veterinary students, veterinarians, and veterinary technicians find answers to common questions quickly. Unlike large textbooks, which are filled with detailed information and meant to serve as reference books, all the books in The Practical Veterinarian series are designed to cut to the heart of the subject matter. Not meant to replace the reference texts, the guides in our series complement the larger books by serving as an introduction to each topic for those learning the subject matter for the first time or as a quick review for those who already have mastered the basics of each subject.

The titles for the books in our series are selected to provide information for the most common subjects one would encounter in veterinary school and veterinary practice. The authors are experienced and established clinicians who can present the subject matter in an easy-to-understand format. This helps both the first-time student of the subject and the seasoned practitioner to assess information often difficult to comprehend.

It is our hope that the books in The Practical Veterinarian series will meet the needs of readers and serve as a constant source of practical and important information. We welcome comments and suggestions that will help us improve future editions of the books in the series.

Shawn P. Messionnier, D.V.M.

Preface

The authors of this text are theriogenologists, that is, specialists who deal with the physiology and pathology of the male and female reproductive systems and the clinical practice of veterinary obstetrics, gynecology, neonatology, and andrology. Some are still in training, and others have been in practice for many years. Some are academicians, some are in industry, and some are private practitioners. A few do exclusively small animal work, whereas most do reproductive work in all species. Theriogenology is well rooted in practice and was the first specialty for which diplomates could be drawn either from practice or from an internship and residency. Theriogenology as a veterinary specialty has close ties to a sister organization for nonboarded practitioners, the

Society for Theriogenology (www.therio.org). The diplomates of the American College of Theriogenologists who have contributed to this text did so to promote our mission of support for veterinary students and practitioners.

As theriogenologists, we strongly recommend that all animals being considered for breeding undergo a thorough prebreeding examination to include testing for brucellosis in dogs; assessment for heritable conditions with hip radiographs, certification of the eyes, and other tests specific to the breed; a complete physical examination; and semen evaluation of males. Many animals should not be bred. If an animal's health will suffer from remaining sexually intact or if that animal is unlikely to produce superior offspring, it should be neutered. Theriogenology is not about breeding animals at any cost; instead, it is about educating clients about how best to manage breeding and reproductive conditions of animals. Theriogenologists have been at the forefront of efforts to curb pet overpopulation and continue to research how best to treat reproductive tract disease in all animals, intact or neutered.

Acknowledgments

The authors wish to thank all those theriogenologists who have gone before, all the researchers who provided us with the information we have collated here for you, all the mentors who assisted us in our training, all the students and clients who have further assisted us in our training, and our families and friends. The editor especially wishes to thank her extremely patient husband and children. If this book leaves a question unanswered, please contact the diplomates of the American College of Theriogenologists (www.theriogenology.org). Our greatest wish is to help you find the answer.

Margaret V. Root Kustritz, DVM, PhD, DACT

1

Disorders of Sexual Development

Sara K. Lyle

AT A GLANCE

- The normal canine karyotype is 78,XX for bitches and 78,XY for dogs. The normal feline karyotype is 38,XX for queens and 38,XY for toms.
- Embryologic development as a female occurs unless genes from the Y chromosome are expressed, which causes differentiation of the indifferent gonad into a functional testis.

- Disorders of sexual development are associated with infertility and include the following:
  - Abnormalities of chromosomal sex = defects in number or structure of sex chromosomes:
    - Klinefelter's syndrome (XXY)
    - Turner's syndrome (XO)
    - Chimera or mosaic (XX/XY, XY/XY)
    - Male calico or tortoiseshell cats: have an abnormal karyotype, containing at least one Y chromosome and more than one X chromosome
  - Abnormalities of gonadal sex = sex reversal; the gonads do not agree with the sex chromosome complement.
  - Abnormalities of phenotypic sex = internal or external genitalia do not agree with gonads and sex chromosome complement.
    - True hermaphrodites (ovarian and testicular tissue present)
    - Pseudohermaphrodites
      Male pseudohermaphrodites (testes and female genitalia)
      Female pseudohermaphrodites (ovaries and male genitalia)

Normal sexual differentiation can be described as occurring in three sequential steps: establishment of chromosomal sex, development of gonadal sex, and development of phenotypic sex. Chromosomal sex is

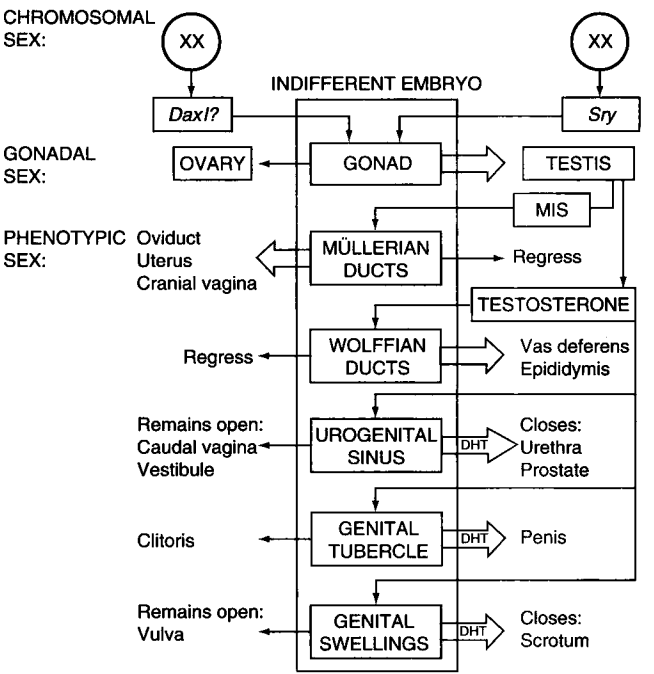
established at fertilization (either XX or XY), and this chromosomal composition is maintained throughout life in all cell lines during mitosis. The early embryo is sexually indifferent; all XX and XY embryos have genital ridges, wolffian (mesonephric) and müllerian (paramesonephric) ducts, a urogenital sinus, a genital tubercle, and genital swellings. Gonadal differentiation is determined by the sex chromosome constitution.

The presence of a Y chromosome results in differentiation of a testis from the genital ridge. In the absence of a Y chromosome, the genital ridge differentiates into an ovary. The gene *Sry*, named for the sex-determining region on the Y chromosome, encodes a protein that initiates testis differentiation (sometimes referred to as the *testis-determining factor*). Recently, autosomal genes that are involved in gonadal differentiation have been identified. *Sox9* is involved in testis differentiation (specifically Sertoli cell differentiation); two normal alleles are necessary for normal testis development in XY males that carry *Sry*. Normal XX individuals, which lack *Sry*, possess two X-linked *Dax1* alleles, which are involved with ovarian differentiation. One hypothesis is that the *Dax1* gene is involved with turning off male-specific genes during gonadal differentiation; it may also play a role in adrenal, pituitary, and hypothalamic development.

Determination of phenotypic sex (differentiation of the tubular reproductive tract and external genitalia)

depends on gonadal sex. The basic embryonic plan is female. If the genital ridges are removed from XX or XY embryos before gonadal differentiation, the female phenotype results. In normal XY individuals, Sertoli cells in the testis secrete müllerian-inhibiting substance (MIS), which causes regression of the müllerian ducts. Leydig cells within the testis secrete testosterone (T), which promotes wolffian duct differentiation into the epididymis and vas deferens. Secretion of these two hormones likely must occur within a critical time window during embryonic development for normal masculinization to result. In the urogenital sinus, genital tubercle, and genital swellings, testosterone is converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase. DHT causes the urethra to close and initiates development of the prostate, penis, and scrotum. Descent of the testes into the scrotum completes phenotypic development in the male. The hormonal and genetic control of testicular descent is not completely understood.

In normal XX individuals, the absence of MIS, T, and DHT allows the müllerian ducts, urogenital sinus, genital tubercle, and genital swellings to develop into female internal and external genitalia. The müllerian ducts develop into the oviducts, uterus, cervix, and cranial vagina; the urogenital sinus develops into the caudal vagina and vestibule; the genital tubercle develops into the clitoris; and the genital swellings develop into the vulva (Figure 1-1).



MIS - müllerian-inhibiting substance
DHT - dihydrotestosterone

Figure 1-1. Normal sexual development in the mammalian embryo. (*Modified from Morrow DA:* Current therapy in theriogenology, *Philadelphia, 1989, WB Saunders.*)

Disorders of Chromosomal Sex

Definition and Pathogenesis

Individuals with abnormal chromosomal sex have defects in either the number or the structure of the sex chromosomes. These defects usually are caused by random events during gamete formation or early embryonic development and are not necessarily related to abnormal genes inherited from the dam or sire. Reported abnormalities of chromosomal sex include XXY syndrome, XO syndrome, XXX syndrome, true hermaphrodite chimeras, XX/XY chimeras with testes, and XY/XY chimeras with testes.

XXY SYNDROME XXY syndrome is called *Klinefelter's syndrome* in humans and is one of the most common sex chromosome abnormalities observed in human beings. The true incidence of this disorder in dogs and cats is unknown; it is the most commonly reported sex chromosome abnormality. Affected dogs have a 79,XXY karyotype, hypoplastic testes, epididymides, vasa deferentia, and male external genitalia that vary from normal to hypoplastic and are sterile. The testes produce MIS and T, which explains the completely male phenotype. The presence of two X chromosomes prevents normal spermatogenesis, resulting in sterility.

Affected cats have a 39,XXY karyotype, with internal and external genitalia and testes similar to those

described for the dog. Of interest is the association of coat color with this chromosomal anomaly in cats. The gene for white coat color is on an autosome, and the genes for orange and nonorange (black or brown) are on the X chromosome, at the same locus. During early embryonic development in normal XX females, one X chromosome is randomly inactivated in each somatic cell to form the Barr body. The result is that each cell can express only an orange or nonorange coat color. Females that are heterozygous for these alleles develop the random patches of the tortoiseshell or calico pattern because only one allele is expressed in a given patch of hair. Normal males with only one X chromosome should be able to express only one coat color—orange or nonorange. Male cats that exhibit the tortoiseshell or calico coat pattern must have two X chromosomes. They either have the karyotype of 39,XXY or their coloring can be the result of chimerism or mosaicism (see following discussion).

XO SYNDROME Affected dogs have a 77,XO karyotype, dysgenetic ovaries (streak gonads), female internal genitalia, and infantile external genitalia, and they are sterile. In humans (Turner's syndrome) and horses this syndrome is associated with somatic abnormalities, the most notable of which is small stature. On the basis of only two reports in the literature (of cases occurring in a Doberman pinscher and an American

Eskimo bitch), this is likely the case in the dog as well. This syndrome was also reported in a 2.5-year-old Burmese cat that had primary anestrus (37,XO) but that did not have somatic abnormalities. Two kittens with an XO karyotype have also been described; the gonadal histology of these kittens was not included in the report. These kittens also had vascular and central nervous system anomalies and did not live past 3 days of age.

XXX SYNDROME A single report of an Airedale terrier bitch with primary anestrus at 4 years of age noted an association with a 79,XXX karyotype. The ovaries lacked follicles, the uterus was small, and the remainder of the genitalia were female. Resting serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were elevated, and progesterone was at the baseline level (consistent with anestrus). In other species, XXX individuals have been reported to be fertile. However, most are infertile with abnormal estrous cycles.

TRUE HERMAPHRODITE CHIMERA Chimerism results when two or more cell populations, each arising from different individuals, are present in a single individual. An example is the fusion of two zygotes with different sex chromosome constitutions, giving rise to a single zygote with an XX/XY chromosome constitution.

Mosaicism results when two or more cell populations with different chromosome constitutions are present but when both of these cell populations arise from within the individual. This usually is attributable to mitotic nondisjunction. True hermaphrodites have both ovarian and testicular tissue present. Any combination can be seen (unilateral ovotestis with a contralateral ovary or testis, bilateral ovotestes, or unilateral ovary and unilateral testis). Three canine cases of true hermaphrodite chimeras have been reported. The karyotype of these individuals was either XX/XY or XX/XXY; all were phenotypic females with enlarged clitorides. There is one report of a feline that was externally male in phenotype, with one scrotal testis and one abdominal ovary. Ipsilateral to the ovary, müllerian duct derivatives were present.

XX/XY CHIMERA WITH TESTES An Old English sheepdog with ambiguous genitalia (cranially displaced vulva containing a hypoplastic penis) possessed a karyotype of XX/XY. Internally, the gonads were aspermatogenic testes, and the tubular tract was a hypoplastic uterus. Several cats with XX/XY chimerism with an external male phenotype have been reported, with variable fertility. It appears that the higher the proportion of XY cells to XX cells is, the greater is the likelihood of fertility. Some tortoiseshell males have this chromosomal anomaly.

XY/XY CHIMERA WITH TESTES Tortoiseshell appearance in males with normal testicular histology and normal fertility is most likely caused by XY/XY chimerism.

Signalment

Disorders of sex chromosomes are random events. Therefore, they can be observed in dogs and cats of any breed. All of these disorders are congenital (present at birth). However, individuals with abnormal genitalia may not be identified until they reach breeding age or may be identified at the time of elective gonadectomy.

History and Clinical Signs

Most animals with abnormalities of chromosomal sex have few clinical symptoms. Detection is most likely if the animal's intended use is in a breeding program. The most common historical complaints are primary anestrus for phenotypic females and inability to sire litters for phenotypic males. The exception to this is an individual that is a chimera with ambiguous external genitalia and the puppy or kitten is presented because of an abnormal vulva or prepuce. Some hermaphrodite chimeras also may have chronic vulvar irritation. It is also possible to see hermaphrodite chimeras or chimeras with testes present with signs of hyper-

estrogenism secondary to Sertoli cell tumor in abdominal testicular tissue.

Physical Examination Findings

In general, the external genitalia of most patients with abnormal sex chromosome constitution is normal or hypoplastic but unambiguous. The penis and prepuce of phenotypic males can vary from normal to hypoplastic, and most will have hypoplastic scrotal testes. Phenotypic females typically have normal or hypoplastic vulvas. Hermaphrodites and chimeras may have ambiguous or unambiguous genitalia. They usually appear to be phenotypic females in the dog, with a normal to enlarged clitoris. In the cat, reports of hermaphrodites or chimeras have been phenotypic males, with or without scrotal testes. Whether this difference in phenotype expression of chimeras between dogs and cats is coincidental or has a genetic basis is unknown.

Diagnostic Tests and Results

A karyotype is necessary to define the error in sex chromosome constitution. This is typically performed on peripheral blood lymphocytes. In all patients with a suspected disorder of sexual development, a careful gross description of the external and internal genitalia

and histopathology of the gonads and tubular tract are necessary to accurately categorize the disorder.

Treatment

Gonadectomy and hysterectomy are recommended. If the clitoris is enlarged, as is seen with some chimeras, amputation is recommended to eliminate continual mucosal irritation.

Prognosis

Because disorders of chromosomal sex are random events occurring during meiosis or mitosis, there is no heritable component to these syndromes. Therefore it is unnecessary to remove siblings or parents of affected individuals from the breeding population. Phenotypic females that are undiagnosed hermaphrodite chimeras or chimeras with testes are potentially at risk for Sertoli cell tumors of abdominal testicular tissue.

Disorders of Gonadal Sex

Definition and Pathogenesis

Individuals with disorders of gonadal sex have either an XX or XY sex chromosomal constitution, but the gonads do not agree with the chromosomal sex. This is referred

to as *sex reversal.* XX sex reversal has been reported to occur only in the dog; affected dogs have a 78,XX chromosome constitution. No cases of XX sex reversal in the cat have been reported, and no cases of XY reversal in either the dog or the cat have been reported. XX sex reversal includes XX true hermaphrodite and XX males. It is possible to see both of these phenotypes within the same family. Eighty percent of human XX males are *Sry* positive as a result of translocation from the Y chromosome to an autosome. However, all cases of canine XX sex reversal to date that have been tested are *Sry* negative. *Sry*-negative XX sex reversal has been described in goats and pigs as an inherited autosomal recessive syndrome. It has been reported to occur in the llama and the horse in isolated cases; the inheritance pattern in these species is unknown. The specific autosomal genes that are responsible for testis induction in the absence of *Sry* are presently unknown. Functionally active MIS is present, but failure of complete müllerian duct regression suggests insensitivity of the target organ to MIS.

Signalment

XX sex reversal has been reported to occur only in the dog. No cases of this syndrome have been identified in the cat. In the American cocker spaniel, XX sex reversal is inherited as an autosomal recessive trait. Inheritance

in the German shorthaired pointer is likely to be autosomal recessive. It has been described as a familial disorder occurring in the English cocker spaniel, beagle, weimaraner, Kerry blue terrier, and Chinese pug. The mode of inheritance in these breeds is unknown but is likely autosomal recessive. Cases of XX sex reversal also have been described in the basset hound, vizsla, soft-coated wheaten terrier, Pomeranian, Doberman pinscher, American pit bull terrier, Border collie, Walker hound, and Afghan hound.

History and Clinical Signs

Affected individuals usually are presented as phenotypic females with primary anestrus, phenotypic females with an abnormal vulva, or males with bilateral cryptorchidism and an abnormal prepuce and penis.

Physical Examination Findings

XX true hermaphrodite individuals have both ovaries and testes. Bilateral ovotestes constitute the most common combination of gonads. The next most common combination is one ovotestis and one ovary. One ovotestis and one testis comprise the least common combination. The amount of testicular tissue present correlates with the degree of masculinization of the

internal and external genitalia. Most individuals are phenotypic females or have a partially masculinized female phenotype that varies from a normal to abnormal vulva, normal-sized or enlarged clitoris (commonly with an os clitoris), uterus, oviducts, epididymides, and vasa deferentia.

XX males have testes, the entire wolffian duct system (epididymides and vasa deferentia), and a prostate. A bicornuate uterus is present, but both oviducts usually are absent. The prepuce usually is abnormal in shape and caudally displaced. Most XX males have a hypoplastic penis, and hypospadias or abnormal curvature of the penis is common.

Diagnostic Tests and Results

A karyotype of 78,XX in conjunction with the presence of testicular tissue (at least one ovotestis or one testis) is needed to verify XX sex reversal. Elevation of testosterone in response to a gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) stimulation test suggests that testicular tissue is present, but negative results of a stimulation test do not completely rule out the presence of testicular tissue. A polymerase chain reaction test for the presence of *Sry* is recommended to accurately describe this disorder. Unfortunately, no laboratory test is available to identify carriers for XX sex reversal.

Treatment

Gonadectomy and hysterectomy are recommended for affected individuals.

Prognosis

On rare occasions, XX true hermaphrodites have reproduced, but it is not recommended to maintain these individuals in a breeding program. Most XX true hermaphrodites and all XX males are sterile. Because this is a heritable trait in breeds that have been closely studied and is most likely a heritable trait in breeds for which breeding trials have not yet been conducted, owners should be counseled that both parents of affected individuals should be removed from the breeding program. At least half of the siblings of affected individuals are expected to be carriers. Because there is no laboratory test that can identify carriers, the best recommendation is to not use any siblings of affected individuals as breeding animals.

Disorders of Phenotypic Sex

Definition and Pathogenesis

In individuals with disorders of phenotypic sex, there is agreement with chromosomal and gonadal sex but disagreement with phenotypic sex (internal or external

genitalia). A female pseudohermaphrodite has an XX chromosome constitution, ovaries, and masculinized internal or external genitalia. A male pseudohermaphrodite has an XY chromosome constitution, testes, and internal or external genitalia feminized to some degree. Descent of the testes into the scrotum completes the development of phenotypic sex. The genetic and hormonal control of testicular descent is not completely understood, and the classification of cryptorchidism as a disorder of phenotypic sex is debatable (see Chapter 16).

FEMALE PSEUDOHERMAPHRODITISM Female pseudohermaphroditism resulting from endogenous androgen exposure (e.g., adrenogenital syndrome in humans) has not been reported as occurring in the dog or cat. Rare reports of female pseudohermaphrodites in the dog suggest that iatrogenic exposure of the fetus to exogenous androgens or progestogens during gestation is responsible for this syndrome. There have been no reports of this syndrome in the cat.

MALE PSEUDOHERMAPHRODITISM Male pseudohermaphrodites include XY males in whom the müllerian ducts fail to regress and individuals with defects in androgen-dependent masculinization.

Persistent müllerian duct syndrome Persistent müllerian duct syndrome (PMDS) is recognized as a form of

male pseudohermaphroditism in the miniature schnauzer in the United States, the basset hound in The Netherlands, and possibly the Persian cat. In the miniature schnauzer, affected individuals are XY males, with bilateral testes, external male genitalia, and all müllerian and wolffian duct derivatives present. PMDS has been shown to be inherited with an autosomal recessive pattern in the miniature schnauzer; only homozygous individuals display the abnormal phenotype. Affected individuals secrete bioactive MIS at the critical time period during embryonic development. This suggests that the defect in animals affected with PMDS is insensitivity of the müllerian ducts to MIS, possibly related to a defect in the MIS receptor.

Defects in androgen-dependent masculinization Animals that possess defects in androgen-dependent masculinization have an XY sex chromosome constitution, bilateral testes, and no müllerian duct derivatives. However, internal and external genitalia that require androgens for masculinization during embryonic development do not develop normally. The resulting abnormal phenotype can vary from complete (severe) to incomplete (mild). These are grouped according to primary defect as follows:

- Defects in androgen production
- Androgen resistance or androgen insensitivity

Failure in conversion of T to DHT (defect in the 5α-reductase enzyme)

Defects in the androgen receptor (testicular feminization)

Defects in androgen production or in the 5α-reductase enzyme have not been reported to occur in the dog or cat.

Hypospadias is the abnormal location of the urinary orifice. The orifice can be located anywhere along the ventrum of the glans penis to the perineum. This defect occurs when there is incomplete masculinization of the urogenital sinus (closure of the urethra). The remainder of the external genitalia of these animals is not ambiguous, but concurrent cryptorchidism, penile hypoplasia, ventral deviation of the penis, and abnormalities of the ventral prepuce have been described.

Testicular feminization syndromes (Tfm) are those in which there are mutations, qualitative or quantitative, in the X-linked androgen receptor gene. Affected animals are XY males with bilateral testes. Because testes are present that secrete normal amounts of T and MIS, no müllerian duct derivatives are present. However, because there is a defect in the androgen receptor gene, androgen-dependent masculinization is either absent or incomplete, despite normal production of T and DHT.

Signalment

FEMALE PSEUDOHERMAPHRODITISM Female pseudohermaphroditism has been described only in dogs. Phenotypic females with enlarged clitorides are most likely to be presented as juveniles because of abnormal genitalia. Phenotypic males can be seen at any age depending on the clinical signs. Because this disorder is caused by iatrogenic sex steroid administration during pregnancy, there is no breed predilection.

PERSISTENT MÜLLERIAN DUCT SYNDROME Persistent müllerian duct syndrome occurs in dogs and cats. Although this disorder is congenital, the age at presentation likely depends on whether the individual has bilateral scrotal testes. Those with unilateral or bilateral cryptorchidism may be seen as juveniles, whereas those with scrotal testes are more likely to be seen at a later date in life because of symptoms related to uterine disease, urinary tract infections, or prostatitis. PMDS is heritable in the miniature schnauzer in the United States and the basset hound in The Netherlands.

HYPOSPADIAS Although congenital, mild forms, in which the orifice is located along the glans penis, may not be recognized until after puberty, more severe forms are more likely to be identified in puppies because of the abnormal location of the urine stream. This syndrome may have a familial basis in the Boston terrier.

TESTICULAR FEMINIZATION SYNDROME Complete Tfm has been reported only in the cat (domestic shorthair). Incomplete Tfm has occurred in the cat and dog. Although the defect is congenital, most affected animals are not presented until they reach breeding age.

History and Clinical Signs

FEMALE PSEUDOHERMAPHRODITISM Dogs with female pseudohermaphroditism are phenotypic males with hematuria, are attractive to male dogs, have swelling of the prepuce (periodic estrus), have signs of cystic endometrial hyperplasia/pyometra, or have urinary incontinence secondary to pooling of urine within the vagina. Mildly affected individuals with external female genitalia either are clinically inapparent or are seen because of an enlarged clitoris or an abnormal vulvar conformation.

PERSISTENT MÜLLERIAN DUCT SYNDROME Dogs with PMDS may be brought for treatment because of unilateral or bilateral cryptorchidism; these cases may also be seen because of signs of hyperestrogenism caused by Sertoli cell tumors. Those with bilateral scrotal testes are more likely to have clinical signs consistent with systemic illness resulting from pyometra, urinary tract infections, or prostatitis.

HYPOSPADIAS Dogs with hypospadias may be asymptomatic or may have a history of inguinal dermatitis secondary to urinary incontinence.

TESTICULAR FEMINIZATION SYNDROME Animals with complete Tfm are seen as phenotypic females with complaints of primary anestrus and sterility. Animals with incomplete Tfm are more often presented because of genital ambiguity.

Physical Examination Findings

FEMALE PSEUDOHERMAPHRODITISM Dogs that are female pseudohermaphrodites have bilateral ovaries and oviducts, a uterus, a cervix, and a cranial vagina. The degree of masculinization of androgen-sensitive tissues ranges from a normal vulva with mild clitoral enlargement to a somewhat normal penis and prepuce with an internal prostate.

PERSISTENT MÜLLERIAN DUCT SYNDROME Dogs with PMDS have oviducts, a bicornuate uterus, a cervix, and a cranial vagina. They also have testes, epididymides, vasa deferentia, and a prostate. Half of affected males are either unilaterally or bilaterally cryptorchid, and the remainder have bilateral scrotal testes. Development of Sertoli cell tumor may be seen in those with abdominal

testes. Cystic endometrial hyperplasia/pyometra may be present in uterine tissue.

HYPOSPADIAS In mildly affected dogs the urethral orifice is located on the ventral aspect of the glans penis (glandular form). In more severely affected dogs, the urethral orifice can be located along the proximal penis, prepuce, scrotum, or perineum (penile, preputial, scrotal, or perineal forms). These latter types reflect a more significant defect in androgenization. Some individuals may be cryptorchid or may have a hypoplastic penis, ventral deviation of the penis, or an abnormally developed prepuce.

TESTICULAR FEMINIZATION SYNDROME Individuals with complete Tfm have bilateral testes (usually abdominal) and no epididymidides, vasa deferentia, oviducts, uterus, cervix, or cranial vagina. A vulva is present externally. Animals with incomplete Tfm have bilateral testes, which can be abdominal, but are more frequently described as having a perineal bifid scrotum. The external genitalia in affected cats have been those of a fairly normal female with a vulvalike opening, perineal hypospadias, a blind-ending vagina, and a penis that resembles a clitoris and develops spines.

Diagnostic Tests and Results

FEMALE PSEUDOHERMAPHRODITISM Individuals suspected of being female pseudohermaphrodites should have their chromosome constitution defined by karyotype; affected dogs have a 78,XX karyotype. Although endogenous androgen exposure has not been reported as occurring in any of the canine cases of female pseudohermaphroditism, it is recommended to rule out an endogenous source of androgens before surgery is performed. Elevation of T to a concentration of 3 ng/ml or more in response to administration of GnRH (2 μg/kg administered intramuscularly; draw blood sample 1 hour later) or hCG (40 IU/kg administered intramuscularly; draw blood sample 4 hours later) suggests the presence of testicular tissue and, therefore, a diagnosis of XX sex reversal. Elevation of serum T in response to adrenocorticotropic hormone stimulation suggests adrenal production of testosterone (similar to adrenogenital syndrome in humans). Histopathologic examination of the gonads and tubular tract confirms the presence of ovaries, oviducts, a uterus, and a cervix. Dogs that have symptoms of urinary tract abnormalities should undergo contrast cystourethrography before undergoing surgical intervention.

PERSISTENT MÜLLERIAN DUCT SYNDROME Dogs with PMDS have a karyotype of 78,XY and gonadal and tubular tract histopathology confirming the presence of testes,

epididymides, vasa deferentia, a uterus, a cervix, and a cranial vagina. Contrast cystourethrography is indicated for dogs with symptoms of urinary tract abnormalities.

HYPOSPADIAS Hypospadias has been reported to occur in association with other types of abnormalities of sexual differentiation (e.g., XX sex reversal). To provide informed genetic counseling to the owner, it is recommended to perform a karyotype and gonadal histopathologic examination on the affected dog.

TESTICULAR FEMINIZATION SYNDROME A diagnosis of Tfm depends on the finding of an XY chromosome constitution, bilateral testes, and female external genitalia. Binding studies of cultured genital fibroblasts from androgen-responsive tissues show reduced or absent binding for T and DHT.

Treatment

FEMALE PSEUDOHERMAPHRODITISM Ovariohysterectomy (OHE) is recommended. Any urinary tract abnormality identified by contrast cystourethrography should be surgically corrected.

PERSISTENT MÜLLERIAN DUCT SYNDROME Castration and hysterectomy are recommended. Occasionally, a small communication from the cranial vagina to the prostate is

present. Removal of as much of the vagina as possible is recommended to avoid urinary tract complications.

HYPOSPADIAS Castration is recommended for all animals with hypospadias. Asymptomatic dogs may not require surgical intervention, whereas animals that are more severely affected may require surgical management. Such management can range anywhere from closure of the defect in glandular forms to penile amputation in those cases in which the location is more proximal (penile, preputial, scrotal, or perineal forms).

TESTICULAR FEMINIZATION SYNDROME Castration of affected animals is recommended. Prevention can be accomplished through genetic counseling to reduce or remove carrier animals from a breeding program. Tfm is an X-linked disorder, and carrier females are fertile. The expected outcome is that 50% of female offspring from carrier females will be carriers, 50% of male offspring will be affected, and 50% of male offspring will be normal. Male offspring with normal genitalia can be presumed not to carry the mutation for Tfm and may remain in the breeding program.

Prognosis

FEMALE PSEUDOHERMAPHRODITISM After surgical intervention, the prognosis is good. The importance of

distinguishing this syndrome from XX sex reversal relates to genetic counseling. With female pseudohermaphroditism resulting from exogenous androgen exposure, there is no heritable component and no need to remove the parents or siblings from the breeding program. With XX sex reversal, it is strongly advised to remove the parents and siblings of affected individuals from a breeding program. In the dog the internal and external genitalia undergo differentiation from days 34-46 from the LH peak that occurs during estrus in the dam. Avoiding in utero exposure of the fetus to androgens or progestogens during this critical period should prevent this syndrome.

PERSISTENT MÜLLERIAN DUCT SYNDROME Prognosis is good after castration and hysterectomy. Complications resulting from pyometra, urinary tract abnormalities, or prostatic disease carry a fair to poor prognosis depending on the duration of disease before diagnosis.

HYPOSPADIAS After castration and surgical correction (if necessary), the prognosis is good. Although dogs that are mildly affected can breed normally, removing all affected individuals from a breeding program is recommended.

TESTICULAR FEMINIZATION SYNDROME The prognosis is good after castration. Genetic counseling should be aimed at eliminating female carriers from the gene pool.

Agenesis and Dysgenesis of the Reproductive Tract

Definition and Pathogenesis

Agenesis is the failure of a structure or organ system to develop because of nonappearance of its primordium during embryonic development. Dysgenesis is a defect in development of a structure or organ. In the reproductive tract of dogs and cats, agenesis or dysgenesis of the gonads, müllerian or wolffian ducts, urogenital sinus, genital tubercle, or genital swellings can be seen. Examples include monorchidism and testicular hypoplasia, ovarian agenesis and ovarian hypoplasia, segmental aplasia of the epididymides, vasa deferentia, oviducts, uterus, and vagina, and penile hypoplasia. In females, failure of fusion of the caudal müllerian ducts or urogenital sinus can give rise to a variety of vaginal anatomic anomalies (see Chapter 13). In affected animals in which chromosomal sex, gonadal sex, and phenotypic sex agree, it is unknown whether there is some genetic, hormonal, or heritable component to these anomalies.

Signalment

All of these defects are present at birth; however, most are not recognized until the animal is used for breeding or are found incidentally at OHE or castration. Some

dogs with vaginal anomalies are treated as juveniles for persistent vaginitis. Agenesis or dysgenesis of any portion of the reproductive tract can be seen in all breeds of dogs and cats.

History and Clinical Signs

Most animals with agenesis or dysgenesis of a portion of the reproductive tract have no clinical symptoms, but if present, the signs will depend on what part of the tract is affected. In females, signs may vary from primary anestrus to small litter size, infertility, or sterility. Clinical signs in males vary from absence of a testis to infertility or sterility.

Physical Examination Findings

The external genitalia of affected animals usually are normal, except in a male with a hypoplastic penis or prepuce. Some females with vaginal anomalies may have perivulvar dermatitis secondary to chronic vaginitis. Internally, a variety of anomalies may be identified, such as complete lack of one or both gonads, unilateral or bilateral gonadal hypoplasia, unilateral aplasia of a uterine horn (uterus unicornis), and segmental aplasia of one or both uterine horns. Several types of vaginal malformations are possible (see Chapter 13).

Diagnostic Tests and Results

It is recommended to determine the karyotype and the type of gonad present in affected individuals. Many cases of agenesis or dysgenesis are secondary to abnormalities of chromosomal or gonadal sex. This information is necessary to provide informed genetic counseling to owners of affected animals. Contrast vaginography can delineate vaginal anomalies. Vaginography performed when the animal is in proestrus or estrus extends cranially to produce a hysterogram, which can evaluate patency of the cervix, uterine body, and uterine horns.

Treatment

Surgical treatment varies with the nature of the disorder. Females with segmental aplasia may have accumulation of intraluminal serous to purulent fluid proximal and ipsilateral to the affected region of the uterus; complete OHE is recommended if breeding potential is not desired. If the animal's intended use is breeding, removal of the affected side only can be attempted. Some surgical facilities have attempted microreconstruction on a few male dogs with epididymal blockages. The results have been extremely variable, and more work is needed in this area. Theoretically, this procedure could be applied to dogs with segmental aplasia of the epididymides or vasa deferentia, provided that the aplastic segment is relatively short.

Prognosis

The prognosis for life is good, whereas the prognosis for fertility varies from fair to poor depending on whether the anomaly of the tract is unilateral or bilateral. Those with unilateral agenesis or dysgenesis may be able to reproduce, although fertility will be reduced when compared with an unaffected individual. Those with bilateral abnormalities are likely to be sterile.

Bibliography

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology,* Philadelphia, 2001, WB Saunders.

McElreavey K, Vilain E, Nacer A et al: A regulatory cascade hypothesis for mammalian sex determination: *Sry* represses a negative regulator of male development, *Proc Natl Acad Sci USA* 90:3368, 1993.

Meyers-Wallen VN: Disorders of sexual development in the dog. In Kirk RW, Bonagura JD (eds): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Meyers-Wallen VN: Inherited disorders in sexual development, *J Hered* 90:93, 1999.

Meyers-Wallen VN: CVT update: inherited disorders of the reproductive tract in dogs and cats. In Kirk RW, Bonagura JD (eds): *Current veterinary therapy XIII,* Philadelphia, 2000, WB Saunders.

Meyers-Wallen VN, Schlafer D, Barr I et al: *Sry*-negative XX sex reversal in purebred dogs, *Mol Reprod Dev* 53:266, 1999.

2

Breeding Management in the Bitch and Queen

Mylissa S.D. Edens and Allen M. Heath

AT A GLANCE

- Estrous cycle of the bitch
  - Proestrus: 9-day average duration; vulvar swelling and serosanguineous vulvar discharge present; female does not allow copulation; vaginal epithelium increasingly cornified

- Estrus: 9-day average duration; female does allow copulation; vaginal epithelium completely cornified
- Diestrus: 60-day average duration; occurs regardless of breeding status; abrupt return to noncornified vaginal epithelium
- Anestrus

- Breeding management of the bitch
  - The average bitch ovulates on the second day of estrus (standing heat), but this varies in normal bitches.
  - The fertile window is from 3 days before to 4 days after ovulation.
  - If only one breeding is possible or if artificial insemination is to be performed, optimal breeding day is 2 days after ovulation.
  - Ovulation day is best determined by measurement of serum progesterone concentration; serum progesterone concentration 2 days before ovulation is 2-3 ng/ml and on ovulation day is 5-8 ng/ml.
- Estrous cycle of the queen
  - Estrus: 7-day average duration; lordosis posture; vocalization; female allows copulation; cats are induced ovulators
    - If not induced to ovulate, go into interestrus; 8-day average duration

  - If induced to ovulate but not pregnant, go into pseudopregnancy; 45-day average duration
  - If induced to ovulate and pregnant, go into diestrus; 63- to 66-day average gestation length
- Anestrus: seasonal (long-day breeders)

The Bitch

The most common cause of infertility in the bitch is inappropriately timed breeding. By knowing the stages of the estrous cycle and the corresponding clinical signs and physiology, one can optimize breeding efficiency and improve fertility.

Onset of puberty in the bitch occurs at 6-23 months of age, with an average of 10-14 months. The bitch is nonseasonally monoestrous. The interestrous interval is 4-13 months, with an average of 7 months. A few breeds, such as the basenji and Mexican Hairless, cycle every 12 months, whereas the German shepherd and rottweiler cycle every 4-5 months.

Stages of the Canine Estrous Cycle

The stages of the canine estrous cycle are proestrus, estrus, diestrus, and anestrus (Figure 2-1).

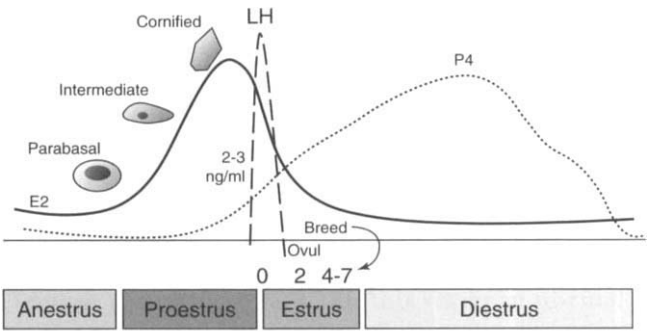


Figure 2-1. Illustration of the hormonal events during the estrous cycle of the bitch. The vaginal epithelial cells seen during proestrus are also shown. *E2,* Estradiol; *LH,* luteinizing hormone; *P4,* progesterone; *Ovul,* ovulation.

PROESTRUS

Duration

- Proestrus lasts for 3-17 days, with an average of 9 days.

Behavior

- This stage is characterized by attraction of male dogs to the bitch. She does not allow the male to mount.

Physical changes

- The vulva is swollen, and a serosanguineous vulvar discharge, originating from the uterus, typically is present.
- Vaginoscopic examination reveals a moist, pink, and edematous vagina. The vaginal lumen often is difficult to visualize (Figure 2-2 and Color Plate 1).

Endocrinology and vaginal cytology

- Follicular development causes a gradual increase in serum estrogen concentration, which peaks 2-3 days before estrus and then rapidly declines during estrus. The rise in estrogen leads to hyperplasia of the vaginal epithelial cells.
- Evaluation of vaginal cytology during early proestrus, when estrogen concentrations are low, reveals parabasal and intermediate epithelial cells (Figure 2-3 and Color Plate 2). Parabasal cells are the healthiest of the vaginal epithelial cells. Red blood cells are commonly present. Neutrophils and bacteria also may be seen.
- As proestrus progresses, estrogen concentrations continue to rise and hyperplasia of the vaginal epithelial cells occurs. The increased thickness of the vaginal epithelium results in the most superficial cells being located farther away from the blood supply. These cells are not viable and are

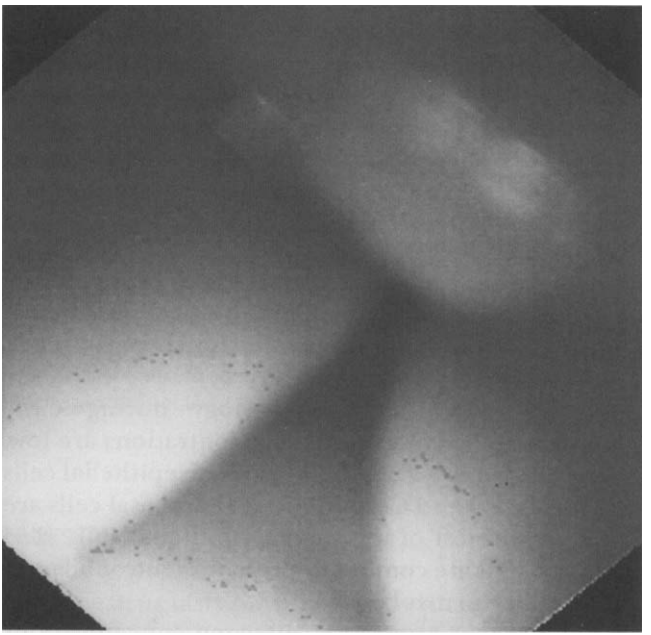


Figure 2-2. Endoscopic view of vaginal mucosal folds during proestrus. The folds are edematous, moist, and pink. The progesterone concentration of this bitch was less than 1 ng/ml.

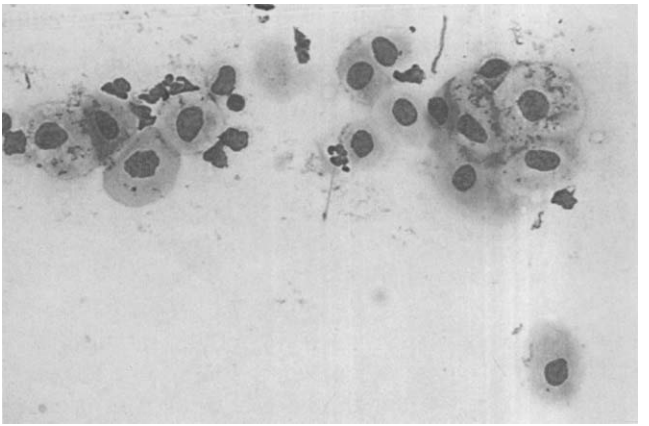


Figure 2-3. Vaginal cytologic result showing parabasal cells. They are round cells with a large nucleus and a small amount of cytoplasm.

called *superficial cells* (Figure 2-4 and Color Plate 3). At the end of proestrus, more than 80% of the epithelial cells are cornified superficial cells. Red blood cells may still be present; however, fewer neutrophils are present compared with early proestrus. Bacteria may be present throughout proestrus.

- Progesterone concentrations in serum during most of proestrus are less than 2 ng/ml.

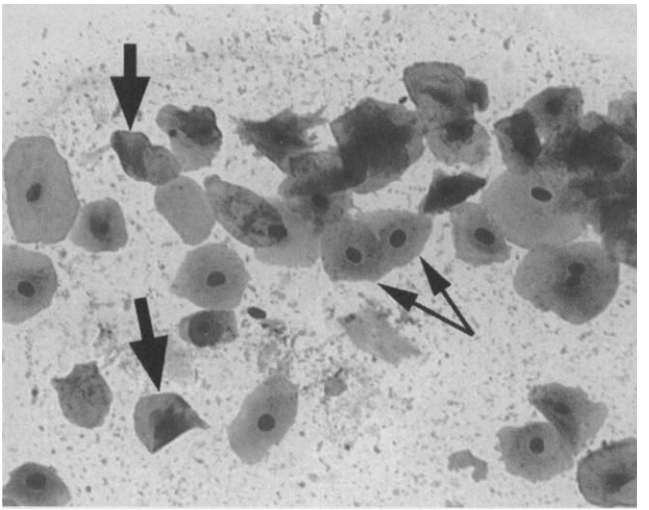


Figure 2-4. Intermediate cells *(thin black arrows)* and superficial cells *(thick black arrows)* seen on vaginal cytologic examination. Intermediate cells have more irregular borders than do parabasal cells, and they have a smaller nucleus and larger cytoplasm. Cornified superficial cells are dead vaginal epithelial cells. They have sharp, angular borders and contain a small, pyknotic nucleus or no visible nucleus.

ESTRUS

Duration

- The duration of estrus is 3-21 days, with an average of 9 days.

Behavior

- This stage is characterized by the bitch allowing the male to mount and standing to be bred.

Physical changes

- The vulva is flaccid, and the vulvar discharge often is straw-colored. However, some normal bitches may continue to have a blood-tinged discharge throughout estrus.
- Vaginoscopic examination shows a crenulated vagina with a more prominent lumen (Figure 2-5 and Color Plate 4). The mucosa may be hyperemic but often is blanched.

Endocrinology and vaginal cytology

- Estrus usually begins at approximately the time of the luteinizing hormone (LH) surge, which has a duration of 24-48 hours. This is coincident with a decline in estrogen concentrations and a rise in progesterone concentrations.
- Luteinization begins before ovulation. Therefore progesterone concentrations at the LH surge are generally 2-3 ng/ml.

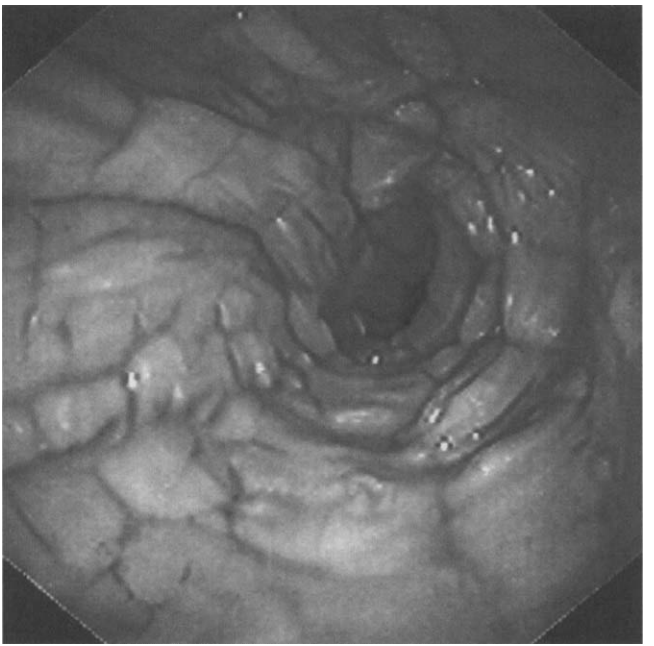


Figure 2-5. Endoscopic view of the vagina of a bitch in estrus. The vaginal vault is crenulated. The serum progesterone of this bitch was 3.8 ng/ml.

- Ovulation of a primary oocyte occurs 2 days after the LH surge (day 0).
- An additional 2-3 days are required for the oocyte to mature to a fertilizable secondary oocyte. The oocytes remain fertile for 2-3 days. Best conception rate and litter size are achieved by breeding 4-7 days after the LH surge.
- During estrus, vaginal cytology specimens contain greater than 90% cornified superficial epithelial cells. Red blood cells are fewer in number, and neutrophils are not present.
- Progesterone concentrations continue to rise, reaching 5.0-8.0 ng/ml at ovulation and 4.0-20.0 ng/ml during the fertile period.

DIESTRUS

Duration Diestrus lasts 56-58 days from ovulation.

Behavior During this period, the bitch will no longer stand to be mounted.

Physical changes

- The vulva is no longer swollen.
- Vaginoscopic examination reveals the mucosa to be pale, with no crenulation seen.

Endocrinology and vaginal cytology

- Serum progesterone concentrations remain elevated throughout diestrus.
- Vaginal cytology changes during a 24- to 36-hour period from full cornification of epithelial cells to 40%-60% parabasal and intermediate cells. Many neutrophils may be seen. In addition, metestrum cells (a parabasal cell with a neutrophil in the cytoplasm) and foam cells (a parabasal cell with vacuoles in the cytoplasm) may be seen.

ANESTRUS

Duration

- Anestrus lasts for 2-9 months.

Physical changes

- It is the period of uterine involution. Involution requires 70 days in the nonpregnant bitch and 90 days in the postpartum bitch.

Endocrinology and vaginal cytology

- Progesterone concentrations decrease just before parturition or gradually after corpus luteum regression in the nonpregnant bitch.
- Vaginal cytologic examination reveals parabasal and intermediate epithelial cells. Red blood cells are not

present. Neutrophils may or may not be present but never should be great in number in a normal anestrous bitch.

Technique for Obtaining Vaginal Cytology Specimens

The vulvar lips are parted with one hand while the other hand is used to pass a 7-inch-long cotton-tipped applicator that has been moistened with saline or tap water. It is important to avoid the ventral clitoral fossa because it generally contains keratinized cells, which can be confused with superficial epithelial cells. The swab is passed in a craniodorsal direction, avoiding the urethral papilla on the floor of the vestibule, until the ischial arch is reached, at which point the swab is directed craniad. The swab is rotated within the vagina a few times and then withdrawn. The swab is gently rolled across a microscope slide in two to three rows. It is important not to smear the swab across the slide because this will distort the epithelial cells. The slide is dried and then stained with a modified Wright-Giemsa stain (Diff-Quik; American Scientific Products, McGraw, Ill.). The time required to stain vaginal epithelial cells is longer than that for blood smears; the slide is placed in each of the solutions of stain for 10-15 seconds each. Once dried, the slide is observed under 40× magnification.

Breeding Management of the Normal Bitch

Although dog sperm is capable of fertilization for as long as 7-9 days after ejaculation, breeding several days before ovulation may result in aged gametes and smaller litters. With good breeding management, the bitch can be inseminated at the optimal time to ensure fertilization of the maximal number of viable oocytes. Breeding management is important when sending the bitch to the stud dog, when using shipped cooled semen, and especially when using frozen semen.

It is important to make sure that both the bitch and the dog are free of *Brucella canis* before breeding (see Chapter 7) and that they are vaccinated, free of internal and external parasites, and in good general health. Testing for hereditary defects may be recommended, depending on predispositions present in that breed.

When deciding when to breed the bitch, behavior, character of the vulvar discharge, vulvar swelling, vaginal cytologic examination, vaginoscopy, and serum progesterone concentrations are all used together to determine the time of the LH surge.

- Vaginoscopy may be used to determine the decline in serum estradiol and estimate the day of the LH surge.

  As estradiol is decreasing, the vaginal folds become crenulated as a result of a decrease in edema. Maximum crenulation occurs 4-7 days after the LH surge, which is the most fertile period. The

degree of crenulation varies among bitches. Vaginoscopy is performed using a rigid endoscope (10-inch Welch Allyn juvenile proctoscope or 3.5-mm 30-degree cystourethroscope), passing it into the vagina as described for collection of vaginal cytology specimens. Alternatively, a clear speculum can be used.

Vaginoscopic changes are much less accurate than is measurement of progesterone in serum for assessment of optimal breeding day.

- Vaginal cytologic examination is performed every 2-4 days. Once the vaginal epithelial cells are greater than 50% cornified, measurement of progesterone in serum is begun.
- Measurement of serum progesterone is performed every 48 hours until serum progesterone concentration is 2-3 ng/ml. This concentration is coincidental with the LH surge. If the progesterone concentration was less than 2 ng/ml on the first day of testing and greater than 3 ng/ml 2 days later, one can assume that the LH surge occurred between those 2 days. Once the serum progesterone has reached 2-3 ng/ml, retesting the progesterone the next day to confirm that it is continuing to rise is recommended.

  In-house enzyme-linked immunosorbent assay (ELISA) kits are available for measurement of serum progesterone concentration. ELISA is easy

to perform and is inexpensive, but it is not as accurate as radioimmunoassay (RIA) or chemiluminescence assay. Progesterone concentrations measured by ELISA are reported as a range. They are adequate to use for fresh or fresh chilled semen breeding procedures but generally are not accurate enough for timing of insemination with frozen semen. When ELISA is used, a false decrease in serum progesterone concentration may be measured if the sample is hemolyzed. A false increase in serum progesterone concentration may occur if the ELISA kits are not warmed to room temperature before use.

Progesterone is the same in all species. Therefore serum progesterone may be determined by RIA or chemiluminescence assay at any endocrinology laboratory or at a human hospital. The RIA and chemiluminescence assays are more expensive to perform but provide an absolute progesterone concentration. These more accurate assays should be used when knowledge of the exact day of ovulation is required, such as when inseminating a bitch with frozen semen or breeding to a subfertile dog.

- LH is the hormone that causes ovulation to occur. Serum LH must be measured every 24 hours to ensure that the day of the LH peak is identified. A value of greater than 1 ng/ml is considered positive. An in-house kit for measurement of LH in serum is

commercially available (Status-LH; Synbiotics, San Diego, Calif.). The manufacturer recommends that positive results be verified by measurement of serum progesterone.

- *Once the LH surge occurs, evidenced by measurement of greater than 1 ng/ml of LH in serum or, ideally, by measurement of 2-3 ng/ml of progesterone in serum, the bitch is bred 4-7 days later.*

  Measurement of serum progesterone concentrations beyond that identifying the LH surge is recommended to ensure that the bitch ovulates and that she forms and maintains normal luteal tissue. Serum progesterone concentration on ovulation day is 5-8 ng/ml.

  If one insemination is performed, it is best done on day 5 or 6 after the LH surge. For two breeding procedures, insemination should be done on days 5 and 7 after the LH surge.

Alterations of the Canine Estrous Cycle

SHORTENING THE INTERESTROUS INTERVAL There are two ways to shorten the interestrous interval in the bitch. One is to shorten diestrus, and the other is to shorten anestrus.

- Diestrus can be shortened by inducing luteolysis with the use of prostaglandin $F_{2\alpha}$. Prostaglandin $F_{2\alpha}$ (Lutalyse; Pharmacia & Upjohn, Peapack, NJ) is

administered subcutaneously or intramuscularly (50-200 μg/kg twice a day) for 4-9 days beginning at day 5 of diestrus or later. The bitch will proceed into anestrus after luteolysis. Possible side effects associated with the use of prostaglandin include emesis, salivation, diarrhea, and respiratory difficulty. The side effects generally are self-limiting. Time until onset of the subsequent proestrus is widely variable.

- Anestrus may be shortened with the use of dopamine agonists, such as bromocriptine or cabergoline. Therapy for shortening of anestrus should not be instituted before day 90 of anestrus to allow normal uterine involution to occur. Bromocriptine (Parlodel; Novartis, East Hanover, NJ; 50 μg/kg given orally twice a day) is given until proestrus begins. Emesis may occur as a side effect. Cabergoline (Dostinex; Pharmacia & Upjohn; 5 μg/kg administered orally once daily) also is given until proestrus begins and reportedly causes fewer side effects than does bromocriptine. Proestrus generally occurs after 17-50 days of treatment with either drug.

IRREGULAR INTERESTROUS INTERVALS

- Split heats may appear as shortened interestrous intervals. *Split heat* is defined as appearance of proestrus signs with no ovulation occurring, then a brief (3-4 week) anestrus period, and then a normal

ovulatory cycle. Split heat generally occurs in young bitches during puberal heat. The bitch is fertile only during ovulatory heat. Repeated vaginal cytologic examination and serum progesterone concentrations can be used to diagnose split heats.

- Silent heats may appear as prolonged interestrous intervals. *Silent heat* is defined as the bitch's having little to no vulvar swelling or discharge despite normal follicular development and ovulation. Bitches undergoing silent heat generally attract male dogs. To diagnose silent heat in bitches and to manage them for breeding, vaginal cytology specimens should be collected and evaluated every 1-2 weeks and serum progesterone concentrations monitored monthly to determine when the bitch is cycling and fertile.
- Ovulation failure can shorten the interestrous interval. Ovulation failure is diagnosed by monitoring serum progesterone concentrations. In anovulatory bitches, serum progesterone concentration does not rise to normal diestrous concentrations (>5-8 ng/ml). The bitch may be treated with human chorionic gonadotropin (hCG; 500 IU/kg) during subsequent estrus to induce ovulation at that cycle.
- Hypothyroidism may be associated with prolonged interestrous intervals or acyclicity. Hypothyroidism is best diagnosed by concurrent measurement of

thyroid-stimulating hormone, which is elevated in serum of hypothyroid dogs, and free thyroxine by dialysis, which is decreased in serum of hypothyroid dogs.

The Queen

Onset of puberty in the queen occurs when she achieves 80% of her adult body weight, if that occurs during the appropriate season of the year. Puberty onset generally occurs when queens are 6-9 months old. Most queens are seasonally polyestrous, with cycling occurring during long day periods. Cycling usually begins in January or February and ends in September in temperate latitudes. The cyclic activity of a queen can be altered artificially. Cats that are maintained in 10 hours of artificial light may cycle year round.

Stages of the Feline Estrous Cycle

The stages of the feline estrous cycle are proestrus, estrus, diestrus, interestrous, and anestrus (Figure 2-6).

PROESTRUS

Duration This stage lasts for 12 hours to 2 days and is difficult to distinguish from estrus.

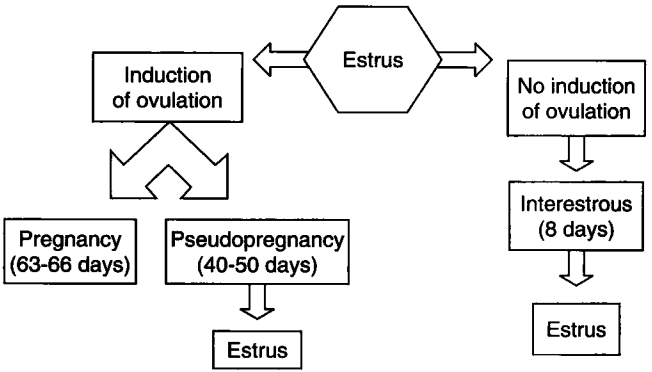


Figure 2-6. Potential outcome of each estrous cycle of the queen.

Behavior During proestrus the tom is attracted to the queen but she does not allow him to breed.

Endocrinology and vaginal cytology Proestrus is the period of follicular growth and rise in serum estrogen concentrations. Vaginal cytologic examination may be performed as in the bitch. The number of superficial epithelial cells increases to greater than 10%, and the number of parabasal and intermediate cells decreases. Red blood cells and neutrophils are not commonly seen. The vaginal mucous becomes

less viscous, and there are less noncellular debris and eosinophilic and basophilic strands of mucus present.

ESTRUS

Duration

- The duration of estrus is variable. Controversy exists regarding whether coitus and induction of ovulation shorten behavioral estrus in queens. In general, estrus lasts an average of 6.5-8 days.

Behavior

- Estrus is the period of sexual receptivity. During estrus the queen vocalizes, rubs her head and neck on objects, becomes more restless, rolls, and assumes the posture called *lordosis,* in which she holds her forequarters to the floor, elevates her hindquarters, and holds her tail to one side.

Endocrinology and vaginal cytology

- At the beginning of estrus, anuclear superficial cells number approximately 10%, with an increase to 40% by the fourth day. Percentage of superficial cells remains at 40%-60%. Percentage of intermediate cells decreases from 40%-10% during estrus. Parabasal cells are fewer than 10% of the epithelial cells.

- Ovulation may occur during estrus. Queens are induced ovulators. The means of induction may be vaginal stimulation or a form of external stimulation, such as petting or visual stimulation. This external stimulus causes release of gonadotropin-releasing hormone (GnRH) from the hypothalamus and subsequent release of pituitary LH. Maximum LH concentration occurs 4 hours after multiple (8-12) copulations. Return to baseline concentrations occurs in 24 hours. Ovulation occurs 24 hours after the release of LH. At least four copulations are required to reliably cause release of endogenous GnRH and LH, and ovulation, in estrous queens.

INTERESTROUS

Duration

- If the queen does not undergo ovulation during estrus, interestrus occurs next. This stage lasts for an average of 8 days, with a range of 2-19 days.

Behavior

- The queen is no longer attractive to the male, and estrous behavior ceases.

Endocrinology and vaginal cytology

- During this period, there is a sharp decline in estrogen concentrations.

- On vaginal cytologic examination the predominant cell types seen are nucleated superficial cells and intermediate cells. Background debris is present.

DIESTRUS

Duration

- The length of diestrus depends on whether the queen is pregnant or pseudopregnant. The duration of pregnancy is 63-66 days. The duration of pseudopregnancy is 40-50 days. This consists of a luteal phase of 36-37 days and then an interestrous period of 7-10 days.

Behavior

- This is a period of reproductive quiescence.

Endocrinology and vaginal cytology

- During diestrus the queen is under the influence of progesterone.

ANESTRUS

Duration

- Seasonal anestrus occurs during the shortening photoperiod, generally from October through December. Lactational anestrus may persist for 2-3 weeks after weaning.

Behavior

- This is a period of reproductive quiescence.

Endocrinology and vaginal cytology

- Vaginal cytology specimens contain fewer than 10% parabasal cells. Intermediate cells constitute 40%-70% of the epithelial cells. Nucleated superficial cells comprise 30%-40% of the cells. Background debris is evident.

Manipulation of the Feline Estrous Cycle

INDUCTION OF ESTRUS Artificial induction of estrus may be needed for synchronizing donors and recipients in an embryo transfer program. Equine chorionic gonadotropin (eCG; 150 IU intramuscularly) is administered, and then hCG (100 IU intramuscularly) is administered 80-88 hours later. A less successful protocol is to given follicle-stimulating hormone (2 mg intramuscularly once daily) for 5-6 days. The queen is mated during the estrus cycle or is induced to ovulate with hCG and is then inseminated. Neither eCG nor FSH is commercially available in the United States at this time.

INDUCTION OF OVULATION Ovulation may be induced pharmacologically with hCG (250 IU given intramuscularly on days 1 and 2 of estrus) or GnRH (25 μg given intramuscularly on any day of estrus). Induction also

may be induced by vaginal stimulation using a cotton swab. Manual stimulation needs to occur 4-8 times at 5- to 20-minute intervals, with each stimulation lasting 2-5 seconds.

Breeding Management of the Normal Queen

The optimal age to breed a cat is between 1.5 and 7 years. The queen is taken to the tom for breeding. The premating period lasts for 10 seconds to 5 minutes and consists of the cats calling to and smelling one another. The mating period includes 1-3 minutes of the male mounting, neck biting, and treading with his hind legs. This is followed by intromission and ejaculation, which take place within 5-10 seconds. During this period the queen emits a characteristic yowl. Coitus is followed by an after-reaction by the queen that lasts 30 seconds to 10 minutes and consists of the queen rubbing herself on the ground, rolling from side to side, and licking her vulva. Subsequent breeding may resume following the after-reaction. Cats may breed as often as 30 times in 24 hours.

Bibliography

Concannon P: A review for breeding management and artificial insemination with chilled or frozen semen. Proceedings of the Society for Theriogenology

Canine Male Reproduction Symposium, Montreal, Quebec, Canada, 1997.

Concannon P, Verstegen J: Estrus induction in dogs: use of gonadotropins, therapies and dopamine agonists. Proceedings of the Society for Theriogenology, Montreal, Quebec, Canada, 1997.

Feldman E, Nelson R: Ovarian cycle and vaginal cytology. In Feldman E (ed), *Canine and feline endocrinology and reproduction,* Philadelphia, 1996, WB Saunders.

Freshman J: Clinical approach to infertility in the cycling bitch, *Vet Clin North Am* 21:427, 1991.

Freshman J: Solve the mystery: infertility in the bitch. Proceedings of the Society for Theriogenology Canine Reproduction Symposium, Baltimore, 1998.

Goodman M: Canine ovulation timing. Proceedings of the Society for Theriogenology Canine Reproduction Symposium, Baltimore, 1998.

Goodman M: Ovulation timing concepts and controversies, *Vet Clin North Am* 31:219, 2001.

Hutchison R: Maximizing conception rates using fresh cooled or frozen canine semen. Proceedings of the Society for Theriogenology Canine Male Reproduction Symposium, Montreal, Quebec, Canada, 1997.

Johnston S: Clinical approach to infertility in bitches with primary anestrus, *Vet Clin North Am* 21:421, 1991.

Johnston S: Using hormone assays in small animal reproduction. Proceedings of the Society for Theriogenology, Kansas City, Mo, 1996.

Johnston S, Olson P, Root M: Clinical approach to infertility in the bitch, *Semin Vet Med Surg* 9:2, 1994.

Olson P, Thrall M, Wykes P et al: Vaginal cytology: part I. A useful tool for staging the canine estrous cycle, *Comp Cont Ed* 6:288, 1984.

Purswell B: Pharmaceuticals used in canine theriogenology. Proceedings of the Society for Theriogenology, Baltimore, 1998.

Root Kustritz M: Unique aspects of feline reproduction. Proceedings of the Society for Theriogenology, Kansas City, Mo, 1996.

3

Artificial Insemination in the Dog

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AT A GLANCE

- Artificial insemination with fresh semen
  - Semen collection: routine (see Chapter 4)
  - Semen handling/storage/shipment: none
  - Insemination: vaginal
- Artificial insemination with chilled semen
  - Semen collection: routine

- Semen handling/storage/shipment: addition of sperm-rich fraction of ejaculate to extender; maintenance at refrigerator temperature; commercial systems available
- Insemination: vaginal or intrauterine
  - Ideally, inseminate within 24 hours of semen collection and extension.
  - Optimal breeding day is 2 days after ovulation (see Chapter 2). Higher conception rates are associated with larger number of breeding procedures.

- Artificial insemination with frozen semen
  - Semen collection: routine
  - Semen handling/storage/shipment: sperm-rich fraction of the ejaculate extended with one or more solutions, at least one of which contains a cryoprotectant such as glycerol; semen is frozen in straws or as pellets in liquid nitrogen; must be maintained and shipped in liquid nitrogen and thawed just before insemination; optimal breeding day for a single insemination is 3 days after ovulation
  - Insemination: intrauterine
    - Surgical: general anesthesia, laparotomy, inject semen through uterine wall with sterile needle and syringe
    - Transcervical
      "Scandinavian" or "Norwegian" rigid catheter: blind technique

Endoscopy: visualization of cervix with endoscope, passage of polypropylene urinary catheter through cervix for semen deposition

Artificial insemination (AI) is required when using fresh cooled or frozen semen and also is beneficial in some situations with fresh semen. Reasons for using AI when breeding with fresh semen include presence of vaginal abnormalities that prevent natural mating, such as strictures, or a bitch that will not allow mating for behavioral reasons, such as being either overly submissive or dominant to the male. Problems of the male dog requiring AI include the male being either overly aggressive or too timid to breed the bitch, and disease conditions that prevent the normal mating actions of mounting and intromission. Finally, AI provides the opportunity to obtain information regarding the quality of semen inseminated into the bitch. Even if a breeding soundness examination shows a male to have acceptable semen quality, only AI ensures that the acceptable semen is actually deposited into the female's vagina.

Fresh Semen

Collection of semen and semen analysis are presented in Chapter 4. When semen is collected for immediate insemination, fractioning of the semen into presperm, sperm-rich, and prostatic portions is not needed. Fresh semen most often is inseminated immediately after

collection with no further dilutions or semen extenders added. However, it is imperative that the ejaculate be examined at least for the presence of motile spermatozoa before insemination. It is not uncommon to collect a milky white sample from a male that appears to be a "good" ejaculate and then have the initial microscopic evaluation reveal that there are no spermatozoa present. A complete evaluation of the ejaculate, including assessment of percentage of progressively motile spermatozoa, total number of spermatozoa in the ejaculate, and percentage of morphologically normal spermatozoa, is preferable to just a cursory examination of spermatozoal motility. If motility is acceptable, it may be advisable to perform the insemination before completing the evaluation because prolonged exposure of sperm to autologous prostatic fluid can decrease sperm motility.

Vaginal Insemination

To perform vaginal insemination, the only equipment needed is an AI pipette and a syringe to infuse the semen. There are reports that the lubricants in syringes with rubber plungers can have detrimental effects on the motility of sperm; however, this occurs only after long incubation periods, and using a syringe with a rubber plunger to inseminate shortly after semen collection should have no detrimental effect on fertility. Pipettes specifically made for AI in the dog are commercially

available (Synbiotics, San Diego, Calif.; Maple Hill Embryos Inc., Woodstock, Ontario, Canada; International Canine Semen Bank, Sandy, Or.). We prefer bovine or equine uterine infusion pipettes or Cassou AI sheaths (available from most veterinary suppliers) that have been cut to appropriate lengths based on the size of the bitch to be inseminated (Figure 3-1). An insemination pipette named the *Osiris pipette* also is commercially available. The Osiris pipette simulates the erect penis in the vagina and is intended to prevent semen leakage after AI (IMV International, Minneapolis, Minn.). No controlled studies have been conducted to determine

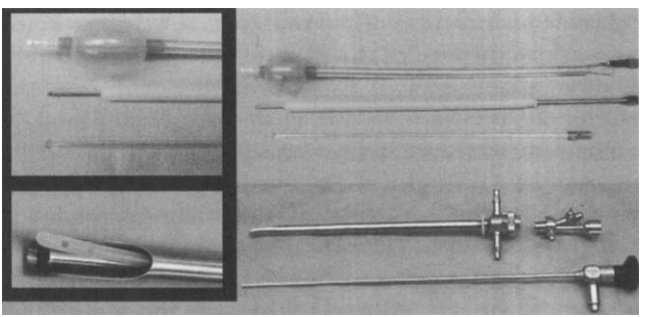


Figure 3-1. From top to bottom, the Osiris, Norwegian, and large animal infusion pipettes and the transcervical endoscope. Insets show close-up views of the delivery ends.

whether conception rates are better with the Osiris pipette than with regular AI pipettes.

Various techniques to perform vaginal AI have been described. Some clinicians recommend washing the vulva before insemination, inserting a finger into the vagina to guide the AI pipette into the vagina, "feathering" the vagina by digital stimulation, and elevating of the hindquarters of the bitch after insemination. We do not use any of these techniques and attain 90% pregnancy rates when inseminating with fresh semen. The method we use is to insert a nonlubricated AI pipette into the vagina at the dorsal commissure of the vulva, being careful not to allow the pipette to enter the urethra. We then pass the pipette over the brim of the pelvis and into the cranial vagina near the external os of the cervix. The insertion of the pipette is sometimes made difficult by the end of the pipette being obstructed by folds of the vagina. If this occurs, we withdraw the pipette slightly, redirect, and move it forward with slight increased pressure. If the vaginal epithelium is very hyperplastic, a finger can be used to guide the pipette into the vagina. The pipette should be inserted such that the end is passed far enough to be at the level of the caudal abdomen. One of us routinely palpates the tip of the pipette in the cranial vagina through the abdominal wall. Next, the bitch is elevated to a 45-degree angle by grasping above the stifles and elevating the hindquarters. The syringe is placed on the pipette and the semen

injected through the pipette. After the semen is injected through the pipette, the syringe is removed. If the semen remains in the pipette, the pipette should be withdrawn 1-2 mm until the semen is observed to freely flow down the pipette. After the semen is allowed to drain through the pipette, a small amount of air is injected through the pipette to flush any remaining semen into the vagina. Care must be taken to ensure the pipette is completely empty of semen; large animal uterine infusion pipettes can hold up to 3 ml of semen, which may be the entire ejaculate.

After AI, we elevate the hindquarters of the bitches for no more than 1 minute. After that time the bitches are allowed to do what they want. This is in direct contrast to many authors who state that bitches' hindquarters should be elevated for at least 10 (if not 20) minutes, that the bitches should be put into crates immediately after the hindquarter elevation time, that the bitches should not be allowed to urinate, and that the bitches should not be allowed to jump or squat. The only thing we avoid is the placement of pressure on the caudoventral abdomen. The only controlled study to assess the effects of elevating the hindquarters showed that elevating the hindquarters for 1 minute resulted in the same pregnancy rate and fecundity as elevating the hindquarters for 10 minutes. In a subsequent study that we conducted (unpublished), not elevating the hindquarters at all after insemination gave the same excellent pregnancy rates.

If the ejaculate has a very small volume, it is sometimes advisable to dilute the ejaculate with semen extender to make the volume easier to handle (see Chilled Semen, Equipment and Techniques). No difference in fertility was obtained when fresh semen was extended 1:1 and vaginal inseminations were performed multiple times compared with natural mating or AI of unextended semen on the same schedule. We recommend breeding every other day during cytologic estrus when the male is present. It has been shown that if AI is performed twice, rather than once, around the time of best fertility (see Chapter 2), the conception rate is significantly improved. There also seemed to be a trend toward increasing pregnancy rates when the number of inseminations increased beyond two.

Conception Rate with Fresh Semen

The number of spermatozoa inseminated depends on the ejaculate obtained; however, it has been estimated that at least 220×10^6 normal spermatozoa per ejaculate need to be inseminated (Table 3-1). Vaginal AI on 3 consecutive days after acceptance of the male by the female using 50×10^6 spermatozoa in fresh semen extended 1:4 for each breeding resulted in lower fertility (20%) than AI on 3 consecutive days using 200×10^6 spermatozoa in fresh semen extended 1:1 (80%) or natural mating (80%).

Table 3-1. Outcome of Vaginal, Intrauterine, or Surgical Artificial Insemination Procedure Using Fresh, Chilled, or Frozen Semen

Method	Timing of breeding (see Chapter 2)	Volume (ml)	Spermatozoa/ breeding procedure	*n*	Conception rate	Conception different from—yes/no	Fecundity
Fresh	Every other day during cytologic estrus	3.6	444×10^6		90%	NA	7.3
Fresh + extender	3 times after acceptance	2	200×10^6	5	80% (4/5)	Natural breeding —no	7.2
Fresh + extender	3 times after acceptance	0.5	50×10^6	5	20% (1/5)	200×10^6— no	8
Fresh	2 times at estrus	Not stated	Not stated	107	66.3	Breeding one time —yes	6.1
Fresh, transcervical	1-2 times during early estrus	Not stated	Not stated	25	84% (21/25)	Vaginal AI —yes, but vaginal AI CR = 25%	5.6

Continued

Table 3-1. Outcome of Vaginal, Intrauterine, or Surgical Artificial Insemination Procedure Using Fresh, Chilled, or Frozen Semen —cont'd

Method	Timing of breeding (see Chapter 2)	Volume (ml)	Spermatozoa/ breeding procedure	*n*	Concep-tion rate	Conception different from—yes/no	Fecundity
Fresh, surgical, uterine	Once on day 4, 5, or 6 after LH peak	1 ml	Not stated	9	100% (9/9)	Vaginal—vaginal AI CR = 35% (infertility diagnosed)	8
Chilled	Every other day during cytologic estrus (average, 4 breeding procedures)	4 ml	351×10^6 normal motile at 24 hr of storage; 258×10^6 total normal motile at 48 hr of storage	20	90 at 24 hr (9/10) 100 at 48 hr (10/10)	Fresh semen AI—no	7
Chilled, extended	3 times after acceptance	2	200×10^6	5	80% (4/5)	Natural breeding —no	4.2

Chilled, extended	3 times after acceptance	0.5	50×10^6	5	20% (1/5)	Natural breeding —yes	4.0
Chilled, extended	Not stated	Not stated	Not stated	Not stated	28%-60%	NA	Not stated
Frozen, vaginal	Days 3 and 5 after LH peak	5 ml	200×10^6	10	60% (6/10)	Surgical AI —no	
Frozen, vaginal	Daily in estrus (1-12)	3-12	$9\text{-}300 \times 10^6$	40	87.5% (35/40)	NA	4.6
Frozen, vaginal	Daily in estrus (1-12)	3-12	$9\text{-}300 \times 10^6$	40	87.5% (35/40)	NA	4.6
Frozen, vaginal	Based on P4 2-5 days after ovulation (2 times)	1 ml	183×10^6	60	60% (40/60)	One vaginal —yes One Norwegian IU—no	3.4
Frozen, vaginal	Based on P4 assays	Not stated	132×10^6	38	52.6 (920/38)	Norwegian IU—no	4.2
Frozen, uterine	Days 3 and 5 after LH peak	5 ml	200×10^6	10	60% (6/10)	Vaginal AI —no	Not stated
Frozen, vaginal	Every other day during estrus (1-12)	Not stated	21×10^6	7	100 (7/7)	NA	3.9
Frozen, vaginal	Every other day during estrus (1-12)	Not stated	105×10^6	7	100% (7/7)	NA	4.7

Continued

Table 3-1. Outcome of Vaginal, Intrauterine, or Surgical Artificial Insemination Procedure Using Fresh, Chilled, or Frozen Semen—cont'd

Method	Timing of breeding (see Chapter 2)	Volume (ml)	Spermatozoa/ breeding procedure	*n*	Conception rate	Conception different from—yes/no	Fecundity
Frozen, uterine, Norwegian	Late estrus 1-2 times	Not stated	Not stated	30	67% (20/30)	Fresh semen IU—no	5.6
Frozen, uterine, Norwegian	2 times 24 and 48 hr after LH?	2 ml	200×10^6	6	83% (5/6)	Transcervical—no	7.6
Frozen, uterine, Norwegian	Based on P4 2-5 days after ovulation (1 time)	1 ml	186×10^6	51	84% (43/51)	One vaginal —yes >1 Norwegian IU—no	7.6
Frozen, uterine, transcervical	2 times 24 and 48 hr after LH peak?	2 ml	200×10^6	5	100% (5/5)	Norwegian IU—no	7.6

Frozen, uterine, transcervical	2 times 24 and 48 hr after LH peak?	2 ml	50×10^6	7	85% (6/7)	200×10^6 —no	7.8
Frozen, uterine, transcervical	Based on P4 2-5 days after ovulation (2.4 breeding procedures)	1 ml	188×10^6 (calculated from data)	19	58% (11/19)	One -vaginal —no Norwegian IU—yes, Norwegian I U higher	6.0
Frozen, surgical, laparotomy	Once at "optimal mating time"	Not stated	$100\text{-}300 \times 10^6$	10	90% (9/10)	NA	3.6
Frozen, surgical, laparoscopy	3 and 5 days after LH peak	2 ml	200×10^6	10	60 (6/10)	Vaginal AI 2 times—no	Not stated

AI, Artificial insemination; *CR,* conception rate; *IU,* intrauterine; *LH,* luteinizing hormone; *NA,* not applicable; *P4,* progesterone.

The effect of volume of fresh semen placed in the anterior vagina has not been critically evaluated. Volumes as low as 2.2 ml and up to 3.6-3.9 ml have been reported to yield good pregnancy rates. It seems that as long as an adequate number of normal spermatozoa are placed into the vagina, the volume of the inseminate does not affect fertility. Excessively large semen volumes inseminated into the vagina could result in the drainage of some of the ejaculate from the vagina; this has not been critically tested.

Lower pregnancy rates have been reported to be associated with artificially inseminated fresh semen compared with natural breeding. These all were retrospective studies of pregnancy rates of different females bred to different males by various veterinarians under a variety of breeding conditions. In the one controlled study that directly compared pregnancy rates of bitches bred by AI using fresh semen versus natural mating, there was no difference in pregnancy rates of bitches mated by AI or natural mating when the same males were used under similar breeding conditions. In the canine colony at our institution, AI with fresh semen is used almost exclusively and the pregnancy rate in normal bitches is 90%.

Transcervical intrauterine insemination and surgical insemination with fresh semen have been reported (see Frozen Semen, Intrauterine Insemination Techniques). Although intrauterine insemination with fresh semen seems to have no benefit under normal

situations, one report did indicate that intrauterine insemination with fresh semen significantly improved the pregnancy rate in bitches that were previously infertile when bred to the same proven males.

Chilled Semen

Chilled shipped semen also is called *chilled extended semen* or *fresh cooled semen.* Breeding with chilled shipped semen requires a well-coordinated effort among the stud and bitch owners, the veterinarian collecting the semen, and the veterinarian inseminating the bitch. The veterinarian managing the bitch must ensure the proper timing of the insemination (see Chapter 2). The veterinarian collecting the semen must prepare it for shipment in such a way to maintain quality during transport.

If the male has never had semen collected, it may not be possible to collect a suitable ejaculate on the exact day the first semen sample is desired. It may take 1-2 weeks to train a male to ejaculate in a veterinary office without an estrual bitch present. Therefore it is ideal for the dog to have had semen collected and to be familiar with the collection procedure long before any semen actually is needed. It also is advisable to prepare a "test shipment" in advance. A semen sample should be extended, stored a minimum of 24 hours in the container that will be used for transport, and evaluated after 24 hours to ensure that extension and storage do not

have detrimental effects on the semen quality. Not all semen will respond to extending, cooling, and storage in a similar fashion, and this test shipment will determine the viability of the sperm after extension and cooling.

Shipping company schedules and venues to which they ship need to be assessed carefully well before the need arises. Some destinations are serviced by shipping companies, and some are best served by counter-to-counter airline shipments. In some cases, shipments cannot be sent out on the appropriate day, and in others, shipments cannot be delivered on the appropriate day.

Equipment and Techniques

When shipping chilled semen, it is imperative that an appropriate semen extender be added to the ejaculate. Semen extenders provide an energy source and buffers that enhance the survival of chilled sperm cells. We have received several chilled ejaculates that were not extended, and the motility was nil at the time of arrival 24-48 hours after the shipment was sent. Extenders can be obtained from commercial sources that are manufactured exclusively for extending canine semen (Synbiotics; CLONE, Chester Springs, Pa.; Camelot Farms, College Station, Tx.; International Canine Semen Bank) or for extending equine semen (Lane Manufacturing, Denver, Colo.; IMV International). Homemade semen extenders also can be prepared, but

proper laboratory techniques are essential for good results. Commercial extenders marketed for canine semen tend to be expensive, and preparation of homemade extenders tends not to be cost effective, requiring exacting quality control that is beyond the capability of most private veterinary clinics. We have found that commercially available equine semen extenders work well for chilling canine semen up to 48 hours and are extremely cost effective. The prepared extender can be portioned into smaller (10-15 ml) aliquots and frozen in a non–frost-free freezer for use within 4 months.

Chilled semen is prepared by diluting freshly collected semen with the desired semen extender. The extender must be prewarmed to 37° C before adding it to the semen or the spermatozoa will suffer cold-shock. The prostatic portion of the ejaculate may have detrimental effects on the storage of canine sperm cells, so some workers advise not collecting any of the prostatic portion of the ejaculate. We found no detrimental effects on pregnancy rate or fecundity when whole ejaculates were extended 1:1 and inseminated after 24 or 48 hours of storage. If the entire prostatic portion of the ejaculate is collected and the semen is extended, the volume of the resulting extended semen may be such that it cannot be completely inseminated without some vaginal reflux. If the volume of the ejaculate is large, the semen can be extended 1:1 in an extender and then centrifuged at 900 × g for 10 minutes. The supernatant can

then be removed and the remaining semen pellet extended to a more desirable volume with fresh extender. When a commercial canine semen extender is used, it is always best to follow the manufacturer's recommendations regarding semen preparation, extension ratios, and chilling procedures. The most desirable concentration for extending and shipping dog semen has not been determined.

Containers designed to ship semen can be obtained from many of the same sources that provide canine semen extenders. Several companies manufacture or provide canine semen shipping containers (Synbiotics; CLONE; Camelot Farms; Bio-Flite, Anaheim Hills, Calif.; International Canine Semen Bank). The containers usually consist of a Styrofoam box, an ice pack, and a receptacle for the semen. These commercially available semen containers maintain semen well enough that acceptable pregnancy rates result. Commercially available shipping containers offer a predictable, attractive, and easy way to ship semen. However, many of these containers are relatively expensive when compared with the disposable equine shipping containers. We found that a commercially available (although no longer marketed) disposable equine shipping container allowed storage of extended canine semen for up to 48 hours, with resulting pregnancy rates equivalent to AI with fresh semen. We have used other brands of disposable equine semen shippers and have had good success in maintaining

semen viability. The advantage of the equine semen shippers is their lower cost than the canine semen shippers. Homemade shipping containers can be used; however, they do not usually have a predictable cooling rate and semen quality may not be maintained as well as with use of commercially available containers.

With fresh cooled semen, little or no semen preparation is required after its arrival before insemination is performed. It is advisable to check the motility of the spermatozoa by placing a drop on a prewarmed slide and, if motile sperm are present, proceeding with insemination in the same manner as with fresh semen. The entire semen sample need not be warmed before AI. It is recommended to save a small aliquot of the semen for evaluation. With knowledge of the concentration of spermatozoa in the semen (millions of spermatozoa per milliliter) and the volume inseminated (milliliter per inseminate), the total number of spermatozoa in the inseminate can be calculated (see Chapter 4). Even with optimal timing, poor-quality semen may not achieve pregnancy. Knowledge of semen quality (total number of spermatozoa, percentage of progressively motile spermatozoa, percentage of morphologically normal spermatozoa) may help in determining the cause, or at least rule out some factors, if the bitch does not become pregnant. Furthermore, if semen quality is not acceptable, the persons who prepared and shipped the semen can be notified and an additional shipment provided if time

permits. Modifications in semen preparation can be made to improve quality after shipping.

Conception Rate with Chilled Semen

Results after using chilled extended semen can be the same as those with natural breeding if sufficient spermatozoa are inseminated at the proper time. Vaginal AI on 3 consecutive days with 200×10^6 spermatozoa/breeding with semen extended 1:1 and stored for 24 hours produced the same pregnancy rates as natural mating using the same breeding schedule (80%). However, using 50×10^6 spermatozoa extended 1:4 and stored for 24 hours resulted in lower fertility (20%). Other reports that summarized data from chilled semen inseminations showed pregnancy rates to vary from 28%-60% depending on the type of extender used. These pregnancy rates are lower when ovulation is timed and the number of breeding procedures is limited to one or two, compared with the 90%-100% conception rates we observed when an average of 3.9 breeding procedures of $250\text{-}350 \times 10^6$ spermatozoa/breeding were performed.

Frozen Semen

An exhaustive review of the preparation of frozen semen is beyond the scope of this chapter. Simply stated, to prepare frozen semen after a good-quality semen sample is

obtained requires a method to standardize the concentration of the sample, a freezing extender with a cryoprotectant, a packaging system, a freezing method, and a storage facility.

Equipment and Techniques

There are many different semen extenders, cryoprotectants and cryoprotectant concentrations, freezing mechanisms, and freezing rates reported. One of the main differences between chilled semen extenders and frozen semen extenders is the addition of a cryoprotectant such as glycerol to the medium. The cryoprotectant helps maintain cell integrity during the freezing and thawing process.

A simple method to freeze canine semen that is currently used by our laboratory is as follows:

- Collect semen and conduct a complete analysis.
- During the evaluation process, dilute the semen 1:1 using a commercially available semen refrigeration extender (Refrigeration Media, TEST Yolk Buffer [TYB] 9972; Irvine Scientific, Santa Ana, Calif.) and centrifuge it for 10 minutes at 900 × gravity.
- After centrifugation, remove the supernatant and resuspend the pellet to a concentration of 400×10^6 spermatozoa/ml using the same refrigeration extender. Place this standardized semen sample in a refrigerator set at 5° C for 1 hour.

- During the hour of cooling, label an appropriate number of 0.5-ml French straws with all the data required by the dog's registry (e.g., name, registration number, breed, date, collection facility).
- After 1 hour, add a commercial freezing extender containing 12% glycerol (Freezing Medium, TEST Yolk Buffer [TYB] with Glycerol 9971; Irvine Scientific), which also has been kept at 5° C, to the cooled semen solution at a 1:1 ratio by volume, to make a final concentration of 200×10^6 spermatozoa/ml in fluid containing 6% glycerol.
- In a 5° C cold box, fill the 0.5-ml straws and seal the ends.
- Place the straws on a screen that is attached to a 3-cm-thick Styrofoam frame, which is floating in liquid nitrogen. After 10 minutes, plunge the straws into the liquid nitrogen before storage.
- Semen is stored at –196° C in commercially available liquid nitrogen tanks. Storage and inventory are critical aspects of frozen semen use. Meticulous records must be kept regarding the location and number of straws frozen from each male. The liquid nitrogen must be routinely monitored to ensure that there is sufficient liquid nitrogen to maintain the temperature at –196° C. If frost starts to accumulate on the tank, the semen should be transferred immediately to a new tank and the damaged tank discarded.

Most dog breed associations are not as concerned about the quality of the frozen semen as they are about identification of the semen. The quality control of the final product depends on the integrity of the freezing facility.

Frozen semen is usually shipped in "dry dewars." These are small tanks that do not contain any liquid nitrogen but keep the sample at –196° C for a short period (e.g., for 1-2 weeks). Because there is no liquid in the tanks, the airlines and shipping companies will allow their shipment, unlike liquid nitrogen tanks. If a liquid nitrogen tank is available at the shipping destination, the semen may be shipped well in advance and transferred to the liquid nitrogen tank at the time of arrival. If, however, a liquid nitrogen tank is not available at the insemination site, shipment should be timed in accordance with the intended date of insemination.

Because most veterinarians will deal with semen that was frozen by someone else, it is important to follow the instructions provided by the freezing facility regarding the thawing procedure, as well as timing the insemination and optimizing the insemination technique. Thawing procedures for frozen semen vary as much as freezing protocols. Our laboratory thaws straws at 50° C for 10 seconds. Some facilities recommend thawing at lower temperatures for a longer time, and some recommend adding thawing media during the thaw. Regardless of the experience of the veterinarian that is

to perform the insemination, it is best to use the freezing facilities' recommendation because they have determined the optimal thaw technique for their frozen semen.

Normally, frozen semen is inseminated directly into the uterus to increase chances of conception. At present, there are three methods to place semen directly into the uterus. These methods are surgical implantation by laparotomy or laparoscopy, use of the Scandinavian or Norwegian catheter, and transcervical vaginal endoscopy.

Intrauterine Insemination Techniques

SURGICAL INTRAUTERINE INSEMINATION Surgical implantation is performed with the animal under general anesthesia. The laparotomy procedure is less complicated than ovariohysterectomy, so almost all veterinarians are capable of performing the surgery. A small ventral midline incision is made and the uterus carefully exposed through the incision using a minimal amount of handling. As the uterus is being exposed, an assistant thaws and prepares the semen according to the instructions provided by the freezing facility. The semen can be placed in a sterile syringe to which is attached a 22-gauge needle. The needle is inserted into the uterine lumen, and once the placement of the needle is assured in the uterine lumen, the semen is injected into the uterus. The uterus is placed back in the abdomen and

the incision closed. It is advised that the hindquarters of the bitch be kept slightly elevated during the procedure because semen can reflux out the cervix if they are not. Laparoscopic insemination affords few advantages over laparotomy other than having a smaller skin incision. However, the cost of equipment needed and the time required to perfect the laparoscopic technique are significant disadvantages.

Controlled studies examining the appropriate volume of surgical inseminates have not been performed. The volume of semen that the uterus can hold is not known, but volumes up to 1 ml have been inseminated into each uterine horn with success. One worker recommends that no more than 4 ml be placed in the uterus. If the semen to be used is stored in low concentrations or if the postthaw motility is poor and several 0.5-ml straws are needed to inseminate $100\text{-}200 \times 10^6$ normal spermatozoa, then the volume of the uterus may be exceeded and the semen may overflow into the vagina and be wasted. If this occurs, it is suggested to centrifuge the sample to increase the concentration by decreasing the volume.

Surgical insemination usually is performed on a single day, 3 or 4 days after ovulation (see Chapter 2), but some veterinarians perform surgery on consecutive days. It is unknown whether multiple surgeries consistently yield higher pregnancy rates. In countries where elective surgeries are not allowed or if the client does not

want to risk anesthesia and surgery, nonsurgical methods of uterine insemination must be used.

TRANSCERVICAL INSEMINATION WITH A RIGID CATHETER

Uterine insemination using the Scandinavian or Norwegian catheter requires a special catheter and considerable skill by the veterinarian. The catheter consists of a large plastic sheath and a smaller stainless steel catheter that fits inside the sheath (see Figure 3-1). There are at least three sizes of catheters made for different size dogs. To perform the insemination, the veterinarian passes the sheath, with the internal catheter retracted, as far into the vagina as possible. The tip of the stainless steel catheter is advanced cranially into the fornix under the cervix. Because the cervical os opens in a dorsoventral direction, the catheter cannot be directly advanced through the cervix. The cervix must be palpated through the abdomen and grasped by the veterinarian. Once the cervix is grasped and the catheter is in the cervical fornix, the veterinarian manipulates the cervix by turning it ventrally so that the cervical os assumes a more horizontal position. As the cervix assumes a horizontal orientation, the catheter is backed out of the fornix and threaded through the cervix. When the catheter encounters the cervix, a "gritty" sensation is felt by the veterinarian. Once the catheter is placed through the cervix, the semen is introduced. The purchase of the catheters is a relatively small expense;

however, attaining the skill to consistently pass the catheter through the cervix requires considerable training, practice, and patience. The possibility of a vaginal or uterine rupture is always present when inexperienced clinicians are attempting this intrauterine insemination procedure.

ENDOSCOPIC TRANSCERVICAL INSEMINATION Another method to inseminate directly into the uterus that does not require surgery is the "New Zealand" method of transcervical uterine insemination. The equipment needed for the New Zealand intrauterine insemination technique is much more expensive than that needed for the other techniques, but the insemination process is much easier to learn than the "Scandinavian" method. The New Zealand method uses a cystoscope to directly view and catheterize the cervix. The equipment needed consists of a 36-cm-long × 5-mm-diameter cystoscope that has a 30-degree viewing angle, a sheath that contains a channel for the catheter, a light source, and an optional camera and television monitor (see Figure 3-1). (Companies that manufacture or sell this equipment include Karl Storz, Goleta, Calif.; MDS, Brandon, Fla.; and Endoscopy Support Services, Brewster, NY.) To set up the equipment, the veterinarian inserts the cystoscope into the sheath and connects the light source to the cystoscope. An 8-French polypropylene urinary catheter, which will be used to introduce the semen into

the uterus, is inserted into the channel on the sheath. The procedure can be viewed directly through the cystoscope or via a television monitor if so equipped. To perform the transvaginal insemination, the estrual bitch ideally is placed on a specially designed hydraulic table that contains a large flat restraining strap placed gently around the bitch's abdomen. The adjustable, hydraulic table allows the bitch's height to be adjusted easily and gives the operator much more comfort when performing the procedure than if the operator has to bend over. Sedation is not required because estrual bitches generally tend to tolerate the procedure very well. The cystoscope, sheath, and catheter combination (hereafter referred to as *the cystoscope*) is inserted through the vulva and into the anterior vagina, as described for performing vaginal artificial insemination. The cystoscope must be directed dorsally to avoid the urethra and then over the pelvis into the vagina. Once the vaginal folds are visualized, the cystoscope is directed cranially while the end of the cystoscope is kept centered in the vagina through manipulation of the viewing end of the cystoscope outside the bitch. Air insufflation, as needed in many endoscopic procedures, is not necessary. The clinician should continue to direct the cystoscope craniad until the dorsal median postcervical fold is visualized on the dorsal aspect of the field of view. The dorsal median postcervical fold is recognizable as a semicylindrical, regular fold oriented longitudinally in the dorsal anterior vagina.

The dorsal median postcervical fold is followed anteriorly until the cervix is visualized as a small reddish rosette on the vaginal wall. The cervix can be visualized because the cystoscope has a viewing angle of 30 degrees directed dorsally. When the cystoscope is inserted past the dorsal median postcervical fold, the operator is actually looking dorsally, so the cervix can then be seen. To attain the correct angle to visualize the cervix, it is best for the operator to lift the external operating end of the cystoscope as far dorsally as possible. This places the distal, viewing end in a better position to view the cervix. When the cervix is visualized, the 8-French catheter is passed into the cervical os and gently rotated to pass it through the cervix. The catheter should pass easily through the cervical os. If not, the angle of the catheter can be adjusted slightly by turning the catheter or adjusting the angle of the cystoscope. If the catheter still does not pass readily into the cervical os, a small fold may have been incorrectly identified as the cervix and the search for the cervix should be continued. Once the catheter is inserted through the cervical os, the semen is injected through the catheter. It is advisable that frozen semen not be thawed until the catheter has been placed through the cervix because in some bitches the cervix cannot be found and surgical insemination may be required. When the semen is injected through the catheter that has been passed through the cervix, there should be no semen reflux out the cervical os. It has

been reported that almost all breeds and sizes of dogs have been inseminated successfully using this technique. If the cervix is not catheterized and deep vaginal insemination is going to be performed regardless of cervical passage, it is best to withdraw the cystoscope and perform routine AI. This is because semen will run out through the sheath of the cystoscope and will not stay in the vagina.

Conception Rate with Frozen Semen

Most AI with frozen semen is performed surgically because early work using vaginal insemination did not yield high pregnancy rates. However, for breeding that is attempted by vaginal AI on repeated days during the estrous cycle with adequate numbers of sperm cells, conception rates of 50% and 60% have been reported. Conception rates associated with frozen semen using vaginal AI have been less than or equal to those using intrauterine insemination. Frequency of insemination and sperm numbers used in these studies with vaginal AI varied, but include 1-12 breeding procedures with 9-300 $\times 10^6$ spermatozoa/insemination, 2 breeding procedures with 132 $\times 10^6$ spermatozoa/breeding, 2 breeding procedures with 200 $\times 10^6$ spermatozoa/breeding, and 1-6 breeding procedures with 183 $\times 10^6$ spermatozoa/breeding. Conception rates of 100% have been attained when as few as 21 $\times 10^6$ spermatozoa or up to

105×10^6 spermatozoa were vaginally inseminated daily during cytologic estrus. Increasing the number of breeding procedures from one to two increased conception rate and fecundity in another study from 34%-60%, but a third insemination had no additive effect.

The intrauterine deposition of frozen semen using the Norwegian insemination technique has yielded pregnancy rates of 67% breeding once or twice with an unstated number of sperm, 74% breeding twice with 132×10^6 spermatozoa/procedure, 83% breeding twice with 200×10^6 spermatozoa/procedure, and 84% breeding one to three times using 186×10^6 spermatozoa/procedure. The transcervical endoscopic technique has yielded pregnancy rates of 100% breeding twice with 200×10^6 spermatozoa/procedure, 85% using as few as 50×10^6 spermatozoa/procedure, and 57% using a total of 452×10^6 spermatozoa in 2.4 breeding procedures/cycle. Increasing the number of breeding procedures from one to three did not increase the conception rate or fecundity using the Norwegian transcervical technique.

Surgical insemination has been the most widely used technique in the United States, although controlled studies examining pregnancy rates are limited. In one study a 90% conception rate was reported when $100\text{-}300 \times 10^6$ spermatozoa were inseminated once during the fertile period. However, another study using laparoscopic surgical AI attained only a 60% conception

rate using one insemination of 200×10^6 spermatozoa. The 60% conception rate was not different from the 100% conception rate attained by vaginal AI twice with 310×10^6 spermatozoa. Many private facilities in the United States currently use surgical insemination and report success rates averaging 83% using a single timed AI. These success rates cannot be confirmed. Because the continued operation of these private facilities relies on success, it can be assumed that these results are being attained.

As with chilled semen breeding, the American Kennel Club (AKC) requires the proper paperwork to be completed, as well as DNA identification of the stud and the bitch before a litter can be registered.

In summary, attention must be paid to the quality of the ejaculate, proper handling of the ejaculate, and the insemination technique used to attain acceptable fertility using artificial insemination. If care is taken, conception rates for AI with fresh, chilled, or frozen semen can approach those associated with natural breeding. The optimum technique to breed with frozen semen that combines the best conception rate and fecundity, lowest sperm dose, ease for the operator, lowest expense for the client and veterinarian, and least trauma to the bitch is not yet known; however, during the next few years, a standard semen dose and insemination technique probably will be widely adopted.

Bibliography

Brittain D, Concannon PW, Flanders JA et al: Use of surgical intrauterine insemination to manage infertility in a colony of research German shepherd dogs, *Lab Anim Sci* 45:404, 1995.

England GC, Allen WE: Factors affecting the viability of canine spermatozoa II: effects of seminal plasma and blood, *Theriogenology* 37:373, 1992.

Farstad W: Bitch fertility after natural mating and after artificial insemination with fresh or frozen semen, *J Small Anim Prac* 25:561, 1984.

Fontbonne A, Badinand F: Canine artificial insemination with frozen semen: comparison of intravaginal and intrauterine deposition of semen, *J Reprod Fertil Suppl* 47:325, 1993.

Gill HP, Kaufman GF, Foote RH et al: Artificial insemination of beagle bitches with freshly collected, liquid-stored, and frozen-stored semen, *Am J Vet Res* 31:1807, 1970.

Hutchison RV: Maximizing conception rates using fresh cooled or frozen canine semen. Proceedings of the Society for Theriogenology, Montreal, Quebec, Canada, 1997.

Linde-Forsberg C: Artificial insemination with fresh, chilled extended, and frozen-thawed semen in the dog, *Semin Vet Med Surg* 10:48, 1995.

Linde-Forsberg C, Forsberg M: Fertility in dogs in relation to semen quality and the time and site of

insemination with fresh and frozen semen, *J Reprod Fertil Suppl* 39:299, 1989.

Linde-Forsberg C, Forsberg M: Results of 527 controlled artificial inseminations in dogs, *J Reprod Fertil Suppl* 47:313, 1993.

Linde-Forsberg C, Holst BS, Govette G: Comparison of fertility data from vaginal vs intrauterine insemination of frozen-thawed dog semen: a retrospective study, *Theriogenology* 52:11, 1999.

Mickelson WD, Memon MA, Anderson PB et al: The relationship of semen quality to pregnancy rate and litter size following artificial insemination in the bitch, *Theriogenology* 39:553, 1993.

Nöthling JO, Gerber D, Shuttleworth T: Intravaginal insemination of bitches with semen frozen in Triladyl with Equex STM paste to which prostatic fluid or modified TALP was added prior to insemination (abstract). 4th International Symposium on Dog and Cat Reproduction, Oslo, Norway, 2000.

Nöthling JO, Gerstenberg C, Volkmann DH: Success with intravaginal insemination of frozen-thawed dog semen: a retrospective study, *J So Afr Vet Assoc* 66:49, 1995.

Pinto CR, Eilts BE, Paccamonti DL: The effect of reducing hindquarter elevation time after artificial insemination in bitches, *Theriogenology* 50:301, 1998.

Pinto CR, Paccamonti DL, Eilts BE: Fertility in bitches artificially inseminated with extended, chilled semen, *Theriogenology* 52:609, 1999.

Silva LD, Onclin K, Lejeune B et al: Comparisons of intravaginal and intrauterine insemination of bitches with fresh or frozen semen, *Vet Rec* 138:154, 1996.

Tsutsui T, Hase M, Tanaka A et al: Intrauterine and intravaginal insemination with frozen canine semen using an extender consisting of orvus paste-supplemented egg yolk tris-fructose citrate, *J Vet Med Sci* 62:603, 2000.

Wilson M: Non surgical intrauterine artificial insemination in bitches using frozen semen, *J Reprod Fertil Suppl* 47:307, 1993.

Wilson MS: Transcervical insemination techniques in the bitch, *Vet Clin North Am* 31:291, 2001.

4

Semen Collection and Evaluation

Walter R. Threlfall

AT A GLANCE

- A breeding soundness examination
  - Complete physical examination, including rectal palpation of the prostate, palpation of the scrotal contents, and examination of the extruded penis
  - Semen collection and evaluation

- Serologic testing for canine brucellosis and possibly measurement of serum thyroid hormone concentrations
- Semen collection
  - A latex cone with attached plastic tube is introduced over the dog's penis, and erection is stimulated manually.
  - Semen is ejaculated in three fractions; the second, or sperm-rich, fraction is the most valuable to the veterinarian.
- Semen evaluation
  - Parameters evaluated include the following:
    - Volume: The volume collected varies with the collector and the dog. There is no minimum acceptable value.
    - Color: Normal semen is milky white.
    - Progressive motility: The normal percentage of spermatozoa moving forward quickly in a straight line is 70% or greater.
    - Morphology: The normal percentage of morphologically normal spermatozoa is 70% or greater. Primary defects are those that occur during spermatogenesis. These may be associated with a worse prognosis than secondary defects, which develop during maturation or transit of spermatozoa outside the testes or are an artifact of collection or preparation of the stained slide.

- Concentration/total number: The concentration varies with amount of prostatic fluid added to the sperm-rich fraction during ejaculation. Total number of spermatozoa in the ejaculate in millions is calculated by multiplying concentration (millions of spermatozoa per milliliter) by volume (milliliter per ejaculate). The normal total number of spermatozoa in the canine ejaculate varies from 300 million to 2 billion.

Breeding Soundness Examination

The purpose of a breeding soundness examination (BSE) is to predict the fertility of a stud dog to the best of one's ability using laboratory and clinical evaluations without breeding trials. This is not as accurate as the mating of the stud to numerous females and determination of conception rates, but it is accurate and conserves much time and expense. Any male of value being mated with the purpose of producing offspring of value should have a BSE performed periodically. This prevents loss of reproductive life in bitches bred to subfertile or infertile males and allows diagnosis of subfertility before it becomes infertility. Male dogs most likely to be presented for BSE are older males, males mated to bitches that failed to whelp after breeding, and young untested males for which the breeder wants proof of fertility before mating. Other dogs that should undergo BSE but

seldom do are males being purchased for breeding purposes and dogs being sold for breeding purposes, especially if those animals are to be shipped abroad. Male dogs that will have semen shipped or frozen also should undergo BSE; this is performed to determine the reproductive health of the male and the quality of the semen before arrangements are made to perform artificial insemination with chilled semen or to freeze semen.

The components of a BSE vary according to the individual performing the procedure; there is no standardized procedure. For that reason, the components included in the examination are listed and then described with the readers' understanding that this is only a guideline. Other elective tests sometimes are indicated.

History

The examination should begin with a complete history of the animal's previous health, uses (e.g., showing, obedience, hunting), and breeding experiences with outcome information. The general history should include any information related to injuries or illnesses that could affect reproduction. It should be remembered that these conditions could have occurred at any time in the animal's life. The length of ownership and the accuracy of any history provided based on information before ownership also are important to know. The results of

diagnostic tests that have been performed previously, if any, and the status of vaccination, deworming, heartworm prevention, and medications administered should be recorded.

Physical Examination

The physical examination begins with visual inspection of the entire animal and examination with auscultation and palpation. Special attention should be paid to those characteristics that are known to be heritable. The clinician should observe the animal's gait as he enters the examination room to note whether front or rear limb abnormalities are present that may decrease his desirability as a breeder. This is the best opportunity that we as veterinarians have to provide genetic counseling to owners regarding the importance of not using males that have genetic abnormalities. It is known that not all owners will heed our advice, and it is our prerogative whether to assist in mating of an animal with genetic flaws.

The next portion of the physical examination is the determination of normalcy of the reproductive system. This examination should include palpation of the scrotum and all scrotal contents, palpation of the penis and prepuce and visual inspection of the extruded penis, and palpation of the prostate. This portion of the examination may be deferred until after the semen has been

collected, depending on the disposition of the male. In some cases it is more difficult to collect semen from very timid males after palpation of the internal and external genitalia.

The veterinarian begins palpation of the scrotum and scrotal contents by locating two fully descended testes. Animals with only one testis in the scrotum should have a very good reason, such as a history of surgical removal of one testis; if no such history exists, the animal is cryptorchid and should not be used for breeding (see Chapter 16). The testes should be palpated for consistency and the size measured. The best way to learn normal consistency of testes is to palpate many testes; excessively firm or excessively soft testes are abnormal in any case. Testicular size can best be measured using calipers obtained from a hardware store, such as those used to measure diameters of metal rods or pipe. The length, width, and height of each testis is recorded. This allows comparison at future examinations to determine objectively any change in testicular size. This information is especially beneficial when attempting to determine the reason for a decrease in fertility as an animal ages.

After palpation of the testes, the veterinarian identifies the tail of the epididymis. The tail of the epididymis is the most prominent portion of the epididymis, and it should be directed posteriorly. If this orientation is not present, torsion of the spermatic cord may be present

(see Chapter 16). The body and head of the epididymis should blend in smoothly to the testis. The size of the epididymis should correspond with the size of the normal testis. The consistency can best be determined again by palpating as many epididymides as possible. The clinician examines the vas deferens, which is the firmest structure within the spermatic cord, proximal to the epididymis. The scrotal skin is examined for abnormal thickness or dermatitis, which could elevate the intrascrotal temperature and impair testicular function.

Palpation of the prostate should be part of the physical examination of every male dog. Prostatomegaly in a castrated male dog invariably was caused by prostatic adenocarcinoma. Prostatomegaly in an intact dog more commonly is associated with benign prostatic hypertrophy or prostatitis (see Chapter 17). The veterinarian should elevate the tail while an assistant restrains the dog. The veterinarian gently inserts a gloved, well-lubricated finger into the anal sphincter, with care to keep the palmar portion of the finger most ventral in the rectum. The pelvic portion of the penis is palpated while the finger is advanced forward. The prostate is the structure surrounding the penis at approximately the depth of the length of the index finger. The prostate may fall forward into the abdomen and not be palpable per rectum in larger dogs or dogs with significant prostatomegaly. In this case an assistant should elevate the anterior portion of the dog or the veterinarian may

use the nonpalpating hand to put pressure on the caudal abdomen, moving the prostate back into the pelvic inlet toward the gloved finger. The prostate should be bilobed, symmetric, and uniform in consistency. Any disparity or enlargement and any changes in consistency from one area to another should be noted.

The penis and prepuce usually are best palpated before semen collection, but this examination can be performed later if the dog is timid and resists this procedure. The clinician palpates the penile shaft through the prepuce, noting any abnormal enlargements, pain, or crepitus in the area of the os penis. The extruded penis should be visually examined both before and immediately after collection, if possible.

Libido

Libido can be determined with or without the presence of a bitch in estrus. If the male has had semen collected previously and is used to the procedure, no female may be necessary. However, assessment of quality of libido should not be reduced in those male dogs requiring a female to be present. Many males that have not had semen collected and have been used for natural service only may not show interest unless a female is present. For some males the bitch needs to be near optimal breeding time (see Chapter 2). If the male continues to demonstrate no interest in the presence of a female in estrus, it

still may be unwise to think there is a libido problem. Many males are intimidated by the surroundings in a veterinary clinic or have no interest in specific females for unknown reasons. Libido assessment is easy if the male is extremely interested in collection with or without a female, but a less-than-expected libido is more difficult to categorize with certainty.

Semen Collection

Manual manipulation to achieve erection and ejaculation is the most commonly used method to collect semen samples from dogs because it is generally very successful and is not stressful to the male. Electroejaculation after the administration of general anesthesia can be used for wild canids but is seldom indicated or used for domestic dogs. The quality of the ejaculate collected by electroejaculation is not as good as that collected by manipulation.

The process for the collection of semen varies greatly from one individual to the next. As long as the basic premise "do no harm to the spermatozoa" is followed, the exact method is insignificant. The method I use is described herein.

The male dog, with or without a teaser bitch, is placed in a suitable quiet environment. Most males, once accustomed to collection, do not require the presence of a bitch but do ejaculate a greater number of spermatozoa

if a bitch is present, especially if the bitch is in estrus. Teaser bitches need not be in estrus in all cases; I have found that a beagle bitch of proper disposition makes an excellent teaser and will stand and permit males of any size to mount, even when she is not in estrus. Some veterinarians have had success using pheromone-like substances said to stimulate the male's interest in a nonestrus bitch. I have not had success with this type of product. The use of cotton sponges impregnated with vulvar secretions of bitches in estrus and maintained in a freezer until needed is more successful in stimulating uninterested males to perform. The female and male also can be permitted to "play" if necessary to develop the male's interest before semen collection.

The veterinarian grasps the male dog's penis and prepuce and prolapses the penis from the prepuce by putting pressure proximal to the bulbus glandis and pushing toward the preputial opening. I believe that it is important to have the bulbus glandis completely outside the preputial opening before attempts to collect semen are made because some males exhibit pain near the time of collection if the bulbus glandis is permitted to remain within the prepuce during complete erection. The bulbus glandis of these animals appears to become larger than the prepuce can accommodate.

Next, a latex cone, the inner liner from a bovine artificial vagina, is introduced over the exposed penis. The latex liner (Nasco, Fort Atkinson, Wisc.) itself serves as

an artificial dog vagina. The latex liners should be soaked overnight in water when first purchased and rinsed in distilled water the next day to ensure removal of any byproducts of the manufacturing process that are detrimental to spermatozoa. The top of the latex cone is folded over and the top of the fold lightly lubricated with a sterile, nonspermicidal lubricant. The clinician then places the thumb and forefinger in the fold of the liner to assist in holding it proximal to the bulbus glandis.

Latex has been reported to decrease spermatozoal motility, but this has been reported to occur only if the spermatozoa remain in contact with the latex for 20 minutes or longer. During the semen collection procedure described subsequently, the contact time of the semen and latex is less than 30 seconds. No obvious change in semen quality has been reported with use of this equipment. Use of other types of containers, such as plastic bags or small cups, may affect semen quality because of the presence of spermicidal residues in those containers. The concern usually is not that a particular container will kill all the spermatozoa but that significant enough quantities will be damaged or killed to significantly reduce semen quality. I have observed one animal that had an allergic reaction to a plastic disposable collection device made for bulls.

The penis is completely protruded from the prepuce, and the latex liner is introduced over the penis. The thumb and forefinger, which are within the cone at

the top of the latex liner, are placed around the penis proximal to the bulbus glandis, and alternating pressure is applied (Figure 4-1). This is all the stimulation that is required to obtain a collection. Manipulation of the distal penis should be avoided because it is a very vascular area and bleeding easily can be induced. It is extremely undesirable to have blood contaminate the ejaculate.

The first fraction of the canine ejaculate is designed to flush the urethra, and it is not advisable to collect this fraction. I prefer to permit this fraction to be ejaculated onto the floor. Some clinicians collect this fraction to measure the volume, but I have not seen a reason to do so. Most males move a great deal while ejaculating the first fraction. When the movement decreases, the cloudy second fraction usually is ejaculated.

The second, or sperm-rich, fraction should be collected into a clear graduated plastic tube attached to the end of the latex liner. During the latter part of the collection procedure, it is very common for the male to step over the hand of the collector and even to turn his body 90-180 degrees from the direction of the bitch. This is acceptable and will not harm the penis or prepuce. The tube must be watched during the collection, and when the ejaculate running down the side of the tube becomes clear, it should be removed immediately. The volume of the second fraction is then recorded; normal values range from 1-3 ml (Figure 4-2).

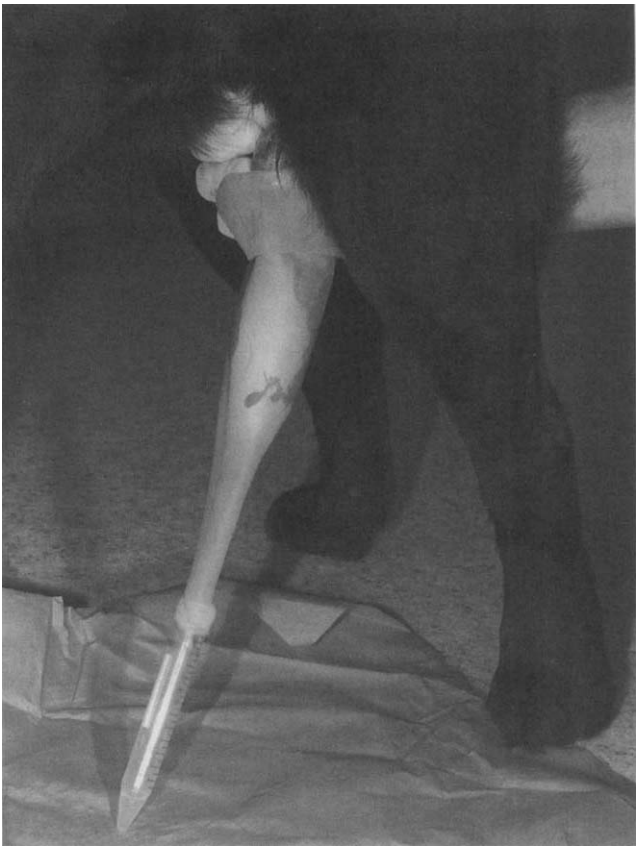


Figure 4-1. Latex liner over penis during semen collection.

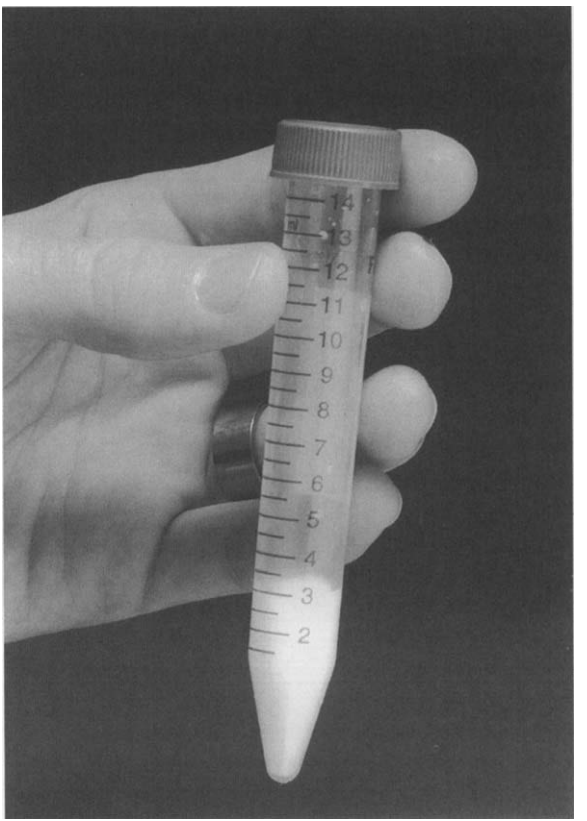


Figure 4-2. Second fraction of the canine ejaculate in a graduated plastic tube.

The clear third fraction of the ejaculate makes up the largest portion by volume; volume collected varies with the length of time the latex liner and clear plastic tube remain over the penis and may be greater than 35 ml. This fraction is detrimental to spermatozoa that are to be shipped or frozen and must be removed as quickly as possible. It also is not recommended to have a large volume ejaculate when performing artificial insemination with fresh semen (see Chapter 3).

Once collection is completed, especially in those breeds with longhair coats, the penis should be observed returning into the prepuce because hair may accompany the penis and cause irritation to the penis and prepuce. Return of the penis to the prepuce can be hastened by walking the male or applying cold packs to the penis and prepuce.

Semen Evaluation

Reported abnormalities of semen quality in dogs include the following: azoospermia, lack of spermatozoa in the ejaculate; oligozoospermia, decreased number of spermatozoa in the ejaculate; teratozoospermia, decreased number of morphologically normal spermatozoa; and asthenozoospermia, decreased motility of spermatozoa (see Chapter 19).

Once collected, the semen should be maintained at a relatively constant temperature. Canine spermatozoa

seem to be more resistant to temperature fluctuations ("cold-shock") than spermatozoa of other domestic species. The ejaculate can be maintained in an incubator for short periods or can do very well when placed on a countertop at room temperature. The temperature of holding, within reason, does not seem to be as important as avoiding fast temperature fluctuations.

VOLUME Volume varies with the amount of the third fraction collected. The volume should be recorded before any samples are removed; this value is needed to calculate the total number of spermatozoa in the ejaculate.

COLOR The color and opacity of the semen sample should be observed immediately after semen collection. Red indicates blood from either the surface of the penis or the prostate. Dark brown indicates older blood from the prostate. Yellow indicates the presence of urine, and white particles may indicate the presence of white blood cells.

MOTILITY Motility is the first criterion examined. The slide and coverslip should be at body temperature for this procedure. Although canine spermatozoa are relatively resistant to cold-shock, exposure to temperatures cooler than body temperatures has a tremendous impact on the observed motility, leading to the possibility of erroneous conclusions if a cold slide is used. A heated

substage on the microscope is beneficial but not mandatory. The motility assessment should be made immediately after placing the slide on the microscope stage if heat is not provided; motility on a slide significantly declines after 1-2 minutes on a lighted microscope.

To assess motility, the veterinarian places one drop of semen on a slide and examines it at 100×-200× magnification. The spermatozoa should be evaluated for speed and direction of movement. The speed should be extremely rapid, so one does not see the narrow side of the spermatozoa, and the spermatozoa should traverse across the microscope field in only 2-3 seconds. The direction of movement should be in a straight line. Circular movement is undesirable and probably related to abnormal morphology. *The general acceptable percentage of progressive motility of canine spermatozoa is 70% or greater.*

The movement of the spermatozoa can be greatly influenced by the prostatic fluid, if it is abnormal. A decrease in progressive motility often is followed by an increase in secondary morphologic abnormalities (see Morphology). When a sample is examined and the majority of the spermatozoa are found to be nonmotile, it may be helpful to examine spermatozoa on the same eosin-nigrosin–stained slide that is used for morphologic examination. If there is a problem with seminal plasma components, the spermatozoa will be alive but immobilized instead of damaged; these immobile but intact spermatozoa may be less likely to take up stain and

appear white on an eosin-nigrosin–stained slide. Damaged spermatozoa appear pink.

MORPHOLOGY The shape of the spermatozoa is an important characteristic because it affects motility. This portion of the examination is best performed by using a stain such as eosin-nigrosin and a light microscope with an oil immersion lens (1000× magnification). A wet-mount slide also can be used, with a phase-contrast microscope, but because of the availability of light microscopes in practice, the former is recommended. To prepare an eosin-nigrosin–stained morphology slide, a drop of stain is placed on a prewarmed glass slide and a drop of semen added. The size or number of drops of each is somewhat dependent on the concentration of the ejaculate. The two drops are mixed with the end of another slide, and the mixture of that slide is streaked across a third slide, similar to the technique for making a blood smear. Permit the slide to dry; it should remain warm.

Another staining technique uses modified Giemsa stain (DiffQuik; Baxter Healthcare, Miami, Fla.). A drop of semen is drawn across a slide, as for a blood smear, and allowed to dry. The slide is then immersed in the three different stain solutions for 5 minutes each, rinsed, and allowed to dry.

The stained morphology slide should be examined under oil immersion; this magnification is recom-

mended to allow for the clear visualization of abnormalities. The veterinarian should count 100 spermatozoa. Spermatozoa are labeled as normal or as having primary or secondary abnormalities. *Acceptable samples generally contain 70% or greater morphologically normal spermatozoa.* Primary or major abnormalities are those that occur in the testes during production. These abnormalities include unusual size and shape of the head, bending of the midpiece, presence of proximal cytoplasmic droplets, and detached heads if they are present in very high numbers. The secondary or minor abnormalities occur during maturation in the epididymis, in transit through the vas deferens or urethra, or as artifacts of sample collection or preparation. These include bent or coiled tails, detached heads, and distal cytoplasmic droplets (Figures 4-3 and 4-4).

CONCENTRATION Determination of the spermatozoa concentration easily can be performed with a Unopette system (Becton-Dickinson, Rutherford, NJ) and a hemacytometer. A No. 5853 Unopette is used as the diluting chamber. The top of the diluting chamber is pierced with the pipette cover. Semen is drawn up into the pipette and dispensed into the chamber. The solution is then placed on a hemacytometer. The loaded hemacytometer should rest for approximately 5 minutes before the onset of counting. This allows the spermatozoa to gravitate to the bottom and makes counting easier. Any

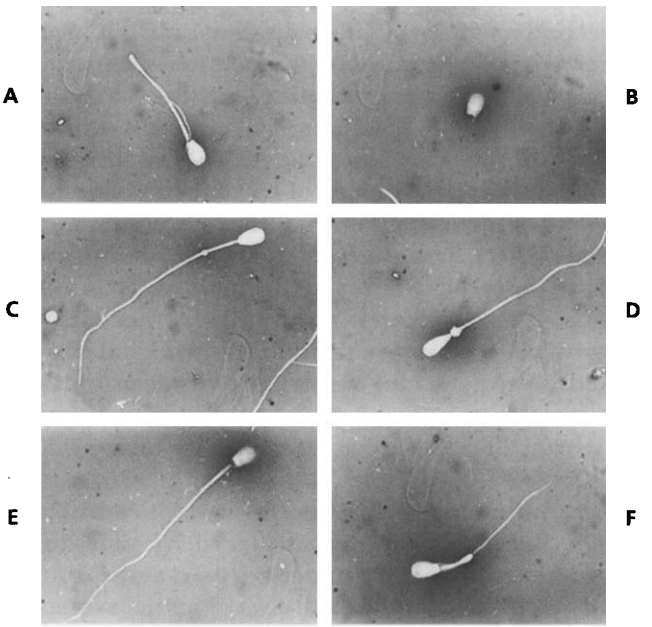


Figure 4-3. Spermatozoal abnormalities. **A,** Bent tail. **B,** Detached head. **C,** Distal cytoplasmic droplet. **D,** Proximal cytoplasmic droplet. **E,** Detached acrosome. **F,** Bent midpiece.

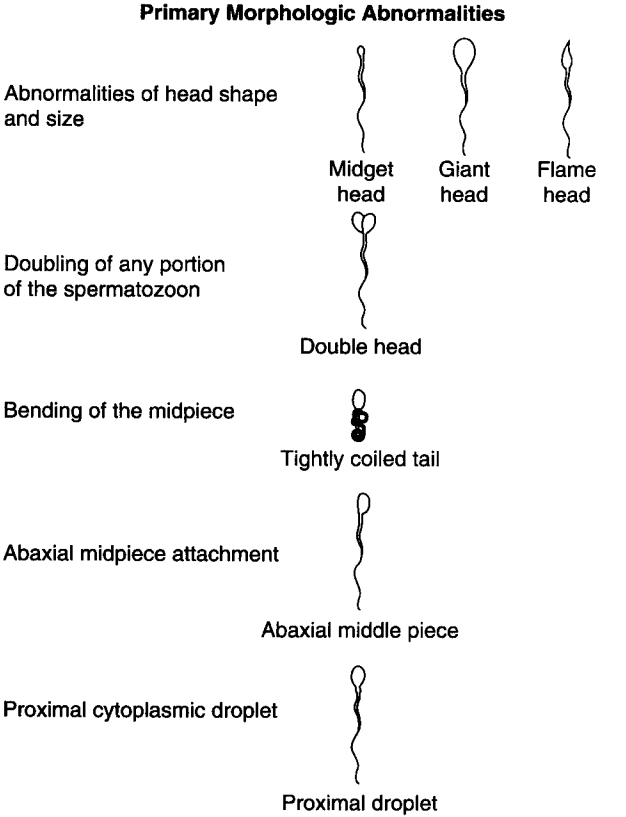


Figure 4-4. Primary and secondary morphologic abnormalities of canine spermatozoa.

Continued

Secondary Morphologic Abnormalities

Detached and separating heads

Separated head

Bending or coiling of the tail

Distally coiled tail

Distal cytoplasmic droplet

Distal droplet

Figure 4-4, cont'd. For legend see p. 117.

three of the large nine squares on the grid can be counted, and the total (S) should be noted. The clinician then multiplies the number obtained by 3 (3S), calculates 10% of that number ($3S \times 0.1$), and adds it back to the total ($3S + [3S \times 0.1]$). The resultant number is divided by 10; this value equals the concentration of spermatozoa in the ejaculate in millions per milliliter (Figure 4-5).

The normal range in concentration varies greatly with the volume collected and the number of spermatozoa being produced by the male. The concentration most important to the veterinarian is that of the second fraction. The average anticipated in this fraction is 125 million/ml, but normal values vary from 20 million to 2 billion spermatozoa/ml. *The total number of spermatozoa in the ejaculate (concentration × volume) averages 1.25 billion spermatozoa per ejaculate, with a range of 300 million to 2 billion.* The great variability is related to age, breed, and size of the male's testes.

pH Assessment of the pH of the ejaculate seems to be of little value. If it is to be used, it must be measured immediately and should be determined by using a very accurate method; this does not include dipsticks.

OTHER FACTORS It must be determined whether there are any other cells present in the sample that would be considered abnormal and that could be detrimental to the spermatozoa. These include epithelial cells, neutrophils, and

Unopette System No. 5853 (Acetic Acid—1:100)

Count any three of the total nine large squares and add them together.

Square 1 =	105 cells
Square 2 =	119 cells
Square 3 =	113 cells
Total	337 cells

Multiply by 3:

$337 \times 3 = 1011$ cells

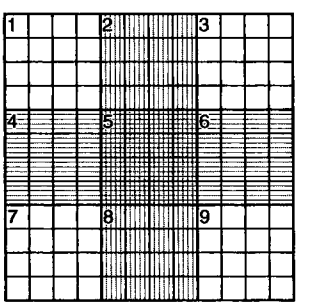
Add 10% of the value obtained above:

$1011 + 101.1 = 1112.1$

Divide by 10 to get the number of million sperm cells per ml of semen:

$1112/10 = 111.21$ million sperm/ml

When counting the sperm in large squares, the number obtained between squares should not vary by more than 20. If a discrepancy exists, recount the squares. If there is still a discrepancy, take a new unopette sample.



If a sperm is touching or crossing a large square border, count only those sperm touching the right or bottom sides of the large squares.
See example below:

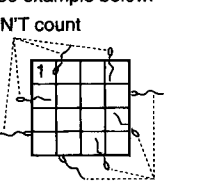


Figure 4-5. Use of the hemacytometer for calculation of concentration of spermatozoa in the ejaculate.

erythrocytes. Examination of the pellet from a centrifuged sample is more accurate than is evaluation of semen diluted with prostatic fluid.

ADDITIONAL COLLECTIONS A second collection obtained within 2 hours after the first may contain up to 50% fewer spermatozoa than the first collection. Males that have not had semen collected for long periods may have a slightly increased number of abnormal spermatozoa in the ejaculate; this is because of the presence of aged spermatozoa, which generally make up approximately 10% of the spermatozoa in that ejaculate. The advantage of collecting the semen to dispose of these aged spermatozoa before shipping or freezing must be weighed against the decrease in total number of spermatozoa obtained if the second sample is collected very soon after the first.

Other Tests

Other tests that should be conducted during a complete BSE include a serologic test for canine brucellosis (see Chapters 7 and 16) and measurement of thyroid hormones (see Chapter 19).

Summary

It is essential to know the quality of the semen being used to inseminate bitches before they are bred. This is criti-

cal because of the long periods of time between estrous periods in bitches and the relatively limited number of heats during the bitch's lifetime. One of the major reasons for performing artificial insemination is so that the semen quality can be ascertained before the insemination occurs. The summary statement of the BSE should take into account all the portions included in the evaluation. Males exhibiting known genetically linked abnormalities should fail this examination, and the reason should be stated. It will be the owner's decision whether to use the animal after the opinion is provided, but by bringing it to the attention of the owner, we will not have endorsed the use of a male that is genetically inferior.

Bibliography

Althouse GC, Ko JC, Hopkins SM et al: Effect of latex and vinyl examination gloves on canine spermatozoal motility, *J Am Vet Med Assoc* 199:227, 1991.

Bartlett DJ: Studies on dog semen: I. Morphological characteristics, *J Reprod Fertil* 3:173, 1962.

Boucher JH, Foote RH, Kirk RW: The evaluation of semen quality in the dog and the effects of frequency of ejaculation upon semen quality, libido, and depletion of sperm reserves, *Cornell Vet* 48:67, 1958.

England GC: Semen quality in dogs and the influence of a short-interval second ejaculation, *Theriogenology* 52:981, 1999.

Froman DP, Amann RP: Inhibition of motility of bovine, canine and equine spermatozoa by artificial vagina lubricants, *Theriogenology* 20:357, 1983.

Goodwin M, Gooding KM, Regnier F: Sex pheromone in the dog, *Science* 203:559, 1979.

Johnson C, Jacobs J, Walker R: Diagnosis and control of *Brucella canis* in kennel situations: morphology stain induced spermatozoal abnormalities. Proceedings of the Society for Theriogenology, San Diego, 1991.

Johnston SD: Performing a complete canine semen evaluation in a small animal hospital, *Vet Clin North Am* 21:545, 1991.

Oettle EE: Sperm morphology and fertility in the dog, *J Reprod Fertil Suppl* 47:257, 1993.

Olar TT, Amann RP, Pickett BW: Relationships among testicular size, daily production and output of spermatozoa, and extragonadal spermatozoal reserves of the dog, *Biol Reprod* 29:1114, 1983.

Purswell BJ, Althouse GC, Root MV: *Guidelines for using the canine breeding soundness evaluation form,* Nashville, 1992, Society for Theriogenology.

Roberts SJ: *Veterinary obstetrics and genital diseases,* Woodstock, 1986, SJ Roberts.

Sekoni VO, Gustafsson BK, Mather EC: Influence of wet fixation, staining techniques, and storage time on bull sperm morphology, *Nord Vet Med* 33:161, 1981.

5

Contraception and Pregnancy Termination

Michelle Anne Kutzler

AT A GLANCE

- Contraception
- Surgical
  - Ovariohysterectomy: *Ovariohysterectomy is the best technique for estrus suppression in bitches and queens.*

- Medical
  - Progestogens: Megestrol acetate (Ovaban; Schering-Plough, Kenilworth, NJ) can be administered during anestrus or during the first 3 days of proestrus. Megestrol acetate is the only drug approved for estrus suppression for bitches in the United States.
  - Androgens: Mibolerone (Cheque; Pharmacia & Upjohn, Peapack, NJ) can be used safely but is not readily available.
  - Immunization against zona pellucida proteins: Immunizing against zona pellucida proteins is experimental.
- Pregnancy termination
  - Surgical
    - Ovariohysterectomy: *Ovariohysterectomy is the preferred technique for pregnancy termination in all bitches and queens not intended for breeding.*
  - Medical
    - Pregnancy diagnosis ideally precedes treatment (see Chapter 8).
    - Treatment with estrogenic compounds ("mismate shots") is not recommended.
    - Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Lutalyse, Pharmacia & Upjohn) can be effectively used any time after the fifth day of diestrus. Lower dosages are more effective later in gestation. At midgestation, the suggested dosage is 250 μg/kg administered

subcutaneously twice a day for 4 days. Side effects include hypersalivation, emesis, and diarrhea. The side effects subside within several hours of administration of the drug and decrease in severity over the course of treatment.

- Prolactin inhibitors: Bromocriptine mesylate (Parlodel; Novartis, East Hanover, NJ) and cabergoline (Dostinex, Pharmacia & Upjohn) can be effectively used after midgestation.
- Dexamethasone: An effective regimen has not been well described. Owners of pregnant dogs that must be treated with glucocorticoids must be cautioned that therapy may terminate pregnancy in their bitches.

Contraception

Definition

- Prevention of pregnancy
- Can be permanent (sterilization) or temporary (estrus suppression)

Surgical Methods for Estrus Suppression

OVARIOHYSTERECTOMY AND OVARIECTOMY

Mechanism of action Removal of the ovaries with or without the uterus results in complete regression and

atrophy of the remaining reproductive tract, provided that all the ovarian tissue is removed and no exposure to exogenous progestogens occurs.

Advantages

- Ovariohysterectomy and ovariectomy produce permanent results.
- Health benefits include prevention of ovarian and uterine disease and reduced risk of mammary neoplasia if performed before the next estrus; this benefit of ovariectomy is lost by the time the bitch reaches 2.5 years of age or has cycled four times.
- *Ovariohysterectomy is the best technique for estrus suppression in most bitches and queens.*

Disadvantages

- Ovariohysterectomy and ovariectomy produce a decreased resting metabolic rate in cats and have been associated with obesity in dogs.
- Urinary incontinence: There is no significant difference in the occurrence of urinary incontinence between animals that have undergone ovariohysterectomy and those that have undergone ovariectomy. Urinary incontinence results from displacement of the bladder neck into the pelvic cavity.

TUBAL LIGATION

Mechanism of action Ligation and removal of a portion of the uterine tube (oviduct) prevents gamete movement in either direction.

Advantages

- Potentially reversible
- Can be performed laparoscopically, which requires less surgical time and postoperative care
- Widely accepted technique in human medicine

Disadvantages

- Does not offer the health advantages of the previous two surgical methods because steroid production is unaltered
- Little information available regarding the use of this technique in small animals

Pharmacologic Methods for Estrus Suppression

PROGESTOGENS

Mechanism of action

- May act locally to prevent follicle growth, estrogen production, and ovulation
- Do not alter circulating levels of luteinizing hormone (LH)

Compounds described

Megestrol acetate (Ovaban; Schering-Plough)

- Megestrol acetate is the only progestogen approved for use for estrus suppression in bitches in the United States. It is not approved for estrus suppression in cats.
- Dosage (canine): If therapy is instituted during anestrus, the recommended dosage is 0.5 mg/kg per os once daily for 32 days. If therapy is instituted within the first 3 days of proestrus, the recommended dose is 2 mg/kg per os once daily for 8 days. If treatment is begun after the third day of proestrus, a fertile estrus may ensue despite therapy. Return to estrus after drug withdrawal is variable (1-9 months).
- Dosage (feline): If therapy is instituted during anestrus, reported dosages are 2.5 mg/cat per os once weekly or 5 mg/cat per os every 2 weeks. If therapy is instituted within the first 3 days of proestrus, the reported dosage is 2.5 mg/cat per os once daily for no longer than 14 days. Return to estrus after drug withdrawal varies from a few days to several weeks.
- The manufacturer does not recommend that megestrol acetate be administered during a bitch's first estrus because it might cause irreversible prolonged anestrus. Similarly, no more than two consecutive estrous cycles should be suppressed in a given bitch to decrease risk of infertility via chemical sterilization. Long-term use in cats is not recommended.

Medroxyprogesterone acetate (Depo-Provera; Pharmacia & Upjohn)

- Dosage (canine): The reported dosage is 2 mg/kg administered intramuscularly every 3 months.

Norethindrone (Primolut N; Schering-Plough)

- Dosage (canine): Reported dosages are 0.075 mg/kg administered orally once daily or 0.15 mg/kg administered orally weekly.

Proligestone (Covinan; Intervet, Millsboro, Del.)

- Dosage (canine): The reported dose is 10 mg/kg administered subcutaneously or intramuscularly every 3 months.
- Dosage (feline): The recommended dose is 1 ml administered subcutaneously every 6 months.

Levonorgestrel (50 μg)/ethinylestradiol (30 μg) (Nordette; Wyeth-Ayerst, Philadelphia)

- The reported dosage is one tablet/30 kg administered orally daily.

Advantages

- Progestogens are effective at suppressing estrus.
- The manufacturer of megestrol acetate reports no adverse effects on fertility when administered as directed.

Disadvantages

- Only megestrol acetate is licensed for use in dogs.

- Side effects depend on the type of progestogen administered, dosage, when treatment is initiated, treatment regimen, and age and species of the patient.

  Uterine side effects include stimulation of the endometrium with increased incidence of cystic endometrial hyperplasia and pyometra. The reported incidence of uterine lesions after treatment with medroxyprogesterone acetate is more than 60%.

  Incidence of mammary carcinoma reportedly is increased in dogs and cats treated with exogenous progestogens. Mammary development may occur in dogs and mammary hypertrophy in cats.

  An increased number of prolactin-secreting cells may be present in the anterior portion of the pituitary gland. Pancreatic changes typical of diabetes mellitus may occur, and systemic insulin resistance may develop.

  If administered during pregnancy, masculinization of female fetuses and fetal death, maceration, or mummification may occur.

  General side effects reported include increased body weight and morphologic changes (acromegaly) consistent with high secretory activity in growth hormone–producing cells. Additional side effects reported in queens include temperament changes (e.g., depression, lethargy, loss of social order),

adrenal cortical suppression, and suppression of fibroblast and T-cell function. Injectable progestogens are not recommended for use in queens.

ANDROGENS

Mechanism of action Negative feedback to the pituitary gland decreases synthesis and release of LH and follicle-stimulating hormone (FSH).

Compounds described

Mibolerone

- Mibolerone is the only androgen approved for estrus suppression in dogs in the United States.
- Dosage (canine): The recommended dosage depends on the weight and breed of the dog:
  30 µg administered orally once daily for 0.5-12 kg of body weight
  60 µg administered orally once daily for 12-25 kg of body weight
  120 µg administered orally once daily for 25-45 kg of body weight
  180 µg administered orally once daily for dogs weighing more than 45 kg and for German shepherd dogs and their crosses
- Therapy must begin 30 days before the next expected estrus. Mibolerone will not arrest proestrus or estrus once it has begun.

- Return to estrus averages 70-90 days, with a range of 7-240 days after drug withdrawal.
- The only commercially available product (Cheque drops, Pharmacia & Upjohn) has been discontinued, but generic suspensions are available through compounding pharmacies.

Testosterone combinations (Durateston, Intervet)

- Dosage (canine and feline): The reported dosage is 0.5-1 ml/10 kg administered intramuscularly every 4 weeks.
- Each milliliter contains 6 mg of testosterone propionate, 12 mg of testosterone phenylpropionate, 12 mg of testosterone isocaproate, and 20 mg of testosterone decanoate.

Testosterone cypionate (Depo-Testosterone, Pharmacia & Upjohn)

- Dosage (canine): The reported dosage is 0.5 mg/kg administered intramuscularly every 5 days.

Testosterone enanthate (Delatestryl; BTG Pharmaceuticals, Iselin, NJ)

- Dosage (canine): The reported dosage is 0.5 mg/kg administered intramuscularly every 5 days.

Boldenone undecanoate (Vebonol, Novartis)

- Dosage (canine): The reported dosage is 1-2 mg/kg administered intramuscularly every 4 weeks.

Methyltestosterone (Android; ICN Pharmaceuticals, Costa Mesa, Calif.)

- Dosage (canine): The reported dosage is 1 mg/kg administered orally twice weekly.

Advantages

- Androgens are effective at suppressing estrus.
- Lack of estrogenic or progestational effects decreases incidence of cystic endometrial hyperplasia/pyometra and mammary neoplasia as side effects.

Disadvantages

- Only mibolerone is licensed for use in dogs.
- Testosterone products are controlled substances (C III).
- Reported side effects include mucoid vaginal discharge with or without concurrent vaginitis, clitoral hypertrophy, increased serum liver enzymes, change in temperament, and increased body weight. Anabolic effects may be seen with administration of high dosages.
- The manufacturer of Cheque drops recommends that mibolerone not be used in Bedlington terriers, in dogs with a history of liver disease, in bitches before their first estrus, in animals intended for breeding, or in pregnant dogs because masculinization of the external genitalia of female pups can occur. *There is no*

safe dose for mibolerone in cats. Cats treated with mibolerone may develop fatal hepatic or thyroid disease.

GONADOTROPIN-RELEASING HORMONE AGONISTS

Mechanism of action

- Gonadotropin-releasing hormone (GnRH) agonists bind to GnRH receptors on pituitary cells, resulting in downregulation and decreased production of FSH and LH.

Compounds described

Deslorelin acetate (Peptech Animal Health, New South Wales)

- Dosage (canine): The reported dose is 3-12 mg/dog administered subcutaneously, once.
- Dosage (feline): The reported dose is 6 mg/cat administered subcutaneously, once.

Advantages

- These compounds can suppress estrus for up to 27 months in the dog and up to 14 months in the cat.
- No long-term effects on fertility have been reported.

Disadvantages

- The initial response to administration of the GnRH agonist is estrus induction, but this can be prevented with simultaneous treatment with a progestogen

(megestrol acetate at 1-2 mg/kg administered orally once daily for 2-3 weeks).

- Minimal edema may develop at the site of drug administration for 3-5 days.

GnRH ANTAGONISTS

Mechanism of action

- GnRH antagonists bind to GnRH receptors on pituitary cells and decrease the secretion of LH and FSH by preventing gene transcription.

Compounds described

Detirelix acetate

- No information is available about appropriate dosages in small animals.

Advantages

- Detirelix acetate produces atrophy of the reproductive organs and inhibits ovulation.
- The effect is reversible. Time to recovery of normal reproductive organ morphology and function is directly related to the dose administered.

Disadvantages

- The drug is very expensive.
- Localized skin erythema and pruritus may develop at the injection site.

IMMUNOCONTRACEPTION

- Definition: injection of a reproductive protein to produce a humoral immune response that leads to infertility for a defined period
- Disadvantages:
  Skin irritation and/or localized pain at the immunization site, similar to that associated with other immunizations
  Continual presence of circulating antigen-antibody complexes, resulting in immune complex glomerulonephritis

Immunization against zona pellucida

Mechanism of action

- Zona pellucida (ZP) is an extracellular glycoprotein matrix that surrounds the oocyte and contains specific receptors for binding of spermatozoa.
- Antibodies directed against ZP prevent binding of spermatozoa and fertilization and impair oocyte development and ovulation.

Advantages

- Immunocontraception will last for several months after vaccination.
- Immunocontraception is reversible in some species.

Disadvantages

- Ovarian pathologic abnormalities described as occurring in dogs vaccinated against porcine ZP proteins include premature ovarian atrophy,

polycystic ovarian disease, and autoimmune oophoritis.
- Ovarian damage leads to immunosterilization, an immune response that leads to the animal's sterilization by destroying oocyte–granulosa cell complexes or causing ovarian follicular failure.
- Vaccine made from readily available porcine ZP proteins is not effective in the cat.

Immunization against GnRH

Mechanism of action

- Antibodies bind to GnRH. These antibody-GnRH complexes are unable to bind to GnRH receptors on pituitary cells, resulting in decreased LH and FSH secretion.

Disadvantages

- It is difficult to present GnRH in an immunogenic form.
- Duration of immune response is variable among individuals.
- Immune response diminishes after booster immunizations.

INDUCTION OF PSEUDOPREGNANCY (FELINE)

Mechanism of action

- During estrus, ovulation can be induced mechanically by vaginal stimulation or pharmacologically by

administration of exogenous hormones. Ovulation occurs within 24-36 hours, and 30-45 days of pseudopregnancy then occurs if the animal is neither mated nor inseminated.

Compounds described

Gonadorelin (Factrel; Fort Dodge, Fort Dodge, Iowa)

- The gonadorelin compound acts as GnRH, causing release of endogenous LH and FSH.
- Dosage: The reported dose is 25 µg administered intramuscularly, once.

Human chorionic gonadotropin

- Human chorionic gonadotropin (hCG) binds to and activates LH receptors in the cat.
- Dosage: The reported dose is 50-500 IU administered intramuscularly, once.

Pregnancy Termination

Definition

- Expulsion from the uterus of the products of conception before the fetus is viable
- Synonym = induced abortion
- Can be achieved by surgical or pharmacologic methods

Objectives

- To induce abortion only if the bitch or queen is pregnant; in one study, 30 (62%) of 48 bitches examined at 30-35 days after a single, unplanned breeding were not pregnant.
- Use a method that is reliable, easy to administer, and efficacious, with a product that is safe for the animal's health and subsequent fertility.
- General considerations include the following:
  Ovariohysterectomy should be performed in all animals not specifically intended for breeding.
  Estrogenic compounds are not recommended for pregnancy termination in dogs and cats.
  Pregnancy diagnosis (see Chapter 8) ideally precedes treatment.
  Drugs available for pregnancy termination in dogs and cats in the United States include $PGF_{2\alpha}$, prolactin inhibitors, and dexamethasone (Table 5-1). No drugs are approved for pregnancy termination in dogs and cats in the United States.

Surgical Methods for Pregnancy Termination

OVARIOHYSTERECTOMY

Advantages

- This is the treatment of choice for mismated animals when future reproductive potential is not important.
- The animal will not become pregnant again.

Table 5-1. Pregnancy Termination Protocols for the Dog and Cat

Stage of gestation	Effective methods	Comments
Fertilization: implantation (from day of LH surge to 21 ± 1 days past LH surge)	• Luteolytic agents ($PGF_{2\alpha}$, high dosages); not effective until after day 5 of diestrus • Inhibitors of progesterone action (mifepristone) • Inhibitors of progesterone secretion (epostane)	• Pregnancy termination is more difficult because of the refractoriness of the corpora lutea to luteolytic treatments. • Pregnancy termination at this stage of gestation may be imposed on nonpregnant animals because pregnancy cannot yet be confirmed.
Implantation: fetal ossification (from about 21-41 ± 1 days past LH surge)	• Luteolytic agents ($PGF_{2\alpha}$, low or high dosages) • Combination of dopamine agonists (bromocriptine, cabergoline) and $PGF_{2\alpha}$ • Inhibitors of progesterone action (mifepristone)	• Pregnancy termination at this stage of gestation is associated with fetal resorption.

Table 5-1. Pregnancy Termination Protocols for the Dog and Cat—cont'd

Stage of gestation	Effective methods	Comments
	• Dexamethasone • Inhibitors of progesterone secretion (epostane)	
Fetal ossification: parturition (from about 41-65 ± 1 days past LH surge)	• Luteolytic agents ($PGF_{2\alpha}$, low or high dosages) • Dopamine agonists or serotonin antagonists (bromocriptine, cabergoline) • Combination of dopamine agonists and $PGF_{2\alpha}$ • Inhibitors of progesterone action (mifepristone) • Dexamethasone • Inhibitors of progesterone secretion (epostane)	• Pregnancy termination at this stage of gestation is associated with fetal expulsion; if performed beyond day 50-55 of gestation, viable fetuses may be delivered.

- Health benefits include prevention of ovarian and uterine disease and reduced risk of mammary neoplasia if performed before the next estrus; this benefit of ovariectomy is lost by the time the bitch reaches 2.5 years of age or has cycled four times.
- Production of unwanted pets is prevented.

Disadvantages

- Surgery may be more complicated because of increased vascularity of the gravid uterus; difficulty of surgery increases with advancing gestation.
- Lactation may result after ovariohysterectomy during pregnancy or diestrus (see Chapter 14).

Pharmacologic Methods for Pregnancy Termination

ESTROGENIC COMPOUNDS

Mechanism of action

- Delay transport of embryos through the uterine tubes
- Induce endometrial congestion and edema, thereby preventing normal embryo migration and implantation
- Exert direct embryotoxic effects
- Alter composition of uterine milk, making uterine environment less hospitable to conceptuses
- Only safe and effective if given after ovulation and before onset of cytologic diestrus

Compounds described

Diethylstilbestrol

- Dosage (canine): The reported dose of repositol diethylstilbestrol (DES) is 0.5 mg/kg administered intramuscularly, once. The reported dosage of orally administered DES is 0.1-1 mg/kg once daily for 5 days beginning 24-48 hours after mating.
- Efficacy with the oral product is very poor. Efficacy with the injectable product is better, but at present, no injectable DES products are commercially available. Preparations may be available from compounding pharmacies.

Estradiol cypionate (Depo-Estradiol, Pharmacia & Upjohn)

- Dosage (canine): The reported dose is 0.02-0.044 mg/kg (not to exceed a total dose of 1 mg) administered intramuscularly, once. The historical recommendation of administration of the drug within 72 hours of mating has no basis in science. To be efficacious and safe, the drug must be administered after ovulation (evidenced by serum progesterone concentration greater than 5 ng/ml) and before onset of cytologic diestrus (evidenced by noncornified vaginal cytology).
- Dosage (feline): The reported dose is 0.125-0.25 mg administered intramuscularly, once, within 40 hours of mating.

Estradiol benzoate

- Dosage (canine): The reported dose is 5-10 μg/kg, to a maximum total dose of 1 mg. The calculated dose is divided into two to three injections, administered subcutaneously at 48-hour intervals beginning 2 days after mating.

Tamoxifen citrate (Novadex; Astrazeneca, Wilmington, Del.)

- Dosage (canine): The reported dosage is 1 mg/kg administered orally twice a day for 10 days.

Disadvantages

- Ineffective at terminating pregnancy if administered before ovulation
- Side effects:

  Cystic endometrial hyperplasia and pyometra are likely to develop if the drug is administered during diestrus; as many as 25% of bitches treated with estradiol cypionate (ECP) developed pyometra in one study.

  Prolonged behavioral estrus may occur.

  Medullary aplasia of bone marrow resulting in thrombocytopenia, leukopenia, aplastic anemia, and death develops in some cases. Bone marrow toxicity is idiosyncratic and dose dependent, with the first clinical signs occurring 5-10 days after treatment. Dogs are more susceptible to bone marrow toxicity from estrogenic compounds than are cats.

Because of the potential for serious adverse effects, estrogenic compounds are not recommended for pregnancy termination in dogs and cats.

LUTEOLYTIC AGENTS (PROSTAGLANDINS)

Mechanism of action

- Prostaglandins induce vasoconstriction that reduces blood flow to the corpora lutea with subsequent cellular degeneration. Prostaglandins also induce myometrial contractility and contribute to cervical dilation.

Compounds described

Dinoprost tromethamine (Lutalyse, Pharmacia & Upjohn)

- Dosage (canine): Reported dosages are 250 μg/kg administered subcutaneously twice a day or 10-50 μg/kg administered subcutaneously every 4-8 hours. Therapy must be instituted on day 5 of diestrus at the earliest. Lower dosages are more effective later in gestation.
- Dosage (feline): Reported dosages are 500 μg/kg administered subcutaneously two to three times a day for 5 days beginning at least 30 days after mating or 500-1000 μg/kg administered once daily for 2 days beginning after 40 days after mating. The lowest effective dosage for cats has not been established.

Cloprostenol (Estrumate, Schering-Plough)

- Dosage (canine): The reported dosage is 1-2.5 μg/kg once daily.

Fenprostalene (Bovilene, Fort Dodge)

- Dosage (canine): The reported dosage is 20 μg/kg once daily.

Advantages

- Luteolysis can be induced as early as 1 week after the onset of cytologic diestrus.
- Efficacy is 80%-100%.
- $PGF_{2\alpha}$ analogs require less frequent injections and are associated with few side effects if administered in appropriate doses.

Disadvantages

- The duration of treatment averages 4 days (range, 3-11 days). Longer duration of treatment is required with administration of lower dosages or treatment administered early in gestation. Treatment should be continued until serum progesterone concentration decreases to less than 2 ng/ml for more than 2 days or until there is no ultrasonographic evidence of fetal viability.
- Length of the interestrous interval may be reduced.
- Side effects include tachypnea, hypersalivation, vomiting, diarrhea, ataxia, urination, anxiety, pupil dilation, and abdominal discomfort. Onset of side effects is

within 5-30 minutes of administration of the drug. Side effects subside within 2-3 hours. Severity of side effects varies depending on individual sensitivity, but generally the side effects are more severe after the initial dose and when prostaglandins are administered at high dosages (greater than 100-150 μg/kg of body weight). Side effects may be alleviated in dogs by walking the dog for 15-30 minutes after drug administration or by concurrent administration of atropine (0.025 mg/kg administered subcutaneously). Side effects are less severe in cats.

DOPAMINE AGONISTS AND SEROTONIN ANTAGONISTS

Mechanism of action

- Inhibition of prolactin secretion results in luteolysis.

Compounds described

Bromocriptine mesylate (Parlodel, Novartis)

- Dosage (canine): Reported dosages are 50-100 μg/kg administered orally or subcutaneously twice a day for 7 days or 25-50 μg/kg administered orally or subcutaneously three times a day for 7 days.

Cabergoline (Dostinex, Pharmacia & Upjohn)

- Dosage (canine): Reported dosages are 5 μg/kg administered orally once daily for 7 days or 1.65 μg/kg administered subcutaneously once daily for 5 days.

- Dosage (feline): Reported dosages are 2.5 μg/kg administered orally once daily for 5 days or 5 μg/kg administered orally once daily for 3 days.

Metergoline

- Dosage (canine): The reported dosage is 400-500 μg/kg administered orally once daily for 5 days.

Advantages

- Efficacy is 100% after 40 days past the LH surge.

Disadvantages

- Efficacy is less than 66% before 40 days past the LH surge.
- Bromocriptine produces side effects similar to those produced by $PGF_{2\alpha}$, especially emesis.

COMBINATIONS OF DOPAMINE AGONISTS AND $PGF_{2\alpha}$

Mechanism of action

- The mechanism of action is the same as for the individual compounds. The synergistic effect allows for reduced dosages of both drugs to be administered, with a resultant decrease in side effects.

Combinations described

Bromocriptine and cloprostenol

- Dosage (canine): The reported dosages are 5 μg/kg body weight bromocriptine administered orally

once daily for 7 days in conjunction with 2.5 μg/kg cloprostenol administered subcutaneously on days 1, 3, and 5 of treatment.

Cabergoline and cloprostenol

- Dosage (canine): The reported dosages are 2.5-5 μg/kg cabergoline administered orally once daily for 7 days in conjunction with 1 μg/kg cloprostenol administered subcutaneously on days 1, 3, and 5 of treatment.
- Dosage (feline): The reported dosages are 5 μg/kg cabergoline administered orally once daily for 8 days in conjunction with 5 μg/kg cloprostenol administered subcutaneously on days 1, 3, and 5 of treatment.

Advantages

- Efficacy is 100% when initiated after 25 days past the LH surge.

INHIBITORS OF PROGESTERONE ACTION

Mechanism of action

- Progesterone receptor antagonists bind to progesterone receptors with higher affinity than that associated with endogenous progesterone, but they do not activate the receptors, preventing progesterone-induced effects.

Compounds described

Mifepristone (RU486) (Mifeprex; Danco Laboratories, New York, NY)

- Dosage (canine): Reported dosages are 2.5 mg/kg administered orally twice a day for 4-5 days or 10-22.7 mg/kg administered subcutaneously, once.
- Dosage (feline): The reported dosage is 20-34.3 mg/kg administered orally or subcutaneously as a single injection.

Aglepristone (RU534) (Alizine; Verbac Laboratories, Carros, France)

- Dosage (canine): The reported dosage is 10 mg/kg administered subcutaneously once daily for 2 consecutive days.

Advantages

- Pregnancy is effectively terminated in the dog within 4-7 days of instituting therapy.
- Few side effects are reported, and those that do occur are not as severe as those that occur after treatment with $PGF_{2\alpha}$.

Disadvantages

- Side effects include mammary gland development and lactation.

- Seems to be less effective in terminating feline pregnancy; pregnancy loss is reported to occur less than 20% of the time.
- Progesterone levels are not diminished during treatment or at the time of abortion.

DEXAMETHASONE

Mechanism of action

- Corticosteroids mediate a luteolytic effect that is not well understood.

Dosage

- Dosage (canine): Reported dosages are 5 mg/kg administered intramuscularly twice a day for 10 days or 0.2-0.5 mg/kg administered orally one to three times a day for 5 days, followed by another 3-5 days during which the dosage is progressively reduced to 0.16-0.02 mg/kg.

Advantages

- Efficacy is very good after 30 days past the LH surge.

Disadvantages

- Transient corticosteroid-induced side effects, including anorexia, polydipsia, and polyuria, are observed using this protocol.

INHIBITORS OF PROGESTERONE SECRETION

Mechanism of action

- Epostane inhibits progesterone synthesis by competitive inhibition of enzymes involved in the conversion of pregnenolone to progesterone (3β-hydroxysteroid dehydrogenase and Δ-4-5-isomerase).

Compound described

Epostane (Hoescht-Roussel, Kansas City, Mo.)

- Dosage (canine): Reported dosages are 15-20 mg/kg administered intramuscularly, once, or 50 mg/kg administered orally once daily for 7 days starting on the first day of cytologic diestrus.

Bibliography

Baldwin C, Evans LE, Peter AT: Evaluation of natural prostaglandin therapy for pregnancy termination in the domestic cat, *Feline Pract* 28:16, 2000.

Banks DH, Stabenfeldt GH: Luteinizing hormone release in the cat in response to coitus on consecutive days of estrus, *Biol Reprod* 26:603, 1982.

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, 1985.

Bowen RA, Olson PN, Young S et al: Efficacy and toxicity of tamoxifen citrate for prevention and termination of pregnancy in bitches, *Am J Vet Res* 49:27, 1988.

Brown JL, Bush M, Wildt DE et al: Effects of GnRH analogues on pituitary-testicular function in free-ranging African elephants *(Luxodonta africana), J Reprod Fertil* 99:627, 1993.

Burke T: Pharmacologic control of estrus in the bitch and queen, *Vet Clin North Am* 12:79, 1982.

Chan SY, Chakraborty PK, Bass EJ et al: Ovarian-endocrine-behavioural function in the domestic cat treated with exogenous gonadotrophins during mid-gestation, *J Reprod Fertil* 65:395, 1982.

Chester AE, Fairchild DG, Depass LR: Acute and subchronic toxicity studies with detirelix, a luteinizing hormone-releasing hormone antagonist, in the rat and monkey, *Fundam Appl Toxicol* 17:505, 1991.

Concannon PW: Biology of gonadotropin secretion in adult and prepubertal female dogs, *J Reprod Fertil Suppl* 47:3, 1993.

Concannon PW: Fertility regulation in the bitch: contraception, sterilization, and pregnancy termination. In Kirk RW (ed): *Current veterinary therapy VIII,* Philadelphia, 1983, WB Saunders.

Concannon PW: Use of DES, gonadotropins, GnRH agonists and dopamine agonists for rapid induction of fertile estrus in dogs. Proceedings of the Society for Theriogenology, Jacksonville, Fl, 1993.

Concannon PW, Hansel W: Prostaglandin F2 alpha induced luteolysis, hypothermia and abortion in beagle bitches, *Prostaglandins* 13:533, 1977.

Concannon PW, Meyers-Wallen V: Current and proposed methods for contraception and termination of pregnancy in dogs and cats, *J Am Vet Med Assoc* 198:1214, 1991.

Concannon PW, Yeager A, Frank D et al: Termination of pregnancy and induction of premature luteolysis by the antiprogestagen, mifepristone, in dogs, *J Reprod Fertil* 88:99, 1990.

Davidson AP: The use of prostaglandins in small animal reproduction. Proceedings of the Society for Theriogenology, San Antonio, 1992.

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, 1988.

Eigenmann JE, Eigenmann RY: Influence of medroxyprogesterone acetate (Provera) on plasma growth hormone levels and on carbohydrate metabolism. II. Studies in the ovariohysterectomized, estradiol-primed bitch, *Acta Endocrionol (Copenh)* 98:603, 1981.

Evans JM, Sutton DJ: The use of hormones, especially progestogens to control estrus in bitches, *J Reprod Fertil Suppl* 39:163, 1989.

Fayrer-Hosken RA, Dookwah HD, Brandon CI: Immunocontrol in dogs, *Anim Reprod Sci* 60-61:365, 2000.

Feldman EC, Davidson AP, Nelson RW et al: Prostaglandin induction of abortion in pregnant bitches after misalliance, *J Am Vet Med Assoc* 202:1855, 1993.

Ferguson RG: The reproductive cycle and progesterone levels in greyhounds. Proceedings of the Australian Greyhound Veterinary Association, Surfers Paradise, Queensland, Australia, October 29, 1988.

Fieni F, Dumon C, Tainturier D et al: Clinical protocol for pregnancy termination in bitches using prostaglandin F2 alpha, *J Reprod Fertil Suppl* 51:245, 1997.

Freshman JL, Olson PN, Amann RP et al: The effects of methyltestosterone on reproductive function in male greyhounds, *Theriogenology* 33:1057, 1990.

Galac S, Kooistra HS, Butinar J: Termination of mid-gestation pregnancy in bitches with aglepristone, a progesterone receptor antagonist, *Theriogenology* 53:941, 2000.

Gannon J: Clinical aspects of the oestrus cycle in the greyhound, *Racing Greyhound* 1:12, 1976.

Gerstenberg C, Nöthling JO: The effects of metergoline combined with PGF2α treatment on luteal function and gestation in pregnant bitches, *Theriogenology* 44:649, 1995.

Gonzalez A, Allen AF, Post R et al: Immunological approaches to contraception in dogs, *J Reprod Fertil Suppl* 39:189, 1989.

Goodrowe KL, Howard JG, Schmidt PM et al: Reproductive biology of the domestic cat with special reference to endocrinology, sperm function and in-vitro fertilization, *J Reprod Fertil Suppl* 39:73, 1989.

Henik RA, Olson PN, Roysychuk RA: Progestogen therapy in cats, *Comp Cont Edu* 7:135, 1985.

Herron MA, Sis RF: Ovum transport in the cat and the effect of estrogen administration, *Am J Vet Res* 35:1277, 1974.

Hubler M, Arnold S, Dobeli M: The use of low dose prostaglandin F-2 alpha in the bitch, *J Reprod Fertil Suppl* 47:555, 1993.

Jackson EK: Contraception in the dog and cat, *Br Vet J* 140:132, 1984.

Janssens LA, Janssens GH: Bilateral flank ovariectomy in the dog: surgical technique and sequelae in 72 animals, *J Small Anim Pract* 32:249, 1991.

Jewgenow K, Rohleder M, Wegner I: Differences between antigenic determinants of pig and cat zona pellucida proteins, *J Reprod Fertil* 119:15, 2000.

Jochle W, Arbeiter K, Post K et al: Effects of pseudopregnancy, pregnancy, and interoestrus intervals of pharmacologic suppression of prolactin secretion in female dogs and cats, *J Reprod Fertil Suppl* 39:199, 1989.

Johnston SD: Canine pregnancy termination with prostaglandin F2 alpha. Proceedings of the Society for Theriogenology, Toronto, Ontario, Canada, 1990.

Johnston SD: Questions and answers on the effects of surgically neutering dogs and cats, *J Am Vet Med Assoc* 198:1206, 1991.

Keister DM, Gutheil RF, Kaiser LD et al: Efficacy of oral epostane administration to terminate pregnancy in mated laboratory bitches, *J Reprod Fertil Suppl* 39:241, 1989.

Kwochka KW, Short BG: Cutaneous xanthomatosis and diabetes mellitus following long-term therapy with megestrol acetate in a cat, *Comp Cont Edu* 6:185, 1984.

Lange K, Gunzel-Apel R, Hoppen HO et al: Effects of low doses of prostaglandin F2 alpha during the early luteal phase before and after implantation in beagle bitches, *J Reprod Fertil Suppl* 51:251, 1997.

Lloyd JB: Alternatives to surgical sterilization of the dog and cat, *Aust Vet Pract* 18:68, 1988.

Mahi-Brown CA, Yanagimachi R, Nelson ML et al: Ovarian histology of bitches induced by active immunization with porcine zonae pellucidae, *Am J Reprod Immunol* 18:94, 1988.

McCann JP, Altszuler N, Hampshire J et al: Growth hormone, insulin, glucose, cortisol, luteinizing hormone, and diabetes in beagle bitches treated with medroxyprogesterone acetate, *Acta Endocrinol (Copenh)* 116:73, 1987.

Misdorp W, Romijn A, Hart AA: Feline mammary tumors: a case-control study of hormonal factors, *Anticancer Res* 11:1793, 1991.

Morris JS, Dobson JM, Bostock DE: Use of tamoxifen in the control of canine mammary neoplasia, *Vet Rec* 133:539, 1991.

Munson L, Bauman JE, Asa CS et al: Efficacy of the GnRH analogue for suppression of oestrous cycles in cats, *J Reprod Fertil Suppl* 57:269, 2001.

Nachreiner RF, Marple DN: Termination of pregnancy in cats with prostaglandin F2α, *Prostaglandins* 7:303, 1974.

Noakes DE, Dhaliwal GK, England GC: Cystic endometrial hyperplasia/pyometra in dogs: a review of the causes and pathogenesis, *J Reprod Fertil Suppl* 57:395, 2001.

Norval M, Else RW, Maingay J: The effect of tamoxifen on tumours induced by cells from a canine mammary carcinoma cell line in athymic mice, *Res Vet Sci* 44:76, 1988.

Okkens AC, Dieleman SJ, Kooistra HS et al: Comparison of long-term effects of ovariectomy versus ovariohysterectomy in bitches, *J Reprod Fertil Suppl* 51:227, 1997.

Olson PN, Johnston SD: New developments in small animal population control, *J Am Vet Med Assoc* 202:904, 1993.

Olson PN, Nett TM: Small animal contraceptives. In Ettinger SJ (ed): *Textbook of veterinary internal medicine,* Philadelphia, 1983, WB Saunders.

Olson PN, Nett TM, Bowen RA et al: A need for sterilization, contraception and abortifacients: abandoned

and unwanted pets. Part III. Abortifacient, *Comp Cont Edu* 8:235, 1986.

Olson PN, Nett TM, Bowen RA et al: A need for sterilization, contraception and abortifacients: abandoned and unwanted pets. Part IV. Potential methods of controlling reproduction, *Comp Cont Edu* 8:303, 1986.

Olson PN, Root Kustritz MV, Johnston SD: Early-age neutering of dogs and cats in the United States, *J Reprod Fertil Suppl* 57:223, 2001.

Onclin K, Silva LD, Verstegen JP: Termination of unwanted pregnancy in dogs with the dopamine agonist, cabergoline, in combination with a synthetic analog of PGF2a, either cloprostenol or alphaprostol, *Theriogenology* 43:813, 1995.

Onclin K, Verstegen J: Practical use of a combination of a dopamine agonist and synthetic prostaglandin analogue to terminate unwanted pregnancy in dogs, *J Small Anim Pract* 37:211, 1996.

Plumb DC: *Veterinary drug handbook,* Ames, Iowa, 1999, Iowa State University Press.

Post K: Induced pregnancy termination in dogs. Proceedings of the Society for Theriogenology, Jacksonville, Fl, 1993.

Prats AE: Ovarian remnant syndrome in the queen, *EVSSAR Newsletter* 4:5, 2001.

Romagnoli SE, Camilla F, Cela M et al: Clinical use of natural prostaglandin F2 alpha to induce early

abortion in the mismated bitch: serum progesterone, treatment outcome and interval to subsequent estrus. Proceedings of the International Symposium on Canine and Feline Reproduction, Liege, Belgium, 1992.

Root MV, Johnston SD, Olson PN: Effect of prepuberal and postpuberal gonadectomy on heat production measured by indirect calorimetry in male and female domestic cats, *Am J Vet Res* 57:371, 1996.

Sankai T, Endo T, Kanayama K et al: Antiprogesterone compound, RU 486 administration to terminate pregnancy in dogs and cats, *J Vet Med Sci* 53:1069, 1991.

Singh V: Active immunization of female dogs against luteinizing hormone releasing hormone (LHRH) and its effects on ovarian steroids and estrus suppression, *Ind J Exp Biol* 23:667, 1985.

Stocklin-Gautschi NM, Hassig M, Reicher IM et al: The relationship of urinary incontinence to early spaying in bitches, *J Reprod Fertil Suppl* 57:233, 2001.

Sutton DJ, Geary NM, Bergman GB: Prevention of pregnancy in bitches following unwanted mating: a clinical trial using low dose of oestradiol benzoate, *J Reprod Fertil Suppl* 51:239, 1997.

Trigg TE, Wright PJ, Armour AF et al: Use of a GnRH analogue to produce reversible long-term suppression of reproductive function in male and female domestic dogs, *J Reprod Fertil Suppl* 57:255, 2001.

Verstegen J, Ballabio R: Interruption of pregnancy in bitches using cabergoline, *Riv Zoot Vet* 20:35, 1993.

Verstegen J, Decoster R, Brasseur M: Influence of estrogens on blood parameters in bitches, *Ann Med Vet* 125:397, 1981.

Verstegen J, Onclin K, Donnay I et al: Luteotropic action of prolactin in dogs and effects of a dopamine agonist, cabergoline, *J Reprod Fertil Suppl* 47:403, 1993.

Verstegen J, Onclin K, Silva LD et al: Abortion induction in queens and bitches using cabergoline, a specific dopamine agonist, *Ann Med Vet* 137:251, 1993.

Verstegen J, Onclin K, Silva LD et al: Regulation of progesterone during pregnancy in the cat: studies on the roles of corpora lutea, placenta and prolactin secretion, *J Reprod Fertil Suppl* 47:165, 1993.

Vickery BH, McRae GI, Goodpasture JC et al: Use of potent LHRH analogues for chronic contraception and pregnancy termination in dogs, *J Reprod Fertil Suppl* 39:175, 1989.

Von Berky AG, Townsend WL: The relationship between the prevalence of uterine lesions and the use of medroxyprogesterone acetate for canine population control, *Aust Vet J* 70:249, 1993.

Wanke M, Loza ME, Monaschi N et al: Clinical use of dexamethasone for termination of unwanted pregnancy in dogs, *J Reprod Fertil Suppl* 51:233, 1997.

Wildt DE, Seager SW: Ovarian response in the estrual cat receiving varying doses of HCG, *Hormone Res* 9:144, 1978.

Wildt DE, Seager SW: Reproduction control in dogs, *Vet Clin North Am* 7:775, 1978.

Wright PJ, Verstegen JP, Onclin K et al: Suppression of the oestrus responses of bitches to the GnRH analogue deslorelin by progestin, *J Reprod Fertil Suppl* 57:263, 2001.

Zone M, Wanke M, Rebuelto M et al: Termination of pregnancy in dogs by oral administration of dexamethasone, *Theriogenology* 43:487, 1995.

6

Prepuberal Gonadectomy (Early-Age Neutering) of Dogs and Cats

Patricia N. Olson

AT A GLANCE

- Prepuberal gonadectomy is surgical sterilization of puppies and kittens at a very early age, usually younger than 16 weeks.
- Pet overpopulation is a very real problem in the United States. If animals could be adopted from

humane organizations already having undergone gonadectomy, they would not be able to repopulate shelters by bearing young.

- No significant short- or long-term side effects have been reported to occur in either dogs or cats; spayed and neutered animals differ from intact animals, but age at time of surgery makes no difference regarding physical and behavioral changes observed.
- Considerations associated with pediatric anesthesia include the following:
  - Pediatric animals are prone to hypothermia and hypoglycemia.
  - Uptake, distribution, and excretion of anesthetic agents are different in pediatric animals than in adult animals, which alters the required dosages.
- The surgical technique used to perform ovariohysterectomy and castration is as for adult animals, bearing in mind the increased fragility of pediatric tissues and the need for meticulous hemostasis.

The neutering of puppies and kittens as early as 7 weeks of age may be considered for several reasons:

- An owner desires to have a young animal neutered before sale or adoption.
- The procedure may be beneficial in curbing the numbers of unwanted pets in the United States (pet overpopulation).
- The health and temperament of an animal neutered before puberty may be improved.

- The surgical and recovery times are often shorter than when animals are neutered at older ages or after body fat has increased.

Population Control

Prepuberal gonadectomy is not a new procedure. For many years veterinarians in the United States have recommended that female dogs and cats be neutered before the first estrus (prepuberally) to reduce the risk of mammary neoplasia and eliminate the possibility of unwanted pregnancies. What is new, however, is the age of prepuberal animals that many veterinarians are now neutering. Traditionally, female dogs and cats not intended for breeding were neutered at approximately 6 months of age and male dogs and cats were neutered at approximately 6-9 months of age. However, in an attempt to reduce the number of unwanted pets in the United States, veterinarians began to question whether it was safe to neuter puppies and kittens at an even younger age.

Although animal shelters often request that neuter contracts be signed at the time of adopting a pet, 10%-50% of all people who sign neuter contracts fail to comply with the terms of their contracts. This compliance failure occurs even when financial reimbursement is available for the neuter surgery. In a survey published by the Massachusetts Society for the Prevention of Cruelty

to Animals and conducted by the Dorr Research Corporation of Boston, 73% and 87% of 500 households with dogs and cats, respectively, stated that their pets were neutered, but nearly 20% of the neutered animals had produced at least one litter of offspring before being sterilized. In fact, in a telephone survey of 343 respondents with 88 dogs and 121 cats, the rate of litter production did not differ significantly between neutered and intact animals, at 0.31 litter per neutered animal and 0.40 litter per intact animal. Many authors have proposed mathematical models for the number of offspring that can be produced over time, even when animals are allowed to reproduce only for a limited period. For example, it has been suggested that when cats are allowed to reproduce for only 1 year, a male and female cat can be the progenitors of more than 70,000 cats in 6 years, more than 400,000 cats in 7 years, and more than 13 million cats in 9 years.

In addition to controlling the numbers of puppies and kittens born, prepuberal gonadectomy could prove beneficial in controlling the number of dogs and cats surrendered to animal shelters and impoundment facilities because of undesirable behaviors. Many "adolescent" animals are abandoned or relinquished to shelters when they begin to exhibit certain hormonally influenced behaviors. The role of animal behavior has been the focus of many researchers and trainers and also has

been the focus of the National Council on Pet Population Study and Policy. In personal interviews, people surrendering their dogs and cats to 12 animal shelters in 4 regions of the United States listed 71 reasons for relinquishment. Many of the reasons cited were categorized as "behavioral," suggesting that future intervention strategies should include those designed to modify animal behavior. Retaining pet animals in their homes is an issue of ethical and economical significance to the veterinary profession. The public expects the veterinary profession to help prevent the euthanasia of millions of healthy dogs and cats each year. In return the veterinary profession experiences increased income as animals are allowed to age in permanent and loving homes. Information collected by Professional Software Inc. (Hills) verified what many pet owners already know: More money is spent per year for veterinary services as a pet ages. Thus retaining a dog or cat in a good home makes ethical and economic sense.

Although numerous scientific studies now suggest that neutering puppies and kittens is safe, veterinarians also want to be assured that the procedure does not result in long-term adverse effects on health or behavior. Early-age neutering has now been performed on puppies and kittens for several decades in the United States; therefore it seems unlikely that the procedure is associated with significant health or temperament risks.

In fact, there are data suggesting that early-age neutering may be associated with health and temperament benefits.

Physical and Health Considerations

Growth

Growth plate closure is delayed in dogs and cats that are neutered before puberty, but this delay generally does not result in animals that are notably taller or larger. Individual dogs of large breeds neutered at 7 weeks may appear "lankier" compared with intact siblings. Owners of individual animals, however, rarely notice this subtle difference. The lanky appearance is not as apparent in smaller breeds of dogs or cats, even when littermates are available for comparison. The rate of bone growth is not affected by gonadectomy, but the growth period is extended because of the delayed growth plate closure. This can result in greater final radial or ulnar length when measured on radiographs.

Weight

Although obesity can occur in both neutered and intact animals and is influenced by a number of factors such as diet and activity level, neutered animals may gain significantly more weight than those remaining intact.

Information regarding 8268 dogs from 11 veterinary practices in the United Kingdom revealed that spayed female dogs were approximately twice as likely to be obese as intact female dogs. However, in a study of working dogs, no change in body mass was found among sham-operated controls, ovariectomized dogs, or ovariectomized dogs with ovarian autografts. Perhaps exercise differences among the working dogs and those presented to the 11 veterinary practices accounted for the different results. Neutered cats are also more likely to weigh more than sexually intact cats. Neutered cats have relatively more falciform fat, higher body condition scores, and higher body mass indices than intact cats. Heat coefficient, a measure of resting metabolic rate, is lower in neutered cats, leading to the recommendation that neutered male cats be fed 28% fewer calories than intact male cats and that neutered female cats be fed 33% fewer calories than intact female cats.

Urethral Function

The incidence of estrogen-responsive urinary incontinence is increased in neutered female dogs. In some spayed dogs, urinary incontinence develops within days of ovariohysterectomy, and in others, not until years later. In one study the average onset was 2.8 years after surgery. Estrogen-responsive urinary incontinence was reported in 34 (4%) of 791 bitches spayed at traditional

ages and in 7 (0.3%) of 2434 of sexually intact bitches. In a study of 269 dogs neutered at animal shelters at various ages (either younger than or older than 24 weeks), follow-up surveys of owners revealed that one dog neutered at the earlier age and two dogs neutered at the later age developed urinary incontinence. In another study of 206 bitches spayed before their first estrus that had been neutered for at least 3 years, urinary incontinence occurred in 9.7% of the bitches. This incidence was approximately half of that for dogs evaluated in the same study that had been spayed after the first estrus. However, compared with late spaying, the clinical signs of urinary incontinence were more distinct after early spaying. To date, there is little information to suggest that urinary incontinence is increased in puppies neutered as early as 7 weeks of age, something many veterinarians initially feared might correlate with an earlier age of spaying. However, continued assessment of the incidence and magnitude of urinary incontinence in puppies neutered at various ages is still important for long-term assessment of the procedure.

The incidence of urinary incontinence is very low in cats and male dogs, regardless of neuter status. When incontinence does occur in the cat, it is more likely to be associated with a neuropathy or with feline leukemia virus infection than with a hormonally mediated disorder.

Physical Characteristics of the Penis, Prepuce, Urethra, and Vulva

The penis, prepuce, and os penis are infantile in puppies who are neutered as early as 7 weeks of age compared with puppies neutered at traditional ages or those remaining sexually intact. The infantile secondary sex characteristics have not been associated with an increased risk of balanoposthitis. Complete penile extrusion is not possible in many cats neutered at traditional or early ages. The clinical significance of failure to extrude the penis in the cat is unknown, although it can increase the difficulty of catheterizing animals that require such a procedure for urethral obstruction. Urethral diameters as determined by contrast retrograde urethrography are similar among neutered and intact cats. In addition, the incidence of urethral obstruction in 263 cats adopted from shelters and neutered at less than 24 weeks of age or more than 24 weeks of age did not differ during the follow-up period of the study.

Vulvas of puppies neutered before puberty appear smaller when compared with intact bitches but can also appear "infantile" in intact bitches during anestrus or in some bitches spayed later in life. Although perivulvar dermatitis has been associated with weight gains and recessed vulvas after ovariohysterectomy, there is little information to suggest that the occurrence is higher in bitches spayed at early ages over those neutered at traditional ages.

Behavioral Implications

Neutering male dogs should most readily affect sexually dimorphic behaviors, such as mounting, copulatory intromission, display of ejaculatory patterns, urine marking, roaming, and aggressive dominance toward owners and other dogs. Because such behaviors can be objectionable to pet owners, owners may request castration after such behaviors develop. Although many of these behaviors are eventually lost or diminished after castration, it may take several weeks for the behavior to abate. Urine marking, mounting, and fighting generally are diminished after castration in roughly half of male dogs. Roaming is reduced in almost all male dogs after castration. Conversely, there seems to be little improvement in dogs exhibiting territorial aggression or fear aggression and limited improvement in aggressive dominance toward owners.

Objectionable sexual behaviors in male cats include fighting, urine spraying, and roaming. Castration is effective in eliminating these problem behaviors in 80%-90% of the affected cats.

Although many studies have evaluated the behaviors of adult animals after castration, some research suggests that objectionable behaviors in puppies and kittens may relate to intrauterine position or exposure to androgens during gestation. To date, there are limited data regarding whether neutering puppies and kittens at 7 weeks of age will reduce the incidence of undesirable sexually dimorphic behaviors.

There is also considerable interest in whether the age of neutering affects the outcome of guide or service dog trainability. In an observational study conducted at a service dog organization, the success of dogs trained to provide assistance to disabled people was measured in relation to age at neutering. The graduation rate was 28% for dogs neutered at traditional ages (age 7 months, $n = 76$ dogs) and 39% for dogs neutered early (age 7 weeks, $n = 77$ dogs). There is always concern that such improved "success" may result from the placebo effect, which is hard to conceal from evaluators because male dogs neutered early have altered secondary sex characteristics, such as an infantile prepuce. Nevertheless, many professional service and guide dog instructors are convinced that more male dogs become reliable guides when neutered early. In an epidemiologic study conducted on a data set at Guide Dogs for the Blind, records were evaluated from dogs born after January 1, 1988, that had successfully been trained for a blind or visually impaired student. The data set consisted of 6393 dogs with a gender distribution of 47.8% females and 52.2% males. Breeds represented were Labrador retrievers, golden retrievers, German shepherd dogs, and Labrador–golden retriever crosses. When two dogs with a difference of 6 months in their ages at the time of neutering (with all other factors being equal) were compared, the dog neutered at a younger age was 1.3 times as likely to experience training success. A prospective

study is now in progress at Guide Dogs for the Blind, with more than 100 Labrador–golden retriever male dogs already entered into the study. Puppies are assigned to groups by birth sequence and are later neutered at 7 weeks, 7 months, or 12 months of age. Dogs in the study will be evaluated for various behaviors throughout puppy raising and formal guide-dog training.

Surgical and Anesthetic Considerations

Anesthetic and surgical considerations for the pediatric patient include the potential for hypoglycemia and hypothermia, a relatively small blood volume, and the delicate nature of the pediatric tissues. Because hepatic glycogen stores are minimal in neonates, prolonged fasting may result in hypoglycemia. Food should be withheld no longer than 8 hours before surgery, with 3-4 hours recommended for the youngest patients (6-7 weeks of age). Animals may be fed a small meal within 1-2 hours after recovery from anesthesia. Hypothermia can be lessened by using warm water blankets and by giving warmed intravenous fluids. Minimizing surgery time also helps lessen the severity of hypothermia. Excessive wetting of the pediatric patient during preparation of the surgical site should be avoided, and the use of warmed scrub solution (chlorhexidine) and avoidance of alcohol are beneficial in helping preserve body heat.

Pediatric tissues are very friable and should be handled carefully. The relatively small blood volume of pediatric patients makes meticulous hemostasis very important. Fortunately, the small size of blood vessels and the presence of minimal abdominal and ovarian bursal fat allow for excellent visualization of the vasculature and make precise hemostasis simple to achieve.

Pediatric ovariohysterectomies are performed similarly to adult ovariohysterectomies. In puppies the abdominal incision is started relatively more caudal to the umbilicus than in adult dogs. Generally, the uterus is more easily exposed in puppies if the incision is started at least 2-3 cm caudal to the umbilicus (resulting in an incision placed nearer the middle third of the distance from the umbilicus to the cranial brim of the pelvis, similar to a feline incision). In kittens the incision is placed in a similar location as in adult cats. As the abdomen is entered, it is common to encounter substantial amounts of serous fluid in both puppies and kittens. It may be necessary to remove some of the fluid using gauze sponges to improve visualization. It is recommended that a Snook ovariohysterectomy hook not be used because of the delicate nature of the tissues. The uterus is easy to locate by looking between the urinary bladder and colon. Uterine tissue is extremely friable; therefore care must be taken to avoid excess traction.

When the abdomen is being closed, it is important to carefully identify the ventral fascia and the overlying

subcutaneous tissue because they can occasionally be difficult to differentiate, particularly in some puppies. The subcuticular layer can be closed with an absorbable suture material in a continuous intradermal pattern to avoid the use of skin sutures. Alternatively, skin sutures can be loosely placed after closure of the subcutaneous tissues.

Puppy castration also is performed with modifications to the techniques used in adult dogs. As with adult canine castrations, it is important to ascertain that both testes have descended before commencing surgery. Because of the small size and mobility of puppy testes, the entire scrotal area can be clipped and surgically prepped to permit the scrotum to be included in the sterile field. This will greatly facilitate testis localization and manipulation and does not cause scrotal irritation, as in adult dogs, because the scrotal sac is not yet well developed. Puppies can be castrated through a single midline prescrotal or scrotal incision. Alternatively, two scrotal incisions can be used, similar to a feline castration. Closed castration is performed via a standard technique. Incisions can be closed using one to two buried interrupted sutures in the subcuticular layer, or incisions can be left open to heal by second intention. Closure of the incisions prevents postoperative contamination with urine or feces and prevents fat from extruding from the incision.

Kitten castration is performed using techniques that are identical to those used in the adult cat. Care should

be used when exteriorizing testes to prevent tearing of the spermatic cord that might occur because of its small size. As with adult cats, incisions are left open to heal by second intention.

In one study of 32 pairs of male and 36 pairs of female puppies neutered at approximately 2 or 7 months of age, recovery from anesthesia was uneventful in both groups and faster in the younger animals. In another study of 1213 dogs and 775 cats that were obtained from two humane organizations and that underwent ovariohysterectomy or castration performed by fourth year veterinary students, the duration of surgery was shorter in animals younger than 12 weeks than in older animals. When a survey was conducted, the veterinary students who participated in the program indicated that their confidence in performing anesthesia and surgery on young animals had increased overall, proving useful in situations in which surgery other than gonadectomy was needed for pediatric patients.

Summary

Prepuberal gonadectomy, or early-age neutering, is safe for puppies and kittens if anesthetic and surgical procedures are modified appropriately. Neutered animals may have a propensity to gain weight compared with intact animals. Thus caloric requirements may need to be adjusted or exercise programs implemented to

maintain ideal body weight for some neutered animals. Although scientific studies to date suggest that there are minimal risks for neutering puppies and kittens as early as 7 weeks of age, long-term studies of the incidence of various disorders associated with early-age neutering should continue to be conducted. The benefits and risks should be carefully evaluated for each species, breed, and individual.

Bibliography

Crenshaw WE, Carter CN: Should dogs in animal shelters be neutered early? *Vet Med* 90:756, 1995.

Edney AT, Smith PM: Study of obesity in dogs visiting veterinary practices in the United Kingdom, *Vet Rec* 118:391, 1986.

Faggella AM, Aronsohn MG: Anesthetic techniques for neutering 6- to 14-week-old kittens, *J Am Vet Med Assoc* 202:56, 1993.

Faggella AM, Aronsohn MG: Evaluation of anesthetic protocols for neutering 6- to 14-week-old pups, *J Am Vet Med Assoc* 205:308, 1994.

Hart BL, Eckstein RA: The role of gonadal hormones in the occurrence of objectionable behaviors in dogs and cats, *App Anim Beh Sci* 52:331, 1997.

Howe LM, Olson PN: *Prepuberal gonadectomy: early-age neutering of dogs and cats,* International Veterinary Information Service, 2000. Available at www.ivis.org.

Lieberman LL: A case for neutering pups and kittens at two months of age, *J Am Vet Med Assoc* 191:518, 1987.

Lindsay SR: *Handbook of applied dog behavior and training,* Ames, Iowa, 2000, Iowa State University Press.

Manning AM, Rowan AN: Companion animal demographics and sterilization status: results from a survey in four Massachusetts towns, *Anthrozoos* 4:192, 1992.

Massachusetts Society for the Prevention of Cruelty to Animals: *Spay/neuter summary,* Boston, 1991, MSPCA.

Olson PN: *Questions and answers on the effects of prepuberal gonadectomy: the case for early neutering,* Englewood, 1996, American Humane Association.

Root MV: The effect of prepuberal and postpuberal gonadectomy on the general health and development of obesity in the male and female domestic cat, doctoral thesis, Minneapolis, 1995, University of Minnesota.

Scarlett JM, Salman MD, New JG et al: Reasons for relinquishment of companion animals in U.S. animal shelters: selected health and personal issues, *J Appl Anim Welfare Sci* 2:41, 1999.

Stocklin-Gautschi NM, Hassig M, Reichler IM et al: The relationship of urinary incontinence to early spaying in bitches, *J Reprod Fertil Suppl* 57:233, 2001.

Thrusfield MV: Association between urinary incontinence and spaying in bitches, *Vet Rec* 116:695, 1985.

7

Infectious Diseases of the Reproductive Tract of the Bitch

Peter R. Morresey

AT A GLANCE

- Infectious diseases of the queen: discussed in detail in Chapter 19
- Canine brucellosis *(Brucella canis)*

- Ingestion is the most common natural route of transmission.
- Agglutination tests are good screening tests; all positive test results should be verified by culture or agar gel immunodiffusion testing.
- Affected dogs often are asymptomatic. The classic sign of infection in female dogs is late gestation abortion. The classic signs in male dogs are scrotal dermatitis, epididymitis, and poor semen quality.
- Antibiotic therapy decreases bacteremia and antibody titers but is unlikely to eradicate the organism from the animal's body.

- Canine herpesvirus (CHV)
  - Most animals are asymptomatic when infected with CHV and readily fight off disease.
  - Animals likely to show clinical evidence of CHV infection are naïve bitches during the last 3 weeks of gestation (stillbirths, mummified pups, macerated pups, and normal pups, all born in one litter) and neonates during the first 3 weeks of life (acute onset of hemorrhagic septicemia and death).
  - Affected bitches usually lose only one litter. Isolate naïve bitches and pups. No vaccine is available.
- Bacteria and mycoplasma
  - These organisms are ubiquitous and are part of the normal vaginal flora; pathogenicity is difficult to determine in a given bitch, especially if she is asymptomatic for reproductive tract disease.

Canine Brucellosis

Definition and Pathogenesis

Brucella canis is the only bacterial organism proven to cause infertility in the bitch. The host range is limited compared with other species of *Brucella.* Only domestic dogs and wild Canidae are susceptible; other domestic animals experimentally are resistant. Canid infections with *Brucella melitensis, Brucella suis,* and *Brucella abortus* have been reported.

Several routes of natural transmission are recognized. Most commonly, ingestion or inhalation via oronasal or conjunctival contact with aborted materials is suspected. Other sources of exposure include vaginal discharges after abortion and during estrus and urine and milk (low concentration). Venereal and transplacental transmission also may occur.

The *Brucella* organism penetrates the mucous membranes of the oral cavity, vagina, and conjunctiva. Phagocytosis occurs at the site of mucosal infection. Transport to lymphatic and genital tissues ensues, and multiplication occurs at those sites. Bacteremia begins 1-4 weeks after infection and lasts 6-64 months. Lymphoreticular hyperplasia and hyperglobulinemia occur. Antibodies are produced but are nonprotective and have little effect on organism quantity. The greatest numbers of *Brucella* organisms are found in lymph nodes, spleen, and gonadal steroid–dependent tissues.

The nongravid, diestrual uterus is not a favored site. *B. canis* usually is confined to mononuclear phagocytes but may enter the placental epithelium. Endarterial circulation filters bloodborne organisms and immune complexes, resulting in a wide range of disease foci and clinical presentations, including the intervertebral discs (discospondylitis), eyes (anterior uveitis), kidneys (glomerulonephritis), and meninges (meningoencephalitis).

The highest concentration of *B. canis* organisms is found in vaginal discharge, making this the most likely source of infection after aborted material. Shedding of organisms into vaginal discharge continues for up to 6 weeks after abortion. Urinary excretion of *B. canis* begins a few weeks after onset of bacteremia and continues for at least 3 months. *B. canis* is short lived outside the host and is readily inactivated by common disinfectants; fomite transmission may occur.

Spontaneous recovery may occur 1-5 years after infection. Bacteremia may persist during this time. Appropriate therapy accelerates recovery. Tissue persistence may occur, and in the absence of bacteremia serum, antibody levels decline. Naturally recovered dogs may have low or negative serum antibody concentrations but are immune to reinfection because of protective cell-mediated immunity. Immunosuppressive therapy increases initial susceptibility to *B. canis* infection but does not alter the clinical course of the disease.

Signalment

No age or breed predisposition has been reported.

History and Clinical Signs

Disease most often is inapparent. Infection with *B. canis* does not interfere with normal estrous cycling. Fever is an uncommon clinical sign. Overt clinical signs usually involve reproductive disturbances in sexually mature animals. Conception failure may occur. Early embryonic loss can occur any time after breeding. There may be inapparent abortion caused by either resorption of embryos early in gestation or ingestion of abortuses by the bitch in late gestation. The most common presentation is abortion in late gestation (45-60 days) without signs of maternal illness in previously healthy females. If pregnancy reaches term, both live and dead pups may be whelped.

Physical Examination Findings

Late-term abortion is characterized by brown or gray-green vaginal discharge that persists for 1-6 weeks. Aborted pups often are partially autolyzed, suggesting death some time before abortion.

Pups born live to infected bitches die within hours to days. If pups survive longer, generalized lymphadenomegaly, hyperglobulinemia, recurrent fever,

leukocytosis, and seizures can occur. Splenomegaly, osteomyelitis, multifocal pyogranulomatous dermatitis, anterior uveitis, and endophthalmitis accompanied by hemorrhage also have been reported.

Diagnostic Tests and Results

Canine brucellosis is a reportable disease in most states of the United States.

BACTERIAL ISOLATION Isolation of *B. canis* is the definitive diagnostic procedure. The organism grows well in aerobic culture on media for other *Brucella* species. However, growth is slow and *Brucella* may be overwhelmed by contaminants.

- Bacterial culture: Preferred samples include aborted material and vulvar discharge.
- Blood culture: Whole blood (5 ml, heparinized, refrigerated) is required for isolation as the organism persists within macrophages. Bacteremia is sustained but may be intermittent in chronic cases. Do not use blood cultures as the sole diagnostic test performed if results are negative.
- Urine: Culture of urine may be positive in dogs infected with *B. canis;* collect urine samples by cystocentesis to avoid commensal overgrowth.
- Necropsy: Fetal necropsy findings include subcutaneous edema, congestion, subcutaneous hem-

orrhage, and serosanguineous peritoneal effusion. The organism is recoverable from internal organs or lymph nodes of pups born live and from stomach content of stillborn pups. If an infected bitch dies or is euthanized, the organism can be recovered from lymph nodes, spleen, liver, bone marrow, gravid or estrual uterus, placenta, and vaginal and uterine fluids.

SEROLOGIC TESTING For all serologic tests, at least 2 ml of serum needs to be collected and refrigerated until analyzed. Compared with bacterial isolation, serologic tests have the advantage of being rapid, easy to perform, and widely available. However, although sensitive, most are nonspecific for *B. canis*. Serologic tests often are negative during the first 3-4 weeks after infection, despite bacteremia. Antibody titers are high in response to bacteremia (1:400-1:3200) but decline and become equivocal as bacteremia ceases (1:50-1:200). Chronically infected females display equivocal antibody titers and negative blood cultures. Recrudescence of bacteremia and elevation in antibody titers occur during proestrus, estrus, pregnancy, and abortion, making these the most reliable times to test. Antibiotics may suppress bacteremia and associated serologic responses. For this reason, it is important to avoid antibiotic treatment of individuals or infected kennel populations before screening is complete.

Agglutination tests

Rapid card agglutination test The rapid card agglutination test (RCAT) is rapid, inexpensive, sensitive, and highly specific (99%) and detects antibodies early. False-positive results occur through cross-reaction with *Bordetella* species, *Pseudomonas* species, and other bacteria. Addition of 2-mercaptoethanol (2-ME) reduces false-positive results by eliminating less specifically reacting heterologous immunoglobulin M (IgM). The RCAT for canine brucellosis is commercially available for in-hospital use (Synbiotics, San Diego, Calif.).

Tube agglutination test. The tube agglutination test (TAT) is widely used, improperly, as a confirmatory test for dogs positive for *B. canis* by the RCAT with 2-ME. False-positive results occur because of the presence of bacteria other than *Brucella* species, as for RCAT. False-positive results also may be caused by hemolysis of the serum sample submitted. Significant titers develop approximately 5-8 weeks after infection. The TAT is available through most diagnostic laboratories.

Both the RCAT and TAT can be considered screening tests. Lipopolysaccharide (LPS) antigens of several bacterial species cross-react with *B. canis*. False-positive results occur more commonly than do false-negative results.

Agar gel immunodiffusion Agar gel immunodiffusion (AGID) is a sensitive test for *B. canis* that uses either cell wall antigen (somatic, sAg) or cytoplasmic antigen (cAg). The

latter is highly specific for *B. canis* infection and can be used as a confirmatory test for dogs that are serologically positive by the RCAT or TAT. The AGID may detect infection with other *Brucella* species, but infection with other than *B. canis* is uncommon in dogs. Testing for sAg is positive earlier than for cAg (5-10 weeks versus 8-12 weeks, respectively). The AGID is available only through the veterinary diagnostic laboratory at Cornell University (Ithaca, NY).

Enzyme-linked immunosorbent assay The enzyme-linked immunosorbent assay (ELISA) is a very specific test but is less sensitive than screening tests. It also is available through the veterinary diagnostic laboratory at Cornell University.

Indirect fluorescent antigen With indirect fluorescent antigen (IFA), the organism is directly visualized in the test substrate. This is a useful screening test, but it is not readily available.

General principles for serologic testing for diagnosis of canine brucellosis

- Very early in infection, bacterial isolation is superior to serologic testing for identification of infected individuals. False-negative culture results may occur because of difficulty growing the organism.
- A single high titer with the TAT may indicate active infection, but confirmation is required.

- Asymptomatic animals with positive agglutination test (RCAT or TAT) results should not be considered positive for brucellosis until the infection is confirmed with blood culture or with cytoplasmic antigen testing (AGID or ELISA).
- With the exception of cytoplasmic antigen testing by AGID or ELISA, all tests measure antibodies to LPS and can therefore give false-positive results. Negative results may occur with recent infection; testing should be repeated in 30 days.

MISCELLANEOUS DIAGNOSTIC TESTS Changes on complete blood count and serum chemistry profiles are nonspecific. Hyperglobulinemia with associated hypoalbuminemia may be present in cases of chronic infection. Some animals infected with brucellosis have positive results on Coombs' test in the absence of anemia. Abnormalities consistent with dickospondylitis or osteomyelitis may be seen radiographically, as may signs of fetal death just before abortion (e.g., gas within or around fetuses, collapse of the axial skeleton, overlap of cranial bones). Urinalysis findings usually are unremarkable despite intermittent bacteriuria.

Treatment

Control in kennel situations often requires euthanasia of infected animals. If animals are to be maintained,

long-term combination drug therapy is necessary, yet it is unable reliably to effect a cure. The intracellular location of *Brucella* organisms makes achievement of effective antibiotic levels difficult. Nonbreeding companion animals should receive an appropriate course of antibiotic therapy and be neutered. Tissue persistence of the organism has been demonstrated after ovariohysterectomy or castration, but shedding of *Brucella* is considered less likely after removal of gonadal steroids.

The most effective antibiotic regimen reported involves a combination of minocycline or doxycycline (25 mg/kg administered orally once daily for 4 weeks or 12.5 mg/kg administered orally twice a day for 4 weeks) with streptomycin (20 mg/kg administered once daily intramuscularly or subcutaneously) for the first 2 weeks. Restricted availability of streptomycin has led to use of the less reliably efficacious gentamicin at the same dosage. Fluoroquinolone therapy has been demonstrated to be efficacious in vitro, but use as a single agent is associated with relapse.

Testing for success of treatment at cessation of therapy alone may miss recurrence of bacteremia and is not recommended. Response is evaluated by retesting with culture and serology 6 months after the completion of antibiotic treatment. Reinstatement in breeding programs of animals that are negative for canine brucellosis after antibiotic treatment is very risky and is strongly discouraged. Clinically normal but persistently

infected females may transmit infection to male and offspring.

Control

- Test newly acquired animals at least twice at 30-day intervals before introduction to the breeding kennel. A low titer may indicate previous or recent infection; repeat serologic testing or attempt isolation of the organism.
- Remove proven positive animals from breeding programs. Recommend neutering or euthanasia of infected animals.
- In an infected kennel, strict hygiene procedures within the colony and isolation from the general population are indicated. Remove all positive animals immediately when found and continue to test all animals in the facility at monthly intervals until three consecutive tests find all animals in the kennel to be concurrently negative. This process may take 6-9 months. Regularly monitor by testing all animals in the kennel at 3- to 6-month intervals.

Prognosis

Prognosis is guarded for cure without recrudescence. Owners must be informed that although relatively

uncommon, human infection is possible with any species of *Brucella*, including *B. canis*.

Canine Herpesvirus

Definition and Pathogenesis

The CHV host range is restricted to domestic and wild Canidae. Biologic behavior is similar to α-herpesviruses infecting other species. Both humoral (B cell) and cell-mediated (T cell) responses occur, the latter being essential for CHV control. Concurrent disease affecting T-cell function may enhance pathogenesis. CHV is readily inactivated by most common disinfectants and lipid solvents and by acidic conditions (pH value less than 5) and heat.

In adult animals, transmission is primarily via oronasal secretions, by either direct or aerosol contact. Venereal spread occurs rarely. Virus replication is limited to the nasopharynx, genital tract, tonsillar tissue, and retropharyngeal and bronchial lymph nodes. CHV persists in the tonsils and parotid salivary glands. Localized genital, respiratory, and conjunctival infection can result in virus shedding despite the presence of circulating antibodies. Latency is established at the site of initial infection, usually the nerve ganglion innervating the oropharyngeal region. In adult dogs, high prevalence is associated with a history of repeated exposure

(80%-100% in show or kennel dogs). Female dogs recently introduced to a new or stressful environment are particularly susceptible. Recrudescence has been reported to occur after corticosteroid administration.

In neonatal pups infection most often is acquired transplacentally or during passage through the birth canal after exposure to cervical and vaginal secretions. Oronasal secretions from recently infected littermates or the dam are responsible for infection after birth. Lack of both normal thermoregulation and immune competence make neonates susceptible to rapidly fulminating disease.

Signalment

All canine population subgroups are susceptible to CHV infection. Infection usually is inapparent in all dogs except pregnant females and neonates (younger than 3 weeks).

History

In adult dogs the classic presentation of a bitch infected with CHV is loss of a previously confirmed pregnancy or birth of abnormal or nonviable pups. Stillborn pups, pups with low birth weight, and healthy pups all may be born in the same litter.

In affected neonates, death that occurs at less than 1 week of age indicates in utero exposure. Between

1 and 3 weeks of age, postnatal infection results in rapidly fulminating disease. Lack of adequate colostral transfer or maintenance in an environment with relatively low ambient temperature may predispose neonates to clinical disease.

Physical Examination Findings

In infected bitches, self-limiting hyperemia and lymphoid nodules may be present on the vaginal mucosa. Occasionally, submucosal hemorrhage may occur. Mild upper respiratory disease with serous nasal discharge also may occur.

Infected neonates may be born dead, mummified, or macerated or may be born alive but weak. Pups born infected at or after birth classically present with signs of acute hemorrhagic septicemia, hypoglycemia, and necrosis of extremities caused by vasculitis with resultant thrombocytopenia. Animals that survive the septicemia may demonstrate interstitial pneumonitis, encephalitis, cerebellar and retinal dysplasia, and segmental renal necrosis.

Diagnostic Tests and Results

SEROLOGIC TESTING CHV is poorly immunogenic, resulting in a poor, short-lived antibody response. Paired serum samples, collected 10-14 days apart, need

to be submitted for testing. Titers that rise and fall rapidly (4-8 weeks) after exposure indicate a diagnosis of spontaneous abortion resulting from CHV. Titers of 1:2-1:32 are considered low, but any titer greater than 1:2 with suggestive clinical signs is diagnostically significant. A fourfold rise in titer is indicative of active infection.

VIRUS ISOLATION, FLUORESCENT ANTIGEN CHV may be recovered from nasal or vaginal swabs or from fetal endothelium, liver, adrenals, lung, spleen, kidney, and lymph nodes during necropsy of infected pups.

HISTOPATHOLOGY Pathognomonic lesions present in formalin-fixed tissues include placental necrosis, visible grossly as multifocal, small, gray-white foci with detectable viral inclusion bodies; necrosis and intranuclear inclusion bodies in liver, spleen, kidney, lung, and heart of aborted fetuses; and necrosis and hemorrhage of the kidney, liver, spleen, lungs, and adrenals and serosanguineous effusion of body cavities in infected neonates.

HEMATOLOGIC AND SERUM CHEMISTRY PROFILE VALUES Nonspecific changes are present in affected adults. Marked thrombocytopenia is present in infected neonates.

Treatment

In adults treatment is unnecessary. Infection is self-limiting in immune-competent animals, and clinical signs, if apparent, spontaneously resolve.

In neonates survival with antiviral (acyclovir) therapy has been reported, but neurologic and myocardial damage may persist. Recovering puppies shed large amounts of virus in secretions for 2-3 weeks. Neonatal prophylaxis in infected kennels may be achieved by intraperitoneal or subcutaneous administration of immune serum from previously infected animals. Viral transfer via fomites can be avoided with good hygiene and isolation protocols. Adequate intake of colostrum and good nutrition must be ensured. In addition, adequate environmental temperature must be maintained because CHV requires low temperatures for replication. Raising the environmental temperature of clinically affected neonates usually does not alter the course of disease.

Control

Eradication is not practical because CHV is endemic in the population. Serologic testing is not useful because the poor antigenicity of CHV precludes development of persistently elevated titers in all exposed animals. No commercial vaccine is available.

Susceptible females should be adapted to the kennel environment for a sufficient period before breeding.

Infection must be avoided during the risk period, which is from 3 weeks before until 3 weeks after whelping. Infection of the litter can be prevented by isolating suspected naïve females during the third trimester and until the pups are 3 weeks old.

Prognosis

Prognosis in adult dogs is good. Previous exposure and abortion do not preclude future successful reproduction. Colostral antibodies from previously infected dams are transferred to subsequent litters, which are protective for clinical disease but not for inapparent infection during the first 3 weeks of life. Recrudescence and abortion in subsequent pregnancies occur rarely.

Overt clinical disease in neonates has a grave prognosis. CHV usually is fatal to pups infected at or immediately after birth.

Bacteria and Mycoplasmal Infections

Bacterial Infection

DEFINITION AND PATHOGENESIS The most important bacterial agent infecting the reproductive tract is *B. canis*. Opportunistic infections by vaginal flora can cause abortion. The organisms most commonly

reported are *Escherichia coli, Streptococcus* species, *Salmonella* species, and *Campylobacter* species. A predisposing cause often is present, such as cystic endometrial hyperplasia (CEH) in older females. The most serious bacterial infection of the female reproductive tract is pyometra (see Chapter 12).

SIGNALMENT Cycling females of breeding age may undergo ascending infection anytime the cervix is open (e.g., proestrus, estrus, postpartum). Susceptibility may increase with repeated progesterone exposure from successive estrous cycles because of the development of CEH.

HISTORY Bacterial infections may occur after a recent estrous cycle or in the presence of a previously diagnosed uterine pathologic abnormality.

PHYSICAL EXAMINATION FINDINGS Overt vulvar discharge may be present. Vaginal cytologic examination reveals high numbers of degenerative neutrophils. Signs of generalized illness, such as fever and lethargy, also may occur.

DIAGNOSTIC TESTS AND RESULTS Samples from the anterior vagina may be collected, with a guarded swab if possible, and submitted for culture. Heavy growth of a single organism is significant. Neutrophilia may be present in

systemically ill bitches. Pups that are born dead or that die as a result of septicemia may be submitted for necropsy and culture of internal organs and stomach content.

TREATMENT Supportive treatment with fluids and antipyretics is required if systemic illness is present. The clinician should encourage uterine evacuation if the cervix is patent. One effective drug regimen is prostaglandin $F_{2\alpha}$ at a dosage of 0.1-0.25 mg/kg administered subcutaneously twice a day. If closed cervix pyometra or presence of a macerated fetus is suspected, surgical intervention is indicated (see Chapter 12). Parenteral therapy with a broad-spectrum antibiotic should be instituted pending culture results; a prolonged treatment course may be necessary to effect a cure.

PROGNOSIS Aerobic bacteria can be eliminated completely only when the vaginal flora is sparse initially. Recolonization occurs immediately upon cessation of antibiotic therapy. Underlying defects of the reproductive tract, such as CEH, must be addressed if present. Severity of associated systemic illness alters management of the patient and increases the risk of a negative outcome.

Mycoplasmal Infection

DEFINITION AND PATHOGENESIS Mycoplasmas (genera *Mycoplasma, Ureaplasma, Acholeplasma*) are present in

natural mucosal flora, are fragile outside the host, and depend on their environment for nutrients. Mucous membranes of the respiratory and urogenital tracts provide ideal conditions for colonization. Lack of a rigid cell wall allows resistance to cell wall–active antibiotics, such as penicillins and lysozyme. Few mycoplasmas have been proven to be pathogenic. Mycoplasmas are involved in inflammation of mucosal or serosal surfaces when organisms are present in sufficient numbers and when host factors are favorable. These organisms may become intracellular, resulting in persistent infections. Canine respiratory and genital tracts contain the same species of mycoplasma; spread can occur by airborne droplets and nasal contact in addition to genital contact.

SIGNALMENT These organisms are part of the normal flora of the reproductive tract of sexually mature females.

HISTORY The owners may report presence of a concurrent bacterial genital infection or previous antibacterial treatment for an unrelated condition.

PHYSICAL EXAMINATION FINDINGS Inflammation of mucosal or serosal surfaces of the reproductive tract may be present and may be evidenced by mucopurulent vulvar discharge characteristic of vaginitis.

DIAGNOSTIC TESTS AND RESULTS Mycoplasma organisms are fragile outside a host. Samples for culture must be refrigerated and shipped on ice within 24 hours. Samples can be frozen and shipped with dry ice if longer transport time is necessary. Vaginal culture specimens can be collected with cotton swabs and grown out on Amies medium (noncharcoal) or Hayfields broth medium. Isolation of mycoplasmas from the vagina does not constitute a basis for diagnosis of disease of the caudal reproductive tract because mycoplasmas often are recoverable as part of a mixed bacterial infection. Isolation of mycoplasma organisms from internal organs of aborted fetuses is significant. Growth of *Mycoplasma* species or *Ureaplasma* species from biopsy specimens recovered in a sterile manner from the uterus, uterine tubes, or ovaries is significant because mycoplasmas are not a normal inhabitant of these areas.

TREATMENT Sensitivity data is not routinely available. Mycoplasmas are susceptible to macrolides, tetracycline, chloramphenicol, lincosamides, fluoroquinolones, and nitrofurantoin. Treatment for an extended period is required to eradicate the organism, and recolonization occurs almost immediately. With antibiotic therapy, susceptible bacteria rapidly are eradicated from the vagina. *Mycoplasma* species and *E. coli* emerge during and after antibiotic therapy. This is an argument against routine prophylactic use of antibiotics in healthy breeding

bitches. Tetracycline and chloramphenicol should be avoided in pregnant bitches.

PROGNOSIS The significance of these organisms in cases of reproductive failure is controversial. *Mycoplasma canis* has been isolated from dogs with endometritis, where it is considered to have an opportunistic role during mixed infections. *Ureaplasma* species are isolated more often from vaginal samples from infertile dogs than from reproductively normal dogs, but growth of *Mycoplasma* species in cultures of the vagina or semen is not significantly different between fertile and infertile dogs.

Bibliography

Carmichael L, Greene C: Canine brucellosis. In Greene C (ed): *Infectious diseases of the dog and cat,* Philadelphia, 1998, WB Saunders.

Carmichael L, Greene C: Canine herpesvirus infection. In Greene C (ed): *Infectious diseases of the dog and cat,* Philadelphia, 1998, WB Saunders.

Evermann J: Diagnosis of canine herpetic infections. In Kirk R, Bonagura J (eds): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Greene C: *Mycoplasma, Ureaplasma,* and L-form infections. In Greene C (ed): *Infectious diseases of the dog and cat,* Philadelphia, 1998, WB Saunders.

Hashimoto A, Hirai K, Okada K et al: Pathology of the placenta and newborn pups with suspected intrauterine infection of canine herpesvirus, *Am J Vet Res* 40:1236, 1979.

Johnson C: Management of *Brucella canis* outbreaks in breeding kennels. In Bonagura J, Kirk R (eds): *Current veterinary therapy XII,* Philadelphia, 1997, WB Saunders.

Linde-Forsberg C, Bölske G: Canine genital mycoplasmas and ureaplasmas. In Bonagura J, Kirk R (eds): *Current veterinary therapy XII,* Philadelphia, 1997, WB Saunders.

Miyoshi M, Ishii Y, Takiguchi M et al: Detection of canine herpesvirus DNA in the ganglionic neurons and the lymph node lymphocytes of latently infected dogs, *J Vet Med Sci* 61:375, 1999.

Nicoletti P: Diagnosis and treatment of canine brucellosis. In Kirk R, Bonagura J (eds): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Okuda Y, Ishida K, Hashimoto A et al: Virus reactivation in bitches with a medical history of herpesvirus infection, *Am J Vet Res* 54:551, 1993.

Poulet H, Guigal PM, Soulier M et al: Protection of puppies against canine herpesvirus by vaccination of the dams, *Vet Rec* 148:691, 2001.

Strom B, Linde-Forsberg C: Effects of ampicillin and trimethoprim-sulfamethoxazole on the vaginal bacterial flora of bitches, *Am J Vet Res* 54:891, 1993.

8

Pregnancy

Ahmed Tibary and Mushtaq Memon

AT A GLANCE

- Gestation length in bitches varies from 59-72 days from the first breeding, 64-66 days from the preovulatory surge in luteinizing hormone (LH), and 56-58 days from the first day of cytologic diestrus.

- Pregnancy diagnostic methods include the following:
  - Abdominal palpation: Abdominal palpation is best performed during days 22-30 of pregnancy. Gestational sacs are not palpable earlier and become confluent later. Mineralized pups may be palpable late in gestation.
  - Abdominal radiographs: Radiographs are definitive for pregnancy only after fetal mineralization has occurred, after days 42-45 of pregnancy. Radiography performed late in gestation is the best technique for assessment of litter size.
  - Abdominal ultrasonography: Gestational sacs can be identified reliably after about day 25 of pregnancy by using abdominal ultrasonography. Beating fetal hearts are first visible between days 22-29, and fetal movement can be identified after about day 28 of pregnancy. Real-time (B-mode) ultrasonography is the best technique for assessment of fetal viability.
  - The following should be considered regarding hormone assays:
    - Measurement of progesterone in serum is not useful for pregnancy diagnosis in bitches, which all have a prolonged luteal phase after estrus, or in queens, which maintain functional corpora lutea after induction of ovulation regardless of pregnancy status.
    - A relaxin assay is available for pregnancy diagnosis in dogs after about day 25 of pregnancy.

- Care of pregnant bitches includes the following:
  - Do not vaccinate or unnecessarily medicate pregnant bitches.
  - Maintain pregnant bitches on a good plane of nutrition and continue regular exercise.
  - Provide extra calories only in the second half of gestation.

A discussion of pregnancy diagnosis in the bitch and queen requires an understanding of behavior and hormonal events associated with the estrous cycle and breeding. Pregnancy length is highly variable in dogs, ranging from 59-72 days. Many dog breeders use the first or last breeding date as a reference point for subsequent pregnancy events. This is the least accurate method for staging pregnancy because there is a large variation in the duration of proestrus and estrus in the bitch. Reduced variability of pregnancy length and increased accuracy in prediction of whelping date can be obtained by determining the first day of diestrus by appearance of neutrophils and a preponderance of noncornified vaginal epithelial cells on vaginal cytologic examination (see Chapter 2). Whelping occurs in most bitches 57 ± 3 days after the first day of diestrus. An even more precise method for staging pregnancy and prediction of due date is the use of the LH peak as the reference date. Gestation length in the bitch is 65 ± 1 days from the LH peak. The day of the LH peak usually is determined based on increase

in plasma progesterone that precedes ovulation in the canine species (see Chapter 2). For this discussion of different techniques of pregnancy diagnosis, we assume, unless otherwise stated, that the reference date used is the day of the LH peak, which occurs 48 hours before ovulation.

Chronology of Fetal Development

Knowledge of chronologic events of the development of the embryo and fetus in the dog and cat, relative to the preovulatory peak of LH and coitus, respectively, drive the veterinarian's choice of the most appropriate pregnancy diagnostic technique (Table 8-1).

Methods of Pregnancy Diagnosis

Pregnancy diagnosis in the bitch and queen can be based on behavioral, physical, or hormonal changes or on precise clinical examination involving palpation or imaging of uterine contents. The main methods of imaging are radiography and ultrasonography. When a method of pregnancy diagnosis is being chosen, the most important criteria are the accuracy of the method, the practicality of the method, and the window of pregnancy during which the method is accurate.

Table 8-1. Chronologic Events of the Development of the Embryo and Fetus in the Dog and Cat Relative to the LH Peak

Event	Dog	Cat
Oocyte present in the uterine tube	3 to 5	4 to 5
Fertilization	3 days	2 days
Migration of early embryo into the uterus	9 to 13	4 to 8
Nonfixed, mobile uterine stages of the blastocyst	9-13 to 20-21	4-6 to 12
Implantation	18 to 20	12 to 14
Formation of embryonic vesicles	18 to 23	20 to 15
Individual gestational sacs	20 to 30-35	15 to 30-35
Confluent gestational sacs	30-35 to 45	30-35 to 40-45
End of embryogenesis	30-32	28-32
Beginning of ossification	40-42	38-40
Beginning of mammary development	30-42	45
Detection of fetal movement with transabdominal palpation	55	45

Modified from Verstegen J, Silva LD, Onclin K: *Ann Med Vet* 140:81, 1996;and Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology,* Philadelphia, 2001, WB Saunders.

External Examination and Behavioral Changes

External signs and behavioral changes are not good indicators of pregnancy status and easily could be confused with pseudopregnancy (see Chapter 14). The pregnant female shows mammary gland development, increase in laxity of the abdomen, and behavioral changes that may be appreciated subjectively. Breeders usually mention abdominal laxity at approximately day 25 of pregnancy and male attraction between days 30-35, but these are not objective criteria for the detection of pregnancy.

Weight gain generally occurs in the pregnant bitch. An average increase in body weight of 36.2 ± 1.7% (range, 20% to 55%) has been reported. However, many bitches may show increased body weight during diestrus without pregnancy, especially if the owner is not providing proper nutrition. Mammary development starts at approximately day 35 of pregnancy but also may be seen in nonpregnant animals.

Abdominal Palpation

Abdominal palpation to detect chorioallantoic swellings (gestational sacs) in the uterus is the oldest clinical method for the diagnosis of pregnancy in the bitch and queen. Its major advantage is that it does not require special equipment. However, this technique is limited in that it is not easily accomplished in all animals and the

window of pregnancy during which it is most accurate is very short. Abdominal palpation is difficult or even impossible in obese animals and in animals that are tense when their abdomen is palpated. This technique has poor accuracy for determination of litter size, particularly during the last trimester of pregnancy.

Pregnancy is diagnosed with abdominal palpation by gently isolating the uterus between the urinary bladder and the rectum immediately cranial to the cervix. Next, the uterine horns are examined from the caudal part to the cranial extremity between the thumb and fingers. Palpation of the gestational sacs is possible as early as 18 days but it is preferably performed during days 22-24 after breeding at the earliest in the bitch and during days 14-20 after breeding at the earliest in the queen. The gestational sacs in the caudal part of the uterine horns often are easiest to detect (Figure 8-1).

The chorioallantoic swellings are palpable for 7-15 days. At 28 days, these swellings average 3-5 cm in diameter. As pregnancy advances, the swellings increase in size, change to an oblong shape, and become confluent and difficult to distinguish. The enlarged fluid-filled uterine horns drop and become difficult to differentiate from other abdominal viscera, making diagnosis very difficult after day 30 of pregnancy. Palpation of mineralized fetuses becomes possible after day 40-45 of pregnancy. In practice, palpation for pregnancy in the bitch should be scheduled to be performed 26-28 days after first mating.

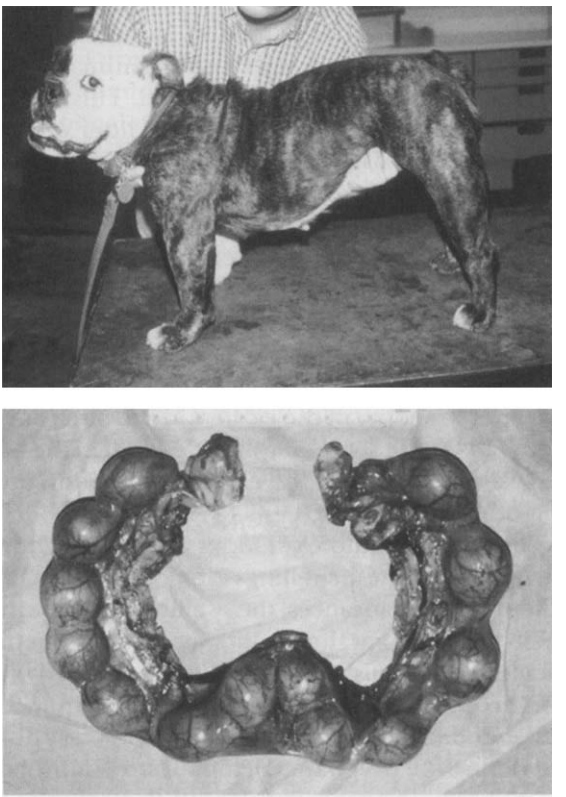


Figure 8-1. *Top,* Technique of abdominal palpation for pregnancy diagnosis in the bitch. *Bottom,* Pregnant bitch uterus presenting 13 gestational sacs.

If no pregnancy can be detected at that time, a second examination should be performed a week later. Abdominal palpation has been reported to be 88% accurate for pregnancy diagnosis in the bitch but only 12% accurate for the determination of fetal number. The most common mistakes are false-positive results from palpation of a stool-filled colon and segmental uterine enlargement resulting from pyometra.

Radiography

Radiography is an accurate method of pregnancy diagnosis, but only during the later stages of pregnancy. Radiography allows accurate determination of litter size, detection of fetal abnormalities, and determination of fetal size. It is therefore the method of choice for a prepartum examination.

Radiography can be used starting at 3 weeks of pregnancy to detect uterine enlargement. Early diagnosis, between the third and fifth week of pregnancy, may require the use of a contrast agent in the abdomen, such as injection of either carbon dioxide gas, room air, or radiopaque solution. This technique allows visualization of the embryonic vesicles but not the fetus itself and is neither practical nor commonly used.

Mineralization of the fetal skeleton is detectable by radiography 42-45 days after the LH peak. In practice, it is better to wait until days 45-50 after the LH peak to

increase the accuracy of diagnosis and determination of fetal number. The vertebral column becomes visible at 6 weeks; shadows from the skull are recognizable from day 45. At 7 weeks, the head, spine, ribs, pelvis, and limbs are sufficiently mineralized and can be visualized in dogs and cats. Determination of fetal number is based on enumeration of skulls and associated vertebral columns (Figure 8-2). Fetal viability should be questioned if radiographs show intrauterine gas pockets or misshapen fetal skeletons. Radiographic signs of fetal death can be seen within 48 hours after the occurrence of fetal death

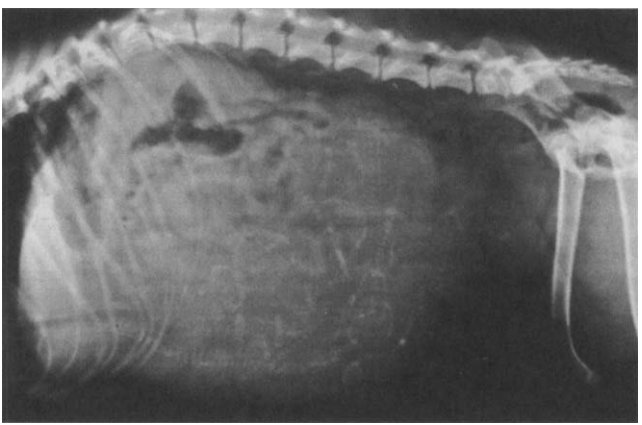


Figure 8-2. Radiograph of near-term pregnancy in a bitch. Note the numerous fetal skulls.

and include depression of the frontal bones of the cranium and abnormal angulations of the spine.

Frequent use of radiology is not recommended during the same pregnancy. Exposure of the fetus to radiation may cause abnormalities, particularly during the organogenesis phase of fetal development (30-35 days). Radiography should be considered when there is a risk of malformation or complication. This examination is best performed after day 55 of pregnancy. Prediction of dystocia resulting from fetomaternal disproportion has been suggested based on measurement of fetal skulls in relation to the maternal pelvis, but the usefulness of this is debatable.

Ultrasonography

Since its introduction in the 1970s, ultrasonography has become the gold standard technique for pregnancy diagnosis in the dog and cat. It is accurate from as early as days 13-15 after mating in the queen and from days 16-18 after fertilization (days 19-21 after the LH peak) in the bitch. This technique is used extensively for early pregnancy diagnosis, evaluation of fetal viability, and tentative determination of fetal number.

Ultrasonographic imaging of the uterus is done transabdominally with a sector or linear transducer with a frequency of 5.0 or 7.5 MHz. Use of a 5.0-MHz transducer is recommended for large-breed dogs. Use of a

7.5-MHz transducer is recommended for pregnancy detection before day 30 in large-breed dogs and for pregnancy diagnosis at any gestational stage in small-breed dogs and cats.

Ultrasonographic examination usually is performed with the bitch in dorsal recumbency. Standing examination also may be performed and has the advantage of reducing the distance between the fetus and the probe. With either positioning, the hair of the abdominal wall needs to be clipped from the pelvis to the umbilical scar, and coupling gel should be applied to improve image quality. Next, the probe is placed on the linea alba just cranial to the pelvic brim. The urinary bladder is visualized as the primary landmark. The uterus usually is detected dorsal to the bladder. The nonpregnant uterus is difficult to visualize, whereas the pregnant uterus is relatively easy to image. Each uterine horn can be examined along its entire length. Diagnosis of pregnancy with ultrasound is possible as soon as the embryonic vesicles can be identified as discrete anechoic spherical structures. The earliest stage possible for the diagnosis of these structures depends on the quality of the equipment, the frequency of the transducer used, patient characteristics (e.g., obesity, nervousness, breed, age), and most important, the expertise of the operator.

ULTRASONOGRAPHIC CHRONOLOGY OF PREGNANCY IN THE BITCH Although diagnosis of pregnancy with use of ultrasonography has been described as being possible as

early as day 10, most authors agree that in the best conditions, with precise timing of the LH peak and use of a 7.5-MHz transducer, detection is not possible until day 17 after the LH peak or approximately 11 days after the first day of diestrus, at the earliest. Authors using the date of first mating as the reference date report detection of pregnancy between days 18-28. Gestational sacs appear 11-25 days after the last mating. When the LH peak is used as a reference, pregnancy diagnosis is highly accurate after days 21-23. It is important to note that when diagnosis is made very early, confirmation examination is needed at a later stage. Pregnancy losses that may occur in the early stages of gestation may not be accompanied by clinical signs of spontaneous abortion. When the date of the LH peak is not known, pregnancy diagnosis should be attempted approximately 30 days from the last mating.

Embryonic vesicles are spherical anechoic structures, 2 mm in diameter. The dorsal and ventral poles of the conceptus are brighter than is the center. The uterus is more echogenic in the areas of contact with the conceptus and less echogenic in areas between the embryonic vesicles. The gestational sacs increase in size and become oblong with increased gestational age. The embryo proper can be detected 23-25 days after the LH peak or 16 days after the onset of diestrus. By day 24 the allantoic sac can be visualized near the embryo. Fetal heartbeats first are detected between days 22-29. First detection of fetal movements has been reported to

occur between days 28-36 of pregnancy. The embryonic limb buds and choroidal plexus of the brain are sufficiently differentiated and identifiable by day 32 of pregnancy. The identification of the head, trunk, and abdomen is possible by day 35 (Figure 8-3). The hyperechoic fetal skeleton is evident from day 33 onward. The heart valves and aorta become easily identifiable at this time as well (Figure 8-4). The diameter of the trunk exceeds that of the head after day 40 of pregnancy. At that stage of pregnancy, other organs become recogniza-

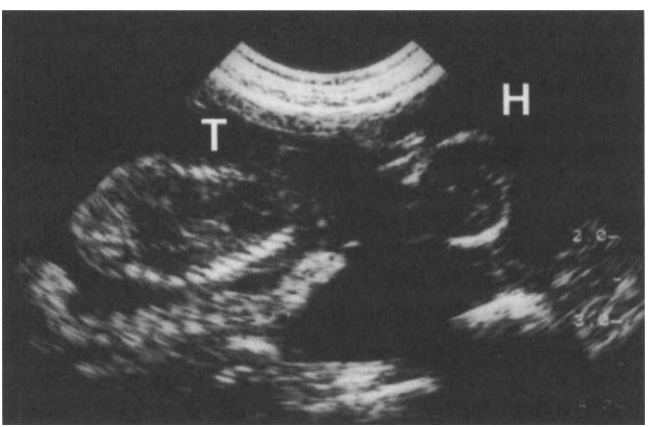


Figure 8-3. Ultrasonogram of the fetus in a bitch. *H*, Head; *T*, thorax.

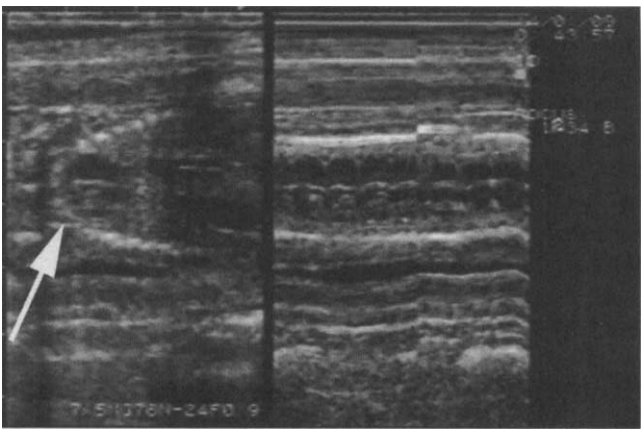


Figure 8-4. M-mode ultrasonogram *(right side)* of the fetus showing heart *(arrow)* activity.

ble; the lungs, diaphragm, liver, and stomach are the most easily detected. The bladder and urachus become identifiable a few days later. During the last 3 weeks of pregnancy, the kidneys, fetal vasculature (including umbilical vessels), and intestines are detected.

An attempt to determine fetal age has been made based on measurement of the biparietal or trunk diameter. However, these measurements have to take into consideration differences related to breed and litter size. In

one study in which crown-rump length, biparietal diameter, and body diameter of fetuses were measured in a variety of breeds of dog, it was demonstrated that the canine fetus grows rapidly in body diameter at 4-5 weeks of gestation and grows rapidly in body length and head diameter about 1 week later. Considerable variation was noted in these measurements among individual bitches and even among littermates. It is preferable to average the measurements of several fetuses to increase the predictive accuracy of stage of pregnancy with this technique.

Attempts also have been made to determine litter size with ultrasonography. The accuracy of fetal numbering has been shown to be only 38% during early pregnancy and even lower later in pregnancy. However, if the estimation is limited to whether the bitch is carrying five pups or more versus four pups or fewer, the predictive value of ultrasonography increases to 100% and 83.2 %, respectively. The best stage for determination of litter size is between days 25-35 after implantation.

One of the problems encountered in early pregnancy detection and fetal numbering is the incidence of fetal loss. Fetal resorption is known to occur throughout pregnancy without effect on the adjacent concepti. Embryonic resorption is recognized by the low volume and increased echogenicity of embryonic fluid, loss of embryonic mass and heartbeat, collapse of the conceptus with thickening of the uterine wall, and reduced size in comparison with adjacent concepti.

ULTRASONOGRAPHIC CHRONOLOGY OF PREGNANCY IN THE QUEEN In the queen, embryonic vesicles are detected earlier than in the bitch. Ultrasonographic diagnosis of pregnancy is highly accurate between 11-16 days after breeding. Heartbeats are detected between days 16-25. The enlarged uterus of pregnancy may be detectable as early as 4 days postcoitus. An anechoic region corresponding to the gestational sac is apparent between 11-14 days. Gestational sacs increase in size from approximately 2 mm on day 11 to approximately 37 mm on day 29. The echogenic fetal pole is visualized between days 15-17 postcoitus. Fetal heartbeats are detected between days 16-20. The fetal membranes become apparent between days 21-24. By days 26-28 the fetal limbs and head are sufficiently differentiated and easily recognized. Fetal movements are apparent between days 28-40.

Diameters of gestational sacs vary considerably at the same stage of pregnancy among fetuses both within and between litters. Ultrasonographic estimation of litter size is best made early in gestation, although the possibility of subsequent fetal resorption still exists and should be considered. Early examinations may completely miss some of the gestational sacs. Fetuses located closest to the uterine bifurcation tend to be the first detected. Fetuses tend to be distributed evenly between the two uterine horns. Nonviable fetuses show no motion and rapidly lose identifiable morphology within 1 day of fetal death.

Ultrasonography routinely is scheduled to be performed approximately 16 days from the last observed mating. The earliest that pregnancy can be confirmed by ultrasonography in cats is between days 11-14 of gestation. If negative results are obtained at that time, the procedure should be repeated 5-7 days later. The chronology of appearance of specific ultrasonographic characteristics of the fetuses can be used to estimate stage of pregnancy with relative accuracy, at least during the first 30 days.

Acute-Phase Proteins

Acute-phase proteins are released as part of an inflammation-like response to the invasion of the uterus by fetal tissue during implantation and placentation. This response seems to be peculiar to the canine species. Acute-phase proteins used for pregnancy testing include C-reactive protein (CRP) and fibrinogen. Elevation in both CRP and fibrinogen can be used as an indicator of pregnancy in the bitch between days 30-50 of pregnancy. However, other inflammatory processes, such as pyometra, may increase plasma fibrinogen and CRP. Increase in CRP also is seen in dogs with hepatitis, acute nephritis, or peritonitis.

CRP belongs to a group of plasma proteins produced by the liver during the acute phase of an inflammatory reaction. Canine CRP is a glycoprotein that can

be detected as early as 24 hours after an inflammatory reaction. A significant increase in CRP is observed in pregnant bitches between days 30-50 of pregnancy. Detection of CRP requires a homologous canine CRP assay because human CRP assays are not very accurate. No such assay is commercially available at this time.

Fibrinogen increases significantly in pregnant bitches after implantation and peaks at approximately midgestation. Fibrinogen concentrations of greater than 280-300 mg/dl are consistent with pregnancy at this stage. Assay of this protein has not proved useful in clinical practice.

Hematologic Changes

Normal packed cell volume (PCV) in the nonpregnant bitch during diestrus is 40%-55%. A decrease in PCV is observed in pregnant bitches starting at approximately day 20 after the LH peak. Around day 35 and near term, PCV may be below 40% and 35%, respectively. However, this parameter is not useful on an individual basis because of the large variability between animals. Pregnant bitches usually have a physiologic normocytic, normochromic anemia. Mean corpuscular volume and hemoglobin concentrations remain unchanged. This anemia probably results from hemodilution because of increased plasma volume. From midpregnancy until day 50, bitches may have a leukocytosis with neutrophilia. It

is important to keep in mind that an increase in white blood cells and decreased PCV during diestrus also may be caused by pyometra or other pathologic processes.

Hormonal Methods

PROGESTERONE The pattern of secretion of progesterone is similar in both the pregnant and nonpregnant bitch and cannot be used as a method for pregnancy diagnosis in the dog. In the queen, progesterone may be used to differentiate pregnancy from pseudopregnancy after day 35 postcoitus. In this species, progesterone levels decrease progressively to basal levels by 40 days in pseudopregnant females but are maintained above 1 ng/ml in pregnant females until the day of parturition.

RELAXIN Relaxin is the only hormone known to be specific for pregnancy in carnivores. Relaxin is produced primarily by the fetoplacental unit. In the bitch relaxin is detected from the third week of pregnancy. Plasma concentration peaks at 4-5 ng/ml at days 40-50 of pregnancy. Plasma relaxin concentrations decrease slowly after parturition but remain at detectable levels for the first 4-9 weeks postpartum.

In the queen relaxin is not present during estrus or pseudopregnancy. It becomes detectable from day 25 of pregnancy, plateaus between days 30-35, and is

detectable until 10-15 days prepartum, after which time plasma concentrations slowly decrease.

Determination of plasma levels of relaxin is accomplished by performing radioimmunoassay or enzyme-linked immunosorbent assay techniques, which are available in a few specialized laboratories. Rapid relaxin tests have been developed for in-clinic pregnancy testing of dogs (ReproCHEK and Witness; Synbiotics, San Diego, Calif.). These tests allow detection of pregnancy starting around day 25 after ovulation. False-positive results occur in the presence of fetal resorption.

PROLACTIN Prolactin concentrations begin to increase significantly in the pregnant bitch 25-30 days after the LH peak, reaching concentrations as high as 60 ng/ml close to parturition. Prolactin cannot be used for pregnancy diagnosis before day 35 after the LH peak in the bitch. In the pregnant queen, plasma prolactin concentration increases from days 20-25 after mating; no increase is observed in the nonpregnant queen.

Summary

- *Abdominal ultrasonography is the best method for pregnancy diagnosis.* It can be used for early detection of pregnancy and evaluation of fetal development and viability and is relatively accurate in estimating fetal number during early pregnancy.

- Abdominal palpation is the easiest and cheapest method for pregnancy diagnosis in cats and dogs. However, its accuracy can be affected by operator experience and the size and body condition of the patient. It should be used between days 24-28 or after day 45 of pregnancy in the bitch.
- Radiography is precise but only after days 42-45 of pregnancy. It does not allow an evaluation of fetal viability but is very accurate for the determination of litter size. It is best used after day 55 after the LH peak.
- Relaxin assay is commercially available and detects pregnancy in dogs after approximately day 27 after the LH peak.
- CRP and fibrinogen are increased in pregnant bitches, but they are not specific to pregnancy. PCV is decreased after implantation in pregnant bitches but cannot be used as a diagnostic tool.

Care of the Bitch and Queen during Pregnancy

Breeders often ask veterinarians questions about proper care of the pregnant bitch or queen. Proper care mainly involves nutrition and prevention of early fetal loss, abortion, and fetal defects. However, care of the pregnant female also should include monitoring during the pregnancy, particularly for those animals that are at risk

for fetal defects or dystocia or have medical conditions that may be exacerbated by pregnancy.

Nutrition during Pregnancy

NUTRITION OF THE PREGNANT BITCH In dogs most fetal growth occurs during the last 3-4 weeks of pregnancy. More than 75% of fetal weight gain occurs between days 40-55 of pregnancy. Therefore adjustment of feeding for pregnancy requirements is not necessary until the last 4 weeks of pregnancy, particularly in bitches that were in good body condition at the time of breeding. Maintenance requirements have been estimated as 132 kcal of metabolizable energy (ME) per kilogram of metabolic weight ($kg^{0.75}$). These requirements are sufficient during the first 4 weeks of pregnancy. Energy intake should be increased by 10% per week during the last 3-4 weeks of pregnancy, to reach levels 25%-30% above the maintenance level by parturition. With this adjustment, most animals will register a 15%-25% increase in body weight by the time of whelping. Protein requirements generally are estimated to be 25%-30% of the total energy, or 71-85 g of protein for each 1000 kcal of ME. From 30%-40% of protein should be of animal origin to prevent amino acid deficiencies. Lipids increase energy density and palatability of the feed and play an important role in fetal development. Lipids should represent 25%-65% of the energy in a diet; this

translates to 30-70 g of fat per 1000 kcal of ME or 120-280 g/kg dry matter in the feed. Carbohydrates generally need not be supplemented during pregnancy, especially if the feed contains enough of the amino acids necessary for glucose formation. Fiber should not exceed 5%.

Increase in uterine size during the last few weeks of pregnancy is an important limiting factor for food intake. Pregnant bitches should be fed food that is high in calories and protein in multiple small boluses. Growth or lactation commercial feed is appropriate (3.6 kcal/kg food dry matter).

Precise nutritional supplementation is difficult in the pregnant bitch because it depends primarily on litter size. Most commercial pet food is well balanced and can be used safely. Excessive caloric intake may result in heavy fetuses and increased risk of dystocia. Weight gained during pregnancy will be lost immediately after whelping. Most balanced commercial feed provides adequate amounts of calcium and vitamins. It is important to avoid excessive supplementation with calcium or vitamins during pregnancy because they can predispose the bitch to eclampsia and dystocia and can cause physical abnormalities in the pups.

NUTRITION OF THE PREGNANT QUEEN Unlike in the bitch, the pregnant queen shows a steady increase in weight beginning the second week of gestation. At about 3 weeks of gestation, many queens undergo a short

period of inappetence lasting 3-10 days that may be accompanied by mild weight loss. Queens will lose one third of the weight gained during pregnancy immediately after parturition. The remaining two thirds are stored as body fat that is gradually lost during lactation. Nutrition of the pregnant queen should be slowly adjusted starting the second week of pregnancy to exceed maintenance levels by 25%-50% at parturition. During the final weeks of gestation, caloric intake should be approximately 220 calories/kg. Commercial food usually is well balanced, but some dry foods may be low in nutrients that produce energy.

Drugs Incompatible with Pregnancy

Most of the drugs used in canine and feline practice have not been tested for their effect on pregnancy and fetal development. The toxic effect on the fetus depends on the dose, duration of use, stage of pregnancy, and ability of the drug to cross the placenta. Drugs that are potentially the most harmful include aminoglycoside antibiotics, which are neurotoxic; chloramphenicol, which decreases bone marrow development; tetracycline, which causes malformations of the bones and teeth; and anesthetics, which may produce respiratory depression in the fetus. Corticosteroids may cause abortion, fetal death, and abnormalities such as cleft palate. Estrogenic and androgenic compounds

may cause malformation of the genitourinary system (see Chapter 10).

Monitoring the Pregnant Female

Owners should be instructed to monitor changes in behavior, food intake, excessive mammary development, excessive abdominal distension, onset of lactation, and abnormal vulvar discharge in pregnant queens and bitches and to report any abnormalities to their veterinarians promptly. High-risk pregnancies include those bitches and queens with systemic disease, obesity, small litter size, or a history of dystocia. A thorough physical examination should be performed at least three times during pregnancy on all animals with high-risk pregnancies, and radiographs should be obtained late in gestation to assess the size of the puppies or kittens and the litter size.

Pregnancy toxemia has been reported to occur in bitches carrying large litters. Ketosis develops during late pregnancy in bitches that are not able to meet the nutritional requirements of pregnancy and develop a negative energy balance. Anorexia during late pregnancy always should be taken seriously. Pregnancy toxemia occurs in all breeds of dog, but Yorkshire terriers and Labrador retrievers seem to be predisposed. Affected bitches must be provided with parenteral nutrition if they will not or cannot eat and the pregnancy ended by elective termination (see Chapter 5) or cesarean section (C-section).

The effect of pregnancy on glucose homeostasis may lead to onset of diabetes mellitus. Animals with diabetes mellitus should not be bred purposefully. Development of gestational diabetes in previously healthy bitches is thought to be the effect of progesterone on carbohydrate metabolism; progesterone stimulates secretion of growth hormone, which antagonizes insulin. Pups from diabetic bitches may be very large at birth because of these metabolic changes, predisposing the bitch to dystocia.

Monitoring of the pregnant bitch also should include prediction of onset of parturition. Determination of the LH peak or first day of diestrus helps reduce the variability in pregnancy length. Nesting behavior is displayed within a week of whelping. Rectal temperature should be recorded three to four times daily, starting at 54 days after mating. A drop of 1° F (usually to less than 99.0° F) indicates onset of parturition within 8-24 hours. In high-risk pregnancies, external monitoring devices based on uterine activity and fetal heart rates should be used (see Chapter 9).

Bibliography

Addiego LA, Tsutsui T, Stewart DR et al: Determination of the source of immunoreactive relaxin in the cat, *Biol Reprod* 37:1165, 1987.

Allen WE, Meredith MJ: Detection of pregnancy in the bitch: a study of abdominal palpation, A-mode

ultrasound and Doppler ultrasound techniques, *J Small Anim Pract* 22:609, 1981.

Barr FJ: Pregnancy diagnosis and assessment of fetal viability in the dog: a review, *J Small Anim Pract* 29:647, 1988.

Biourge V, Istasse L, Gielen M et al: Alimentation du chien. II. Les besoins du chien a l'entretien [in French], *Ann Med Vet* 131:81, 1987.

Bondestam S, Alitalo I, Karkkainen M: Real-time ultrasound pregnancy diagnosis in the bitch, *J Small Anim Pract* 24:145, 1983.

Brown RG: A comparison of certified and non-certified pet foods, *Can Vet J* 38:707, 1997.

Cartee RE, Rowles T: Preliminary study of the ultrasonographic diagnosis of pregnancy and fetal development in the dog, *Am J Vet Res* 45:1259, 1984.

Concannon PW: Canine pregnancy and parturition, *Vet Clin North Am* 16:453, 1986.

Concannon PW, Gimpel T, Newton LE: Postimplantation increase in plasma fibrinogen concentration with increase in relaxin concentration in pregnant dogs, *Am J Vet Res* 57:1382, 1996.

Concannon PW, McCann JP, Temple M: Biology and endocrinology of ovulation, pregnancy and parturition in the dog, *J Reprod Fertil Suppl* 39:3, 1989.

Concannon PW, Powers ME, Holder W et al: Pregnancy and parturition in the bitch, *Biol Reprod* 16:517, 1977.

Concannon P, Whaley S, Lein D et al: Canine gestation length: variation related to time of mating and fertile life of sperm, *Am J Vet Res* 44:1819, 1983.

Davidson AP, Nyland TG, Tsutsui T: Pregnancy diagnosis with ultrasound in the domestic cat, *Vet Radiol* 27:109, 1986.

DeCoster R, Beckers JF, Beerens D et al: A homologous radioimmunoassay for canine prolactin: plasma levels during the reproductive cycle, *Acta Endocrinol (Copenh)* 103:473, 1983.

Diez M, Istasse L: Les alimens industriels chez le chien: caracteristiques et utilisation dans differents etats physiologiques [in French], *Ann Med Vet* 139:309, 1995.

Eckersall PD, Conner JG: Bovine and canine acute phase proteins, *Vet Res Commun* 12:169, 1988.

Eckersall PD, Harvey MJ, Ferguson J et al: Acute phase proteins in canine pregnancy (Canis familiaris), *J Reprod Fertil Suppl* 47:159, 1993.

England GC: Ultrasonic assessment of abnormal pregnancy, *Vet Clin North Am* 28:849, 1998.

England GC: Ultrasound evaluation of pregnancy and spontaneous embryonic resorption in the bitch, *J Small Anim Pract* 33:430, 1992.

England GC, Allen WE: Diagnosis of pregnancy and pyometra in the bitch using real-time ultrasonography, *Vet Annu* 30:217, 1990.

England GC, Allen WE: Studies of canine pregnancy using B-mode ultrasound: diagnosis of early pregnancy and

the number of conceptuses, *J Small Anim Pract* 31:321, 1990.

Gentry PA, Liptrap RM: Influence of progesterone and pregnancy on canine fibrinogen values, *J Small Anim Pract* 22:185, 1981.

Gunzel-Apel AR, Hayer M, Mischke R: Dynamics of haemostasis during the oestrus cycle and pregnancy in bitches, *J Reprod Fertil Suppl* 51:185, 1997.

Holme DW: Practical use of prepared food for dogs and cats. In Edney AT (ed): *Dog and cat nutrition,* Oxford, 1982, Pergamon Press.

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology,* Philadelphia, 2001, WB Saunders.

Kronfeld DS: Optimal ranges of nutrients for dogs and cats, *Tisch Dierg Suppl* 1:425, 1986.

Kronfeld DS: Optimal regimens based on recipes for cooking in home or hospital or in proprietary pet food. In Anderson RS (ed): *Nutrition and behaviour in dogs and cats,* Oxford, 1982, Pergamon Press.

Lamm AM: Ultrasound diagnosis of pregnancy in the dog, *Acta Radiol* Suppl 319:293, 1972.

Lawler DF, Bebiak DM: Nutrition and management of reproduction in the cat, *Vet Clin North Am* 16:495, 1986.

Lewis LD, Morris ML: *Small animal clinical nutrition,* Topeka, 1984, Mark Morris Associates.

Lewis LD, Morris ML, Hand MS: *Small animal clinical nutrition III,* Topeka, 1987, Mark Morris Associates.

Loveridge GG: Body weight changes and energy intake of cats during gestation and lactation, *Anim Technol* 37:7, 1986.

Mailhac JM, Chaffaux S, Legrand JJ et al: Pregnancy diagnosis in the cat: use of echography, *Rec Med Vet* 156:899, 1980.

McCann JP, Altszuler N, Hampshire JE: Growth hormone, insulin, cortisol, luteinizing hormone, and diabetes in beagle bitches treated with medroxyprogesterone acetate, *Acta Endocrinol (Copenh)* 104:167, 1987.

Moser D: Feeding to optimize canine reproductive efficiency, *Probl Vet Med* 4:545, 1992.

Mosier JE: Nutritional recommendations for gestation and lactation in the dog, *Vet Clin North Am* 7:683, 1977.

Nett TM, Akbar AM, Phemister RD et al: Levels of luteinizing hormone, estradiol and progesterone in serum during the oestrus cycle and pregnancy in the beagle bitch. Proceedings of the Society for Experimental Biology and Medicine, 1975.

Nguyen P: Feeding of the cat: 2. Commercial cat food and homemade food, *Point Vet* 28:241, 1996.

Okkens AS, Bevers MM, Dieleman SJ et al: Evidence for prolactin as the main leutotrophic factor in the cyclic dog, *Vet Q* 12:193, 1990.

Okkens AS, Silva LD, Donnay I et al: Luteotrophic action of prolactin in dogs and the effects of a dopamine

agonist, cabergoline, *J Reprod Fertil Suppl* 47:403, 1993.

Papich MG: Effects of drugs on pregnancy. In Kirk RW, Bonagura JD (eds): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Ralston SL: Feeding for breeding. Proceedings of the Society for Theriogenology, Toronto, Ontario, Canada, 1990.

Rivers B, Johnston GR: Diagnostic imaging of the reproductive organs of the bitch: methods and limitations, *Vet Clin North Am* 21:437, 1991.

Shille VM: Reproductive physiology and endocrinology of the female and male. In Ettinger SJ (ed): *Textbook of veterinary internal medicine,* Philadelphia, 1989, WB Saunders.

Shille VM, Gontarek J: The use of ultrasonography for pregnancy diagnosis in the bitch, *J Am Vet Med Assoc* 187:1021, 1985.

Sloan JM, Path MR, Olivier IM: Progesterone-induced diabetes in the dog, *Diabetes* 24:337, 1975.

Smith MS, McDonald LE: Serum levels of luteinizing hormone and progesterone during the estrous cycle, pseudopregnancy and pregnancy in the dog, *Endocrinology* 94:404, 1974.

Steinetz BG, Bullesbach EE, Goldsmith LT et al: Use of synthetic canine relaxin to develop a rapid homologous radioimmunoassay, *Biol Reprod* 54:1252, 1996.

Steinetz BG, Goldsmith LT, Brown MC et al: Use of serum relaxin for pregnancy diagnosis in the bitch. In Bonagura JD, Kirk RW (eds): *Current veterinary therapy XIII,* Philadelphia, 2000, WB Saunders.

Steinetz BG, Goldsmith LT, Lust G: Plasma relaxin levels in pregnant and lactating dogs, *Biol Reprod* 37:719, 1987.

Stewart DR, Stabenfeldt GH: Relaxin activity in the pregnant cat, *Biol Reprod* 32:848, 1985.

Stowater J, Memon M: Ultrasonic features of the dog uterus and fetus, *J Reprod Fertil Suppl* 39:329, 1988.

Stowater J, Memon M, Tidwell A et al: Ultrasonographic features of feline fetal development. Proceedings of the Society for Theriogenology, Orlando, 1988.

Tiedmann K, Henschel E: Early radiographic diagnosis of pregnancy in the cat, *J Small Anim Pract* 14:567, 1973.

Toal RL, Walker MA, Henry GA: A comparison of real-time ultrasound, palpation and radiography in pregnancy detection and litter size determination in the bitch, *Vet Radiol* 27:102, 1986.

Verstegen J, Onclin K, Silva LD et al: Regulation of progesterone during pregnancy in the cat: studies on the roles of corpora lutea, placenta and prolactin secretion, *J Reprod Fertil Suppl* 47:165, 1993.

Verstegen J, Silva LD, Onclin K: Mise au point sur le diagnostic de gestation chez les carnivores domestiques [in French], *Ann Med Vet* 140:81, 1996.

Wolter R: *L'alimentation du chien et du chat,* Paris, 1982, Maison Alfort.

Yaeger AE, Concannon PW: Association between the preovulatory luteinizing hormone surge and the early ultrasonographic detection of pregnancy and fetal heartbeats in beagle dogs, *Theriogenology* 34:655, 1990.

Yaeger 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, 1992.

9

Parturition and Dystocia

Jane A. Barber

AT A GLANCE

- Induction of parturition is mediated by the fetuses. If the litter is very small or the fetuses are dead, labor may not be initiated.
- In bitches, serum progesterone concentration must be less than 2 ng/ml for labor to begin. Decline in serum progesterone is reflected by a transient

decrease in body temperature. Monitoring rectal temperature three to four times daily after approximately day 54 of pregnancy or directly measuring serum progesterone concentration can help to determine whether parturition is imminent.

- Stages of labor are as follows:
  - Stage I: The cervix dilates, duration averages 6-12 hours, dam is restless, and pants.
  - Stage II: The offspring are passed, duration depends on litter size, and coordinated abdominal contractions are evident.
  - Stage III: The placentas pass, duration depends on litter size, and dam usually alternates passage of pups and placentas.
- Labor can be monitored with external devices that record uterine contractions and can be used to detect fetal heartbeats.
- Postpartum care includes the following:
  - Do not allow bitches and queens to eat the placentas. They are of no benefit and may cause gastroenteritis.
  - Lochia is normal postpartum vaginal discharge. It varies in color from brick red to green-black, has no odor, and may persist for up to 3 weeks.
  - Abnormalities of the dam seen postpartum include mastitis (see Chapter 14), metritis and subinvolution of placental sites (see Chapter 12), and eclampsia.

- Eclampsia is hypocalcemia. It is most common in small-breed dogs nursing large litters and typically occurs at 2-3 weeks of lactation. Clinical signs include ataxia, restlessness, and disorientation. Clinical progression to seizures, hyperthermia, and death may occur. Calcium is administered intravenously or subcutaneously for immediate treatment; calcium and vitamin D, to be administered orally, are sent home with the dog.

- Dystocia is abnormal birth. Causes include the following:
  - Maternal factors: uterine inertia, inadequate size of birth canal
  - Fetal factors: fetal oversize, abnormal orientation of the fetus as it enters the birth canal
- Determine whether dystocia is present by doing the following:
  - History: Stage I labor for more than 12 hours, strong stage II labor for more than 30 minutes, weak stage II labor for more than 4 hours before first neonate born, more than 2 hours between offspring, history of dystocia, abnormal vulvar discharge passed.
  - Physical examination: Digital vaginal examination reveals puppy or kitten obstructing birth canal with or without abnormal orientation, bitch systemically ill, abnormal vulvar discharge present (e.g., frank hemorrhage, pus, green discharge before any neonates born [indicates placental separation]).

- Diagnostic tests: Tests include abdominal radiographs and possibly abdominal ultrasonography.
- Treatment options for dystocia include the following:
  - Manipulation: difficult because of small size of bitch or queen
  - Medical therapy
    - Oxytocin: 0.25-4 IU administered intramuscularly; repeat at 20- to 30-minute intervals; give no more than three doses if no effect seen
  - Surgical therapy
    - Cesarean section (C-section)
    - Resuscitation of neonates

      Remove fluid from oral and nasal cavities.

      Rub puppy or kitten vigorously to stimulate respiration and warm them.

      Administer oxygen, respiratory stimulant (doxapram), or anesthetic reversal agent (naloxone).

Endocrinology of Parturition

Corticosteroids

Normal parturition in the bitch results from a complex cascade of endocrinologic events. Although the exact mechanisms leading to initiation of parturition are not completely known, this hormonal cascade

is thought to be initiated by the fetuses. During the last 5-10 days of gestation, the fetuses become stressed, perhaps from crowding or from hypoxia that occurs when fetal oxygen requirements exceed placental delivery capability. In response to fetal stress, the fetal pituitary gland secretes adrenocorticotropic hormone (ACTH). Fetal ACTH then induces secretion of glucocorticoids, primarily cortisol, from the fetal adrenal gland. Cortisol acts on the placenta to cause decreased production of progesterone concurrent with an increased production of estrogen 12-48 hours before parturition.

Prostaglandins and Progesterone

Both fetal cortisol and placental estrogen promote synthesis and release of prostaglandins locally in the placenta and endometrium. Prostaglandins probably also are produced in the myometrium, cervix, and fetal membranes. Prostaglandins are potent luteolytic agents. In the bitch the ovary is the sole source of progesterone, which is necessary for pregnancy maintenance. Parturition cannot occur in bitches until the serum progesterone concentration falls to less than 1-2 ng/ml. Endogenous or exogenous prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can be used to induce luteolysis, cause maternal progesterone levels to plummet, and initiate parturition in many species, including the dog.

Estrogens

Estrogens enhance prostaglandin synthesis. The increase in estrogen secretion near parturition leads to increased prostaglandin synthesis. In addition, oxytocin stimulates direct prostaglandin release from the uterus.

Estrogen also increases myometrial contractility by sensitizing the uterus to oxytocin through an upregulation of oxytocin receptors. Elevated estrogen concentrations also induce oxytocin release from the posterior lobe of the pituitary gland. Oxytocin, in turn, further stimulates prostaglandin production in the endometrium. Prostaglandins, in addition to being luteolytic, also act directly on the myometrium to increase uterine contractility. Meanwhile, progesterone-mediated inhibition of myometrial contractility decreases as progesterone concentrations decline.

Oxytocin

Uterine contractions propel the fetus caudally. The cervix dilates in response to the presence of estrogen, $PGF_{2\alpha}$, uterine contractions, and the presence of the fetal head. Stretching of the cervix stimulates a nervous impulse (Ferguson's reflex), leading to the secretion and release of oxytocin, which reinforces and maintains the ecbolic effect of prostaglandin. Uterine contractions and dilation of the cervix then feed back

on the pituitary gland to promote further oxytocin release. Oxytocin release may regulate labor; the bitch can voluntarily inhibit its release if she is frightened or stressed.

Relaxin and Prolactin

Other pregnancy-specific endocrine changes in the bitch include progressive increases in relaxin and prolactin during the second half of pregnancy. Prolactin, secreted from the posterior pituitary gland, is elevated by days 30-35 and peaks 1-2 days before whelping. Prolactin then decreases for 1 or 2 days after parturition, before increasing again in response to suckling by neonates during the postpartum period.

Relaxin is a peptide hormone produced by the fetoplacental unit. Relaxin levels in the bitch increase during the third week of pregnancy, peak by midgestation, and remain elevated to term. Relaxin concentrations then decline at parturition and reach nondetectable levels 1-6 weeks postpartum. Relaxin probably functions to promote myometrial quiescence during late gestation, to elongate the collagenous interpubic ligament facilitating vaginal delivery, to alter the cervical structural collagen to soften the cervix, and to induce oxytocin receptors in the myometrium.

Normal Parturition or Eutocia

Onset of Parturition

Accurately predicting the whelping date can be difficult. Predictions based on breeding dates can be inaccurate; gestation length can range from 57 days from the last breeding to 72 days from the first breeding. When gestation length in the bitch is timed from the luteinizing hormone (LH) surge, parturition occurs 64-66 days afterward. Similarly, when ovulation date is used, gestation length ranges from 62-64 days. After breeding, daily vaginal cytology smears can be used to accurately predict the parturition date. Parturition occurs 57 ± 1 days after the onset of cytologic diestrus (see Chapter 2). Ultrasonographic determination of gestational age based on fetal head and trunk measurements is limited by normal variation in puppy size by breed and litter size. Radiography performed late in gestation may be useful; fetal teeth can be observed radiographically 3-8 days prepartum. Onset of lactation, nesting behavior, and vulvar relaxation occur days to weeks before parturition and are not useful.

Assessment of the normal decline in serum progesterone concentration before whelping is an accurate indicator of imminent parturition in normal dogs. In the bitch serum progesterone concentration drops below 1-2 ng/ml 18-30 hours before parturition.

From 10-14 hours after the progesterone drops, rectal temperature transiently falls below 100° F and recognizable signs of labor usually begin within 18-24 hours. The mechanism of hypothermia is poorly understood, but it is known that moderate doses of progesterone are thermogenic in humans and it is hypothesized that the temperature drop reflects an inability of the thermoregulatory mechanism to compensate for the rapid removal of progesterone associated with luteolysis. The prepartum rectal temperature drop in the bitch usually can be detected if the temperature is recorded three to four times daily beginning about 54 days after breeding. After the drop occurs, temperature returns to normal (101° to 102° F) before parturition onset. Direct measurement of progesterone concentrations in serum also can be used to assess the likelihood of imminent parturition.

In queens, gestation length ranges from 62-71 days from the first breeding. Average gestation length in queens is 66-67 days. Queens show no changes in behavior, such as nesting, or in mammary development, such as lactation, before queening. Serum progesterone concentration does not consistently decrease before queening, so the rectal temperature drop described for bitches is not useful. Measurement of crown-rump length on radiographs may be used to estimate gestational age.

Stages of Labor

STAGE I Stage I of labor begins with the onset of uterine contractions and ends when the cervix is fully dilated. Although it is comparable to the long, first stage of labor in humans, it is not recognized easily in the bitch. Uterine contractions, occurring at regular but progressively shorter intervals, are not visible externally, and the anatomy of the bitch prevents digital palpation and detection of cervical dilation. The duration of stage I labor in the bitch is 6-12 hours but may last up to 24 hours. The bitch may seem restless and nervous and may refuse food. Other behaviors of stage I labor include shivering, panting, vomiting, chewing, scratching at the floor, and pacing. Most bitches seek seclusion and exhibit nesting behavior, but some eat normally and are more gregarious. Probably the most characteristic external sign is panting. Vulvar discharge, if present, should be clear and mucoid.

STAGE II Stage II, the stage of delivery, begins with full dilation of the cervix and ends with expulsion of the fetus or fetuses. The onset of the second stage of labor is marked by visible efforts to expel puppies with abdominal muscle contractions, with the bitch either on her side or in a squatting position. Puppies are delivered, on average, every 30-60 minutes. With passage of a pup through the birth canal, the outer chorioallantoic membrane either ruptures spontaneously or is torn by the

bitch. The mother normally licks each newborn vigorously, thereby removing the inner alloamniotic membrane and stimulating respiration.

The order of puppy delivery typically alternates between uterine horns. Parturition usually is complete in 3-6 hours, although normal deliveries can take up to 24 hours, especially with large litters. The interval between pups is irregular, and the bitch may deliver a few pups and then rest for several hours before completing delivery.

STAGE III During stage III of labor, the fetal membranes are expelled, usually within 15 minutes of delivery of a fetus. Normally, the parturition process alternates between stages II and III until delivery of all fetuses and placentas is complete. The bitch severs the umbilical cord with her teeth and consumes the placenta unless it is removed. Placental ingestion may result in mild diarrhea or vomiting and has no known benefit. Normally, one placenta is expelled for each pup delivered. Careful observation, including counting of placentas as they are expelled, ensures that no fetal membranes are retained in the uterus.

Monitoring Labor

Detection of labor onset is usually limited to the client's observation of behavior change and detection of a drop

in rectal temperature. Because the drop is abrupt and transient, it easily can be missed if temperature measurements are not made at least three to four times daily. Likewise, the standard approach to parturition management involves the client's monitoring the progression of labor and the condition of neonates. Intrapartum monitoring, now the accepted standard of practice in human medicine, is credited for dramatically reducing morbidity and mortality in human obstetrics. Labor monitoring provides a means for both early detection and timely intervention in cases of abnormal labor and/or fetal distress. Two parturition management services are available commercially to veterinary practitioners (Better Your Breeding; Biomedical Systems Veterinary Division, Denver, Colo.; WhelpWise; Veterinary Perinatal Specialties, Wheat Ridge, Colo.).

TOCODYNAMOMETRY Both labor-monitoring services offer computer-modemed tocodynamometry combined with Doppler ultrasound fetal heart rate monitoring. The tocodynamometer detects and records intrauterine pressures. An external tocosensor is loosely belted around the bitch's abdomen. Recorded data are transmitted via modem to trained technicians who interpret the data and consult with the attending veterinarian. Monitoring can be performed either in the home setting or in the veterinary hospital.

Beginning 3-7 days before the expected delivery date, uterine activity is recorded twice daily for 1 hour each time. A measurable prelabor pattern of uterine contractions is established. An organized pattern of uterine activity characterized by increased contraction frequency and strength signals the onset of stage I of labor. Thus the onset of labor can be accurately identified even in bitches that do not experience a prepartum drop in rectal temperature. Once labor has begun, uterine activity is monitored constantly throughout the parturition process.

In human medicine a clear correlation exists between the length and quality of labor and the fetal viability and vigor. If labor is prolonged or dysfunctional, bad fetal outcomes may result. In the bitch the uterus exhibits characteristic patterns of contractility, with variations in contraction frequency from 0-12 per hour and strength from 15-40 mm Hg, with spikes to 60 mm Hg. During active labor, contractions usually last 2-5 minutes. Recognizable patterns of uterine activity exist before the onset of labor and during the active stages of labor. Tocodynamometry confirms normal labor-related uterine activity and detects uterine inertia or abnormal patterns of uterine contraction. Weak or prolonged labor patterns are associated with fetal distress.

FETAL HEART RATE MONITORING The parturition management services also incorporate the use of a small,

handheld ultrasonic Doppler unit that audibly detects fetal heart rates. Clients can be instructed in its use so that fetal heart rate monitoring, like tocodynamometry, can be performed in the home. An attempt should be made to identify a normal heart rate for each fetus. Individual fetuses can be monitored during labor to identify fetal compromise. Once labor has begun, hourly fetal heart rate determinations are recommended. Normal fetal heart rate at term gestation is 170-230 beats per minute (bpm). Transient accelerations occur with fetal movement. Fetal heart rates of fewer than 150-160 bpm indicate stress. Fetuses with heart rates of fewer than 130 bpm have poor survival if not delivered within 2-3 hours, and fetuses with heart rates of fewer than 100 bpm require immediate intervention.

One study of 50 whelpings reported that outcomes varied significantly as a factor of management technique used. Perinatal mortality rates were 33% in the group with traditional (non–data-based) interventions and 6% in the group with data-based interventions. The same rate for C-section as for therapy, 30%, was reported for each group. Intrapartum fetal death rate increased as length of labor increased; an even higher correlation was observed in cases in which stage I labor exceeded 18 hours.

Combined use of tocodynamometry and fetal heart rate monitoring removes much of the guesswork from labor management. Both fetal distress and uterine iner-

tia can be detected. Evaluation of uterine contractions provides a direct indication for oxytocin administration when contraction frequency is waning. Similarly, calcium gluconate administration is indicated to improve the strength of uterine contractions. Knowledge of uterine activity also can be used to titrate medications to achieve a more physiologic pattern of uterine contraction. As little as 0.25 IU of oxytocin may be sufficient to stimulate a normal contraction pattern. This small dose of oxytocin reduces stress to the bitch and her unborn pups. During a uterine contraction, blood flow (and oxygen delivery) to the fetuses is reduced. Although fetuses tolerate this for short periods, labor patterns made up of long, dysfunctional contractions may contribute to fetal mortality. Similarly, injudicious administration of oxytocin to a bitch that already is having maximal uterine contractions can result in fetal hypoxia. Assessment via uterine monitoring provides objective data that are useful in making decisions regarding choice and dosage of therapeutic agents. Labor monitoring also can identify contraindications to drug use and prevent further fetal compromise in certain cases of dystocia.

Dystocia

Dystocia refers to abnormal or difficult parturition. An increased incidence of dystocia is reported in certain dog breeds, including the bulldog, Chihuahua,

dachshund, miniature poodle, Pekingese, Pomeranian, and Yorkshire terrier. In cats dystocia is uncommon. Persian cats, however, may be predisposed. Maternal and fetal risk factors can occur singly or collectively to cause dystocia. Correct identification of all contributing factors allows the veterinarian to decide on necessity and type of intervention.

Causes of Dystocia

MATERNAL FACTORS

- Uterine inertia: Uterine inertia is described as one of the most common causes of dystocia in bitches and queens. Uterine inertia is lack of normal, sequential uterine contractions with subsequent failure to expel fetuses through the birth canal. Primary uterine inertia is characterized as being either complete or partial. It is complete if stage I labor fails to proceed to stage II labor and partial when parturition begins normally and proceeds to stage II labor but the contractions cease before fetal expulsion occurs. Although the causes of primary uterine inertia are unclear, the condition has been associated with an inherited breed predisposition; overstretching of the uterine musculature in large litters; inadequate uterine stimulation as a result of insufficient oxytocin release in bitches with small litters or

malpositioned pups; uterine torsion; systemic imbalances such as obesity, hypocalcemia, hypoglycemia, or septicemia; age-related changes; psychogenic causes; and exhaustion. In secondary uterine inertia, parturition starts and proceeds normally to stage II labor, but stage II labor becomes excessively prolonged as a result of fetal obstruction. Maternal exhaustion ensues, and uterine contractions eventually cease.

- Inadequate birth canal size: Congenital causes of an unusually small birth canal include an inherited breed predisposition and reproductive immaturity. An example of an acquired cause is narrowing of the pelvic canal after spontaneous healing or surgical repair of a pelvic fracture.
- Obstruction of a normal birth canal: Uterine torsion is more common in the queen than in the bitch but is rare in both species. Vaginal sources of obstruction include anatomic anomalies, prolapse, and masses (see Chapter 13).
- Lack of normal abdominal press: Any condition that weakens abdominal musculature decreases functional abdominal press and precludes normal whelping. Examples include a ruptured diaphragm; tracheal perforation; obesity; and administration of drugs such as progesterone, paralytic agents, anesthetic agents, and muscle relaxants.

FETAL FACTORS Fetal oversize results in obstructive dystocia. Oversize may be of the entire body, as with a singleton fetus, fetal monster, or anasarca fetus. Relative oversize of the fetal head occurs with hydrocephalus.

Orientation of the fetus as it encounters the cervix and enters the birth canal can predispose to dystocia. Both cranial and caudal presentations are normal in the bitch and queen. About 40% of puppies are born in a caudal presentation. The normal caudal presentation, with hips extended, should be differentiated from the abnormal breech position, in which the hips are flexed with the hind limbs extended beneath the pup's body (Figure 9-1). Transverse presentations, although rare, usually occur with bicornual pregnancy of a single fetus and often result in obstructive dystocia (see Figure 9-1). Other, less common abnormal positions include lateral and ventral neck deviation, ventral neck flexion with a cranial "poll" presentation, and the "dog sitting" posture in which the pup presents cranially with its hips flexed and hind limbs extended beneath its body.

Historical Criteria

Differentiating dystocia from normal parturition can be a diagnostic challenge. When available, accurate historical data are extremely useful. Diagnosis also requires clinical evaluation, laboratory assessment, or imaging.

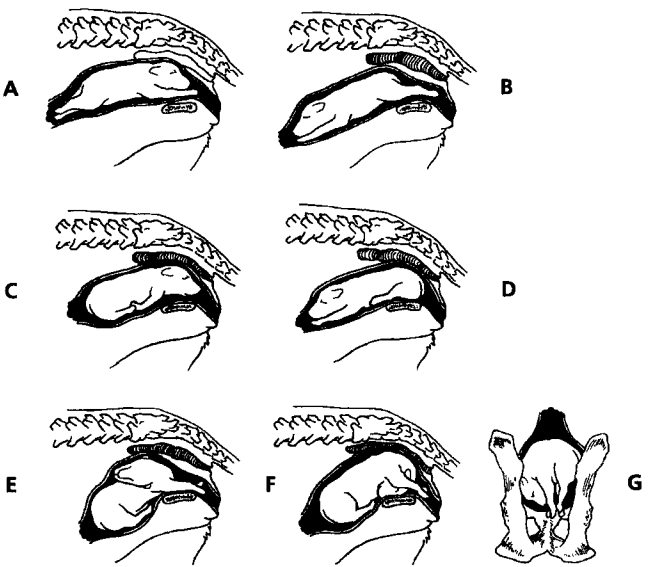


Figure 9-1. Fetal presentations, postures, and positions. **A,** Normal cranial presentation. **B,** Normal caudal presentation. **C,** Forelimbs are retained under the body. **D,** Rear limbs are retained under the body in a breech posture. **E,** Lateral deviation of the neck. **F,** Ventral deviation of the neck. **G,** Transverse presentation. (*From Johnston SD, Root Kustritz MV, Olson PN:* Canine and feline theriogenology, *Philadelphia, 2001, WB Saunders.*)

A diagnosis of dystocia is made when any of the following occur:

- Gestation length is prolonged. Rule out false pregnancy with abdominal radiographs. Gestation length calculated from breeding dates is the most common but least reliable predictor of delivery date. A gestation length of more than 72 days after breeding is always considered prolonged. Prolonged gestation is confirmed more accurately when gestation length is more than 67 days from the LH surge, more than 65 days from ovulation, or more than 60 days from onset of cytologic diestrus. Gestation also is prolonged when a bitch fails to deliver a pup within 36 hours of serum progesterone levels decreasing to less than 2 ng/ml or within 24 hours of the bitch's rectal temperature dropping below 100° F.
- Stage I labor lasts more than 12 hours.
- Vigorous stage II labor fails to produce a pup or kitten within 30 minutes.
- Weak or intermittent stage II labor fails to produce the first pup or kitten within 4 hours.
- No further pups or kittens are born within 2 hours of a successful delivery.
- Partial delivery of a pup or kitten occurs.
- A vulvar discharge is present for 2 hours without delivery of the first pup. Green-black vulvar discharge indicates that at least partial separation of at least one

placenta has occurred. Passage of clear, watery fluid usually represents amniotic or allantoic fluid and should not be confused with passage of urine.

- Evidence of fetal compromise is present. Distressed pups do not move when viewed by ultrasonography and have heart rates that consistently are of fewer than 180 bpm.
- Evidence of fetal death is present, either on ultrasound (no heartbeat or fetal movement) or radiographs (e.g., intrafetal or perifetal gas, overlapping of skull bones, collapse of axial skeleton).
- Maternal compromise is present and prevents normal progression of labor. Examples include cases of septic metritis, pregnancy toxemia, uterine torsion, uterine rupture, and herniation of a gravid uterine horn through the inguinal canal. In each case the bitch or queen may present with signs of abdominal pain, weakness, and collapse, which may progress rapidly to shock.

Diagnostic Criteria

- Confirm that the bitch is pregnant and not experiencing false pregnancy. Pregnancy can be confirmed with abdominal palpation, digital vaginal examination, radiography, or ultrasonography.
- Confirm that the bitch is at term. Document that the bitch experienced a drop in rectal temperature

to less than 99° F. If that information is unavailable, a progesterone assay can be performed to verify that progesterone levels are less than 2 ng/ml. Term gestation is more difficult to confirm in the queen because there may be no prepartum rectal temperature drop and serum progesterone concentrations may still be above baseline at the time of parturition.

- Confirm that labor has begun and is not progressing normally. A minimum database includes a complete physical examination and a thorough digital vaginal examination. Laboratory assessment minimally includes a packed cell volume, blood urea nitrogen, serum glucose, and serum calcium. A complete blood count, serum biochemical panel, and urinalysis often are helpful. Radiographic and ultrasonographic imaging may document risk factors or confirm a diagnosis of dystocia. Doppler units offer an accurate and inexpensive means of assessing fetal heart rates when conventional ultrasonography is not available. Tocodynamometry can characterize uterine activity.

Treatment of Dystocia

Manipulative, medical, or surgical treatment for dystocia may be indicated, depending on the contributing causes and their chronicity. The goal of any treatment is

to deliver live, vigorous puppies or kittens without compromising the health of the dam.

MANIPULATIVE TREATMENT Manipulative treatment is used to deliver a pup lodged in the birth canal when the pup is only slightly oversized, when there is a correctable malposition or when uterine inertia prevents delivery of the final pup. Small birth canal size restricts manipulative attempts in the queen.

- Perform manipulations with the bitch in a standing position.
- Use plenty of lubrication.
- Use two fingers to gently perform manipulations.
- Do not grasp distal extremities or the tail. Degloving injuries are common.
- A gauze sponge may aid in obtaining a secure grasp of the pup.
- Exercise extreme caution when using instruments such as the Snook ovariohysterectomy hook, sponge forceps, and clamshell forceps, unless the pup already has died. Undesirable sequelae associated with instrument use include mutilation of the pup and laceration of the bitch. Do not use instruments in the queen.
- Concurrent abdominal or rectal palpation may aid manipulative efforts.

MEDICAL TREATMENT Medical treatment primarily involves labor augmentation through the use of ecbolic

agents, which increase uterine contractions. Medical management is indicated when the bitch is in good health and condition, the cervix is relaxed and dilated, fetal size is appropriate, and labor has not been prolonged. Ideally, tocodynamometry is performed to document the need for and monitor the response to the administration of ecbolic agents.

- *Ecbolic agents absolutely are contraindicated in cases of obstructive dystocia.*
- Oxytocin is the most commonly used ecbolic agent. Oxytocin increases the frequency of uterine contractions. Start with a low dosage (0.25-0.5 IU per dog) and do not exceed 4 IU per dose per dog. Higher dosages can cause uterine tetany, during which placental blood supply is reduced and fetal compromise is likely to occur because of hypoxia. Following a "three strikes, you're out" philosophy, a bitch should be taken to surgery if three doses of oxytocin, administered at 30-minute intervals, do not result in delivery of a pup.
- Calcium, in the form of 10% calcium gluconate, often is administered in conjunction with oxytocin. Calcium increases the strength of uterine contractions. Ionized calcium, required for myometrial contraction, may be depleted when total serum calcium concentration is normal. The recommended subcutaneous dose is 1-5 ml per dog.

- Other medical treatments include fluid replacement with balanced electrolyte solutions in dehydrated patients and oral or intravenous administration of glucose if hypoglycemia is detected. Low dosages of phenothiazine tranquilizers have been used to facilitate examination or manipulation in nervous patients and to overcome voluntary inhibition of stage II labor. Their use is discouraged because of risk of fetal depression after placental passage of these drugs.

SURGICAL TREATMENT Surgical treatment usually consists of a C-section (hysterotomy); concurrent ovariohysterectomy can be performed safely. Rarely, an episiotomy is required to relieve obstruction in a primigravid bitch with an insufficient vulvar cleft.

Anesthetic considerations

- Although local or epidural regional anesthesia can be used for C-sections, general anesthesia most often is used because it offers total immobilization and complete analgesia. A variety of anesthetic protocols can be used successfully, with the following recommendations:

  Premedicate with either glycopyrrolate (0.011 mg/kg given intramuscularly or subcutaneously) or atropine (0.04 mg/kg given intramuscularly or subcutaneously) to counteract vagal stimulation occurring secondary to visceral manipulation.

Prepare the surgical site by clipping the ventral abdomen from the xiphoid to the pubis and performing the initial scrub of the area before anesthetic induction.

Administer isotonic fluids, with or without 2.5% dextrose, intravenously. The addition of dextrose is beneficial for patients that may be developing hypoglycemia. A patent intravenous line also is invaluable for compromised patients; emergency drugs may be required.

Oxygenate the patient before induction by administering oxygen through a mask. This helps prevent fetal hypoxemia.

Induce anesthesia with one of the following:

- Propofol (5.5-7.0 mg/kg administered intravenously)
- Diazepam (0.2 mg/kg administered intravenously) and oxymorphone (0.05-0.1 mg/kg administered intravenously)
- Ketamine (5-10 mg/kg administered intravenously) combined with diazepam (0.2 mg/kg administered intravenously)

Intubate quickly and maintain anesthesia with isoflurane. Use of enflurane, halothane, or methoxyflurane also is described.

Puppy vigor after C-section, as assessed by presence of spontaneous breathing, movement, and vocalization, is enhanced by use of isoflurane as the

inhalant anesthetic and decreased with use of thiobarbiturates or ketamine for induction.

Complete surgical preparation of the patient as rapidly as possible.

Anecdotal reports suggest that intravenous administration of 0.5-2.0 ml doxapram to the bitch before incising the uterus may promote spontaneous respiration in the pups.

Surgical technique

- Perform the C-section quickly and efficiently because long induction to delivery times are associated with increased fetal hypoxia and depression. The recommended surgical technique for hysterotomy is as follows:

Use a ventral midline approach, taking care to avoid the engorged, hypertrophied mammary tissue.

Exteriorize the uterus by carefully lifting each uterine horn through the incision. Pack off the area with saline-moistened laparotomy sponges to prevent abdominal contamination with fetal fluids.

Make a dorsal midline incision in a relatively avascular area of the uterine body, taking care not to inadvertently lacerate an underlying pup.

Usually, it is possible to remove all pups through this incision, beginning with the presenting pup in the uterine body. Bring each fetus to the incision by gently "milking it down" the uterine horn. If

necessary, additional incisions through the uterine horns can be made to facilitate rapid removal of pups.

Once each fetus nears the hysterotomy incision, grasp it and apply gentle traction to remove it from the uterus. A saline-soaked sponge often aids the surgeon in obtaining a secure hold of the pup.

Remove the fetal membranes from the pup's head and wipe its nose with a gauze sponge.

Gently separate the zonary placenta from the endometrium and pass the pup with its accompanying placenta to an assistant.

Deliver all pups in a similar fashion.

Before closing, palpate the uterus in its entirety to be certain that all pups and placentas have been removed. Inspect the uterine lumen to be certain that hemorrhage into the uterine lumen is not excessive. Diagnostic procedures, such as bacterial culture of the uterine lumen or uterine biopsy, may be performed at this time.

Administration of oxytocin (0.25-2.0 IU intramuscularly or murally intrauterine) may promote uterine involution and reduce hemorrhage.

Close the hysterotomy incision in two layers routinely and cleanse the uterus with warmed sterile saline solution before replacing it in the abdomen. Close the abdomen in a routine manner.

Neonatal Resuscitation

Resuscitation of the neonate is necessary when the bitch fails to or is unable to perform this task or when a puppy does not respond to typical maternal manipulations. Equipment needed includes the following:

- A box prewarmed with a heating pad or hot water bottles
- Gauze sponges
- Warm towels
- Cotton-tipped swabs
- A suction device (airway suction apparatus or rubber bulb syringe)
- Hemostatic forceps
- Scissors
- Suture
- Diluted iodine solution
- Supplemental oxygen source

After delivery of the neonate, the fetal membranes need to be removed, beginning with those covering the nose and mouth. Use of gauze sponges aids in removal. Next, fluids need to be removed from the mouth and nose using swabs or gentle suction. Puppies and kittens are often wrapped in warm towels and "swung" with their heads down, using centrifugal force to clear the mouth and nose of fluid. This is effective in some cases but should be done carefully and gently, with the head and neck of the neonate fully supported in the resuscitator's hands to prevent trauma. As the initial drying and

suctioning is being performed, the puppy should be assessed to determine whether it is breathing and whether it has a heartbeat. A cyanotic puppy with a good strong heartbeat with a rate of more than 120-150 bpm should be supplemented with oxygen in addition to receiving continued stimulation. Once a pup is breathing normally, the umbilical cord can be ligated and cut 2-3 cm from the body wall. The stump should be disinfected with dilute iodine solution, and the neonate should be examined for the presence of congenital defects such as cleft palate, deformed limbs, or imperforate anus. The neonates are then placed in a prewarmed box until the dam can assume their care. Thermoregulation in puppies and kittens at birth is poor, and it is essential to get the neonate dried off and into a warm environment.

If the puppy or kitten is apneic and has a slow or weak heartbeat, ventilation must be established before cardiac compressions are begun. Ventilatory efforts must come first because it is unlikely that cardiac message will be effective in a hypoxic animal. Supplemental oxygen can be administered by mask, pharmacologic respiratory stimulants can be administered, or the Jen Chung acupuncture site can be stimulated by inserting a 25-gauge needle into the nasal philtrum at the base of the nares and twisting once to reach the periosteum. For cardiac massage, in most breeds the chest compressions should be applied across the lateral chest wall. In some

barrel-chested breeds (e.g., Chinese pugs, French bulldogs), sternal compression may be more effective.

Gaining intravenous access in a newborn puppy is not easy because of the puppy's small size and fragile vessels. The most ready access is through the umbilical vein. Some drugs may be administered intramuscularly with the caveat that this will not be very effective if the circulation is poor. Some drugs have been applied sublingually, but there is little information regarding their uptake by this route in the neonate. Drugs may be administered via a transpulmonary route. This entails endotracheal intubation and the placement of the drug into the lower airway. This requires technical expertise and a very gentle technique to prevent trauma to the airway. Drugs placed in the trachea rather than the lower airways will have much lower uptake. Because the bones of the puppy are soft, it is easy to obtain a "venous" access by use of an intraosseous technique. A 22- to 25-gauge needle can be inserted into the medullary canal of the femur via the greater trochanter or the tibia via the tibial crest (see Chapter 10). Intracardiac injections should be avoided because of the risk of damage to the lungs and heart.

Drugs used for neonatal resuscitation include the following.

- Doxapram is a respiratory stimulant that may increase ventilatory effort once it has begun. However, it probably cannot initiate ventilatory effort in an apneic, hypoxic puppy or kitten. It can

be administered intramuscularly or sublingually if intravenous access is not available. Doxapram is not used routinely for human neonatal resuscitation.

- Naloxone is beneficial to the neonate only when the dam has received an opioid anesthetic. Administer naloxone only if the puppy is showing signs of respiratory depression.
- Atropine is administered for its parasympatholytic effect. However, atropine is no longer recommended for resuscitation of newborn humans. If bradycardia is thought to be drug induced, it is better to antagonize the specific drug if possible.
- Epinephrine increases mean blood pressure and improves myocardial oxygen delivery. It is recommended for neonatal cardiac arrest in humans. Endotracheal administration should be avoided because it can cause intense vasoconstriction of the tracheal mucosa, thereby inhibiting uptake into the systemic circulation.
- Glucose can be used to correct neonatal hypoglycemia that can occur with dystocia or maternal malnutrition. Hypoglycemic neonates should receive glucose only after initial resuscitation is complete. Oral glucose supplementation also may be necessary if there is a delay in the dam's recovery.
- Sodium bicarbonate may be beneficial in treating neonatal acidosis and should be considered for the depressed puppy that has had a marginal oxygen

supply for some time. Because sodium bicarbonate will react with hydrogen ions to produce carbon dioxide, it is essential that adequate ventilation be achieved before its administration. It must be given slowly intravenously (1-2 mmol/kg). The standard preparation of sodium bicarbonate is hypertonic (2000 mOsm/L); it can be diluted with sterile water (1 ml of bicarbonate in 5.7 ml of water) to render an isotonic solution.

Periparturient Disorders of the Bitch and Queen

Care of the Postpartum Bitch

When delivery is complete, soiled bedding in the birthing box should be replaced with clean bedding. A thorough cleansing followed by liberal rinsing of the dam's perineal and ventral abdominal regions also is recommended. Because fetal fluids and placental tissues offer an optimal environment for bacterial growth, attention to hygiene reduces the risk for bacterial contamination and infection of both the dam and her neonates.

Owners often request that a "clean-out shot" of oxytocin be administered to the bitch. This usually is unnecessary. Owners should be instructed that suckling by the pups stimulates endogenous oxytocin release in the

bitch. Oxytocin therapy may be indicated when the bitch has no pups to nurse or when placentas are believed to have been retained.

Postpartum management primarily consists of careful monitoring to allow for early detection of periparturient disorders, should they arise. Periparturient disorders include mastitis (see Chapter 14), metritis and subinvolution of placental sites (see Chapter 12), and eclampsia.

Owners need to evaluate the postpartum bitch or queen daily. After a period of rest immediately postpartum, the dam should appear bright, alert, and attentive to her newborns. She should resume eating and drinking. As lactation progresses and peaks, she may consume two to three times her normal food intake. Mammary glands should be inspected daily for discoloration and evidence of purulent discharge and should be palpated for heat, pain, and changes in consistency. Milk should be uniformly white or slightly yellow. It should not be of varying consistency and should not be greenish yellow, pink, or red. The bitch should not experience pain when milk is expressed. Normal postpartum endometrial desquamation is evidenced as a vulvar discharge, called *lochia,* which gradually diminishes and ceases within 3 weeks. Lochia typically is brick red to green-black and without significant odor. If lochia turns creamy or develops a foul odor, it is abnormal. Little or no discharge is observed in postpartum queens. Rectal temperature

may be elevated for 2-3 days postpartum but should not exceed 103° F.

Eclampsia (Postparturient Hypocalcemia, Puerperal Tetany)

DEFINITION AND PATHOGENESIS Eclampsia is associated with ionized calcium depletion in the extracellular compartment of the body. Depletion of membrane-bound calcium increases membrane permeability and results in spontaneous muscle depolarization.

SIGNALMENT Hypocalcemia occurs more commonly in toy breeds of dogs and is rare in the queen.

HISTORY AND CLINICAL SIGNS Although hypocalcemia can occur before parturition or during any part of lactation, by far, most cases present 1-4 weeks postpartum, when lactational demands are greatest. Signs include restlessness, nervousness, whining, panting, muscle tremors, dilated pupils, and hyperthermia. If hypocalcemia is left untreated, within hours, signs can progress to recumbency, extensor rigidity, convulsions resulting from cerebral edema, and death.

DIAGNOSTIC TESTS AND RESULTS A total serum calcium concentration of less than 7 mg/100 ml indicates hypocalcemia. However, a diagnosis usually is made

before obtaining laboratory results on the basis of the history and clinical signs. Some patients, when presented early in the course of the disease, may have a normal total calcium concentration. Hypoglycemia may occur concurrently with hypocalcemia, but it alone cannot cause muscle rigidity. A differential diagnosis list would include other causes of seizure activity, such as toxicosis, epilepsy, and other neurologic disorders. A history of recent parturition aids the clinician in a diagnosis of eclampsia.

TREATMENT Treatment of eclampsia consists of the following:

- Immediate calcium supplementation can be intravenously administered slowly to effect. Commercial calcium solutions vary widely in their concentrations of elemental calcium. For example, a 10% calcium chloride solution contains about three times as much elemental calcium (in milligrams per milliliter) as does a 10% solution of calcium gluconate. Concurrent monitoring of heart rate by auscultation, or preferably by electrocardiography, is strongly recommended. Intravenous administration of calcium should be discontinued if progressive bradycardia or tachycardia develops or if dysrhythmias occur. If muscle tremors have not subsided when intravenous administration of calcium is discontinued, additional calcium can be administered

by either subcutaneous or intramuscular routes. Care must be taken to ensure that preparations are labeled and safe for the intended route of administration.

- Hypoglycemia, if present, can be corrected with intravenous dextrose solutions.
- The body temperature of hyperthermic patients can be reduced with cool water baths and alcohol soaks to areas of rapid heat exchange, such as the ear pinnae and footpads.
- The puppies can be removed from the bitch for at least 24 hours and, if possible, weaned.
- Calcium supplementation can be dispensed at the time of discharge. The recommended dosage of oral calcium is 1-3 g/day with 10,000-25,000 IU of vitamin D, which enhances uptake of calcium from the intestinal tract. Calcium supplementation should continue until the end of lactation.

Glucocorticoid therapy for treatment of eclampsia is no longer recommended. It is contraindicated because it further compromises the patient by decreasing calcium absorption in the intestine and increasing renal excretion of calcium.

PROGNOSIS It is important that the owner understand the need for continued monitoring of serum calcium concentration, particularly if puppies continue to nurse. Hypocalcemia may recur in the current lactation, and the

bitch will be at risk for another episode of eclampsia with subsequent litters. It should be emphasized that oversupplementation of calcium during pregnancy may be counterproductive because it may hinder the bitch's ability to rapidly mobilize calcium from bone at peak lactation. Dietary recommendations include feeding balanced diets with calcium/phosphorus ratios in the 1:1-1.2:1 range to pregnant bitches. Dog foods that contain high amounts of phytates, which are contained in soybean meal, should be avoided. The phytates combine with ionized calcium and render the calcium unavailable physiologically.

Bibliography

Al-Bassam MA, Thomson RG, O'Donnell L: Normal postpartum involution of the uterus of the dog, *Can J Comp Med* 45:217, 1981.

Bebchuk TN, Probst CW, Bojrab MJ et al (eds): *Current techniques in small animal surgery,* Philadelphia, 1998, Lippincott Williams & Wilkins.

Bennet D: Canine dystocia: a review of the literature, *J Small Anim Pract* 15:101, 1974.

Concannon PW: Physiology of reproduction. In Burke TJ (ed): *Small animal reproduction and fertility,* Philadelphia, 1986, Lea & Febiger.

Concannon PW, McCann JP, Temple M: Biology and endocrinology of ovulation, pregnancy and parturition in the dog, *J Reprod Fertil Suppl* 39:3, 1989.

Copley K: Canine parturition management. Proceedings of the Society for Theriogenology, Vancouver, British Columbia, Canada, 2001.

Darvelid AW, Linde-Forsberg C: Dystocia in the bitch: a retrospective study of 182 cases, *J Small Anim Pract* 35:402, 1994.

Davidson A: Periparturient problems in the bitch. Proceedings of the Society for Theriogenology, Montreal, Quebec, Canada, 1997.

Ekstrand C, Linde-Forsberg C: Dystocia in the cat: a retrospective study of 155 cases, *J Small Anim Pract* 35:459, 1994.

Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction,* Philadelphia, 1978, WB Saunders.

Frand MN, Honig KL, Hageman JR: Neonatal cardiopulmonary resuscitation: the good news and the bad, *Pediatr Clin North Am* 45:587, 1998.

Friedman E: Use of labor pattern as a management guide, *J Reprod Med* 2:57, 1968.

Gaudet DA: Retrospective study of 128 cases of canine dystocia, *J Am Anim Hosp Assoc* 21:813, 1985.

Gaudet DA, Kitchell BE: Canine dystocia, *Comp Cont Ed* 7:406, 1985.

Ginsberg HG, Goldsmith JP: Controversies in neonatal resuscitation, *Clin Perinat* 25:1, 1998.

Holladay JR: Routine use of doxapram hydrochloride in neonatal pups delivered by caesarian section, *Vet Med Small Anim Pract* 66:28, 1971.

Johnston SD: Management of the postpartum bitch and queen. In Kirk RW (ed): *Current veterinary therapy VIII*, Philadelphia, 1983, WB Saunders.

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology*, Philadelphia, 2001, WB Saunders.

Johnston SD, Smith FO, Bailie NC et al: Prenatal indicators of puppy viability at term, *Comp Cont Ed* 5:1013, 1983.

Jones RL: Special considerations for appropriate antimicrobial therapy in neonates, *Vet Clin North Am* 17:577, 1987.

Laliberte L: Pregnancy, obstetrics and postpartum management of the queen. In Morrow DA (ed): *Current therapy in theriogenology*, Philadelphia, 1986, WB Saunders.

Leuthner SR, Jansen RD, Hageman JR: Cardiopulmonary resuscitation of the newborn, *Pediatr Clin North Am* 42:893, 1994.

Monheit AG, Stone ML, Abitol MM: Fetal heart rate and transcutaneous monitoring during experimentally induced hypoxia in the fetal dog, *Pediatr Res* 23:548, 1988.

Moon-Massat PF, Erb HN: Perioperative factors associated with puppy vigor after delivery by cesarean section, *J Am Anim Hosp Assoc* 38:90, 2002.

Root MV, Johnston SD, Olson PN: Estrous length, pregnancy rate, gestation and parturition lengths, litter

size, and juvenile mortality in the domestic cat, *J Am Anim Hosp Assoc* 31:429, 1995.

Schmidt PM: Feline breeding management, *Vet Clin North Am* 16:435, 1986.

van der Weyden GC, Taverne MA, Dieleman SJ et al: Physiological aspects of pregnancy and parturition in dogs, *J Reprod Fertil Suppl* 39:211, 1989.

10

Neonatology

Margaret V. Root Kustritz

AT A GLANCE

- Physical examination of neonates
  - The owner should weigh the pups or kittens daily. Neonates should maintain or gain weight every day, doubling birth weight by 7-10 days of age. Loss of weight often is the first sign of a disorder.

- Eyes open at 5-14 days of age. Ear canals open at 6-14 days of age.
- Rectal temperature is lower than that of adults until 7 weeks of age. A rectal temperature of less than 95° F is abnormal at any age.
- Normal behavior during the first 2-3 weeks of life includes suckling and sleeping. Abnormalities include lying apart from the littermates or dam and crying excessively.

- Common abnormalities of neonates
  - Congenital defects can be present.
  - Septicemia is systemic infection. Entry of organisms most commonly occurs through the umbilicus. *Escherichia coli* is the most common organism isolated. Treat with antibiotics. Blood or urine can be cultured to guide therapy; penicillins and cephalosporins are appropriate empirical choices pending culture results. Adjunctive therapy with fluids and oxygen may be required.
  - Neonatal isoerythrolysis occurs in kittens born to a dam with a blood type different from their own. Antibodies ingested in the colostrum destroy the neonate's red blood cells (RBCs). Treat by removing the kitten from its dam and performing a blood transfusion; the dam serves as a good donor because her RBCs are not affected by her serum antibodies.
  - Canine herpesvirus causes fulminating hemorrhagic necrosis of the internal organs in puppies

exposed during the first 3 weeks of life. Treatment generally is unrewarding.
  - Internal and external parasites can be present.
  - "Fading" puppies and kittens are sometimes observed. Causes include septicemia, inadequate environment, low birth weight, congenital abnormalities, maternal neglect or trauma, lack of ingestion of colostrum, and inadequate milk.
- Feeding of orphan puppies and kittens
  - Use a commercial milk replacer.
  - Tube feeding is quick but may be associated with aspiration of milk replacer and excessive suckling of littermates, inanimate objects, or self.
  - Warm the milk replacer to 95°-100° F. Feed four times daily for the first 2 weeks of life and then three times daily until weaning at about 6 weeks of age.
  - Stimulate urination and defecation until the pup or kitten is mobile and can void spontaneously, at about 3 weeks of age.

Physical Examination of Neonates

Rectal Temperature

Rectal temperature varies with age and environment. Normal rectal temperature is 96.0° ± 1.5° F (35.6° ± 0.7° C) during the first week of life, is 98.6°-100.0° F (37.0°-38.2° C) during the second and third weeks of

life, and gradually rises to adult levels by the age of 7 weeks.

Behavior

During the first 2-3 weeks of life, puppies and kittens spend most of their time sleeping. They huddle together or near the dam and will not ordinarily sleep apart from littermates or the dam until 5-6 weeks of age. When awake, neonates suckle vigorously. During examination, normal puppies and kittens easily can be encouraged to suckle the examiner's fingers. The veterinarian should immediately examine all pediatric animals that are observed to lie apart from littermates or the dam, cry excessively, are extremely restless, or are recumbent.

Eyes and Ears

Eyelids separate in puppies and kittens from 5-14 days of age. Abyssinian kittens may exhibit eyelid separation substantially earlier (0-5 days of age). Corneal cloudiness resulting from overhydration resolves within 2-3 weeks of eyelid separation. Menace and pupillary light responses develop slowly but should be present by 21 days of age. Shirmer tear testing for adequacy of lacrimation can be performed any time after eyelid separation. The external auditory canals open at approximately 6-14 days of age. A thorough otoscopic

examination is possible in animals as young as 4 weeks. Assessment of hearing in the clinic is difficult. Percussive hearing tests, such as hand clapping out of the animal's field of vision, are inaccurate because distracted animals with normal hearing may not react (false-negative result) and deaf animals may react as a result of pressure changes (false-positive result). A brainstem auditory evoked response (BAER) test measures electrical potential from the cochlea, cochlear nerve, and brainstem in response to auditory stimuli. Functional maturity of hearing and an accurate BAER test should be possible by 4-6 weeks of age. If the test result is negative, the animal is functionally deaf.

Skin and Hair Coat/Hydration Status

Assessment of the entire skin and hair coat should be performed. Dermatologic conditions such as flea infestation and dermatophytosis are relatively common in young animals. The umbilicus falls off or is removed by the dam at 2-3 days of age. The umbilicus should be examined for evidence of infection. Traumatic inflammation of the appendages may occur if littermates are suckling on each other. Skin turgor is difficult to use as a measure of dehydration, but dehydrated pediatric animals exhibit more wrinkling and less turgor of the skin and exhibit deepening of the red color of the ventral abdomen and muzzle. Other signs of dehydration

include dryness of the eyes and oral mucous membranes and visible yellow color of the normally dilute urine.

Neurologic System

Neurologic examination of pediatric patients is possible at birth, but neurologic function does not mature until 6-8 weeks. Normal neonatal puppies and kittens can crawl; suckle; vocalize if distressed; and respond to odor, touch, and pain. Withdrawal reflexes are present but slow at birth. Flexor dominance is present until about 4 days; onset of extensor dominance varies from days 5-21. Postural reactions, including nonvisual placing, extensor postural thrust, and hemiwalking, are not fully developed until 6-8 weeks and generally develop in the forelimbs before the hind limbs.

Musculoskeletal System

Assessment of the musculoskeletal system includes evaluation of movement and palpation of the bones and joints. Puppies and kittens can lift their heads at birth. They crawl for the first 7-14 days of life and should be able to support weight on their forelimbs by 10 days. Locomotion is present by 3 weeks, allowing assessment of gait. Muscle tone should be evaluated; decreased muscle tone often precedes other signs of illness. Radiographic assessment of bone and joint abnormalities is difficult in puppies and

kittens because mineralization of bone is decreased. The quality of the radiographs can be enhanced by decreasing kilovolts peak (kVp) to half that for an adult of the same thickness or, for puppies, by using 2 kVp for each 1 cm of soft tissue measured for values up to 80 kVp.

Thoracic Cavity

The heart may be difficult to auscult in puppies and kittens because of the small size of the heart and rapid heart rate. Heart rate is greater than 220 beats per minute during the first week of life. Cardiac murmurs of grades I-III/VI, most commonly heard at the base of the heart on the left side, are often functional murmurs caused by anemia, hypoproteinemia, fever, or sepsis. Congenital cardiac abnormalities usually are associated with murmurs of grades III-VI/VI and abnormal peripheral pulses. Lung sounds are auscultated easily with a stethoscope with a pediatric head. Respiratory rate is 10-35 breaths per minute during the first week of life and reaches adult levels by 4 weeks.

Oral and Abdominal Cavities

The deciduous teeth are present by 6 weeks. Permanent teeth erupt, displacing the deciduous teeth, at 4-6 months. Abdominal palpation should permit recognition of the left kidney, small intestines, colon,

and urinary bladder. If the spleen is palpable, splenomegaly is present. Hepatomegaly is present if the liver margin is palpable beyond the ribcage.

Common Disorders of Neonates

General Information

PREWEANING MORTALITY/GENERAL SIGNS OF ILLNESS Mortality of puppies and kittens is high, even in well-managed colonies. The greatest incidence of mortality occurs within the first week of life and averages 27.3% in kittens and 26.0% in puppies. Overall mortality rates by weaning at 8-12 weeks of age average 23.2% in kittens and 18.7% in puppies. It is recommended that all dead puppies and kittens be submitted for necropsy.

Behavior that suggests illness of a pediatric patient includes separation from the dam, indicating either fever or culling by the dam; crying for more than 20 minutes at a time; and decreased activity. On physical examination, decreased muscle tone, pale to cyanotic mucous membranes, lack of normal bowel sounds, panting or labored breathing, rough hair coat, and diarrhea may be evident.

Systemic Disorders

ANOXIA Length of the anoxic episode is not associated with outcome in neonates. Anoxia is associated with

bradycardia and hypoventilation in very young animals and often is accompanied by hypothermia and a subsequent lower oxygen demand.

CONGENITAL DEFECTS Congenital defects, those present at birth, may be hereditary or developmental, or may result from exposure of the dam to teratogenic substances. The fetuses are most susceptible to noxious influences during the first third of pregnancy, during organogenesis. The congenital defect most commonly reported in a survey of 51 kittens was cleft palate. A survey of 1679 pups that were 8-16 weeks old identified cryptorchidism, patellar luxation, cardiac abnormalities with murmurs, cleft palate, and umbilical and inguinal hernias as the most common congenital defects.

Excellent reviews of congenital and hereditary defects in puppies and kittens exist. If a particular defect occurs in more than one litter from a particular dam or sire or if frequency of the defect increases with inbreeding, a hereditary basis should be suspected and the animals involved removed from the breeding program. Gross anatomic abnormalities are visible at necropsy. Microanatomic and biochemical abnormalities may be identified by special testing available at the University of Pennsylvania. Availability of tests for hereditary disorders is consistently updated on the American Kennel Club Canine Health Foundation website (http://www.akcchf.org/research/genetic.htm).

Effects of drugs during pregnancy in dogs and cats often are extrapolated from work in other species (Box 10-1). Teratogens present during the first 26 days after conception often cause cephalic, ocular, otic, or cardiac abnormalities, whereas those present during the transition period immediately after day 26 are more likely to cause palate, cerebellar, or urogenital defects. Defects of the central nervous system, cardiovascular system, and respiratory tract, depending on degree, often are incompatible with life. Queens infected with or, presumably, vaccinated with modified live virus vaccine for panleukopenia may give birth to kittens with cerebellar hypoplasia.

"FADING" PUPPIES AND KITTENS Causes of fading in puppies and kittens depend on a definition of the term. Taken at its broadest meaning, the term *fading puppies and kittens* includes those that are either born weak and fail to thrive or are vigorous but then weaken, dying by approximately 1 week of age. The causes are many and include septicemia, inadequate environment, low birth weight, congenital abnormalities, maternal neglect or trauma, lack of ingestion of colostrum or inadequate milk, and neonatal isoerythrolysis (described as occurring in cats only). In most cases more than one of these factors probably is causative, with primary factors such as hypothermia, deficient colostrum ingestion, and immunodeficiency predisposing the animal to secondary infection, with subsequent hypoglycemia and dehydration

leading to cardiopulmonary failure. Treatment of fading puppy and kitten syndrome depends on identification of the underlying cause.

HYPOTHERMIA Rewarming of hypothermic puppies and kittens should be gradual, taking 30 minutes to 2 hours. The neonate should be turned and rectal temperature monitored frequently. Rectal temperature should not exceed 101° F (36.3° C) because this will cause dehydration. Rewarming can be accomplished with careful use of surface heat, such as circulating hot water pads and hot water bottles, warmed inspired air, or warmed fluids administered intravenously or intraosseously.

Body temperature of less than 94°-95° F (34.5°-35.0° C) is associated with failure to suckle and ileus (i.e., visceral paralysis). Milk products should not be given orally until body temperature is returned to normal because hypothermic animals are incapable of digesting milk and are predisposed to aspiration if the stomach is distended. Calories can be provided by parenteral or oral administration of glucose-rich solutions, which do not require normal peristalsis for absorption.

LOW BIRTH WEIGHT/POOR GROWTH RATE The puppy or kitten should have been weighed at birth and should be weighed daily thereafter. Kittens should weigh approximately 3.5 oz (100 g) at birth. Puppy birth weight varies by breed; estimates for various breeds are 4.2 oz (120 g) for Pomeranians, 9 oz (250 g) for beagles, 17 oz (490 g) for

Box 10-1. Teratogenicity of Drugs Used in Veterinary Practice

Type	Representative Drugs and Effects
Anticonvulsants	Primidone: cardiac defects, cleft palate, skeletal abnormalities
Antiinfectives	Griseofulvin: microphthalmos (kittens), cleft palate (puppies) Ketoconazole: stillbirths (dogs) Tetracycline and aminoglycoside antibiotics: bone and teeth malformations (tetracycline), deafness and renal disease (aminoglycosides) Metronidazole: described only as being teratogenic in laboratory animals; no specific disorders are described; not recommended for use in animals
Antiinflammatories	Aspirin: embryonic death Dimethyl sulfoxide (DMSO): described as being teratogenic in laboratory animals; manufacturer does not recommend its application to breeding animals Glucocorticoids: anasarca in brachycephalic breeds
Antineoplastic agents	All: embryotoxic and can produce a variety of malformations in offspring that survive; examples of antineoplastic drugs commonly used in small animal oncology include cisplatin, cyclophosphamide, methotrexate, and vincristine

Box 10-1. Teratogenicity of Drugs Used in Veterinary Practice—cont'd

Type	Representative Drugs and Effects
Hormones	Diethylstilbestrol and estradiol cypionate: feminization of males Testosterone and mibolerone: masculinization of females Progesterone: masculinization of females
Sedatives	Diazepam and midazolam: congenital defects in laboratory animals and human beings; not recommended for use during early pregnancy in dogs and cats
Vitamins	Excess vitamin A: cleft palate, kinked tails, cardiac defects (kittens) Excess vitamin D: tissue calcinosis, enamel hypoplasia, cardiac defects

greyhounds, and 22 oz (625 g) for Great Danes. Low birth weight is correlated with poor survivability in puppies and kittens. Birth weight is not influenced by sex of the neonate and is more likely an indicator of inadequate intrauterine nutrition or congenital abnormalities than of

prematurity. A guarded prognosis should be made regarding neonates with lower than average weight at birth.

Slight weight loss may occur in the first 24 hours of life. Puppies that lose more than 10% of their birth weight during the first day of life have a poor prognosis. After that, the puppy or kitten should gain weight daily, doubling the birth weight by 7-10 days of age.

Pediatric animals with poor growth rates should be assessed for adequacy of nursing. The veterinarian should ensure that they are not being excluded from nursing by competition with stronger littermates, that the nipples are not hyperkeratotic, that mastitis is not present, and that there are no congenital abnormalities in the patient precluding effective suckling (e.g., cleft palate). During and after weaning the animal should be fed a high-quality food that is dense with energy-providing nutrients. Animals with slow growth and poor body condition may have disorders such as a portosystemic shunt, renal failure, megaesophagus, exocrine pancreatic insufficiency, or cardiac disease. Animals with slow growth and normal body condition may have disorders such as hypothyroidism, diabetes mellitus, or adrenal disease.

NEONATAL ISOERYTHROLYSIS (KITTENS) Neonatal isoerythrolysis is an acute disease of kittens that can occur during the first days of life. Cats have naturally occurring antibodies against the blood type they are lacking; development does not require previous pregnancy or transfu-

sion. There are two main feline blood types: A and B. Type A cats have weak anti-B antibodies, whereas type B cats have strong anti-A antibodies. Kittens with blood type A born to type B queens become acutely ill after ingestion of colostrum and absorption of her anti-A antibodies.

Clinical signs in the kittens include anemia, icterus, tail tip necrosis from hemagglutination in peripheral capillaries and localized thrombus formation, weakness, tachypnea, tachycardia, hemoglobinuria, and sudden death. Severity of signs may vary within the litter, presumably because of variability in antibody uptake.

The mortality rate is high in affected kittens, even with prompt intervention. The kittens should be removed from the dam and given blood transfusions if necessary. The dam is a good blood donor because she has no antibodies to her own RBCs.

Prevention involves avoiding incompatible matings (type B queens to type A toms). Estimation of proportion of incompatible matings by breed is 0.25% for domestic shorthaired cats, and as high as 14%-25% for Persians and Abyssinians. The type B blood type is most prevalent in the Devon and Cornish Rex and British Shorthair breeds (Table 10-1). Blood typing can be performed in-house (DMS Laboratories, Flemington, NJ; 800-567-4367) or at commercial laboratories.

SEPTICEMIA Septicemia, also called *neonatal sepsis*, is systemwide infection with one or more bacterial organisms.

Table 10-1. Frequency of Type A and B Blood in Cats in the United States

Breed	Type A (%)	Type B (%)
Abyssinian	81	19
Birman	82	18
British Shorthair	41	59
Devon Rex	57	43
Himalayan	80	20
Persian	76	24
Scottish Fold	85	15
Somali	78	22
Domestic Shorthair	99	1

Entry occurs most commonly via the umbilicus. The animal may be predisposed to septicemia by inadequate ingestion of colostrum or concurrent disease of the neonate or dam. Gram-negative organisms are most commonly involved, with *E. coli* being the most prevalent isolate.

Clinical signs vary with the organs affected. Reported syndromes include gastroenteritis with foamy vomitus, liquid diarrhea, reddening of the anus, rapid dehydration, and death; pyelonephritis with abdominal pain, fever, dehydration, and hematuria; omphalitis; conjunctivitis; pneumonia with respiratory distress and cyanosis; and nonspecific weakness, vocalization, and dehydration. Acute respiratory distress syndrome,

characterized by life-threatening noncardiogenic pulmonary edema, may occur secondary to septicemia, as may sloughing of the extremities, perhaps because of concurrent disseminated intravascular coagulation, tissue hypoxemia, or vasculitis.

Putative diagnosis is based on clinical signs, presence of normocytic normochromic anemia, thrombocytopenia and mild to moderate neutrophilia with a left shift on complete blood count, and hypoglycemia revealed by serum chemistry profile. Hypoglycemia may develop because of impaired glycogenolysis and gluconeogenesis, decreased liver perfusion resulting from congestion of major organs, and increased use of glucose by bacteria and leukocytes. Definitive diagnosis requires blood culture. Blood cultures can be performed by diluting 1 ml of whole blood with 5-10 ml of enrichment broth and examining the broth culture 6-18 hours later. Urine culture may be positive in some septicemic animals. Septicemia commonly is diagnosed at necropsy.

Treatment of septicemia requires fluid therapy to counter dehydration and hypoglycemia (balanced electrolyte solution with 5% dextrose and potassium chloride [KCl] supplement if serum potassium concentrations are less than 2.5 mEq/L), oxygen therapy for management of tissue hypoxemia, and appropriate antibiotic therapy. Penicillins and cephalosporins are appropriate empirical choices, pending culture and sensitivity results.

Infectious Diseases

Severity of clinical signs with infectious disease is dependent on nutrition, thermoregulation, concurrent parasitism, developmental or hereditary defects of the immune system, and acquisition of passive immunity.

PUPPIES

Herpesvirus Herpesvirus is an opportunistic virus that most readily infects bitches in late gestation, causing stillbirths and abortion, or infects puppies during passage through the birth canal or during the first 3 weeks of life, causing acute neonatal viremia. Puppies exhibit abdominal pain and constant crying and die within 24-48 hours of onset of signs. Diagnosis usually is made at necropsy, during which hemorrhagic necrotizing changes are observed as petechiation of the kidneys, liver, and intestinal mucosa. Excessive pleural and abdominal fluid may be present, and inclusion bodies may be identified in hepatocytes. Treatment generally is unrewarding. The optimal temperature for incubation of the virus in tissue culture is 95.0°-98.6° F (35.0°-37.0° C); replication may be inhibited in infected puppies by maintaining body temperature above 101.0°-102.2° F (36.3°-37.0° C).

Infectious canine hepatitis Puppies may be infected with infectious canine hepatitis, an adenovirus, in utero or

during passage through the birth canal. This disease usually is diagnosed at necropsy; characteristic intranuclear inclusion bodies are present in hepatocytes. Infectious canine hepatitis resulting from vaccination occurs rarely.

Canine distemper Canine distemper is uncommon because of vaccination. It usually is diagnosed at necropsy in puppies; thymic atrophy, bronchopneumonia, and characteristic intranuclear inclusion bodies are observed.

KITTENS

Upper respiratory infection A complex of respiratory diseases occurs in cats and is caused by viruses, such as rhinotracheitis and calicivirus; bacteria, including *Bordetella bronchiseptica* and *Chlamydia psittaci;* and rarely, fungal organisms. Queens often are asymptomatic until stressed by queening, at which time organisms are shed and the less immunocompetent kittens become infected. Clinical signs vary from mild conjunctivitis and serous oculonasal discharge to sneezing, tenacious oculonasal discharge, self-trauma related to pawing at the face, and respiratory distress. The condition usually is self-limited and resolves in 10-14 days. Antibiotic therapy may hasten resolution of clinical signs. Appropriate vaccination within the colony is important for control.

Calicivirus infection may be associated with mononuclear cell infiltration of joints, causing a lameness that usually is self-resolving; this is sometimes termed *limping kitten syndrome.*

Panleukopenia Kittens infected with feline distemper parvovirus in utero develop cerebellar hypoplasia. Those infected as neonates exhibit acute onset of vomiting and diarrhea, fever, rapid dehydration, leukopenia, and death. The disease is rarely caused by vaccination.

Feline leukemia Feline leukemia virus can be transmitted to susceptible kittens while nursing or through any other close contact with infected animals. Infected kittens undergo lymphoid depletion with thymic atrophy. Clinically, they exhibit failure to thrive and secondary sepsis.

Feline infectious peritonitis Feline infectious peritonitis rarely is described as a cause of mortality in very young kittens. Diagnosis via serologic examination is difficult because of cross-reaction with the relatively nonpathogenic enteric coronavirus (FECV). Animals infected with FECV, including kittens, shed virus before becoming viremic, lessening the value of serologic testing as a means to decrease introduction of infected animals into the cattery.

Disorders of Specific Systems

DERMATOLOGIC DISORDERS Fleas are a common external parasite of young animals. Severe flea infestation can cause anemia; clinical signs include pale mucous membranes, lethargy, tachycardia, collapse, and death. Fleas also can transmit tapeworms. The preferred treatment for fleas in animals younger than 2 months is thorough bathing and grooming with a flea comb. Lufenuron (Program, Sentinel [with milbemycin]; Novartis, East Hanover, NJ) is an orally administered product approved for use in kittens 4 weeks or older to prevent and control fleas. Sentinel also controls roundworms, whipworms, and hookworms and prevents heartworms. Nitenpyram (Capstar, Novartis) is an oral product appropriate for use in animals in facilities with a heavy flea infestation; the youngest age at which it can be used safely has not been defined. Selamectin (Revolution; Pfizer, New York, NY), imidacloprid (Advantage; Bayer, Shawnee Mission, Kan.), and fipronil (Frontline; Merial, Iselin, NJ) are topical flea control products reportedly safe for use in kittens older than 6, 8, and 12 weeks, respectively. Selamectin also controls heartworms, roundworms, hookworms, and ear mite infestations.

Dermatophytosis is relatively common, especially in catteries. *Microsporum caninum* is the most common isolate. The kittens exhibit progressive crusty alopecia and may or may not be pruritic. The organism lives in dead skin and in the hair shafts of infected animals. It is difficult

to eradicate from the environment and is zoonotic. If griseofulvin is used for treatment, kittens may exhibit side effects of anorexia, vomiting, diarrhea, anemia, leukopenia, and ataxia. Griseofulvin is slowly metabolized in kittens and may be hepatotoxic. Vaccination against *M. caninum* reportedly is variable in efficacy in catteries.

GASTROINTESTINAL DISORDERS/PARASITES *Toxic milk syndrome* is a term often used to describe increased vocalization and abdominal distention in 3- to 14-day-old puppies or kittens. This is more likely to be caused by hypothermia, with secondary ileus, or to overfeeding than to abnormalities of the dam's milk.

Diarrhea often occurs secondary to overfeeding of neonates or disruption of the normal gastrointestinal environment with changes in diet or with antimicrobial therapy. Diarrhea may be difficult to diagnose in young animals with zealous dams and may be first observed as lack of weight gain and dehydration. Treatment is supportive care with fluid therapy and assessment of the feeding schedule.

Intestinal parasites are common in young animals, especially those born in warm climates or in closely managed facilities. In puppies, roundworms *(Toxocara canis)* and hookworms *(Ancyclostoma caninum)* are very common. Roundworms can pass transplacentally in late gestation. Within infected puppies the larvae may migrate to the lungs and liver, causing a nonproductive cough

and poor weight gain. Hookworms are passed via the dam's milk and can cause significant blood loss in infected pups within 8 days of infection, although oocysts are not shed in the feces until approximately 14 days after infection. Puppies 2 weeks or older are treated with pyrantel pamoate (5-10 mg/kg administered orally once daily for 2-3 weeks). Prevention involves treatment of the bitch with fenbendazole (50 mg/kg administered orally once daily) from the fortieth day of gestation to 14 days postpartum. Kittens also can be infected with roundworms; treatment is with pyrantel pamoate, as described previously. Use of piperazine is not recommended in kittens.

Coccidiosis can occur in either puppies or kittens. Infection usually is asymptomatic and self-limited. If diarrhea occurs, treatment may be instituted with sulfadimethoxine (30 mg/kg once daily or 15 mg/kg twice a day in puppies, 30 mg/kg once daily in kittens weighing at least 1 kg) until signs regress. Similarly, infection with *Giardia* species usually is asymptomatic and self-limited. If necessary, puppies and kittens can be treated with either metronidazole (30 mg/kg given orally once daily for 7-10 days or 25 mg/kg given twice a day for 5 days and then 10 mg/kg given twice a day) or fenbendazole (50 mg/kg administered orally once daily for 3-7 days).

Hemobartonella felis may be an incidental finding on complete blood counts in kittens or may be a cause of

RBC destruction, anemia, and icterus. It can be treated with doxycycline (10 mg/kg once daily) and decreasing dosages of prednisone.

Toxoplasma gondii is an uncommon cause of a syndrome in kittens characterized by neurologic signs, fever, respiratory disease, anemia, lymphadenopathy, and death within 3-12 days. The mode of transmission is undefined, but congenital infection seems likely because the disease has been definitively diagnosed in kittens as young as 2 weeks. Treatment is with triple sulfonamides, pyrimethamine, and folic acid; efficacy is questionable. Infected kittens, their feces, and their bedding should be isolated and all contaminated materials destroyed. Toxoplasma oocysts are resistant to commonly used disinfectants. A zoonotic potential exists; pregnant women should not handle infected cats or contaminated materials.

NEUROLOGIC DISORDERS Neurologic disorders may be congenital, such as hydrocephalus and cerebellar ataxia, or may be acquired, such as traumatic injury to the spinal column, lead toxicity, or parasitism. Localization of neurologic disorders can be accomplished in neonates as in adults (Box 10-2).

OPHTHALMIA NEONATORUM/JUVENILE CATARACTS Ophthalmia neonatorum is conjunctivitis and infection behind closed eyelids in kittens and puppies younger

Box 10-2. Localization of Neurologic Dysfunction in Puppies and Kittens

Sign	Location
Head tilt	Vestibular defect, ipsilateral
Seizures, abnormal mental status	Cerebrum
Intention tremor, ataxia, limb incoordination	Cerebellum
Gait and posture deficits without cranial nerve deficits	
Forelimbs only	Peripheral
All four limbs	
UMN all	Upper cervical spine or diffuse
LMN front, UMN rear	Lower cervical spine or diffuse
Pelvic limbs only	
UMN	T2-L3
LMN	L4-S1
Dilated anus, flaccid tail	S1-S3

LMN, Lower motor neuron; *UMN,* upper motor neuron.

than 10-14 days. It is common in catteries with endemic herpesvirus (rhinotracheitis) infection. Treatment is gentle separation of the eyelids and application of topical ophthalmic ointment.

Cataracts may develop in puppies and kittens fed either commercial or homemade milk replacers. These small focal cataracts usually resolve spontaneously after weaning.

"SWIMMER" PUPPIES Swimmer puppies and kittens have dorsoventral compression and lateral widening of the thoracic cavity. The cause is unknown; hereditary and environmental factors (e.g., slippery flooring) may be involved. Treatment is taping of the limbs in an adducted position or provision of a nonslip surface or an uneven surface, such as egg carton foam. Nandrolone laurate (10 mg) can be administered twice over a 2-week interval, presumably to promote growth of muscle and joint-associated connective tissue.

Normal Laboratory Values in Neonates

Sample Collection

Venipuncture in pediatric patients can be difficult because the animal may be intractable and the veins are very small and collapse easily. Blood usually is most easily collected from the external jugular vein, although the cephalic vein can be used in larger puppies. The femoral vein is too friable for venipuncture in pediatric patients. The area over the vein should be moistened with water, not alcohol. Puppies or kittens may be restrained either by pulling the forelimbs down over the edge of a table while extending the neck as for larger animals or by laying the animal on its back, pulling the forelimbs toward the abdomen, extending the neck, and introducing the needle toward the thoracic inlet. Appropriate

equipment is a 22- to 25-gauge needle attached to a 3-ml syringe. Hemolysis and venous collapse are less likely to occur if the needle is well seated into the vein and the blood is drawn slowly.

The recommended minimum database for assessment of sick pediatric patients is a hematocrit and total protein, blood glucose concentration, blood urea nitrogen (BUN), and urine specific gravity and sediment. The clinician should contact the laboratory to find the minimum sample size needed. Blood volume in an animal weighing 1 pound (454 g) is 25-40 ml. In-house analyzers need smaller samples and have faster turnaround times than do commercial laboratories but may have less rigorous quality control.

The blood sample should be placed in a small-diameter tube to ensure adequate mixing with any anticoagulant present and to permit greater depth of serum or plasma to aspirate after centrifugation. Often, a greater volume of plasma than of serum is obtained from a particular sample; green-topped (heparinized) tubes are preferred to red-topped (serum) tubes. Purple-topped (ethylenediaminetetraacetic acid [EDTA]) tubes should not be used because EDTA alters the size and shape of RBCs. The plasma sample should be removed from the clot as quickly as possible to prevent an artifactual decrease in glucose caused by RBC metabolism and increase in phosphorus caused by hemolysis.

Normal Laboratory Values

COMPLETE BLOOD COUNT (TABLE 10-2) Hematocrit is normal at birth but declines during the early weeks of life because of decreased production and shortened RBC life span. An increase in polychromasia, nucleated RBCs, Howell-Jolly bodies, and Heinz bodies (kittens) is evident. Hematocrit is normal by 8 weeks. Neutrophilia resulting from stress during venipuncture may occur.

SERUM CHEMISTRY PROFILE (TABLE 10-3) Alanine aminotransferase concentrations are decreased in young animals. Alkaline phosphatase (AP) concentrations are increased by as much as two times adult concentrations and remain elevated throughout rapid growth, peaking in kittens at 7 months of age. Total protein (TP) is decreased in young animals as plasma volume expands faster than hepatic protein synthesis develops; TP is normal by 6-9 months. Fasting and postprandial bile acid concentrations are as adult concentrations by 4 weeks.

Glomerular filtration rate is reduced in puppies and kittens, varying from 20% of adult values at birth to 100% of adult values by several weeks of age. BUN varies with relation of time of sampling to ingestion of the most recent meal but is still a more sensitive indicator of renal function than is creatinine in young animals.

Neonates have maternal blood glucose concentrations at birth and then experience a decrease in blood

Table 10-2. Complete Blood Count Values for Puppies (P) and Kittens (K)

Age (wk)	Hematocrit (%)		WBC ($\times 10^3/\mu l$)		Differential ($\times 10^3/\mu l$)											
	P	K	P	K	Neuts		Bands		Lymphs		Monos		Eos		Basos	
					P	K	P	K	P	K	P	K	P	K	P	K
2	29-53	34-37	7-23	9-10	3-10	5-7	0-1	0-0.1	2-7	3-4	0-1	0	0-2	0-2	0	0
4	27-37	26-27	9-26	14-17	4-13	6-8	0-0.3	0-0.2	1-8	6-7	0-2	0	0-1	1	0	0
6	26-36	26-28	13-27	16-19	4-18	8-11	0-0.3	0-0.3	3-17	6-7	0-3	0	0-1	1	0	0
8	31-39	29-31	13-17	16-20	6-12	6-8	0-0.3	0-0.3	3-7	8-11	0-2	0	0-1	1	0	0

Basos, Basophils; *eos,* eosinophils; *lymphs,* lymphocytes; *monos,* monocytes; *neuts,* neutrophils; *WBC,* white blood cell number.

Table 10-3. Serum Chemistry Profile Values in Puppies (P) and Kittens (K)

Age (wk)	ALT (IU/L)		AP (IU/L)		Albumin (gm/L)		Total protein (g/L)		Glucose (mmol/L)	
	P	K	P	K	P	K	P	K	P	K
2	10-34	11-24	176-560	68-269	20	20	40	40-50	6-8	4-7
4	20-22	14-26	135-201	90-135	10-20	20	40	50	5-6	6
6	16-17	—	125-132	—	40-50	20	30-40	40-50	7	<7
8	9-24	—	144-177	—	20-30	20	40-50	50	8-15	<7

ALT, Alanine aminotransferase; *AP*, alkaline phosphatase; *BUN*, blood urea nitrogen.

glucose to approximately 45 mg/dl during the first 4-6 hours of life, with stabilization at approximately 70 mg/dl by 72 hours of life. *Significant hypoglycemia* is a blood glucose concentration of less than 50 mg/dl. Pathologic hyperglycemia is uncommon in pediatric patients. Juvenile-onset diabetes mellitus has been reported to occur in several breeds of dog and in one cat as young as 6 weeks, with blood glucose concentrations of 200-700 mg/dl; glucosuria; and clinical signs of disease, including weight loss, polyuria, polydipsia, polyphagia, cataracts, and concurrent infections.

Serum sodium concentrations may be decreased during the first several days of life but normalize quickly. Phosphorus is elevated during rapid bone growth and is normal by 8-12 months of age; normalization may take longer in giant breeds.

Table 10-3. Serum Chemistry Profile Values in Puppies (P) and Kittens (K)—cont'd

BUN (mmol/L)		Creatinine (μmol/L)		Sodium (mmol/L)		Chloride (mmol/L)		Potassium (mmol/L)		Calcium (mmol/L)	
P	K	P	K	P	K	P	K	P	K	P	K
—	<11	—	—	—	—	—	—	—	—	—	—
—	<11	—	44	—	149-153	—	120-124	—	4-5	—	2-3
3	<11	88-354	53	148	151-156	105	119-125	5	5-6	3	3
—	<11	—	62	—	150-152	—	119-125	—	4-5	—	2-3

URINALYSIS Urine specific gravity is decreased in pediatric patients, at 1.006-1.017. Physiologic proteinuria is present during the first days of life as colostral antibodies are absorbed and excreted in the urine. Nonpathologic glucosuria may be present until renal function matures.

Treatment of Neonates

Emergency Resuscitation

For puppies and kittens that very recently died or that arrest during examination, the following steps should be taken:

- A = airway: Aspirate secretions from the oral cavity and respiratory tract; intubate if possible.
- B = breathing: Provide 100% oxygen via endotracheal tube or mask or room air with an Ambu bag.

- C = circulation: Direct chest compressions should approximate normal heart rate. If intrathoracic cardiac compression is attempted, it should be instituted within 2 minutes of starting cardiopulmonary resuscitation. Other techniques that may be used include intermittent abdominal compression, epinephrine (0.04-0.4 mg/kg intratracheal or intracardiac), or stimulation of the Jen Chung acupuncture site, in which a 25-gauge needle is inserted into the nasal philtrum at the base of the nares and twisted once to reach the periosteum.

Fluid Therapy

Pediatric animals require more water relative to body mass compared with adults and sustain relatively greater surface losses, with a fluid requirement of approximately 200 ml/kg day. Pediatric animals have decreased cardiac capacity and underdeveloped renal function and thus cannot tolerate large volume replacement.

Fluids can be administered via intraperitoneal, subcutaneous, intravenous, or intraosseous routes. Absorption of fluids from the intraperitoneal space is slow, especially in hypovolemic animals, and fluids must be administered via strict aseptic technique. Similarly, subcutaneous administration of fluids is made difficult by the limited amount of fluid that can be provided in

the subcutaneous space; sporadic absorption can occur, even in only moderately dehydrated animals. Isotonic fluids without glucose should be administered subcutaneously to prevent sloughing of skin over the injection site. Intravenous and intraosseous routes are preferred for administration of fluid to neonatal puppies and kittens.

Intravenous catheters for bolus or continuous infusion of fluids are best placed in the external jugular vein. Catheters 23 to 25 gauge can be placed in the cephalic vein, but the small size of the veins and short legs of pediatric animals make cephalic placement and maintenance of fluid flow difficult. One recommended protocol for intravenous rehydration in pediatric patients is 4-10 ml/kg/hr 0.45% saline, with or without 5% dextrose.

Intraosseous administration of fluids is an alternative to intravenous therapy in animals in which placement of an intravenous catheter is impossible or would take an excessive amount of time. An 18- to 22-gauge spinal needle is passed through the soft cortical bone at the trochanteric fossa of the femur or greater tubercle of the humerus. The needle is inserted parallel to the long axis of the bone into the intramedullary canal. Fluid flow rates of up to 11 ml/min can be achieved with gravity, and the fluid is readily absorbed. Catheter maintenance is as for intravenous placement.

Antibiotic Therapy

Absorption, distribution, metabolism, and excretion of drugs are altered in pediatric patients. Absorption of drugs injected intramuscularly is sporadic because of the small size and poor vascularity of the muscle mass. Drugs administered orally are absorbed differently than in adults because of increased gastric pH, slow gastrointestinal transit time, and decreased intestinal blood flow in young animals. Distribution of drugs is altered by the small amount of total body fat and increased total body water and by the decreased concentration of circulating proteins in pediatric patients. Water-soluble medications must be given at a higher dosage to counter dilution, and fat-soluble drugs need to be given at a lower dosage. Finally, metabolism and excretion are altered by immature hepatic and renal function (Table 10-4).

Therapy can be altered either by decreasing the adult dosage by 30%-50% or by lengthening the dosage interval. Treatment of the lactating dam is not an effective way to deliver medications to nursing puppies or kittens; although deposition of drugs into milk varies with lipid solubility, only about 1%-2% of the dam's dose will reach the young.

Antibiotics that should be avoided in young animals are aminoglycosides (nephrotoxic and ototoxic), tetracycline (dental and skeletal abnormalities, hepatotoxic, and nephrotoxic), trimethoprim/sulfonamides and chloramphenicol (inappetence and hematopoietic

Table 10-4. Antibiotic Therapy in Pediatric Patients

Drug	Kittens	Puppies
Amoxicillin	22 mg/kg PO q8-12h	20 mg/kg PO q8h
Ampicillin	20-40 mg/kg PO q6-8h 10-20 mg/kg IV, IM, SC q6-8h	
Amoxicillin/ clavulanate	12.5-25.0 mg/kg PO q12h	20-30 mg/kg PO q8h
Cefazolin	25-35 mg/kg IV, IM q8h	10-30 mg/kg PO q8h
Cephalexin	10-30 mg/kg PO, SC, IV q8-12h	
Cefotaxime	20-80 mg/kg IV, IM, SC q12h	25-50 mg/kg PO q8h

abnormalities), and fluoroquinolones (cartilage abnormalities in rapidly growing animals).

Feeding Orphan Kittens and Puppies

Orphans

Support of orphaned puppies and kittens involves maintaining an adequate environmental temperature, feeding a well-balanced milk-based diet for the first 3 weeks of life, and stimulating urination and defecation.

MAINTENANCE OF BODY TEMPERATURE Pediatric animals do not have well-developed thermoregulatory systems and rely on an external heat source, ideally the dam, for maintenance of normal body temperature. Orphans should be maintained in an environment at about 85° F (29.5° C) for the first 2 weeks of life and about 80° F (26.5° C) for the subsequent 2 weeks. Warmed and cooler areas should be available so that the puppies and kittens can crawl to an area appropriate in temperature. Among surface sources of heat, circulating hot water blankets are best. Hot water bottles should be wrapped in towels and frequently changed. Regular heating pads may heat unevenly and more easily burn the fragile skin of neonates. Heat lamps are an excellent, safe way to provide radiant heat.

INGESTION OF COLOSTRUM If possible, it needs to be ascertained whether the puppy or kitten has ingested colostrum. If the owner is unsure whether a puppy has ingested colostrum, blood can be drawn from the puppy of interest and a littermate that is known to have nursed, and serum AP and gamma-glutamyl transpeptidase concentrations can be compared between the two. Concentrations remain high for up to 10 days in puppies that have ingested colostrum. If a puppy or kitten has not ingested colostrum, antibodies can be provided by subcutaneous administration of serum from the dam or another immunocompetent animal in the household.

Amount and frequency of serum to be administered are undefined.

BODY WEIGHT The puppy or kitten should have been weighed at birth and should be weighed daily thereafter. Slight weight loss may occur during the first 24 hours of life. After that, the puppy or kitten should gain weight daily, doubling the birth weight by 7-10 days. Kittens should gain 1.8-3.5 oz (50-100 g) weekly. Puppies should gain 0.05-0.1 oz (1-2 g) per pound (2-4 g/kg) of anticipated adult weight daily, about a 10% increase per day. Monitoring of body weight is an early indicator of adequacy of food and water intake in hand-fed animals.

DIET Milk is the primary diet of puppies and kittens up to 3 weeks of age. In addition to providing passive immunity and nutrients, milk, comprised of approximately 85% water, is the primary source of fluid for neonates. It is vital for maintenance of normal hydration and fluid volume and for provision of nonnutritive bioactive agents, such as enzymes, hormones, and growth factors.

An effort always should be made to find another lactating bitch or queen to support orphan animals. No commercial or homemade milk replacer contains all the components of a bitch's or queen's milk. The protein content and percentage of calories from protein in milk are positively correlated with growth rate in animals. Cow's milk is lower in protein than either bitch's or

queen's milk. Goat's milk, a commonly described supplement for orphans, also is a poor substitute for bitch's and queen's milk.

Commercial milk replacers are recommended over homemade diets. Commercial formulas are balanced, are of high nutrient density, are low in fiber, and contain protein of high biologic value. Feline milk replacers must contain a source of taurine for optimal growth to occur. Homemade diets are best used in emergency situations (Box 10-3).

Problems reported with feeding of either commercial or homemade milk replacers to puppies and kittens include small, focal cataracts resulting from deficiencies in vitamins or amino acids that resolve after weaning and slower growth rate as a result of lack of enzymes necessary for fat digestion. By several months of age, hand-raised puppies and kittens achieve the same size as littermates that were allowed to nurse. The high lactose content of cow's milk and decreased caloric density of formulas make it difficult to provide orphans with an adequate number of calories without inducing diarrhea. If feeding induces diarrhea, the formula can be diluted 1:2 with electrolyte solution until the neonate can tolerate it.

Figuring dietary requirements The amount to be fed varies with the caloric density of the formula and the age and weight of the animal. Kittens should receive 100-175 kcal

Box 10-3. Emergency Homemade Milk Replacers for Puppies and Kittens

Puppies	Kittens
1. 3 parts evaporated milk (not skimmed) to 1 part water	1. 1/2 cup (120 ml) whole milk, 1 egg yolk, 1 drop liquid infant vitamins
2. 1 cup (240 ml) whole milk, 1 tsp (5 ml) vegetable oil, 1 drop liquid infant vitamins	2. 1/2 cup (120 ml) condensed milk, 1/2 cup (120 ml) water, 1/2 cup plain yogurt, 3-4 egg yolks
3. 1/2 cup (120 ml) whole milk, 1/2 cup (120 ml) water, 1-2 egg yolks, 2 Tums (calcium supplement), 1 tsp (5 ml) vegetable oil	
4. 1 cup (240 ml) whole milk, 1 Tbsp (15 ml) vegetable oil, a pinch of salt, 3 egg yolks, 1 drop liquid infant vitamins	

per pound (220-380 kcal/kg) daily, split into four to six feedings. Puppies should receive 105-120 kcal per pound (230-260 kcal/kg) daily, split into four feedings. Feeding frequency can be decreased to three times daily after the orphan reaches 2 weeks of age.

Feeding neonatal puppies and kittens The formula should be warmed to 95°-100° F (35°-38° C) when feeding 1- to

2-week-old animals. If the animal has been experiencing inappetence, it may be beneficial to feed half the calculated dose for 1-2 days. Equipment for feeding of pediatric animals includes spoons, droppers, bottles, or tubes.

- Spoon and dropper feedings are dangerous because the limited gag reflex of puppies and kittens easily permits aspiration of formula into the lungs.
- Bottle feeding poses less risk of aspiration and more readily satisfies the neonate's need to suckle. Small bottles marketed for animals or bottles intended for premature human infants can be used. The hole in the nipple should allow milk to ooze slowly. The bottle should never be squeezed to force expulsion of milk while the animal is nursing.
- Tube feeding is quick. Caution must be used to ensure proper placement of the tube into the gastrointestinal tract and to prevent overflowing and regurgitation. The animal should be held horizontally on its ventrum. The feeding tube varies in diameter and length with age. A 5-Fr feeding tube should be used in animals weighing less than 300 g and an 8- to 10-Fr feeding tube should be used in animals weighing more than 300 g. Measure the length of the feeding tube by marking off 75% of the distance from the animal's last rib to the tip of its nose. This length ensures placement in the stomach without kinking of the tube within the gastrointestinal tract. Length should be rechecked and adjusted

weekly. The warmed formula is gently expelled through the tube with a syringe. Monitor gastric distention; average stomach capacity in neonates is approximately 0.7 fl oz (4 tsp) per pound (40 ml/kg). The genital area should be massaged with a cotton ball or soft cloth moistened with warm water after feeding to promote urination and defecation.

Weanlings

Weaning, introduction of solid food, begins at 3 weeks of age in puppies and at 3-4 weeks in kittens. A growth food appropriate for the species, not human baby food, should be introduced. Young animals need more nutrients to allow for normal growth and development but take longer to ingest food and have a smaller digestive capacity, necessitating feeding of a highly digestible food that is dense with energy-producing nutrients. Food should be offered as a gruel initially, formed by thoroughly blending 1 part dry food to 3 parts water or 2 parts canned food to 1 part water for puppies, and 1 part dry food to 3 parts formula or 2 parts canned food to 1 part formula for kittens. Fewer problems with postprandial gastric distention occur if the food is thoroughly soaked. Fresh water always should be provided. Gradually, less water or formula is mixed with the food until the puppy or kitten is eating dry food exclusively. Weaning usually is complete by 6-8 weeks of age. By the

time the animal is weaned, it should have a body weight that is approximately 6-10 times its birth weight.

Bibliography

Addie DD, Toth S, Thompson H et al: Detection of feline parvovirus in dying pedigree kittens, *Vet Rec* 142:353, 1998.

Anvik JO: Clinical considerations of canine herpesvirus infection, *Vet Med* April:394, 1991.

Atkins CE: Disorders of glucose hemostasis in neonatal and juvenile dogs: hyperglycemia, *Comp Cont Ed* 5:851, 1982.

Atkins CE: Disorders of glucose hemostasis in neonatal and juvenile dogs: hypoglycemia: part I, *Comp Cont Ed* 6:197, 1984.

Berman E: Fetal and neonatal growth and development. In Morrow DA (ed): *Current therapy in theriogenology*, Philadelphia, 1986, WB Saunders.

Blunden TS: The neonate: congenital defects and fading puppies. In Simpson GM, England GC, Harvey M (eds): *BSAVA manual of small animal reproduction and neonatology*, Cheltenham, England, 1998, British Small Animal Veterinary Association.

Bounous DI, Boudreaux MK, Hoskins JD: The hematopoietic and lymphoid systems. In Hoskins JD (ed): *Veterinary pediatrics: dogs and cats from birth to six months*, Philadelphia, 1995, WB Saunders.

Bucheler J: Fading kitten syndrome and neonatal isoerythrolysis, *Vet Clin North Am* 29:853, 1999.

Canine Consumer Report: *A guide to hereditary and congenital diseases in purebred dogs,* Vacaville, 1994, Association of Veterinarians for Animal Rights.

Casal ML: Feline paediatrics, *Vet Annu* 35:210, 1995.

Center SA, Hornbuckle WE, Hoskins JD: The liver and pancreas. In Hoskins JD (ed): *Veterinary pediatrics: dogs and cats from birth to six months,* Philadelphia, 1995, WB Saunders.

Dial SM: Hematology, chemistry profile, and urinalysis for pediatric patients, *Comp Cont Ed* 14:305, 1992.

Dubey JP, Carpenter JL: Neonatal toxoplasmosis in littermate cats, *J Am Vet Med Assoc* 203:1546, 1993.

Earle KE: Calculations of energy requirements of dogs, cats and small psittacine birds, *J Small Anim Pract* 34:163, 1993.

Fettman MJ, Allen TA: Developmental aspects of fluid and electrolyte metabolism and renal function in neonates, *Comp Cont Ed* 13:392, 1991.

Fisher EW: Neonatal diseases of dogs and cats, *Br Vet J* 138:277, 1982.

Gelens HC, Ihle SL: Failure to grow, *Vet Clin North Am* 29:989, 1999.

Giger U, Casal ML, Niggemeier A: The fading kitten syndrome and neonatal isoerythrolysis. Proceedings of the ACVIM Forum. Lake Buena Vista, Fl, 1997.

Harpold LM, Legendre AM, Kennedy MA et al: Fecal shedding of feline coronavirus in adult cats and kittens in an Abyssinian cattery, *J Am Vet Med Assoc* 215:948, 1999.

Haskins ME, Casal ML: Interpreting gross necropsy observations in neonatal and pediatric kittens. In August JR (ed): *Consultations in feline internal medicine,* Philadelphia, 1997, WB Saunders.

Hosgood G: Surgical and anesthetic management of puppies and kittens, *Comp Cont Ed* 14:345, 1992.

Hoskins JD: Clinical evaluation of the kitten: from birth to eight weeks of age, *Comp Cont Ed* 12:1215, 1990.

Hoskins JD: Congenital defects of cats, *Comp Cont Ed* 17:385, 1995.

Hoskins JD: Fading puppy and kitten syndromes, *Feline Pract* 21:19, 1993.

Hoskins JD: Pediatric health care and management, *Vet Clin North Am* 29:837, 1999.

Hoskins JD: Pediatrics in small animals. Proceedings of the Metropolitan Animal Hospital Association Spring Conference, St Paul, Minn, 1993.

Hoskins JD: The puppy's first veterinary examination: physical examination and preventive health program, *Vet Tech* 12:521, 1991.

Hoskins JD, Taboada J: Congenital defects of the dog, *Comp Cont Ed* 14:873, 1992.

Jemmett JE, Evans JM: A survey of sexual behaviour and reproduction of female cats, *J Small Anim Pract* 18:31, 1977.

Jezyk PF: Metabolic diseases: an emerging area of veterinary pediatrics, *Comp Cont Ed* 5:1026, 1983.

Johnson CA, Grace JA: Care of newborn puppies and kittens, *Kal Kan Forum* 9, 1987.

Johnson CA, Grace JA, Probst MR: The effect of maternal illness on perinatal health, *Vet Clin North Am* 17:555, 1987.

Jones RL: Special considerations for appropriate antimicrobial therapy in neonates, *Vet Clin North Am* 17:577, 1987.

Kirk CA: Feeding the neonate: how to turn the young and the restless into the bold and the beautiful. Proceedings of the Society for Theriogenology, Nashville, 1999.

Kraft H, Hartmann K, Dereser R: Age-related variations in clinicochemical values for dogs and cats, *Tierarzt Prax* 23:502, 1995.

Laliberte L: Neonatal and orphan kitten care. In Morrow DA (ed): *Current therapy in theriogenology,* Philadelphia, 1986, WB Saunders.

Lawler DF: Neonatal puppies and kittens: physical and environmental evaluation, *Vet Tech* 14:337, 1993.

Lawler DF: The role of perinatal care in development, *Semin Vet Med Surg (Small Anim)* 10:59, 1995.

Lawler DF, Monti KL: Morbidity and mortality in neonatal kittens, *Am J Vet Res* 45:1455, 1984.

Lewis LD, Morris ML, Hand MS: *Small animal clinical nutrition,* Topeka, 1992, Mark Morris Associates.

Macintire DK: Pediatric intensive care, *Vet Clin North Am* 29:971, 1999.

Marks SL: Cardiopulmonary resuscitation and oxygen therapy, *Vet Clin North Am* 29:959, 1999.

Merton DA: Selective breeding in the dog and cat: part II. Known and suspected genetic diseases, *Comp Cont Ed* 4:332, 1982.

Mohrman RK: *The care of newborn orphan puppies,* St Louis, 1980, Ralston Purina Pet Nutrition and Care Research.

Moore PH: Care and management of the neonate. In Simpson GM, England GC, Harvey M (eds): *BSAVA manual of small animal reproduction and neonatology,* Cheltenham, England, 1998, British Small Animal Veterinary Association.

Piechotta D, Kolb E: Concentrations of sodium, potassium, calcium, magnesium, inorganic phosphate, copper, zinc and alkaline phosphatase in the plasma of cats of different ages, *Tierarzt Umschau* 49:439, 1994.

Poffenbarger EM, Olson PN, Ralston SL et al: Canine neonatology: part II. Disorders of the neonate, *Comp Cont Ed* 13:25, 1991.

Poffenbarger EM, Ralston SL, Chandler ML et al: Canine neonatology: part I. Physiologic differences between puppies and adults, *Comp Cont Ed* 12:1601, 1990.

Povey RC: Reproduction in the pedigree female cat: a survey of breeders, *Can Vet J* 19:207, 1978.

Root MV, Johnson KH, Allen WT et al: Diabetes mellitus associated with pancreatic endocrine insufficiency in a kitten, *J Small Anim Pract* 36:416, 1995.

Root MV, Johnston SD, Olson PN: Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and juvenile mortality in the domestic cat, *J Am Anim Hosp Assoc* 31:429, 1995.

Ruble RP, Hird DW: Congenital abnormalities in immature dogs from a pet store: 253 cases (1987-1988), *J Am Vet Med Assoc* 202:633, 1993.

Shores A: Neurologic examination of the canine neonate, *Comp Cont Ed* 5:1033, 1983.

Sturgess K: Infectious disease of young puppies and kittens. In Simpson GM, England GC, Harvey M (eds): *BSAVA manual of small animal reproduction and neonatology,* Cheltenham, England, 1998, British Small Animal Veterinary Association.

Walkowicz C, Wilcox B: *Successful dog breeding,* New York, 1994, Howell Book House.

Willis EL: Flea control as we enter the next century, *Vet Forum* 40, 2000.

11

Ovarian Disorders of the Bitch and Queen

Craig A. Smith

AT A GLANCE

- Ovarian cysts
  - The most common type of ovarian cyst is the functional follicular cyst, which secretes estrogen. Presenting complaints reported include persistent proestrus/estrus, prolonged anestrus, and infertility. Ovarian cysts may be visible with ultrasound. The

primary rule-out for an estrogen-secreting follicular cyst is a granulosa cell tumor. Treat follicular cysts with ovariohysterectomy (OHE) or by inducing luteinization with gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG).

- Ovarian remnant syndrome
  - Cats and dogs with ovarian remnant syndrome (ORS) have signs of heat (e.g., vulvar discharge, breeding behavior), despite having undergone OHE.
  - Diagnose by demonstrating cornified vaginal cytology when the animal is showing signs of heat (proves estrogen secretion is occurring; see Chapter 2).
  - Bitches ovulate spontaneously. Wait until the bitch enters diestrus and measure serum progesterone concentration; a concentration greater than 2 ng/ml indicates that luteinized tissue is present.
  - Queens do not ovulate spontaneously. When they are in estrus, induce ovulation by administering GnRH or hCG. Measure progesterone in serum 2-3 weeks later; a concentration greater than 2 ng/ml indicates that luteinized tissue is present.
  - Treat by performing exploratory laparotomy and removing remnant tissue from one or both ovarian pedicles. Identification of the ovarian remnant is enhanced by performance of surgery during estrus

or diestrus, when the ovarian remnant is made visible by presence of follicular or luteal tissue, respectively.

- Ovarian neoplasia
  - Ovarian neoplasia is uncommon in dogs and cats. Granulosa cell tumor is the most common type; these may produce estrogen, mimicking follicular cysts. Treat with OHE.

Ovarian Cysts

Definition and Pathogenesis

Cystic structures can develop from tissues located either within or near the ovaries. Data are sparse regarding the relative prevalence of ovarian and parovarian cystic structures; in one study, approximately 90% of aged female dogs had ovarian or parovarian cysts.

Follicular cysts occur in queens and bitches. They are reported to be the most common lesion found in ovaries of queens. The incidence appears to be lower in bitches than in queens. Ovarian follicular cysts were identified in 3% of 216 apparently normal beagles in one study. However, in another study, 63 (15.8%) of 400 bitches had ovarian cysts. As in cattle, the apparent cause for the development of follicular cysts is an aberrant or ineffective ovulatory mechanism that results in the

persistence of mature graafian follicles. Follicular cysts may be partially luteinized, which is characterized grossly by tan or orange tissue that lines the inner surface of the cysts. Because of their ability to produce sex hormones, follicular and luteal cysts are commonly referred to as *functional ovarian cysts.* Heritability of follicular ovarian cysts in bitches and queens is unknown.

Cystic remnants of the mesonephric and paramesonephric ducts and tubules are common in ovaries of bitches and queens. Cystic remnants of the mesonephric tubules can be found at either pole of the ovary. Those that develop from the cranial mesonephric tubules are called *cystic epoophoron,* and those that develop from the caudal mesonephric tubules are called *cystic paroophoron.* Usually more than one cyst develops as a result of distention of the remnants of the mesonephric tubules.

Cystic subepithelial structures arise from normal infoldings of the epithelial cells that cover the ovaries. Cells lining the surface of the ovaries undergo hyperplasia and cystic distention. A single cyst or several clusters of cystic structures may be visible on the surface of the ovary. These cystic structures occur in bitches and humans, but there have been no reports of this condition in queens.

Cystic rete ovarii most commonly are observed in queens but also can develop in bitches. As their name implies, these structures develop from the rete ovarii,

which are derived from mesonephric tubules that penetrate the developing, undifferentiated gonads during embryogenesis. The rete ovarii tend to dilate as bitches age and may be grossly visible as cystic structures arising from the medullary area of an ovary. In queens, cystic rete ovarii develop slowly. Cells of the rete ovarii are secretory, and it has been hypothesized that these cysts arise in cats as a result of accumulation of secretion in the blind-ended rete. These cysts can become large and may compress the surrounding normal ovarian stroma.

Ovarian neoplasms may contain cystic structures. They usually are easily distinguished from other cystic ovarian structures by their greater size and complex internal structure (see Ovarian Neoplasia).

Signalment

Cystic ovarian structures are more common in older bitches and queens. There does not appear to be a breed predilection.

Previous pregnancies may affect development of follicular cysts. In a study of 400 bitches 2-15 years of age, 41 had a solitary follicular cyst and 22 had multiple ovarian cysts. Of the 41 bitches with a solitary cyst, 29 were nulliparous and only 7 were multiparous. On the other hand, 18 of the 22 dogs with multiple cysts were nulliparous. Controversy exists regarding the effects of parity on the frequency of luteal cysts. One source indicated that

luteal cysts were more common in older multiparous bitches. However, in the study of 400 bitches described previously, luteal cysts were found in 9 dogs; 6 of these bitches were nulliparous.

History and Clinical Signs

Most ovarian cysts are nonfunctional structures or produce quantities of estrogen and progesterone that are too small to influence behavior or physiology in affected bitches and queens. These animals appear to be clinically healthy. Ovarian and parovarian cysts often are coincidental findings during abdominal surgery (usually routine OHE) in healthy bitches and queens.

Prolonged anestrus is the most common clinical sign associated with ovarian cysts of all types. Although anestrus occurs with functional follicular and luteal cysts, it is unknown whether it is attributable to the effects of progesterone or associated with insufficient quantities of sex hormones. It has been hypothesized that anestrus is associated with the mass of a cyst rather than its ability to produce hormones; cystic rete ovarii may cause prolonged anestrus as a result of compression of ovarian stroma. Prolonged anestrus may not be recognized in bitches, which exhibit a relatively prolonged anestrus as part of the normal estrous cycle, or in queens during the seasonal anestrus associated with short day length (see Chapter 2).

Persistent or prolonged proestrus or estrus is the most commonly reported clinical sign of functional follicular ovarian cysts in affected bitches and queens. Follicular cysts can produce normal or excessive amounts of testosterone or estrogen, resulting in behavioral signs of estrus and nymphomania. Behavioral changes are accompanied by physiologic signs of estrus, such as bloody vulvar discharge.

Infertility is another historical complaint associated with ovarian cysts. Large cysts of the rete ovarii can compress normal ovarian tissue and cause infertility in cats. Infertility in bitches and queens with luteal cysts may result from the prolonged influence of progesterone, which may lead to the development of cystic endometrial hyperplasia (CEH) and pyometra (see Chapter 12).

Finally, pressure from an ovarian cyst may result in signs of pain. Although this is a nonspecific sign, bitches and queens that have signs of pain in the region of the ovaries that cannot be attributed to other causes should be evaluated for an ovarian cyst.

Physical Examination Findings

Most bitches and queens with ovarian cysts are healthy, and results of physical examinations are unremarkable. The exception is the bitch with a functional follicular cyst that is secreting quantities of estrogen sufficient to cause physical changes typical of proestrus and estrus,

such as serosanguineous vaginal discharge and vulvar swelling (see Chapter 2).

Diagnostic Tests and Results

Vaginal cytologic examination can be helpful in bitches and queens that have signs of prolonged proestrus or estrus. Presence of cornified vaginal epithelium indicates an estrogenic influence. However, it does not necessarily indicate that the source of the estrogen is an ovarian cyst because some ovarian tumors also secrete estrogens.

Serial measurement of estrogen in serum may be useful in diagnosing follicular or luteal ovarian cysts but is less commonly used for diagnosis than is vaginal cytologic examination, which acts as a bioassay for estrogen. A concentration of estrogen in serum greater than 20 pg/ml is consistent with follicular activity in bitches and queens. Thus in a bitch or queen with prolonged proestrus or estrus, persistently increased concentrations of estrogen are indicative of a source of chronic estrogen production, which is most likely to be a follicular cyst. One-time measurement of serum estradiol concentration is not advised because measurement of serum estrogen in a single sample may be inaccurate, serum lipid concentrations may affect assay results, the sensitivity of the assay may be too high to allow accurate interpretation of the test results, and estradiol concentrations less than 20 pg/ml in serial samples may be inconclusive.

Similarly, serial evaluation of progesterone concentrations in serum may be effective in identifying the presence of a luteal cyst. A progesterone concentration greater than 2 ng/ml for more than 80 days indicates prolonged luteal function in the bitch, which is most likely to occur because of the presence of a luteal cyst.

Ultrasonography is the best option for the diagnosis of ovarian cysts. Again, serial evaluations are most likely to yield the best results. Follicles grow during proestrus and estrus and reach a maximum diameter of 11 mm (mean, 6 mm) at the time of ovulation in larger bitches. Ovulatory follicles in queens are slightly smaller. Therefore the presence of multiple persistent follicular structures greater than 10 mm in diameter is suggestive of ovarian cysts. Cystic follicular structures also tend to have a more distinct capsule compared with normal ovulatory follicles.

Surgical options for the diagnosis of ovarian cysts include exploratory laparotomy and laparoscopy. Exploratory laparotomy allows examination of the ovaries directly but is invasive. I am not aware of any reports on the use of laparoscopic techniques for the identification of ovarian cysts in dogs and cats.

Treatment

Surgical removal of ovarian cysts often is the best option. Complete OHE is preferred. Partial ovariectomy may be

performed in bitches and queens that have great value as breeding animals. Tissue removed during surgery should be submitted for histologic examination.

Medical treatment for bitches and queens with follicular cysts is aimed at inducing luteinization of the structures. Although induction of ovulation is the ultimate outcome, it is doubtful that these structures are ovulatory and formation of luteal tissue is sufficient for clinical resolution of the disorder. Administration of GnRH or hCG can be used to simulate luteinization. In bitches, reported drug regimens include a single intramuscular injection of GnRH in a dose of 50-100 μg, a single intramuscular injection of hCG in a dose of 100-1000 IU, intramuscular injection of 500 IU of hCG once daily for 3 consecutive days, and injection of 1000 IU of hCG, half of which is administered intravenously and half of which is administered subcutaneously. If luteinization and a decrease in clinical signs have not occurred by 1 week after drug administration, treatment with GnRH or hCG can be repeated. In queens, intramuscular administration of 25 μg of GnRH or hCG (500 IU once or 250 IU on 2 consecutive days) stimulates formation of luteal tissue.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes luteolysis in bitches and queens with luteal cysts. I am unaware of reports of the use of $PGF_{2\alpha}$ for the treatment of luteal cysts, but a dosage regimen involving multiple injections (similar to that used for pregnancy termination) should be effective (see Chapter 5).

Prognosis

Prognosis is excellent for affected bitches and queens after OHE. A guarded prognosis must be made for bitches and queens treated with GnRH or hCG to induce formation of luteal tissues or animals treated with $PGF_{2\alpha}$ to induce luteolysis. Although it is likely that the treatment will successfully resolve the follicular or luteal cysts, it is possible that the condition will recur during subsequent estrous cycles. Furthermore, subsequent exposure to estrogen during proestrus and progesterone during diestrus potentiates development of CEH and pyometra.

Ovarian Remnant Syndrome

Definition and Pathogenesis

ORS is the presence of functional ovarian tissue after routine OHE of a bitch or queen. The piece of ovarian cortex that remains after surgery continues to develop and becomes functional, producing estrogen and progesterone that cause signs of proestrus, estrus, and less commonly, pseudocyesis (false pregnancy). Signs of reproductive activity cause consternation in owners, who assumed that this facet of their pet's life was irrevocably altered.

Causes of ORS include surgeon error and the presence of anomalous ovarian tissue. Most OHEs are

elective surgeries in healthy animals and typically are not associated with pathologic conditions of the reproductive tract. Failure to completely remove ovarian tissue during OHE may be attributed to poor visibility of the surgical field or improper placement of clamps and ligatures, rather than adhesions or other lesions that might obscure the location of the ovaries.

It has been hypothesized that some cases of ORS result from a small piece of ovarian tissue inadvertently being dropped into the abdominal cavity during ovarian removal, with the remnant subsequently developing vascular attachments to the peritoneum or other abdominal structures. This is an unlikely scenario because almost all ovarian remnants are found at the ovarian pedicles. In one study of 46 cases of ORS, none of the remnants were associated with an abnormal abdominal location, such as the mesentery.

Age of the animal at the time of OHE may play a role because it may be difficult to completely discern ovarian structures in extremely young animals (6-8 weeks) through a small abdominal incision. However, in a study of 46 cases of ORS, none were reported to have occurred in animals that were spayed when they were younger than 4 months.

Even the most experienced and competent surgeons have occasionally had an animal with an ovarian remnant and recurrence of estrous cycles after routine OHE, leading to the hypothesis that another possible

cause for ORS may be presence of an accessory ovary or extraneous ovarian tissue in the proper ligament of the ovary. The presence of anomalous ovarian tissue of this type has been reported in cats and women. Removal of the normal ovary during routine OHE allows the previously atrophic accessory ovary to become functional.

Signalment

Some clinicians believe ORS is more common in cats than in dogs. A study of 46 cases of ORS included 29 cats and only 17 dogs. There does not appear to be a breed predilection for this condition in dogs or cats. Furthermore, there does not appear to be an increased incidence of ORS in animals in which OHE may be difficult, such as deep-chested breeds of dogs, obese dogs and cats, dogs and cats with pyometra, or dogs and cats that have been spayed during gestation or as part of a cesarean section.

History and Clinical Signs

By definition, the most commonly reported clinical sign is manifestation of estrus in an ovariohysterectomized animal. In bitches this may include swelling of the vulva, serosanguineous vulvar discharge, flagging, and attraction of males. Signs in queens may include vocalization, rolling, treading, lordosis, and attraction of males.

Affected bitches and queens may permit copulation, but pregnancy cannot result from the mating. Owners should be questioned carefully regarding the administration of exogenous hormones that could account for the clinical signs observed in their pets.

Onset of signs of estrus may be as soon as a few days or as late as many years after OHE. Subsequent estrous cycles recur at anticipated intervals for a particular species.

In a few cases the only reported clinical signs were behaviors of false pregnancy and lactation. In rare situations these clinical signs were reported despite the owners' not having observed signs of estrus before presentation. Serum progesterone concentration always is elevated in bitches after an ovulatory estrus but is not elevated in queens unless ovulation is induced. Because clinical signs of false pregnancy are attributable to the decrease in serum progesterone at the end of diestrus, false pregnancy associated with ORS is reported more commonly in bitches than in queens.

Physical Examination Findings

Bitches and queens with ORS are typically healthy and do not have signs of systemic disease. Physical changes associated with estrus or false pregnancy may be present.

Diagnostic Tests and Results

Vaginal cytologic examination, hormonal analyses, and if necessary, exploratory abdominal surgery are the diagnostic methods that are most useful in identifying the cause for the clinical signs.

Vaginal cytologic examination is a fast, easy, and inexpensive method for evaluating animals with a medical history and clinical signs consistent with ORS. A vaginal cytology specimen should be collected when the animal is exhibiting behaviors or physical signs of proestrus or estrus (see Chapter 2). Cornification of vaginal epithelial cells is consistent with secretion of estrogen. In queens the vaginal cytologic changes during estrus may be more subtle than those observed to occur in bitches. However, an increase in cornified vaginal epithelial cells and a distinct lessening of background mucus and debris are consistent with estrogenic influences on the vaginal mucosa in queens.

Hormonal analysis can be used to confirm the results of vaginal cytologic examination or can be used in animals in which vaginal cytologic examination is not diagnostic. The medical history and results of cytologic evaluation should be used to determine the appropriate hormonal analysis to perform.

A serum estradiol concentration greater than 20 pg/ml is consistent with follicular activity in bitches and queens. Unfortunately, a diagnosis based on the evaluation of a single sample can be incorrect or inconclusive

because the sample could have been obtained at the wrong part of the estrous cycle. In bitches serum estradiol concentrations increase during proestrus, reach a peak near the end of proestrus, and decrease rapidly during estrus (standing heat). Onset of breeding behavior and results of vaginal cytologic evaluations do not accurately indicate the onset of estrus in bitches; a serum sample with a low estradiol concentration may be incorrectly interpreted as an indication that the estrus behavior of the bitch was not associated with follicular activity when, in fact, the dog was in estrus at the time of sample collection. A similar condition exists in queens because cats typically continue to display behavioral signs of estrus for a few days after serum estradiol concentrations decrease. The use of serial vaginal cytologic evaluations is a good bioassay for the detection of estrogen concentrations and represents a more reliable diagnostic test than does assay of a single sample for serum estradiol.

Analysis of serum progesterone concentrations is useful for establishing a diagnosis of ORS in bitches. A progesterone concentration greater than 2 ng/ml is indicative of luteal function in a bitch. The luteal phase of the bitch lasts for approximately 2 months, but it behooves veterinarians to measure progesterone concentrations 2-3 weeks after the end of behavioral estrus. Measurement of a serum progesterone concentration greater than 2 ng/ml in a bitch that had exhibited estrus

2-3 weeks prior allows one to unequivocally diagnose ORS.

In cats, because they are induced ovulators, serum progesterone concentrations will not be increased unless the cat has been induced to ovulate by copulation, manual stimulation, or administration of exogenous hormones. In queens that have been induced to ovulate, serum concentrations of progesterone are greater than 2 ng/ml for approximately 35-50 days after ovulation. Administration of GnRH (25 μg administered intramuscularly) or hCG (250 IU administered intramuscularly) to a cat during behavioral or cytologic estrus stimulates ovulation of follicles, formation of corpora lutea, and an increase in serum progesterone concentrations. Serum progesterone concentrations should be measured 2-3 weeks after injection of GnRH or hCG. Serum progesterone concentrations greater than 2 ng/ml are diagnostic of functional ovarian tissue.

It seems logical that ultrasonographic examination performed during proestrus or immediately after the onset of estrus, when the preovulatory follicles are at their largest diameter, or during diestrus when the corpora lutea are present would be a valuable diagnostic tool. I am unaware of any reports of reliable use of ultrasonography in the diagnosis of ORS in bitches or queens. It is difficult to identify ovarian structures in sexually intact animals, and the fact that the ovarian remnant usually is quite small precludes successful use of this technique.

Exploratory surgery is a final diagnostic option. Exploratory laparotomy enables thorough examination of the ovarian pedicles and abdominal structures and may be coupled with treatment.

Treatment

Surgical removal of the ovarian remnant is the preferred method of treatment. Many clinicians prefer to perform exploratory laparotomy when the bitch or queen is in estrus, when the follicular structures present on the ovarian remnant make it easy to identify. Other clinicians prefer to perform exploratory laparotomy during diestrus, when the corpora lutea present on the ovarian remnant make it easy to detect and when hemorrhage is decreased compared with that seen when surgery is performed during estrus. If surgical exploration is to be attempted during diestrus in a queen, the cat should receive GnRH or hCG during behavioral estrus, the presence of the ovarian remnant should be verified by measurement of serum progesterone concentration greater than 2 ng/ml several weeks after induction of ovulation, and laparotomy should be performed at that time. Surgical exploration during diestrus in bitches should be performed 20-50 days after estrus. Surgery should not be performed during anestrus in affected bitches or queens because there is a lower probability that the ovarian remnant will be identified during that phase of the estrous cycle.

Each side of the abdomen should be completely explored from the caudal pole of the kidney to the region of the ovarian pedicle. Residual ovarian tissue is located most commonly in the region of the ovarian pedicles. In one study about half of affected animals had bilateral ovarian remnants that were located in the region of the ovarian pedicles. In that study unilateral ovarian remnants were equally distributed on the right and left sides. However, most studies have indicated that unilateral ovarian remnants are predominantly identified in the region of the right ovarian pedicle. It is believed that the right ovary is most often affected because its location is more cranial than that of the left ovary.

An increase in pedicle vascularity may provide an indication of an ovarian remnant. The ureters should be identified and avoided. Ovarian tissue should be excised carefully and submitted for histopathologic examination. In some cases ovarian remnants may not be evident. When an ovarian remnant cannot be identified, any granulation tissue and adhesions in the region of the ovarian pedicles should be excised and submitted for histologic examination.

In addition to remnants of the ovaries, remnants of the uterus may be evident in some animals. These tissues should be removed. Parovarian cysts may be found, and clinicians often remove them; however, these cystic structures do not secrete sex hormones,

and their removal is not necessary for a successful outcome.

Although some owners may not opt for additional surgery to correct the ovarian remnant condition in their bitch or queen, the number of medical treatments is limited (see Chapter 5). Mibolerone (Cheque; Pharmacia & Upjohn, Peapack, NJ) is approved for use in bitches for prevention of estrus, but the drug currently is not available in the United States. Megestrol acetate (Ovaban; Schering-Plough, Kenilworth, NJ) is approved by the U.S. Food and Drug Administration for the prevention of estrus in dogs when administered orally at a dosage of 0.25 mg/lb (0.11 mg/kg) beginning 32 days before the onset of anticipated proestrus. It can also be used to stop the progression of estrous events when administered orally at a dosage of 1.0 mg/lb (0.45 mg/kg) for 8 days beginning during early proestrus. However, this drug is not intended for long-term use.

No drugs currently are approved for use in cats for the prevention of estrus. Megestrol acetate and medroxyprogesterone acetate have been used to prevent estrus in queens. However, queens are extremely susceptible to the negative side effects of progestogens, including diabetes mellitus, adrenocortical suppression, development of CEH, mammary hypertrophy, and mammary neoplasia.

Repeated manual stimulation of the vagina (four or five sessions) with a moistened cotton-tipped swab may

induce ovulation in a queen that is displaying signs of estrus as a result of ORS. Also, administration of GnRH or hCG to a queen, as described for diagnosis of ORS, may result in ovulation and cessation of estrus. However, resolution of the condition is short lived. In pseudopregnant queens, induced corpora lutea regress in approximately 35-50 days and estrus recurs.

Finally, it should be mentioned that some owners may opt to refrain from any additional treatment of their animals. Owners must be willing to tolerate the clinical signs associated with recurrent estrous cycles and must be cautioned that there may be an increased incidence of uterine stump pyometra and mammary tumors in untreated bitches and queens.

Prognosis

The prognosis for animals after surgical removal of ovarian remnants is excellent. However, multiple surgeries may be required to identify ovarian remnants. In one study, 9 of 11 queens with ORS were treated successfully with removal of an ovarian remnant during a single surgery. In the other two cats, the initial exploratory laparotomies did not reveal any ovarian remnants but ovarian remnants were identified and removed during second exploratory laparotomies. None of the 11 cats had subsequent recurrence of estrous cycles. Necessity for repeat surgery can be minimized only by performing

exploratory laparotomy when the ovarian remnant contains structures, either follicles or corpora lutea, that make its identification easier.

Ovarian Neoplasia

Definition and Pathogenesis

Ovarian neoplasms are reported to constitute 1%-2% of all tumors in dogs. However, in one study of 400 randomly selected bitches that were necropsied, approximately 70% had ovarian neoplasms. Ovarian neoplasia is less common in queens. Ovarian neoplasms almost always are of primary ovarian origin. Metastasis of other tumor types to the ovaries is rare and appears to be most common for lymphomatous tumors, which have a predilection for luteal tissue.

Primary ovarian tumors typically are not prone to metastasis. It has been estimated that up to 30% of ovarian tumors metastasize, with malignancy dependent on the tumor type. Malignant teratomas and adenocarcinomas have the highest rate of metastasis. Ovarian tumors usually metastasize locally, involving the peritoneum, adjacent kidney, or adjacent mesentery, but metastasis to bone, the lungs, and the retroperitoneal space has been reported.

Ovarian tumors are classified by the tissue of origin. Primary neoplasms may arise from surface epithelium,

sex cord–gonadostromal tissue, germ cells, or mesenchymal tissues.

EPITHELIAL TUMORS Papillary adenomas, papillary carcinomas, cystadenomas, and cystadenocarcinomas are tumors that arise from the ovarian surface epithelium. They are common in bitches, accounting for approximately 50% of the ovarian tumors reported. These tumors most often affect both ovaries but can be unilateral. They may appear as a cauliflower-like mass. Adenocarcinomas usually are larger and more invasive than benign papillary adenomas and cystadenomas. Metastasis may occur through detachment of papillary fronds, which seed the abdominal cavity.

SEX CORD–GONADOSTROMAL TUMORS Three types of tumors arise from the sex cord–stromal (gonadostromal) tissues in bitches. These are the granulosa cell tumor, thecoma, and luteoma.

Although granulosa cell tumors are the most common ovarian tumors reported to occur in most species of domestic animals, they are the second most common ovarian tumors to occur in bitches and account for 40%-50% of the ovarian tumors reported to occur in bitches. Granulosa cell tumors usually are unilateral and are functional. Metastasis apparently is uncommon, reported to occur only about 20% of the time.

Thecomas and luteomas are uncommon tumors that arise from sex cord–gonadostromal tissues. Thecomas typically consist of bundles of interlacing, spindle-shaped cells. Luteomas often appear grossly as yellow-orange masses that resemble luteal cells.

GERM CELL TUMORS Dysgerminomas and teratomas are the two neoplasms that arise from germ cells. Dysgerminomas are reported to be the third most common ovarian neoplasm in bitches and account for 6%-12% of all ovarian neoplasms in bitches. They have been reported to occur in cats, but incidence has not been reported for queens. Dysgerminomas usually are unilateral, round, and soft.

Teratomas are found more commonly in the bitch and cow than in other domestic species. They arise from a totipotent single germ cell that has completed its first meiotic division but not its second. They are characterized by tissues of two or more embryonic germ layers. It is not unusual to find sebaceous material, hair, adipose tissue, bone, teeth, and respiratory epithelium in teratomas. Potential for metastasis of teratomas in bitches is relatively high.

MESENCHYMAL TUMORS Ovarian tumors of mesenchymal origin are rare in bitches and queens, although leiomyomas have been reported. Other potential neoplasms in this category include fibromas,

neurofibromas, myomas, hemangiomas, and lymphangiomas.

Signalment

The frequency of ovarian neoplasms increases with age in bitches and queens with the exception of teratomas, which typically are found in younger dogs (younger than 6 years). Ovarian tumors have been reported as occurring most commonly in small or medium size dogs, but this is believed to reflect the longevity of the dogs and the increased likelihood of tumor development in older bitches rather than a predilection for ovarian neoplasia in these breeds per se.

History and Clinical Signs

Some ovarian tumors are responsive to hormonal stimuli; administration of diethylstilbestrol has been shown to induce ovarian neoplasms in bitches, as has treatment with mibolerone. Owners should be questioned carefully about contraceptive drugs received by the bitch.

Ovarian tumors can be associated with a wide range of clinical signs. Small tumors may be inapparent. Many ovarian neoplasms are functional, most commonly secreting estrogen. Estrogenic effects that may be apparent include nymphomania, prolonged estrus, symmetric nonpruritic alopecia, a pendulous abdomen, and

swelling of the vulva. Some dogs are reported to have clinical signs of hyperadrenocorticism. CEH and pyometra are associated with hormone-producing neoplasms in bitches. These bitches may be lethargic and have polyuria and polydipsia. In queens, approximately half of those with granulosa cell tumors had either prolonged estrus or CEH in one study. Animals with malignant tumors that have metastasized may have systemic signs of disease.

Physical Examination Findings

Bitches and queens with ovarian neoplasia may appear normal, may have findings consistent with estrogenic stimulation (e.g., swollen vulva, serosanguineous vulvar discharge), or may be critically ill. An abdominal mass may be palpable in bitches and queens with ovarian neoplasia. Abdominal distention resulting from increased intraabdominal fluid also may be present.

Diagnostic Tests and Results

Vaginal cytologic examination and serum hormone analyses can be performed in bitches and queens with signs of prolonged estrogenic stimulation. Although results of these examinations can provide supportive evidence, they usually are not definitive for a diagnosis of a functional ovarian tumor.

Abdominal palpation may be used to identify fluid or masses in the abdominal cavity. The location of these masses may be suggestive of an ovarian tumor, but the results are not definitive for diagnosis of an ovarian tumor. Similarly, radiography can be used to identify ovarian masses and displacement of abdominal structures. However, lack of detail usually does not allow definitive diagnosis.

Ultrasonography is the most effective technique for diagnosis of ovarian neoplasia in bitches and queens. The kidneys can be used as a reference point, and the location and appearance of ovarian masses can be used tentatively to diagnose an ovarian tumor (Figure 11-1). It is possible that bitches or queens may have ovaries that appear normal during ultrasonography despite containing a small functional neoplasm. Ovarian tumors may be solid or may contain cystic structures (Figure 11-2). It may be difficult to differentiate them from nonneoplastic cystic ovarian structures, such as follicular or luteal cysts, but ovarian neoplasms generally are larger than other ovarian cystic structures and they are often septate, containing multiple fluid-filled spaces. Approximately 85% of ovarian tumors are unilateral, and the left ovary is reported to be involved more often than the right. The apparently nonneoplastic ovary should be examined closely because it often is cystic. Ultrasonography also should be used to examine the uterus at the time of diagnosis of an ovarian tumor

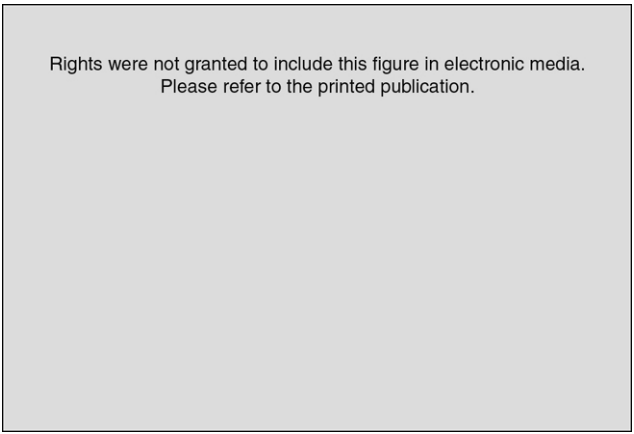


Figure 11-1. Transverse ultrasonographic image of a solid right ovarian adenocarcinoma (between *arrows*) ventral and caudal to the right kidney *(R)* in a bitch. (*From Diez-Bru N, Garcia-Real I, Martinez EM et al:* Vet Radiol Ultrasound *39:226, 1998.*)

because of the association between ovarian tumors and the CEH-pyometra complex.

Exploratory laparotomy can be used for examination of abdominal masses, to confirm the presence of an ovarian tumor, and to look for gross metastases. Treatment, by surgical excision, can be performed concurrently. Laparoscopy seems to offer an effective means for diagnosis, but I am unaware of reports on the use of laparoscopy to diagnose ovarian tumors.

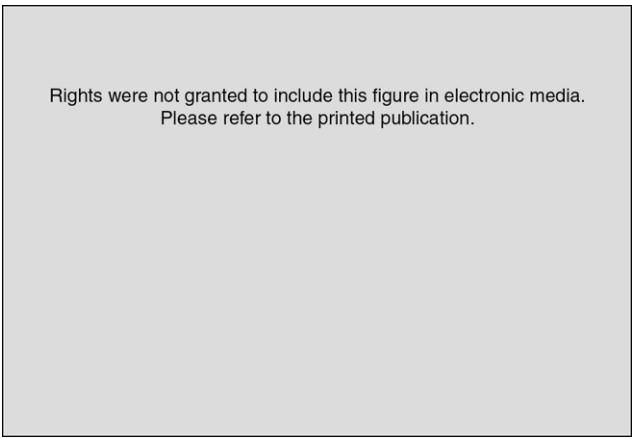


Figure 11-2. Sagittal ultrasonographic image of a solid mass with a cystic component (between *plus symbols*) in the right ovary of a bitch with bilateral ovarian adenocarcinomas. The right kidney *(RD)* is used as an anatomic landmark. The mass is delineated by free fluid in the abdomen *(F)*. Notice an anechoic lesion *(small arrow)* and far enhancement *(large arrow)*. (*From Diez-Bru N, Garcia-Real I, Martinez EM et al:* Vet Radiol Ultrasound *39:226, 1998.*)

Hematologic and serum biochemical analyses should be performed in critically ill animals. Although not diagnostic for ovarian tumors, they can provide valuable information regarding the status of the patient that will be necessary for initiation of treatment. Similarly,

biochemical analysis and cytologic examination of a sample of fluid obtained from the distended abdomen of an affected bitch or queen can yield information that may alter the course of treatment.

Treatment

Surgical removal of ovarian neoplasms is the preferred treatment. A complete OHE should be performed and the excised ovarian and uterine tissues submitted for histologic examination.

Grossly enlarged lymph nodes and obviously abnormal tissues should be removed, if possible, in bitches and queens in which metastasis is evident. In animals in which metastasis is suspected, it is prudent to remove area lymph nodes, which should be submitted for histologic examination. OHE and debulking of neoplastic tissue should be performed in animals that are to receive chemotherapy.

Medical treatment of ovarian neoplasms is not effective. Chemotherapy and radiation therapy appear to have little affect against primary ovarian tumors. However, chemotherapeutics, such as cisplatin, can be effectively used in animals with metastases from an ovarian tumor.

Prognosis

Benign ovarian neoplasms treated with OHE are associated with a good to excellent prognosis. Malignant

ovarian neoplasms are associated with a grave prognosis in dogs and cats. Prognosis varies depending on the degree of metastasis and the physical condition of the affected animal. There are reports of dogs that were treated with chemotherapeutics after the surgical removal of a malignant ovarian tumor and any obvious metastatic tissue that survived for 8 months to more than 1 year after surgery.

Bibliography

Altera KP, Miller LN: Recognition of feline parovarian nodules as ectopic adrenocortical tissue, *J Am Vet Med Assoc* 189:71, 1986.

Andrews EJ, Stookey JL, Helland DR et al: A histopathological study of canine and feline ovarian dysgerminomas, *Can J Comp Med* 38:85, 1974.

Basaraba RJ, Kraft SL, Andrews GA et al: An ovarian teratoma in a cat, *Vet Pathol* 35:141, 1998.

Boyd JS, Renton JP, Harvey MJ et al: Problems associated with ultrasonography of the canine ovary around the time of ovulation, *J Reprod Fertil Suppl* 47:101, 1993.

Cellio LM, Degner DA: Testosterone-producing thecoma in a feral cat, *J Am Anim Hosp Assoc* 36:323, 2000.

Cheng NA: Aberrant behaviour in a bitch with a granulosa-theca cell tumour, *Aust Vet J* 70:71, 1993.

Dehner LP, Norris HJ, Garner FM et al: Comparative pathology of ovarian neoplasms. 3. Germ cell tumours of canine, bovine, feline, rodent and human species, *J Comp Pathol* 80:299, 1970.

Diez-Bru N, Garcia-Real I, Martinez EM et al: Ultrasonographic appearance of ovarian tumors in 10 dogs, *Vet Radiol Ultrasound* 39:226, 1998.

England GC, Allen WE: Real-time ultrasonic imaging of the ovary and uterus of the dog, *J Reprod Fertil Suppl* 39:91, 1989.

Faulkner RT, Johnson SE: An ovarian cyst in a West Highland White Terrier, *Vet Med Small Anim Clin* 75:1375, 1980.

Fayrer-Hosken RA, Durham DH, Allen S et al: Follicular cystic ovaries and cystic endometrial hyperplasia in a bitch, *J Am Vet Med Assoc* 210:107, 1992.

Feldman EC, Nelson RW: Canine female reproduction: ovarian abnormalities. In Feldman EC, Nelson RW (eds): *Canine and feline endocrinology and reproduction,* Philadelphia, 1987, WB Saunders.

Finn-Bodner ST: Reproductive tract sonography of the dog and cat. Proceedings of the Society for Theriogenology, Vancouver, British Columbia, Canada, 2001.

Foley GL: Ovarian neoplasms of domestic animals. Proceedings of the Society for Theriogenology, Reproductive Pathology Symposium, Montreal, Quebec, Canada, 1997.

Gelberg HV, McEntee K: Feline ovarian neoplasms, *Vet Pathol* 22:572, 1985.

Gelberg HB, McEntee K, Heath EH: Feline cystic rete ovarii, *Vet Pathol* 21:304, 1984.

Greenlee PG, Patnaik AK: Canine ovarian tumors of germ cell origin, *Vet Pathal* 22:117, 1985.

Hayes HM Jr, Young JL Jr: Epidemiologic features of canine ovarian neoplasms, *Gynecol Oncol* 6:348, 1978.

Johnston SD: Clinical approach to infertility in bitches with primary anestrus, *Vet Clin North Am* 21:421, 1991.

Jones TC, Hunt RD: The genital system: diseases of the ovary. In Jones TC, Hunt RD (eds): *Veterinary pathology,* Philadelphia, 1983, Lea & Febiger.

Jubb KV, Kennedy PC, Palmer N: The female genital system: the ovary. In Jubb KV, Kennedy PC, Palmer N (eds): *Pathology of domestic animals,* New York, 1985, Academic Press.

Lein DH: Infertility and reproductive diseases in bitches and queens, *Veterinary obstetrics and genital diseases (theriogenology),* Woodstock, 1986, SJ Roberts.

Little S: Uncovering the cause of infertility in queens, *Vet Med* 96:557, 2001.

Miller DM: Ovarian remnant syndrome in dogs and cats: 46 cases (1988-1992), *J Vet Diagn Invest* 7:572, 1995.

Nagashima Y, Hoshi K, Tanaka R et al: Ovarian and retroperitoneal teratomas in a dog, *J Vet Med Sci* 62:793, 2000.

Norris HJ, Garner FM, Taylor HB: Comparative pathology of ovarian neoplasms. IV. Gonadal stroma tumours of canine species, *J Comp Pathol* 80:399, 1970.

Olsen J, Komtebedde J, Lackner A et al: Cytoreductive treatment of ovarian carcinoma in a dog, *J Vet Intern Med* 8:133, 1994.

O'Shea JD, Jabara AG: The histogenesis of canine ovarian tumours induced by stilboestrol administration, *Vet Pathol* 4:137, 1967.

Patnaik AK, Greenlee PG: Canine ovarian neoplasms: a clinicopathologic study of 71 cases, including histology of 12 granulosa cell tumors, *Vet Pathol* 24:509, 1987.

Pluhar GE, Memon MA, Wheaton LG: Granulosa cell tumor in an ovariohysterectomized dog, *J Am Vet Med Assoc* 207:1063, 1995.

Purswell BJ, Parker JA, Bailey TL et al: Persistent estrus caused by functional granulosa cell tumor of the left ovary, *J Am Vet Med Assoc* 215:193, 1999.

Root Kustritz MV, Rudolph KD: Ovarian remnant containing a teratoma in a cat, *J Am Vet Med Assoc* 219:1065, 2001.

Rowley J: Cystic ovary in a dog: a case report, *Vet Med Small Anim Clin* 75:1888, 1980.

Schlafer DH: Non-neoplastic lesions of canine and feline ovaries. Proceedings of the Society for

Theriogenology, Reproductive Pathology Symposium, Montreal, Quebec, Canada, 1997.

Seaman WJ: Canine ovarian fibroma associated with prolonged exposure to mibolerone, *Toxicol Pathol* 13:177, 1985.

Telfer E, Gosden RG: A quantitative cytological study of polyovular follicles in mammalian ovaries with particular reference to the domestic bitch *(Canis familiaris), J Reprod Fertil* 81:137, 1987.

Trasti SL, Schlafer DH: Malignant teratoma of the ovary, *J Am Vet Med Assoc* 214:785, 1999.

Vaden P: Surgical treatment of polycystic ovaries in the dogs (a case report), *Vet Med Small Anim Clin* 73:1160, 1978.

Wallace MS: Ovarian remnant syndrome. In Kirk RW, Bonagura JD (eds): *Current veterinary therapy XI,* Philadelphia, 1992, WB Saunders.

Wallace MS: The ovarian remnant syndrome in the bitch and queen, *Vet Clin North Am* 21:501, 1991.

Wallace SS, Mahaffey MB, Miller DM et al: Ultrasonographic appearance of the ovaries of dogs during the follicular and luteal phases of the estrous cycle, *Am J Vet Res* 53:209, 1992.

Yamini B, VanDenBrink PL, Refsal KR: Ovarian steroid cell tumor resembling luteoma associated with hyperadrenocorticism (Cushing's disease) in a dog, *Vet Pathol* 34:57, 1997.

12

Uterine Disorders

Margaret V. Root Kustritz and Jane A. Barber

AT A GLANCE

- Metritis
  - Metritis is infection of the uterus with no underlying uterine pathologic abnormality. Metritis occurs postpartum. Predisposing causes include prolonged delivery, dystocia, and retained fetuses or placentas. The primary clinical sign is purulent vulvar discharge. The bitch or queen also may be depressed

and anorectic and may neglect her offspring. Treatment includes stabilization of the patient with fluids and antibiotic therapy based on culture and sensitivity testing of the vulvar discharge. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) may be used to promote clearance of intrauterine fluid. Ovariohysterectomy (OHE) is recommended in severely ill bitches or queens, after stabilization.

- Subinvolution of placental sites (SIPS)
  - SIPS is abnormal repair of the endometrial placental sites. This disorder is most common in the young bitch after she has whelped her first litter. The primary clinical sign is lochia (postpartum vulvar discharge) persisting beyond 3 weeks postpartum. The bitch and pups are not ill. Diagnosis is by exclusion; rule-outs include metritis, vaginitis, cystitis, and brucellosis. Treatment is supportive. OHE is recommended for bitches that become anemic enough to require transfusion and for bitches not intended for future breeding.
- Cystic endometrial hyperplasia (CEH) and pyometra
  - Pyometra is a bacterial infection overlying CEH, an endometrial pathologic abnormality that develops after repeated exposure to estrogen and then progesterone, as happens in the normal estrous cycle of bitches and those queens that are induced to ovulate. Bitches and queens present clinically with

pyometra in diestrus or anestrus. *Escherichia coli* is the most common bacterial organism recovered. The uterus fills with purulent fluid; clinical signs are dependent on cervical patency. If the cervix is open, purulent vulvar discharge is present and the bitch or queen shows few signs of systemic illness. If the cervix is closed, abdominal distention occurs as the uterus becomes larger and the bitch or queen shows systemic signs of illness such as fever, depression, leukocytosis with a left shift, and azotemia caused by secondary renal disease. Diagnosis requires demonstration of fluid distention of the uterus in the absence of pregnancy, ideally with an abdominal ultrasound. A complete blood count, serum chemistry profile, cytologic examination, and culture of vulvar discharge also should be performed. *OHE is the treatment of choice for all dogs and cats with pyometra.* Medical therapy is best administered to bitches or queens with open cervices, great breeding value, and no evidence of azotemia. Medical therapy includes treatment with an antibiotic chosen by culture and sensitivity testing of vulvar discharge and with $PGF_{2\alpha}$, which causes uterine contractions and promotes clearance of intrauterine fluid. The reported success rate of breeding after medical treatment for pyometra is approximately 50%. Reported recurrence rates vary from 10%-77%.

- Uterine neoplasia
  - Uterine neoplasia is uncommon in bitches and queens. Benign leiomyoma is the most common uterine tumor reported. OHE is the treatment of choice.

Metritis

Metritis is inflammation of the uterus, involving both the endometrium and the myometrium. Acute metritis is associated with prolonged delivery, dystocia, and retained fetuses or placentas. A dilated postpartum cervix renders the uterus vulnerable to ascending infection by normal vaginal flora, and retained tissues or lochia can serve as an ideal culture medium.

Signalment

Metritis occurs in the postpartum bitch or queen. No age or breed predisposition has been reported.

History, Clinical Signs, and Physical Examination Findings

Presenting signs of metritis include depression, anorexia, fever, vomiting, and neglect of puppies or kittens. A differentiating finding is the presence of a malodorous, red-brown, purulent or sanguinopurulent

vulvar discharge. Severe cases may present with hypovolemic, septic, or endotoxemic shock.

Diagnostic Tests and Results

Vaginal smears from bitches or queens with metritis contain large numbers of degenerate neutrophils. Serologic testing for canine brucellosis should be performed (see Chapter 7). Abdominal radiographs may reveal uterine enlargement; ultrasonograms may reveal intrauterine fluid or tissue.

Treatment

Initial treatment is directed at stabilizing the patient:

- Treat shock, if present.
- Administer balanced electrolyte solution intravenously to treat dehydration.
- Administer dextrose solution intravenously if hypoglycemia has occurred as a result of septicemia or toxemia.
- Correct other metabolic imbalances as indicated from serum chemical profile results.

General therapy for metritis includes the following:

- Initiate broad-spectrum antibiotic therapy pending results of bacterial culture. One common empirical choice is amoxicillin/clavulanate (Clavamox; 14 mg/kg administered orally twice a

day). If hysterotomy is performed, obtain aerobic and anaerobic culture samples directly from the uterus. Obtain cultures from the cranial vagina using guarded swabs from patients that will not be undergoing surgery. Antimicrobial therapy should be modified on the basis of culture results, if indicated.

- Consider use of ecbolic agents to clear the uterus of retained tissue and promote expulsion of fluid. Ecbolic agents must be used with caution to avoid rupture of a compromised, devitalized uterus. Oxytocin is most commonly used, but its effectiveness is limited to the immediate postpartum period. $PGF_{2\alpha}$ (25-250 μg/kg administered subcutaneously twice a day for 3-8 days) also is used as an ecbolic agent. Prostaglandin offers a potential advantage over oxytocin in that it will stimulate uterine activity after the immediate postpartum period has passed. However, side effects of prostaglandin administration include vomiting, defecation, hypersalivation, and restlessness.
- Once the patient is stabilized, surgical therapy, including OHE or hysterotomy, can be considered. OHE is indicated when retained fetal or placental tissues are present, when the uterus is devitalized or ruptured, when the animal will not be used for breeding in the future, and for patients that are systemically ill. In valuable breeding

animals, hysterotomy and uterine lavage can be performed if the uterus contains no devitalized areas.

Prognosis

Metritis is simple infection of a uterus with no underlying pathologic abnormality and should be curable in most animals with appropriate antibiotic and supportive therapy. Bitches and queens treated medically that have no signs of systemic illness and that recover completely should have normal subsequent fertility.

Subinvolution of Placental Sites

Definition and Pathogenesis

SIPS is abnormal repair of the denuded uterine lining. Normal uterine involution involves invasion of the endometrial placental sites with decidua-like cells, replacement of these cells by collagen during the first 3-4 weeks postpartum, and eventual development of normal endometrium and uterine glands. In dogs with SIPS, lack of thrombosis of uterine vessels is apparent and hemorrhage that does not allow progressive healing to occur continues.

Signalment

Although SIPS may occur at any age and in any breed, most cases are seen in primiparous bitches younger than 3 years.

History and Clinical Signs

Historically, affected bitches have experienced uncomplicated parturition and both dams and pups did well. In bitches with SIPS, hemorrhage from the uterus may not be detected until normal, postpartum lochia gradually wanes at about 3 weeks postpartum. With SIPS, uterine hemorrhage persists for weeks and may even continue into the next proestral period. The discharge is serosanguineous to frankly hemorrhagic and may be either persistent or intermittent.

Physical Examination Findings

Physical examination results of bitches with SIPS and their pups usually are normal. Mild normocytic, normochromic anemia may be present; this is a normal finding in postpartum bitches.

Diagnostic Tests and Results

A presumptive diagnosis of SIPS can be made when a persistent hemorrhagic discharge is present in an

otherwise healthy bitch. Rule-outs include metritis, vaginitis, cystitis, trauma, vaginal neoplasia, brucellosis, proestrus, and coagulopathy. Histologic confirmation based on biopsy specimens is the definitive diagnostic test for SIPS and rarely is performed. Placental sites in bitches with SIPS are twice the size of those in normal bitches and contain large masses of collagen and hemorrhage and dilated endometrial glands.

Treatment

In most cases, SIPS resolves spontaneously and requires no treatment. OHE is indicated if hemorrhage is severe and in bitches not intended for future breeding. Occasionally, secondary problems such as anemia, metritis, or peritonitis may develop that require medical or surgical treatment. Ecbolic therapy with prostaglandin is not recommended.

Prognosis

When spontaneous remission occurs, reproductive potential is not compromised nor are affected bitches predisposed to SIPS in subsequent pregnancies.

Cystic Endometrial Hyperplasia and Pyometra

Definition and Pathogenesis

CEH is a progressive pathologic change of the uterine lining. Pyometra is infection overlying CEH. *Pyometra* and *metritis* are not synonymous (Table 12-1).

CEH develops after repeated exposure of the estrogen-primed uterus to progesterone. It has been demonstrated experimentally that exposure to progesterone alone can cause CEH but that lower doses of progesterone are required if the uterus is exposed to estrogen first. Development of CEH is not associated with an increase in progesterone receptors or with unusually high serum concentrations of progesterone. In the bitch it has been demonstrated that CEH worsens with repeated cycles because of the prolonged diestrus that

Table 12-1. Differentiating Pyometra from Metritis

	Pyometra	Metritis
Underlying uterine pathologic abnormality	Cystic endometrial hyperplasia	None
Infection: primary or secondary	Secondary	Primary
Timing relative to estrous cycle events	Diestrus or anestrus	Postpartum

occurs after every estrus in this species. In cats development is associated with retention of functional corpora lutea, as may be seen after induction of ovulation without subsequent pregnancy (see Chapter 2). Bitches and queens that have received estrogen for pregnancy termination or progesterone for estrus suppression may be predisposed to development of CEH and subsequent pyometra. Incidence of pyometra is not increased in bitches with a history of false pregnancy (see Chapter 14). The pathogenesis of pyometra is as follows:

- Bitches and queens may have CEH with no clinical evidence of disease. CEH is assumed to be associated with decreased fertility, perhaps by altering the environment into which the spermatozoa are introduced during coitus or the concepti enter after fertilization or by not providing a normal surface for placentation.
- The cervix opens during proestrus and estrus, allowing bacteria to ascend from the vagina into the normally sterile uterus. This happens during every cycle, and in healthy bitches, these bacteria are expelled or destroyed without causing disease.
- In some bitches with CEH, bacteria readily colonize the hyperplastic endometrium. *E. coli* is the most common organism associated with canine and feline pyometra. The cervix closes as the bitch enters diestrus and under the influence of progesterone, uterine contractility decreases, secretory

activity of the uterine glands increases, and movement of neutrophils into the endometrium and uterine lumen is depressed.

- Intrauterine fluid accumulates as excessive bacterial growth occurs. The cervix may or may not become patent again.

  In animals in which the cervix becomes patent, uterine drainage is established. Ongoing infection is associated with mild systemic disease.

  In animals in which the cervix does not become patent, the uterus distends with purulent fluid. The large endometrial surface provides continual antigen exposure to the cells of the immune system. Secondary systemic disease develops as antibody production leads to formation of circulating antigen-antibody complexes that can cause membranous glomerulonephritis. Endotoxemia resulting from lipopolysaccharides released from the cell wall of dying gram-negative organisms causes renal tubular dysfunction and immunosuppression.

  Septicemia may develop with secondary hypoglycemia, hypotension, tissue hypoxia and ischemic injury, and development of septic shock.

Three conditions are described in which accumulation of sterile fluid occurs in the uterus: hydrometra, mucometra, and hematometra. *Hydrometra* is accumulation of serous fluid, *mucometra* is accumulation of

mucoid fluid, and *hematometra* is accumulation of blood within the uterus. These conditions may or may not be associated with CEH. It is supposed that hydrometra and mucometra develop as a result of increased secretory activity of the endometrial glands in the presence of a functionally closed cervix. It is unknown whether these conditions are steps on the way to the more clinically evident pyometra. Hematometra has been described to occur in an animal suffering from rodenticide toxicity, secondary to uterine torsion, and in a young animal with severe CEH and pyometra.

Signalment

CEH and pyometra are reported to occur most commonly in dogs 8 years or older and in cats older than 5 years. Disease may occur in younger animals, especially those that historically had received estrogen for pregnancy termination or progesterone for estrus suppression. No breed predisposition is reported. CEH and pyometra may be more common in nulliparous than in multiparous bitches. Pyometra of the uterine stump can occur in spayed bitches and queens.

History and Clinical Signs

Pyometra occurs during diestrus or anestrus. Clinical signs most commonly are reported to occur in bitches

about 8 weeks after estrus, with a reported range of 1 week to 4 months after estrus. The clinical signs present and the degree of illness reported vary with cervical patency (Table 12-2).

Physical Examination Findings

In animals with open cervix pyometra, vulvar discharge usually is readily apparent. The character of the discharge varies from thick, creamy, and yellow-green to thin, watery, and red-brown. The discharge usually has a foul odor. In animals with nonpatent cervices, vulvar dis-

Table 12-2. Clinical Manifestations of Open Versus Closed Cervix Pyometra

	Open cervix pyometra	Closed cervix pyometra
Purulent vulvar discharge	Yes	No
Abdominal distention	Usually none; if present, mild	Yes
Fever/depression/ anorexia?	Sometimes present, usually mild	Usually present, often severe
Vomiting/diarrhea/ polyuria-polydipsia	Sometimes present	More often present, may be severe

charge is less likely to occur and abdominal distention often is present. The uterus often is palpably enlarged. Caution needs to be used when performing abdominal palpation; too vigorous palpation potentially could rupture a friable uterus distended with purulent fluid (Figure 12-1). Dogs and cats with pyometra often are febrile, but those with severe disease and septicemia may be hypothermic.

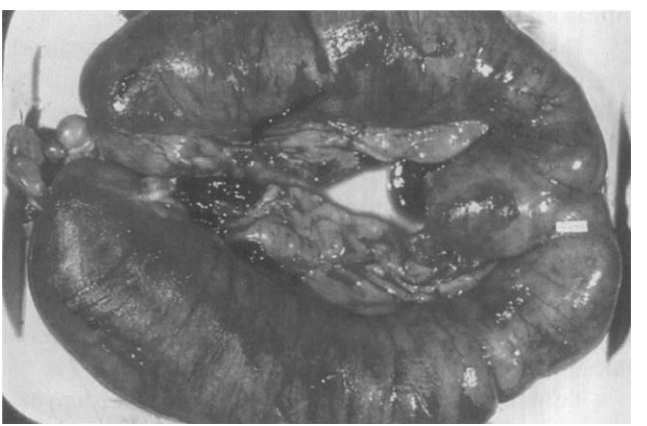


Figure 12-1. Distended uterus from a dog with closed cervix pyometra.

Diagnostic Tests and Results

The hallmark for diagnosis of pyometra is verification of the presence of accumulation of purulent fluid in the uterus. Uterine enlargement must be confirmed, either by abdominal palpation, radiography, or ultrasonography. Remember that pyometra most commonly occurs during the same time period as pregnancy in dogs and cats and that uterine enlargement is the only radiographically visible sign of pregnancy until fairly late in gestation (see Chapter 8). In animals that were bred and are at a time in their cycle when pregnancy and pyometra are both likely causes of uterine enlargement, abdominal ultrasound is the preferred imaging technique.

Pregnancy diagnosis with abdominal ultrasound is definitive any time after 24 days from ovulation (see Chapter 8). CEH may be visible on abdominal ultrasound as a fluffy thickening of the internal uterine wall on cross section; irregular cystic elevations may be evident. If the uterine horns are distended with fluid, the wall will be compressed and CEH will not be visible. The character of the intrauterine fluid cannot be definitively identified with ultrasound. These same changes may be seen in animals with uterine stump pyometra, with the abnormal tissue appearing adjacent to the urinary bladder.

Leukocytosis often is present in animals with pyometra, with elevation in white blood cell (WBC) number and degree of the left shift varying with cervical patency. Dogs and cats with open cervix pyometra and adequate

drainage of uterine fluid generally have normal to mildly elevated WBC numbers, whereas those with closed cervix pyometra may have extremely elevated WBC numbers with a left shift and possible lymphopenia secondary to endotoxemia. Normocytic normochromic anemia of chronic infection may be present.

Vulvar discharge, if present, should be collected for cytologic examination and aerobic culture before instituting antibiotic therapy. Purulent vulvar discharge is characterized by large numbers of degenerative polymorphonuclear leukocytes (PMNs) (Figure 12-2). If

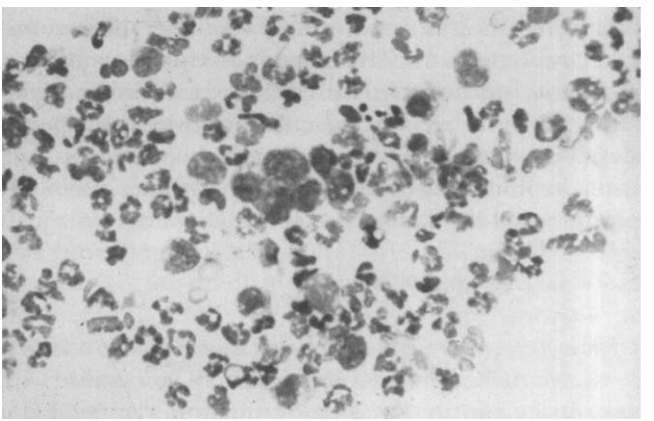


Figure 12-2. Cytology of vulvar discharge from a dog with open cervix pyometra.

vulvar discharge is not evident, a guarded swab can be used to collect a culture sample from as far craniad in the vagina as possible. Collection of intrauterine specimens by infusion and aspiration of sterile saline through a polypropylene urinary catheter passed transcervically with endoscopic guidance has been described but is not routinely used clinically. *E. coli* is the most common bacterial isolate from canine and feline pyometra. Other aerobic organisms that are part of the normal vaginal flora also may be seen, such as *Streptococcus* species, *Staphylococcus* species, *Pasteurella multocida,* and *Pseudomonas aeruginosa.*

Animals with infection caused by gram-negative organisms, such as *E. coli,* and subsequent endotoxemia and circulating antigen-antibody complexes may have elevations in blood urea nitrogen and creatinine. Septic animals may be hypoglycemic. Animals with tissue perfusion injury secondary to septicemia, hypotension, and tissue hypoxia may have changes on a serum chemistry profile related to damage to specific organs.

Treatment

OHE is the treatment of choice for all bitches and queens with pyometra. Although the infection may be curable with appropriate antibiotic therapy, the underlying CEH is irreversible and its presence predisposes the bitch or queen to pyometra at each subsequent cycle. Severity of

disease before OHE was positively correlated with outcome in one study; animals with uterine rupture, sepsis, or severe secondary systemic disease, such as renal failure, were less likely to survive surgery than were those animals that were stable before undergoing surgery. This emphasizes the necessity of rapid diagnosis and treatment of pyometra in dogs and cats. Antibiotic therapy should be instituted before surgery and continued for 7-10 days after OHE; a common empirical antibiotic choice is ampicillin at a dosage of 20 mg/kg administered orally three times a day.

Antibiotic therapy alone is not effective in treating pyometra. If OHE is not to be performed, antibiotic therapy must be combined with use of an ecbolic agent to help clear the uterus of purulent fluid. Adjunctive therapies that have been reported include acupuncture, estrogens, androgens, oxytocin, and antiprogestins. Of these, antiprogestins, such as the pregnancy termination agent RU486, hold the most promise. Unfortunately, these drugs are not available for use in veterinary medicine at this time. The most common and efficacious ecbolic agent used for medical therapy of canine and feline pyometra is $PGF_{2\alpha}$. Criteria for medical therapy for pyometra in dogs and cats include the following:

- The animal is of breeding age and is a valuable component of a planned breeding program.
- The animal's cervix is patent. Medical therapy of closed cervix pyometra has been described, with

the idea that contraction of the fluid-filled uterus will put pressure on the internal cervical os and promote opening of the cervix and uterine drainage. However, if the cervix does not open, purulent fluid may be forced through the uterine tubes or uterine rupture may occur, with subsequent peritonitis.

- Secondary systemic disease, such as renal failure, is not present. If medical therapy is not effective and the animal undergoes OHE several days after medical therapy was instituted, the likelihood of a positive outcome is greatly reduced.

If all of these criteria are met and the owner understands that underlying CEH will not be affected by treatment, the following protocol is used.

- Using a guarded swab, collect a specimen from the cranial vagina for aerobic culture and sensitivity testing. Institute empirical therapy with ampicillin (20 mg/kg administered orally three times a day).
- Determine uterine size with a repeatable technique (palpation, radiography, or ultrasonography).
- Measure serum progesterone concentration. A serum progesterone concentration greater than 2 ng/ml indicates that the animal has functional luteal tissue. Elevated progesterone inhibits myometrial contractility.
- Begin treatment with $PGF_{2\alpha}$ (Lutalyse; Pharmacia & Upjohn, Peapack, NJ) at a dosage of 0.1-0.5 mg/kg

administered subcutaneously. The drug can be dosed one to three times daily. If functional luteal tissue is present, the drug must be given at least twice daily to effect luteolysis and allow sufficient myometrial contractility to ensure expulsion of uterine fluid. The drug should be given for 2-7 days or until uterine size nears normal. Clinical response to the drug, such as obvious increase in volume of vulvar discharge or improvement in attitude or appetite of the animal, may not be evident for up to 48 hours after institution of treatment. Side effects of $PGF_{2\alpha}$ therapy include hypersalivation, emesis, and diarrhea. These signs are less severe with lower dosages and decrease in severity with each treatment. I (MVRK) have seen one animal go into shock after treatment with $PGF_{2\alpha}$; maintenance of a catheter for ready intravenous access and in-hospital observation for 20-30 minutes after treatment are recommended.

- The animal is maintained on appropriate antibiotic therapy for a total of 4 weeks. Vulvar discharge may persist for weeks after therapy.
- At the next proestrus an anterior vaginal culture sample should be collected and the animal placed on an appropriate antibiotic to minimize recurrence of pyometra. The animal should be bred and OHE performed as soon as the bitch or queen has produced the desired offspring.

Prognosis

Prognosis for survival for dogs and cats diagnosed with pyometra is fairly good; the reported survival rate is 83%-84%. For animals treated medically, reported recurrence rate varies from 10%-77%. Of those dogs bred after medical treatment, 40%-68% were bred successfully at least once. Some animals had recurrence of pyometra after a successful pregnancy. In bitches with recurrent disease, the organism identified at the first and later incidences of pyometra was the same, suggesting that medical treatment is not associated with curing the infection as much as with masking persistent infection by reducing it to a subclinical level.

Uterine Neoplasia

Definition and Pathogenesis

Primary uterine neoplasia is uncommon in bitches and queens, with a reported prevalence of less than 1% in both species. Benign uterine tumors reported include leiomyoma, fibroleiomyoma, lipoma, and fibroma. Leiomyoma is the most common uterine tumor in bitches and queens. Endometrial polyps are benign, nonneoplastic, focal proliferations of endometrial tissue that may be confused with uterine neoplasms. Malignant uterine neoplasia also has been reported, with adenocarcinoma being the most common tumor type reported.

Signalment

Uterine neoplasia usually occurs in aged intact animals but has been reported to develop at the uterine stump in ovariohysterectomized animals. No breed predisposition has been reported for cats. Boxers were overrepresented in one retrospective study of canine uterine neoplasia.

History and Clinical Signs

Many animals with uterine neoplasia are asymptomatic. Uterine neoplasms are found incidentally during routine physical examination, during abdominal palpation for pregnancy diagnosis, or at the time of routine OHE. The most common clinical sign in symptomatic animals is vulvar discharge. The character of the discharge varies from serosanguineous to sanguinopurulent. Abdominal distention, dysuria, and/or hematuria may be observed. Signs of systemic illness may be present in animals with metastases from malignant uterine neoplasms, such as vomiting and anorexia associated with liver metastases and dyspnea associated with lung metastases.

Physical Examination Findings

A firm abdominal mass often is palpable. Abdominal distention may preclude thorough abdominal palpation. Vulvar discharge may be present. Abnormalities may

occur secondary to metastatic disease, such as icterus or increased respiratory sounds.

Diagnostic Tests and Results

Abdominal radiography or ultrasonography can be used to localize the origin of the palpable abdominal mass. Tumor type cannot be differentiated by echogenic characteristics alone. A serum chemistry profile and thoracic radiographs may be useful for assessment of metastatic disease.

Treatment

OHE is the treatment of choice. In valuable breeding animals, removal of the affected uterine horn only may be attempted. Any excised tissue should be submitted for histopathologic examination to determine tumor type and malignancy and completeness of surgical removal. Chemotherapy, either for the primary tumor or for metastatic disease, has not been described.

Prognosis

Prognosis for life is excellent in bitches or queens with benign neoplasia. Prognosis for future fertility depends on the tumor type and treatment used; leiomyomas are reported not to interfere with pregnancy, but often the

animal has undergone OHE by the time the diagnosis of tumor type is made. Unilateral uterine horn excision does not interfere directly with the ability of the bitch or queen to conceive but may reduce her ability to carry the pregnancy to term because of the limited endometrial surface available for placentation. Prognosis for animals with malignant uterine neoplasia is dependent on completeness of surgical removal of the tumor and amount of metastasis.

Bibliography

Asheim A: Pathogenesis of renal damage and polydipsia in dogs with pyometra, *J Am Vet Med Assoc* 147:736, 1965.

Brodey RS, Roszel JF: Neoplasms of the canine uterus, vagina, and vulva: a clinicopathological survey of 90 cases, *J Am Vet Med Assoc* 151:1294, 1967.

Chen YM, Wright PJ, Lee CS: A model for the study of cystic endometrial hyperplasia in bitches, *J Reprod Fertil Suppl* 57:407, 2001.

Dickie MB, Arbeiter K: Diagnosis and therapy of the subinvolution of placental sites in the bitch, *J Reprod Fertil Suppl* 47:471, 1993.

Dow C: The cystic hyperplasia-pyometra complex in the bitch, *Vet Rec* 69:1409, 1957.

Dow C: The cystic hyperplasia-pyometra complex in the bitch, *Vet Rec* 70:1102, 1958.

Dow C: The cystic hyperplasia-pyometra complex in the cat, *Vet Rec* 74:141, 1962.

Faldyna M, Laznicka A, Toman M: Immunosuppression in bitches with pyometra, *J Small Anim Pract* 42:5, 2001.

Feldman EC, Nelson RW: Diagnosis and treatment alternatives for pyometra in dogs and cats. In Kirk RW (ed): *Current veterinary therapy X*, Philadelphia, 1989, WB Saunders.

Gilbert RO, Nothling JO, Oettle EE: A retrospective study of 40 cases of canine pyometra-metritis treated with prostaglandin F-2α and broad-spectrum antibacterial drugs, *J Reprod Fertil Suppl* 39:225, 1989.

Gonzalez CG, Sanchez BC, Velez HM et al: Neoplasms of the reproductive system in bitches: retrospective study over 6 years, *Vet Mexico* 28:31, 1997.

Hardy RM, Osborne CA: Canine pyometra: pathophysiology, diagnosis and treatment of uterine and extrauterine lesions, *J Am Anim Hosp Assoc* 10:245, 1974.

Heiene R, Moe L, Molmen G: Calculation of urinary enzyme excretion, with renal structure and function in dogs with pyometra, *Res Vet Sci* 70:129, 2001.

Johnson ME: Hydrometra in the dog: case report, *J Am Anim Hosp Assoc* 20:243, 1984.

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology*, Philadelphia, 2001, WB Saunders.

Lawler DF, Evans RH, Reimers TJ et al: Histopathologic features, environmental factors, and serum estrogen, progesterone, and prolactin values associated

with ovarian phase and inflammatory uterine disease in cats, *Am J Vet Res* 52:1747, 1991.

Meyers-Wallen VN, Goldschmidt MH, Flickinger GL: Prostaglandin F2α treatment of canine pyometra, *J Am Vet Med Assoc* 189:1557, 1986.

Nelson RW, Feldman EC, Stabenfeldt GH: Treatment of canine pyometra and endometritis with prostaglandin F2α, *J Am Vet Med Assoc* 181:899, 1982.

Noakes DE, Dhaliwal GK, England GC: Cystic endometrial hyperplasia/pyometra in dogs: a review of the causes and pathogenesis, *J Reprod Fertil Suppl* 57:395, 2001.

Padgett SL, Stokes JE, Tucker RL et al: Hematometra secondary to anticoagulant rodenticide toxicity, *J Am Anim Hosp Assoc* 34:437, 1998.

Pearson H: The complications of ovariohysterectomy in the bitch, *J Sm Anim Prac* 14:257, 1973.

Potter K, Hancock DH, Gallina AM: Clinical and pathologic features of endometrial hyperplasia, pyometra and endometritis in cats: 79 cases (1980-1985), *J Am Vet Med Assoc* 198:1427, 1991.

Root Kustritz MV: Postpartum disorders of the bitch and queen, *Pedigree Breeder Forum* 5:3, 1996.

Schultheiss PC, Jones RL, Kesel ML et al: Normal bacterial flora in canine and feline uteri, *J Vet Diag Invest* 11:560, 1999.

Tello L, Martin F, Valdes A et al: Comparative study of ultrasonographic, radiographic, and postoperative

characteristics of 50 bitches with pyometra, *Arch Med Vet* 28:137, 1996.

Watts JR, Wright PJ, Lee CS et al: New techniques using transcervical uterine cannulation for the diagnosis of uterine disorders in bitches, *J Reprod Fertil Suppl* 51:283, 1997.

Wheaton LG, Johnson AL, Parker AJ et al: Results and complications of surgical treatment of pyometra: a review of 80 cases, *J Am Anim Hosp Assoc* 25:563, 1989.

13

Vaginal Disorders

Beverly J. Purswell

AT A GLANCE

- Vaginal anatomic anomalies
  - The vaginal anatomic anomalies most commonly reported are vaginal septa and circumferential vaginal strictures. Abnormal embryologic development of the genitourinary tract most often occurs just cranial to the urethral papilla, where the paramesonephric ducts join the urogenital sinus.

Presence of anatomic anomalies is associated with pain when breeding, chronic urinary tract infections, urinary incontinence, and vaginitis. Diagnosis is by digital vaginal examination, vaginoscopy, or contrast vaginography. Surgical reconstruction is the treatment of choice.

- Vaginitis
  - Vaginitis can occur in prepuberal bitches (juvenile or "puppy" vaginitis) or in spayed adult bitches (adult-onset vaginitis). Primary bacterial infection of the vagina is unlikely to occur. Vaginitis most often occurs secondary to anatomic anomalies or chronic urinary tract infections; idiopathic adult-onset vaginitis also occurs. Secondary bacterial infection with resident flora occurs after the primary inflammation. Diagnosis is by visual inspection (vaginal hyperemia) and demonstration of purulent vaginal discharge. The underlying cause, if known, is treated and symptomatic therapy provided. Symptomatic medications reported include antibiotics, diethylstilbestrol, and glucocorticoids.
- Vaginal fold prolapse
  - Prolapse of edematous vaginal folds occurs during proestrus and estrus in some large- or giant-breed bitches. Serum estrogen concentrations are not abnormal in these dogs, but the response of the vaginal epithelium to estrogen is exaggerated, such

that the vaginal tissue cannot be contained within the vaginal vault and protrudes through the vulvar lips. Diagnosis is by visual inspection. Treatment is removal of the estrogen stimulus; the condition resolves spontaneously as the bitch enters diestrus or ovariohysterectomy can be performed. Severely traumatized prolapsed tissue can be surgically resected. Relapse during subsequent cycles is common.

- Vaginal neoplasia
  - Vaginal neoplasia is extremely uncommon and usually is readily apparent as a mass protruding from the vulvar lips. Animals with masses within the vaginal vault often present with hemorrhagic vulvar discharge. Surgical excision is the treatment of choice for all tumors except transmissible venereal tumor, which is best treated with vincristine.

Vaginal Anatomic Anomalies

Definition and Pathogenesis

Anatomic anomalies of the vagina and vestibule include any developmental malformations of the vulva, vestibule, and vagina (Table 13-1). Anatomic anomalies form during the embryologic development of the reproductive tract. The urogenital sinus and genital folds,

Table 13-1. Anatomic Anomalies of the Distal Reproductive Tract of the Bitch

Vagina	Vaginovestibular junction	Vulva
1. Medial septum (double vagina) 2. Segmental hypoplasia or aplasia 3. Diverticulum	1. Imperforate hymen a. Dorsal ventral band b. Annular stricture (360 degrees)	1. Dorsal commissure band 2. Infantile vulva 3. Inverted vulva 4. Vulvar cleft 5. Perineal dysgenesis 6. Rectovaginal fistula 7. Vestibulovulvar stricture

genital tubercle, and genital swellings become the vestibule, the clitoris, and the vulva, respectively. The paired paramesonephric (müllerian) ducts give rise to the uterine tubes (oviducts), uterus, cervix, and vagina. The caudal end of the vaginal canal opens into the vestibule by the joining of the paramesonephric ducts to the urogenital sinus at the vaginovestibular junction (Figure 13-1). This is the area of formation of the hymen. The urogenital sinus also gives rise to the urinary bladder and the urethra, which enters the vestibule just caudal to the vaginovestibular junction.

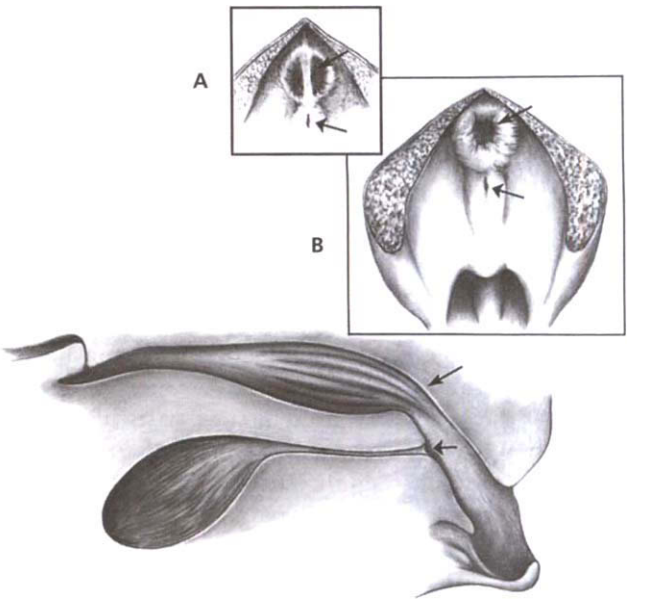


Figure 13-1. Anatomic relationship between the normal vestibule and vagina in the bitch. **A,** Dorsal ventral band; *broad arrows,* urethral opening; *narrow arrows,* vaginovestibular junction. **B,** Annular stricture at vaginovestibular junction, cranial to the urethral opening. (*Modified from Ettinger SJ, Feldman EC [eds]:* Textbook of veterinary internal medicine, *Philadelphia, 2000, WB Saunders.*)

Any aberration in the developmental process results in physical anomalies in the distal reproductive tract.

Medial septal defects of the vagina are a result of the two paired paramesonephric ducts not joining completely. The septum can divide the vagina longitudinally, giving the effect of a "double vagina," or may persist as a single, dorsoventral band or pillar of tissue just cranial to the urethral papilla.

Circumferential vaginovestibular strictures are aberrations in the joining of the paramesonephric ducts with the urogenital sinus. There normally is a narrowing at the vaginovestibular junction, the cingulum, which should not be misinterpreted as an anomaly. The normal narrowing of the vaginovestibular junction is especially evident during anestrus and in spayed bitches, particularly those bitches spayed prepuberally. A common misconception is the narrowing of the vaginovestibular junction being confused with the cervix when palpated digitally during dystocia. Vestibulovulvar strictures are reported less commonly; these are aberrations of the genital folds joining the genital swellings. Anomalies of the clitoris, particularly enlargements, are indicative of hermaphroditic conditions (see Chapter 1).

Signalment

Any bitch of any age can be affected by developmental anomalies of the reproductive tract.

History and Clinical Signs

The incidence of vaginal anomalies is difficult to determine because often there are no clinical signs associated with these anomalies. The history of affected bitches may include chronic urinary tract infections, urinary incontinence, or vaginitis. Vulvar discharge or vulvar irritation may be present with some anomalies in either intact or spayed bitches. Anomalies of the vestibule, vaginovestibular junction, or the vagina may result in infertility or in the inability to tie with the male dog at breeding because of physical barriers or pain at copulation (dyspareunia). Some of the anomalies may not be apparent until dystocia occurs, such as a dorsoventral band at the vaginovestibular junction upon which a puppy is trapped as it passes through the birth canal.

Physical Examination Findings

Anomalies involving the perineum and vulva will be evident grossly. Open vulvar clefts and perineal dysgenesis will be seen as defects in the perineal area. Some prepubertal bitches and bitches spayed for many years have infantile and inverted vulvae that may be partially or totally obscured by skin folds. This problem is exacerbated by obesity.

Skin irritation, discharge, and discolored hair may be evident around the vulva as a result of the disruption of normal urination in bitches with vulvar or

vestibulovulvar anomalies or as a result of urinary incontinence or vaginitis in bitches with anomalies that are not grossly evident.

Diagnostic Tests and Results

Anatomic anomalies of the distal reproductive tract are diagnosed by digital vaginal examination. Tight bands at the dorsal commissure and remnants of the hymen at the vaginovestibular junction, forming either a dorsoventral band or annular ring (see Figure 13-1), easily are detected with a digital examination. Careful restraint of the animal must be used during the digital examination because of the sensitivity of the dorsal commissure and the sometimes sudden resentful bites that may occur in response to pain at palpation. On digital palpation the mucosa of the vestibule may feel grainy, especially opposite the urethral opening, which is evidence of chronic inflammation and the lymphoid follicles that result from this inflammation.

Double vaginas, rare vaginal anomalies such as segmental hypoplasia or aplasia, and vaginal diverticula are diagnosed by vaginoscopy and contrast radiography. Vaginoscopy may require sedation of the animal. Contrast vaginography can be performed well only in heavily sedated animals. Vaginography is recommended if surgical repair of the anomaly is to be undertaken to

ensure complete knowledge of the abnormal vaginal anatomy before a surgical approach is attempted.

Treatment

Anomalies of the reproductive tract that are incidental findings do not require treatment, particularly if there are no concurrent clinical signs. If therapy is necessary, surgery is the treatment of choice. Conditions that necessitate correction include desire of the client to breed the animal and clinical signs that distress the animal or the client, such as urinary incontinence. Medial vaginal septa (double vagina) can be corrected surgically, although the approach and technique may be difficult because of the anatomic location of the vaginal vault. Vaginal hypoplasia and vaginal aplasia may not be correctable.

Vaginovestibular problems vary in their response to treatment. Dorsoventral bands easily are corrected with surgery. A midline episiotomy may be necessary to gain adequate exposure to the vaginovestibular junction. Annular stricture rings of the vaginovestibular region are difficult to correct surgically without recurrence. The stricture may be excised completely with the mucosa closed perpendicular to the initial incision in an attempt to enlarge the lumen. Another approach is to make a series of radial incisions perpendicular to the lumen. Both procedures may require

frequent and repeated postoperative digital dilation to prevent recurrence of the stricture. Clinical signs, such as vulvar discharge and irritation, may persist after attempted surgical correction, making treatment a frustrating exercise in some patients. Vaginectomy, also called *vaginal ablation,* is another possible therapy. It alleviates all problems associated with the vagina and usually is attempted after other therapies have failed to relieve the clinical signs. Bitches with annular strictures of the vaginovestibular junction or tight bands at the dorsal commissure that cannot be bred naturally may be bred artificially and reevaluated immediately before parturition. Relaxation of these strictures and bands usually is sufficient to allow normal delivery.

Infantile and inverted vulvae are corrected surgically by removing the excess skin folds around the vulva. In intact bitches, spontaneous resolution may occur when the bitch is allowed to go through estrus. The problem may recur with age after subsequent ovariohysterectomy (OHE) if vulvar atrophy occurs, especially in obese bitches. Surgical alteration of the perivulvar area (episioplasty) usually alleviates the chronic urinary tract infections and inflammation of the perivulvar skin and the vestibule by allowing more complete urine voiding. Vulvar clefts and perineal dysgenesis may be corrected surgically if deemed necessary by the clinical signs associated with the defects.

Prognosis

Prognosis varies with the anomaly and the desired outcome of the client. Anomalies of the vaginovestibular junction, vestibule, and vulva easily are corrected and are associated with a good prognosis. Prognosis after correction of anomalies of the vaginal vault must be guarded because of the difficulty of the surgical approach to correct these anomalies and the ultimate effect on reproduction.

Vaginitis

Definition and Pathogenesis

Vaginitis is any inflammatory process that involves the mucosal lining of the vestibule or vagina. Puppy vaginitis is a purulent vulvar discharge in a prepuberal bitch, usually present without any discomfort to the animal. The cause of puppy vaginitis is unknown but may be related to immaturity of the vaginal canal and epithelium or immaturity of the immune system. Inflammation of the vestibule and vagina in the adult bitch usually is caused by some predisposing condition, although idiopathic adult-onset vaginitis does occur. Canine herpesvirus may cause vaginitis, although the vaginal lesions seen with canine herpesvirus may cause no clinical signs in the affected animal. Anatomic anomalies may interfere with urine voiding, thus causing inflammation of the vestibule and

distal vagina because of urine pooling and resultant urine scalding. Urinary tract infections may lead to inflammation of the vestibule in the area of the urethral papilla. Vaginitis can be brought about by alterations in the normal bacterial flora after antibiotic therapy or by an immunosuppressive systemic condition, such as diabetes mellitus. Any foreign body in the vaginal canal causes inflammation. Vaginal neoplasia may be mistaken for vaginitis because of the presence of vulvar discharge.

Signalment

Any bitch of any age, spayed or intact, can be affected by vaginitis. However, adult-onset vaginitis is more commonly reported to occur in spayed than in intact bitches.

History and Clinical Signs

Vaginitis is associated with purulent or mucopurulent vulvar discharge and irritation to the animal, as evidenced by excessive licking of the perineal area.

Physical Examination Findings

Discharge or evidence of discharge can be observed at the vulvar lips and in the surrounding hair. Vulvar hyperemia and clitoral hypertrophy secondary to licking may be present.

Diagnostic Tests and Results

Vaginal cytologic examination reveals large numbers of neutrophils in addition to noncornified epithelial cells from the vagina and vestibule in anestrous or spayed bitches. Lymphocytes and macrophages may be present in smaller numbers in the more chronic cases. Vaginoscopy demonstrates hyperemia and possibly follicular hyperplasia, indicative of chronic inflammation. Vaginoscopy is helpful in identifying the source of the discharge and in ruling out other causes of vulvar discharge, such as uterine disease or presence of a vaginal foreign body.

Treatment

Treatment for vaginitis primarily involves identifying and treating the predisposing cause, if possible. Urinary tract infections can be identified by urinalysis and bacterial culture performed on urine samples collected by cystocentesis. Anatomic anomalies should be identified and corrected. Infantile inverted vulvae are noted for causing bitches discomfort and inflammation in the area of the perineum and vestibule. Surgical correction of these conditions often eliminates the clinical signs. If a foreign body is present, it should be removed.

Puppy vaginitis is a self-limiting condition. Most cases require no treatment or only topical therapy with a medicated douche to minimize clinical signs. Puppy

vaginitis resolves on its own either before or during the first estrus. Controversy exists as to whether allowing dogs with clinical signs of puppy vaginitis to go through one heat period prior to OHE hastens resolution of vaginitis.

Systemic antibiotics alter the normal bacterial flora of the vagina and have been shown to select for opportunistic pathogens, such as *Escherichia coli* and *Mycoplasma* species. Local antibiotic therapy, including douching, is not recommended because usually it is not effective, it may cause irritation and worsening of the vulvar licking, and it may further alter the normal bacterial flora of the vagina. If the animal has a concurrent immunosuppressive condition, however, antibiotic therapy may be beneficial if used with discretion. Whenever antibiotic therapy is considered, bacterial cultures of the vagina should be obtained using a guarded swab, thus avoiding the heavily contaminated vestibule and skin. If heavy growth of a single organism is present, systemic antibiotic therapy can be chosen based on the organism's sensitivity pattern.

If no underlying cause for vaginitis is identified, treatment with diethylstilbestrol (DES) may be considered in the spayed bitch. Protocols similar to those described for urinary incontinence are used, decreasing the frequency of doses until a minimal effective dosage regimen is reached. One such protocol is 1 mg of DES administered orally once daily for 5 days and

then 1 mg administered twice weekly for 2-3 weeks with a further decrease to once weekly treatment unless signs recur.

Other symptomatic therapies that may be used to treat vaginitis include glucocorticoids and androgens. Glucocorticoids should not be used in dogs with urinary incontinence. Treatment with testosterone, as for estrus suppression (see Chapter 5), may be beneficial.

Surgical therapy is reported to hasten resolution of idiopathic adult-onset vaginitis and of vaginitis associated with perivulvar dermatitis. Episioplasty (vulvoplasty) is removal of tissue dorsal to the vulvar cleft with subsequent eversion of the vulva from enveloping perivulvar folds. In one study, episioplasty was reported to effect a cure in 18 of 18 dogs with vaginitis.

Prognosis

The success of resolution of vaginitis depends on the cause. Puppy vaginitis resolves spontaneously if left untreated. Most cases of adult vaginitis resolve once the predisposing condition has been corrected. The most difficult cases of adult vaginitis to treat are those resulting from anatomic anomalies because the anomaly itself may be difficult to correct. Idiopathic adult-onset vaginitis resolves eventually, but the patient may require weeks to months of symptomatic therapy.

Vaginal Fold Prolapse (Vaginal Hyperplasia, Vaginal Hypertrophy, Vaginal Prolapse)

Definition and Pathogenesis

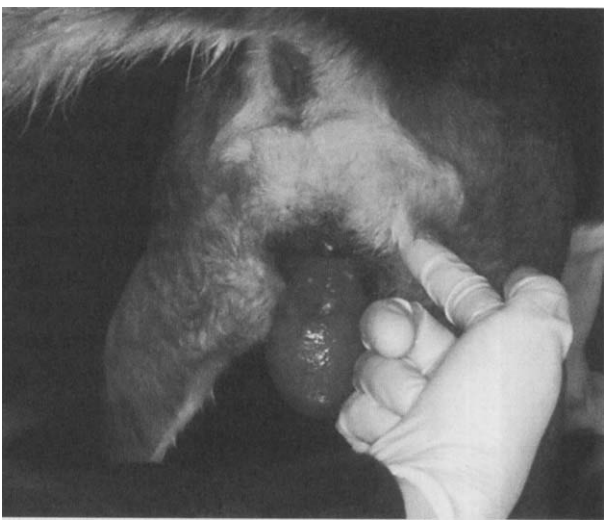
Vaginal fold prolapse, historically and incorrectly referred to as *vaginal hyperplasia* or *vaginal hypertrophy,* is the extrusion of mucosal tissue through the vulvar lips. This protrusion of mucosal tissue is associated with the influence of estrogen on the vaginal epithelium. Estrogen causes thickening of the vaginal squamous epithelium and edema of the underlying tissue in normal bitches during proestrus and estrus. In cases in which the vaginal tissue prolapses through the vulvar lips, it is thought to be an exaggerated response to estrogen. There is evidence that the condition is hereditary in some lines and breeds of dogs. True vaginal prolapse (dislocation of normal vaginal tissue through the vulvar lips) occurs rarely in the bitch and usually is associated with dystocia, tenesmus, or forced extraction of the male during the genital tie.

Signalment

Prolapse of vaginal tissue occurs almost exclusively in intact bitches during proestrus and estrus. Occasionally, the prolapse continues throughout pregnancy or recurs at parturition.

History and Clinical Signs

Vaginal tissue prolapse occurs in the intact bitch during proestrus and estrus, and it may occur during the first estrous cycle or any cycle thereafter (Figure 13-2, *A*). This condition is not associated with dysuria. The normal serosanguineous vulvar discharge of proestrus or estrus usually is present.



A

Figure 13-2. Vaginal fold prolapse in the bitch. **A,** Peduncular vaginal fold prolapse; note standing (flagging) response of estrus.

Continued

Physical Examination Findings

The protruding mucosal tissue most commonly is seen as a solid tumorlike mass protruding from the vulvar lips (Figure 13-2, *B*). This mass originates from the ventral vaginal floor immediately anterior to the urethral papilla and may be peduncular (see Figure 13-2, *A*). Occasionally, the prolapse is circumferential and protrudes as a doughnut-shaped mass (Figure 13-2, *C*).

B

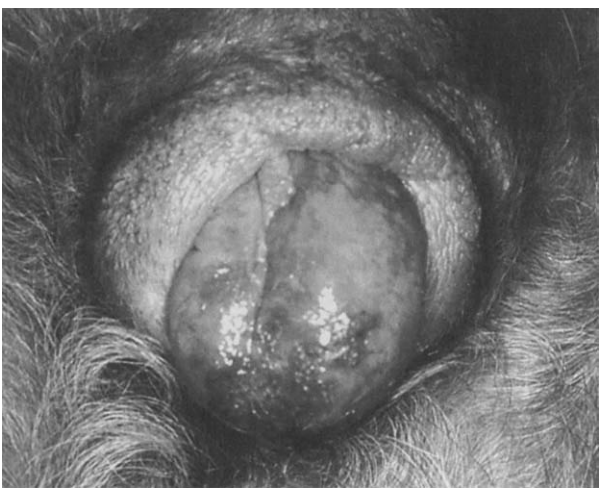
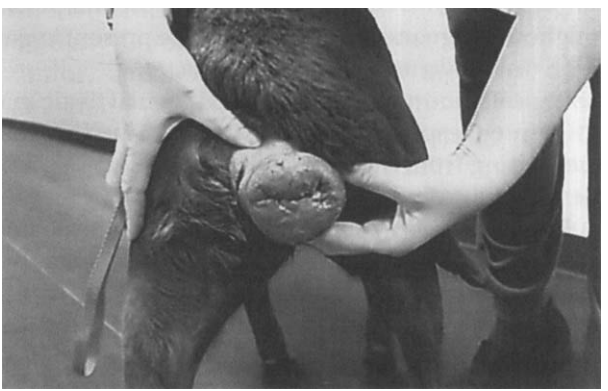


Figure 13-2, cont'd. **B,** Vaginal fold prolapse; note excoriation of mucosa.

Once prolapsed, the mucosal tissue will become excoriated because of exposure to the external environment.

Diagnostic Tests and Results

The influence of estrogen can be confirmed by cornification of the vaginal epithelial cells, the presence of the characteristic serosanguineous estrous discharge, and the presence of estrous behavior.



C

Figure 13-2, cont'd. **C,** Vaginal fold prolapse; 360-degree doughnut-shaped prolapse. (*From Ettinger SJ, Feldman EC [eds]:* Textbook of veterinary internal medicine, *Philadelphia, 2000, WB Saunders.*)

Treatment

Ovariectomy or OHE permanently corrects this condition by removing the gonadal source of estrogen. The prolapsed tissue regresses shortly after removal of the ovaries.

In breeding bitches, treatment may not be necessary or desirable because of the self-limiting nature of the condition. In most cases the prolapsed tissue regresses spontaneously with the onset of diestrus. If natural breeding is not possible because of the size of the prolapse, artificial insemination may be performed to obtain a pregnancy. Occasionally, a prolapse will persist throughout pregnancy. Prolapses that are present at parturition usually do not result in dystocia.

Surgical removal of the prolapsed vaginal tissue may be attempted, especially in cases of larger peduncular masses arising from the ventral floor of the vagina. The surgery is performed through a midline episiotomy incision. Recurrence is possible after surgical removal, during the subsequent proestrus or estrus. Surgery may not be advisable in cases of circumferential prolapses because of the extent of the tissue involved and the potential of strictures of the birth canal.

Prognosis

Vaginal fold prolapse is a self-limiting condition that resolves spontaneously; the prognosis for life is

excellent. Presence of the prolapse may preclude natural service but does not affect fertility.

Vaginal Neoplasia

Definition and Pathogenesis

For this discussion, the term *vaginal neoplasia* includes any tumor arising from the vestibule or vagina. Vaginal neoplasia represents 2.5%-3% of all canine tumors and 41% of tumors involving the canine reproductive tract. From 70%-80% of these tumors are benign. Leiomyoma is the most common benign tumor, and leiomyosarcoma is the most common malignant vaginal tumor. The pathogenesis of vaginal neoplasia is unknown but may be related to the effect of ovarian hormones. Transmissible venereal tumor (TVT) arises from an allogeneic cellular transplant that contains a number of chromosomes different from that seen in canine cells (TVT, $2n = 59 \pm 5$; normal dog cell, $2n = 78$).

Signalment

Any bitch at any age can be affected with vaginal neoplasia. The risk increases with age. TVT is more common in young, free-roaming, intact bitches.

History and Clinical Signs

The presenting signs of vaginal neoplasia may include perineal enlargement, a mass protruding from the vulva, vulvar discharge, tenesmus, or dysuria. Any vaginal mass in a spayed bitch and any vaginal mass in an intact bitch unrelated to the estrous cycle should be considered potentially neoplastic. Any hemorrhagic vulvar discharge in a spayed bitch or hemorrhagic vaginal discharge unrelated to the estrous cycle or pregnancy in the intact bitch should be investigated as a potentially neoplastic process.

Physical Examination Findings

Tumors prolapsing from the vulva can be differentiated from vaginal fold prolapse by evaluating the stage of estrous cycle and its relationship to the onset of signs, location of the mass, and failure of the mass to regress postestrus. Vaginoscopy can be used to identify masses within the vaginal canal that may be responsible for a hemorrhagic vulvar discharge, tenesmus, or dysuria. Benign leiomyomas often are peduncular. TVT may be vegetative and ulcerated.

Diagnostic Tests and Results

Biopsy or excisional biopsy samples from the mass should be submitted for histopathologic examination to confirm the tissue type involved.

Treatment

Surgical excision, when possible, is the treatment of choice for most vaginal tumors. TVT responds readily to chemotherapy; vincristine at a dosage of 0.025 mg/kg or 0.6 mg/m^2 administered intravenously weekly causes complete regression in two to seven treatments. As with any chemotherapeutic regimen, surgical debulking of neoplastic tissue before starting drug therapy may be beneficial. OHE is recommended at the time of surgical removal of vaginal masses because of the possibility of hormonal influence on the tumor.

Prognosis

The prognosis for complete resolution of vaginal and vulvar tumors is good, provided that metastasis has not occurred with the malignant tumors.

Bibliography

Burke TJ, Smith CW: Vulvo-vaginal cleft in a dog, *J Am Anim Hosp Assoc* 11:774, 1975.

Hammel SP, Bjorling DE: Results of vulvoplasty for treatment of recessed vulva in dogs, *J Am Anim Hosp Assoc* 38:79, 2002.

Holt PE: An evaluation of positive contrast vagino-urethrography as a diagnostic aid in the bitch, *J Small Anim Pract* 25:531, 1984.

Holt PE, Sayle B: Congenital vestibulo-vaginal stenosis in the bitch, *J Small Anim Pract* 22:67, 1981.

Johnston SD: Vaginal prolapse. In Kirk RW (ed): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology,* Philadelphia, 2001, WB Saunders.

Kyles AE, Vaden S, Hardie EM et al: Vestibulovaginal stenosis in dogs: 18 cases (1987-1995), *J Am Vet Med Assoc* 209:1889, 1996.

Lightner BA, McLoughlin MA, Chew DJ et al: Episioplasty for the treatment of perivulvar dermatitis or recurrent urinary tract infections in dogs with excessive perivulvar skin folds: 31 cases (1983-2000), *J Am Vet Med Assoc* 219:1577, 2001.

McEntee K: *Reproductive pathology of domestic mammals,* San Diego, 1990, Academic Press.

Memon MA, Pavletic MM, Kumar MS: Chronic vaginal prolapse during pregnancy in a bitch, *J Am Vet Med Assoc* 202:295, 1993.

Meyers-Wallen VN, Patterson DF: Disorders of sexual development in dogs and cats. In Kirk RW (ed): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Root MV, Johnston SD, Johnston GR: Vaginal septa in dogs: 15 cases (1983-1992), *J Am Vet Med Assoc* 206:56, 1995.

Schaefers-Okkens AC: *Vaginal edema and vaginal fold prolapse in the bitch, including surgical management,* International Veterinary Information Service, 2001. Available at www.ivis.org.

Withrow SJ, Susaneck SJ: Tumors of the canine female reproductive tract. In Morrow DA (ed): *Current therapy in theriogenology,* Philadelphia, 1986, WB Saunders.

Wykes PM, Soderberg SF: Congenital abnormalities of the canine vagina and vulva, *J Am Anim Hosp Assoc* 19:995, 1983.

14

Mammary Disorders

Harry Momont and Jane A. Barber

AT A GLANCE

- False pregnancy
  - False pregnancy (pseudocyesis) is a syndrome characterized by mammary development and lactation and behaviors of whelping and mothering. It occurs in intact bitches approximately 2 months after estrus. It is caused by the normal decline in serum

progesterone concentrations at the end of diestrus and is not associated with reproductive tract disease. Spontaneous remission usually occurs in 1-2 weeks; wrapping engorged mammary glands with an elastic bandage may hasten resolution of clinical signs.

- Mastitis
  - Mastitis most commonly occurs in postpartum bitches. It occurs rarely in postpartum queens and in bitches undergoing false pregnancy. Diagnosis is by visual inspection; affected mammary glands are red, turgid, and painful. Treat with an appropriate antibiotic, based on culture and sensitivity of fluid expressed from the affected glands. Puppies may be allowed to nurse from adjacent normal glands.
- Mammary hypertrophy
  - Mammary hypertrophy is nonneoplastic enlargement of one or more mammary glands in cats under the influence of progesterone. Ovariohysterectomy (OHE) of diestrous queens or cessation of administration of exogenous progestogens is curative.
- Mammary neoplasia
  - In dogs, mammary masses may be either benign (fibroadenoma = mixed mammary tumor) or malignant (adenocarcinoma). In cats, mammary neoplasia virtually always is malignant adenocarcinoma. Bitches and queens spayed early in life have a decreased incidence of mammary neoplasia when they get older compared with animals that are left

intact. Siamese cats are predisposed as a breed; no breed predisposition is reported in dogs. Diagnosis is confirmed with excision biopsy and submission for histopathologic examination. Surgical removal is the treatment of choice. More extensive surgery (mastectomy versus removal of the mass only) may be associated with decreased incidence of local recurrence but is not associated with increased survival time. Effect of concurrent OHE on tumor recurrence or survival time is equivocal.

False Pregnancy

Definition and Pathogenesis

False pregnancy (also called *pseudopregnancy* or *pseudocyesis*) is a normal consequence of canine reproductive physiology. The healthy, cycling bitch enters diestrus after ovulation (see Chapter 2). This diestrus, or luteal phase, is in many respects physiologically and hormonally indistinguishable from pregnancy. It also is similar in length to pregnancy, usually lasting approximately 2 months. As a result, all normal nonpregnant bitches experience false pregnancy after estrus. The clinical presentation of false pregnancy actually involves the development of signs and behaviors associated with the end of the luteal phase and may be more correctly thought of as false whelping. The objectionable or overt signs of clinical false pregnancy are

the result of declining progesterone concentrations at the end of diestrus coupled with elevated concentrations of estrogen and prolactin. Mammary gland development can occur in response to the prolonged progesterone stimulation during diestrus, and lactation is initiated by the rise in prolactin.

False pregnancy has been described as an atavistic or vestigial pack behavior. The tendency for dogs to cycle together results in subordinate bitches ending diestrus at about the same time that a dominant bitch would end her pregnancy. The onset of a synchronous lactation would allow the subordinate females to serve as nursemaids for the genetically superior pups of the dominant bitch.

Signalment

Overt false pregnancy can occur in intact postpuberal bitches of any age. It usually occurs 1-3 months after estrus. It also may occur after OHE of the diestrual bitch or after cessation of exogenous progestogen therapy. Spayed bitches suffering from ovarian remnant syndrome also may experience false pregnancy.

History and Clinical Signs

Most dogs with clinical false pregnancy were in estrus 2-3 months before presentation, were recently spayed, or recently completed progestogen therapy. The develop-

ment of clinical signs is variable and may not occur during every cycle. Clinical signs include mammary gland enlargement and galactorrhea (inappropriate lactation), change in appetite, nesting behavior, and adoption or mothering of objects or animals. Abdominal distention sometimes may be observed, and many clients are convinced that their bitch is pregnant. The rare bitch may become extremely agitated or aggressive. The duration of false pregnancy varies greatly but may last as long as 4-6 weeks.

Physical Examination Findings

Most physical examination findings are normal. The only common exception is galactorrhea, an enlargement of the mammae with the presence of a milklike secretion. Mastitis may occur rarely and should be treated as for the lactating bitch. Finally and obviously, the bitch is not pregnant.

Diagnostic Tests and Results

Serum progesterone concentrations are low or declining. No pups are seen on radiographs or ultrasonograms (see Chapter 8). Serum relaxin concentrations are undetectable, as opposed to being elevated in the pregnant bitch after 3-4 weeks of gestation. Thyroid function should be evaluated in any bitch with clinical signs

suggestive of hypothyroidism and galactorrhea; elevated thyroid releasing hormone concentrations in the hypothyroid bitch may stimulate prolactin release.

Treatment

No treatment is necessary for dogs with mild signs of false pregnancy. In the absence of nursing pups the condition usually is self-limited. Physical stimulation of the mammae by the bitch or owner should be avoided because it may encourage milk let-down and prolong the lactation. For this same reason, it may be wise to remove any adopted object from the bitch. A mild restriction of food and water also may hasten the end of the lactation, as may wrapping of the mammary glands with an elastic bandage. Owners who object to mild signs of clinical false pregnancy should be cautioned that a recurrence after the next estrus is possible if not likely. Spaying the bitch after the remission of clinical signs will prevent a recurrence of the problem.

In cases in which mammary development is excessive or the behavior is particularly objectionable, more aggressive treatment measures may be necessary. Hormone replacement therapy with androgens, estrogens, or progestogens has been used with variable success. All hormonal therapy entails significant risk to developing fetuses, so pregnancy must be definitively ruled out before any treatment is begun. The only prod-

uct approved for treatment of false pregnancy in the United States is megestrol acetate (Ovaban; Schering-Plough, Kenilworth, NJ). It is a progestogen that suppresses the signs of false pregnancy initiated by the progesterone withdrawal at the end of diestrus. The recommended dosage of megestrol acetate is 2.5 mg/kg/day given orally for 8 days. Recurrence of clinical signs after therapy has been discontinued is common. Progestogen therapy should be avoided in dogs with diabetes mellitus or mammary neoplasia. The serious side effects associated with estrogen therapy in the dog precludes its use as a treatment for a condition as relatively benign as false pregnancy. Androgen therapy is a safer choice, and the weak, synthetic androgen mibolerone at a dosage of 16 μg/kg/day, has been used to treat false pregnancy. This product unfortunately is not consistently available in the United States.

Drugs that block the secretion of prolactin, either serotonin antagonists or dopamine agonists, also have been effective for treatment of false pregnancy in the bitch. None presently are approved in the United States for this purpose. The serotonin antagonist, cabergoline (Dostinex; Pharmacia & Upjohn, Peapack, NJ), at an oral dosage of 5 μg/kg/day for 5-7 days, has been reported to effectively suppress signs of false pregnancy in most dogs. Bromocriptine (Parlodel; Novartis, East Hanover, NJ), a dopamine agonist, at an oral dosage of 30 μg/kg/day for 16 days, also has been reported to

effectively treat the signs of false pregnancy. Emesis is a common side effect of bromocriptine therapy.

If an affected bitch is so aggressive as to require sedation, dopamine antagonists such as acepromazine or haloperidol should be avoided because they will cause an increase in prolactin release. A 4- to 5-day course of diazepam can be effective. Clients should exercise caution with aggressive dogs and limit their exposure to people and other dogs until the condition improves.

Prognosis

False pregnancy is not associated with infertility or any other diseases of the reproductive system. It should be regarded as a normal condition resulting from the unique physiology of diestrus in the dog. Recurrence during subsequent cycles is possible and can be prevented with OHE.

Mastitis

Definition and Pathogenesis

Mastitis is inflammation of the mammary gland or glands, invariably associated with bacterial infection. Risk factors for developing mastitis include poor

sanitary conditions, trauma inflicted by offspring, and systemic infection. Mastitis may be acute or chronic.

Signalment

Mastitis occurs almost exclusively in the postpartum bitch. It occurs less commonly in the postpartum queen. Rarely, mastitis has been observed in the lactating, pseudopregnant bitch.

History, Clinical Signs, and Physical Examination Findings

Mastitis may be localized (e.g., involving a single gland sinus), diffuse within a single gland, or diffuse within multiple glands. The presenting bitch may be asymptomatic or may be critically compromised. Milk from mastitic glands may appear normal grossly or may be of abnormal color or consistency.

In acute mastitis the affected glands are hot and painful. If acute mastitis progresses to septic mastitis, the bitch shows signs of systemic illness such as fever, depression, anorexia, lethargy, and neglect of pups. Abscessation of mammary glands may develop, and the bitch may present with either a turgid, fluctuant, painful mammary mass or an ulcerated, draining lesion with associated gangrene of mammary tissue. In chronic or

subclinical mastitis the sole presenting complaint may be failure of offspring to thrive.

Diagnostic Tests and Results

Diagnosis is made based on visual inspection. Microscopic examination of milk may reveal inflammatory cells. Culture of milk or fluid expressed from the affected glands yields moderate to heavy growth of organisms common on the skin, such as *Escherichia coli* and *Staphylococcus* species.

Treatment

Treatment of mastitis involves the following:

- Stabilize the condition of the systemically ill patient by using fluid therapy targeted at correcting metabolic disturbances.
- Express milk from affected glands twice daily if pups are not nursing. Pups may or may not be allowed to continue nursing. The decision is based on the conditions of both the dam and the neonates, the safety of the chosen antibiotic for the neonates, and the capability of the owner to hand-raise the puppies. Pups usually are removed if abscessation is present.
- Apply warm compresses to the affected glands twice daily.

- Keep the mammary glands and environment clean; reduce potential sources of trauma by trimming the toenails of puppies and kittens.
- Surgically debride gangrenous tissue, and place drains in mammary abscesses.
- Institute appropriate antimicrobial therapy, based on culture and sensitivity testing. Factors to consider when choosing an appropriate antibiotic include the following:

  The integrity of the blood-milk barrier: In acute mastitis the blood-milk barrier is disrupted, allowing most antibiotics to reach effective tissue levels. In chronic mastitis the blood-milk barrier is reestablished and antibiotics are distributed on the basis of pH partitioning. Simply stated, this means that weak bases, such as clindamycin and erythromycin, tend to concentrate in an acidic milk environment.

  The lipid solubility of antimicrobial agents: Even an antibiotic of appropriate pH will fail to achieve effective concentrations if it is not lipid soluble. Clindamycin and erythromycin are examples of antibiotics with high lipid solubility.

  The safety of the drug in nursing neonates: Choose "gut-friendly" agents that do not adversely affect normal bacterial colonization of the neonatal gastrointestinal tract. Indiscriminate use of

ampicillin, in particular, has been associated with overgrowth of pathogenic bacteria in neonates.

Tetracyclines: Avoid use of tetracyclines if the bitch will continue nursing her pups. When given to juveniles, tetracyclines can cause bone deformity and enamel dysplasia.

Therapeutic agents: Cephalexin (5-15 mg/kg administered orally three times a day) and amoxicillin/clavulanate (Clavamox; 14 mg/kg administered orally two to three times a day) are recommended as initial therapeutic agents pending culture results.

Prognosis

Mastitis usually resolves with appropriate antibiotic therapy. Function of the mammary gland is not compromised unless it is severely damaged, as with abscessation. Bitches may have mastitis during every lactation, presumably because of mammary gland anatomy that allows ready introduction of environmental bacteria through the teats. The pros and cons of breeding these females and their daughters should be discussed with the owner.

Mammary Hypertrophy

Definition and Pathogenesis

Feline mammary hypertrophy (also called *mammary hyperplasia* and *mammary fibroadenomatosis*) is a benign fibroglandular proliferation of the mammae. Progesterone or progestogens are assumed to play a role in the pathogenesis of this disorder; most affected cats are either pregnant, in the luteal phase of the cycle, or being treated with exogenous progestogens at the time of presentation.

Signalment

The disease most often occurs in intact queens younger than 2 years. However, cats of any age, reproductive status, or gender can be affected if treated with exogenous progestogens. More rarely, older pregnant queens also may be affected.

History and Clinical Signs

Affected queens may or may not have a history of having been mated. They usually are pregnant or are in the luteal phase of the estrous cycle (see Chapter 2). The

condition most commonly occurs as a spontaneous disorder of young, intact females. Its occurrence in older, neutered or male animals should suggest the possibility of exogenous progestogen therapy. One or more mammary glands (often all glands) undergo diffuse enlargement over 2-4 weeks.

Physical Examination Findings

In most cases all of the mammary glands are firm and enlarged. Mammary masses range from 1-5 cm in diameter and can affect one, several, or all glands. In severe cases ulcerative necrosis of the skin may occur. The condition is not accompanied by lactation, but normal lactation can occur after delivery of kittens, even without resolution of the hypertrophy.

Diagnostic Tests and Results

Affected cats generally are under the influence of progesterone or a progestogen. The pregnancy status of the queen can be established by palpation, ultrasonography, or radiography at the appropriate stage of gestation (see Chapter 8). An assay for serum progesterone can be used to determine whether there is functional luteal tissue in queens with no evidence of pregnancy. Exogenous progestogens may or may not

be detected by a progesterone assay, and the possibility of their use should always be carefully explored in the history. Histopathologic examination of affected tissue reveals a circumscribed, unencapsulated benign fibroglandular proliferation. The diagnosis in the young queen with diffuse mammary enlargement generally is evident from the history and physical examination and does not require biopsy. Cats with discrete, localized swellings, especially older animals, should be managed as for mammary neoplasia (see following discussion). This should include excisional biopsy of the complete mass and radiographs to check for evidence of metastasis.

Treatment

In young queens, mammary hypertrophy spontaneously resolves after progesterone declines at the end of pregnancy or diestrus. Surgical removal of the hypertrophied glands is not recommended. Recovery can be hastened with OHE or ovariectomy, or medical induction of luteolysis can be attempted. In older cats or those with a history of progestogen therapy, the masses should be removed and examined for evidence of neoplasia. Progestogen therapy should be discontinued because it may contribute to the potential for development of neoplasia in the hypertrophied mammary tissue.

Prognosis

Complete recovery usually occurs in young queens after luteal regression. OHE prevents recurrence of mammary hypertrophy. In older cats the prognosis is dictated by the histopathologic and radiographic findings.

Mammary Neoplasia

Definition and Pathogenesis

Mammary neoplasia is the most common tumor in female dogs and the third most common tumor in female cats. In dogs, primary mammary tumors have an equal likelihood of being benign (fibroadenoma = mixed mammary tumor) or malignant (adenocarcinoma). Virtually all mammary tumors in queens are highly malignant adenocarcinomas.

Pathogenesis of mammary neoplasia in dogs and cats is not completely understood. Bitches and queens that are spayed before their first estrous cycle have a dramatically decreased risk of developing mammary neoplasia when they get older, compared with animals that are left intact. Overall, intact female dogs have a four times greater risk and intact female cats have a seven times greater risk of developing either benign or malignant mammary tumors when they are older, compared with ovariectomized animals. Dogs spayed before their first estrous cycle have 0.5% the risk, those spayed

after one estrous cycle have 8.0% the risk, and those spayed after two cycles have 26.0% the risk of developing mammary neoplasia compared with intact females. There is no apparent sparing effect of pregnancy and lactation on development of mammary neoplasia in dogs and cats, as may occur in women.

Receptors for estrogen and progesterone have been identified in normal and in neoplastic mammary tissue, but no correlation has been identified between type or number of receptors present and type or severity of mammary neoplasia. Often, the most aggressive, anaplastic tumors have no definable hormone receptors. Administration of exogenous progestogens may be associated with mammary tumor development in both bitches and queens.

Body conformation and diet have been associated with incidence of mammary neoplasia in bitches. Bitches judged to have been thin at 9-12 months of age had a decreased incidence of mammary neoplasia, whereas those bitches judged to have been obese at 1 year of age had an increased incidence of mammary neoplasia compared with age-matched control animals. One study demonstrated increased incidence of mammary neoplasia in dogs that were fed homemade diets, especially those containing large amounts of beef or pork. In feline mammary tumors, virus particles have been identified by electron microscopy in up to one third of tumors; the significance of this is unknown.

Signalment

In dogs, mammary neoplasia most commonly occurs in bitches older than 6 years. Mammary neoplasia is most common in bitches left intact or spayed after 2.5 years of age or after four estrous cycles and is more common in purebred dogs than in crossbred dogs. Mammary neoplasia has been reported to occur in male dogs but incidence is extremely low.

In cats, mammary neoplasia is most common in queens older than 6 years, with peak incidence at 10-11 years of age. As in the bitch, intact queens are more likely to develop mammary neoplasia as are female cats spayed after having gone through heat. Siamese cats are predisposed compared with domestic shorthair and other purebred cats. The incidence of mammary neoplasia in male cats is extremely low.

History and Clinical Signs

In both dogs and cats, mammary masses may be incidental findings of physical examinations performed before routine vaccinations or the animals may be presented because the masses were noted by the owners. Appearance of neoplastic mammary masses is unrelated to the time of the last estrus. Rarely, masses ulcerate and cause pain and licking of the mass and adjacent mammary area. Systemic signs may be present if metastasis has occurred and vary with site of metastasis.

Physical Examination Findings

One or more masses may be present. Masses most commonly occur in the caudal pair of mammae in dogs and occur with equal frequency in all four pairs of mammae in cats. Mammary masses usually are firm and freely movable and may be either smooth or nodular. The most common sites of metastasis of malignant tumors are the axillary and inguinal lymph nodes and the lungs. Other reported sites of metastasis include the abdominal organs, the eyes, the pleurae, and the skin. Metastases may not be evident on physical examination.

Diagnostic Tests and Results

Palpable characteristics of the mass are not correlated with tumor type or malignancy. Fine-needle aspirate, scraping of ulcerated lesions, or cytologic examination of fluid from affected glands may yield useful information if evidence of malignancy is present. Lack of neoplastic cells, however, does not imply that malignancy is not present. Definitive diagnosis requires excision of abnormal tissue and submission for histopathologic examination. Radiographs of the chest and a serum chemistry profile should be obtained, and possibly an abdominal ultrasound should be performed, to check for metastasis before surgery.

Treatment

Surgery is the treatment of choice for all mammary tumors and often is necessary for definitive diagnosis of tumor type. Surgery of various extents has been described, including lumpectomy (removal of the mass only), simple mastectomy (removal of the gland containing the mass), en bloc dissection (removal of the gland containing the mass, intervening lymphatics, and regional lymph nodes), and unilateral mastectomy (removal of the entire chain of glands on the side with the mass, with or without the regional lymph nodes). No definitive studies exist defining which surgery is best. More aggressive surgery is not associated with longer postsurgical survival time but may be associated with a decreased incidence of local recurrence. Because of the highly malignant nature of mammary neoplasia in cats, more extensive surgery may be advisable. Concurrent OHE may or may not be associated with increased survival time or decreased incidence of tumor recurrence; if tumor removal and OHE are performed at the same time, the OHE should be performed first to prevent seeding of mammary tumor cells into the abdominal cavity.

Chemotherapy can be used as an adjunct to surgery. Chemotherapeutic agents described for use in the dog include doxorubicin alone and a combination of cyclophosphamide, vincristine, and methotrexate. In the cat, reported response of mammary neoplasia to chemotherapy has been poor. Radiation therapy also has

been described; again, response has been poor enough to preclude its routine use as a therapy. Hormonal therapies as described in women have not been reported to be useful in dogs and cats.

Prognosis

In dogs, prognosis is very good with benign masses and poor to good with malignant masses, with poorest prognosis associated with tumors greater than 2-3 cm in diameter or the presence of metastasis. Seventy-five percent of dogs with malignant mammary tumors survive less than 2 years after surgical removal of the primary tumor. In cats, prognosis is associated with tumor size; after surgery the median survival time for cats with tumors less than 2 cm in diameter is 3 years, for cats with tumors 2-3 cm in diameter is 2 years, and for cats with tumors greater than 3 cm in diameter is 6 months. Cats with pulmonary metastases rarely survive longer than 2 months after diagnosis.

Agalactia

Agalactia (failure to produce or secrete milk) can be primary or secondary. Primary, or true, agalactia (as a result of anatomic or physiologic abnormality) is extremely rare in the bitch. There is no treatment for primary agalactia, and puppies must be hand-reared.

Secondary agalactia, more commonly referred to as *poor milk let-down,* can result from poor nutrition, stress, anxiety, premature delivery, progestogen therapy, or systemic illness (including mastitis, metritis, and endotoxemia). Treatment of secondary agalactia is directed at resolving the underlying, primary problem. For the anxious, primiparous bitch, efforts should include providing a quiet, comfortable environment and reassurance by the owner. Some bitches may benefit from administration of phenothiazine tranquilizers, which may calm the bitch and which stimulate prolactin release. In some cases, administration of oxytocin may be required for milk let-down to occur. Administration either may stimulate endogenous oxytocin release after one or more treatments or may be required at every desired nursing. If repeated dosing is required, use of oxytocin nasal sprays, available through human pharmacies, is preferred over repeated injections.

Galactostasis

Galactostasis is accumulation of milk within a mammary gland secondary to delayed passage of milk from the gland. It can occur when there is a teat abnormality, when puppies are abruptly weaned, or with concurrent illness. It also can occur when milk production exceeds milk consumption or when there are no puppies available to nurse. Treatment is directed at decreasing milk

production and reducing inflammation. Food intake is decreased, and cool compresses are applied to the affected glands. Administration of glucocorticoids, diuretics, and analgesics may be beneficial. Wrapping the mammary area with an elastic bandage promotes negative feedback to the pituitary gland, decreasing prolactin release, and protects sensitive mammary glands from trauma. Engorged mammary glands should not be milked out; this prolongs prolactin secretion and milk production.

Bibliography

Allen SW, Mahaffey EA: Canine mammary neoplasia: prognostic indicators and response to surgical therapy, *J Am Anim Hosp Assoc* 25:540, 1989.

Bender AP, Dorn CR, Schneider R: An epidemiologic study of canine multiple primary neoplasia involving the female and male reproductive systems, *Prev Vet Med* 2:715, 1984.

Brodey RS, Goldschmidt MH, Roszel JR: Canine mammary gland neoplasms, *J Am Anim Hosp Assoc* 19:61, 1983.

Chastain CB, Schmidt B: Galactorrhea associated with hypothyroidism in intact bitches, *J Am Anim Hosp Assoc* 16:851, 1980.

Donnay I, Rauis J, Woutes-Ballman P: Receptors for oestrogen, progesterone and epidermal growth

factor in normal and tumorous canine mammary tissues, *J Reprod Fertil Suppl* 47:501, 1993.

Dorn CR, Taylor DO, Frye FL et al: Survey of animal neoplasms in Alameda and Contra Costa counties, California. I. Methodology and description of cases, *J Natl Cancer Inst* 40:295, 1968.

Dorn CR, Taylor DO, Schneider R et al: Survey of animal neoplasms in Alameda and Contra Costa counties, California. II. Cancer morbidity in dogs and cats from Alameda County, *J Natl Cancer Inst* 40:307, 1968.

Fidler IJ, Brodey RS: The biological behavior of canine mammary neoplasms, *J Am Vet Med Assoc* 151:1311, 1967.

Hahn KA, Adams WH: Feline mammary neoplasia: biological behavior, diagnosis and treatment alternatives, *Feline Pract* 25:5, 1997.

Hahn KA, Richardson RC, Knapp DW: Canine malignant mammary neoplasia: biological behavior, diagnosis, and treatment alternatives, *J Am Anim Hosp Assoc* 28:251, 1992.

Hayden DW, Johnston SD, Kiang DT et al: Feline mammary hypertrophy/fibroadenoma complex: clinical and hormonal aspects, *Am J Vet Res* 42:1699, 1981.

Hayes HM, Milne KL, Mandell CP: Epidemiologic features of feline mammary carcinoma, *Vet Rec* 108:476, 1981.

Hayes HM, Mooney S: Feline mammary tumors, *Vet Clin North Am* 15:513, 1985.

Janssens LA: Treatment of pseudopregnancy with bromocriptine, an ergot alkaloid, *Vet Rec* 119:172, 1986.

Jochle W, Arbeiter K, Post K et al: Effects on pseudopregnancy, pregnancy and interoestrus intervals of pharmacological suppression of prolactin secretion in female dogs and cats, *J Reprod Fertil Suppl* 39:199, 1989.

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology*, Philadelphia, 2001, WB Saunders.

Misdorp W: Canine mammary tumours: protective effect of late ovariectomy and stimulating effects of progestins, *Vet Q* 10:26, 1988.

Misdorp W, Hart AA: Canine mammary cancer. I. Prognosis, *J Small Anim Pract* 20:385, 1979.

Olson JD, Olson PN: Disorders of the canine mammary gland. In Morrow DA (ed): *Current therapy in theriogenology*, Philadelphia, 1986, WB Saunders.

Perez Alenza D, Rutteman GR, Pena L et al: Relation between habitual diet and canine mammary tumors in a case-control study, *J Vet Intern Med* 12:132, 1998.

Priester WA: Occurrence of mammary neoplasms in bitches in relation to breed, age, tumour type, and geographical region from which reported, *J Small Anim Pract* 20:1, 1979.

Schneider R, Dorn CR, Taylor DO et al: Factors influencing canine mammary cancer development and postsurgical survival, *J Natl Cancer Inst* 43:1249, 1969.

Sonnenschein EG, Glickman LT, Goldschmidt MH et al: Body conformation, diet, and risk of breast cancer in pet dogs: a case-control study, *Am J Epidemiol* 133:694, 1991.

Steinetz BG, Goldsmith LT, Harvey HJ et al: Serum relaxin and progesterone concentrations in pregnant, pseudopregnant, and ovariectomized, progestin-treated pregnant bitches: detection of relaxin as a marker of pregnancy, *Am J Vet Res* 50:68, 1989.

Voith VL: Functional significance of pseudocyesis, *Mod Vet Pract* 61:75, 1980.

Weijer K, Hart AM: Prognostic factors in feline mammary carcinoma, *J Natl Cancer Inst* 70:709, 1983.

15

Contraceptive Techniques for Male Dogs and Cats

Richard Fayrer-Hosken

AT A GLANCE

- Surgical
  - Castration is the contraceptive method of choice for male dogs and cats that are not intended for breeding. It is 100% effective as a contraceptive and protects the animal from androgen-dependent disorders, such as prostate disease.

  - Vasectomy has been described but has limited use because vasectomized animals still are exposed to negative consequences of lifelong exposure to androgen.
  - Testicular/epididymal sclerosing agents can be used.
- Medical (All medical contraceptive techniques for male dogs and cats are experimental at this time.)
  - Gonadotropin-releasing hormone (GnRH) agonists
  - Immunocontraceptive vaccine

In many countries, especially third world countries, the need for contraception techniques in male dogs and cats is approaching crisis levels. Several successful strategies are used in individual animals, but no population-level techniques have been developed. Surgical, medical, and immunologic contraception strategies are reported.

Male Reproductive Physiology

Spermatogenesis is stimulated and sustained by hypothalamic GnRH, which causes release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland. FSH stimulates the Sertoli cells within the testes to produce androgen-binding protein (ABP), which binds testosterone in the seminiferous

tubules, helping maintain the high intratesticular testosterone concentrations necessary for spermatogenesis. The Sertoli cells also produce inhibin, which is postulated to control FSH secretion by a simple negative feedback mechanism. LH, also called *interstitial cell–stimulating hormone,* stimulates the Leydig (interstitial) cells to produce testosterone. Testosterone secretion is precisely regulated by negative feedback to the pituitary and subsequent decrease in serum concentration of LH. Increases in serum testosterone decrease LH secretion within approximately 1 hour, which in turn decreases serum testosterone concentrations within 1 hour to several hours.

Testosterone, bound to ABP in the seminiferous tubules, is essential for initiation of meiosis of spermatogonia (primitive germ cells). Testosterone also is necessary for epididymal maturation of spermatozoa, development of secondary sex characteristics, and normal libido. Secretion of testosterone is pulsatile, so serum concentration of testosterone, measured as a single sample, can vary from 0.4-10.0 ng/ml.

Spermatogenesis encompasses the development of the spermatozoon from the spermatogonium. All domestic mammals have specifically defined periods that are required for complete spermatogenesis, called *spermatogenic phases.* In dogs and cats a complete spermatogenic phase is about 60 days in length.

The bulk of spermatogenesis occurs within the blood-testis barrier (BTB). The BTB consists of the basement membrane of the seminiferous tubule, the Sertoli cells, and the tight junctions apposing the Sertoli cells. The primordial germ cells lie only partially within the BTB and thus make them potential candidates for immunologically targeted contraceptive strategies.

Contraceptive Methods

Surgical

Two methods of surgical control that are well established are castration and vasectomy. These are permanent methods of contraception and are easily performed on individual animals. Testicular or epididymal sclerosing agents also have been described.

CASTRATION

- Definition: Castration is the removal of both testes.
- Mechanism of action: All spermatogenic tissue, the primary source of testosterone production, is removed.
- Pros:
  Permanent contraception
  Decrease in androgen-dependent behaviors (e.g., roaming, urine spraying, intraspecies aggression)

and diseases (e.g., benign prostatic hypertrophy, perianal adenomas)

- Cons:

  Permanent contraception

  Requires general anesthesia

  Side effects associated with decline in serum testosterone include obesity, testosterone-responsive urinary incontinence, decrease in secondary sex characteristics (cats), possible predisposition to prostatic neoplasia (dogs)

Castration is the recommended contraceptive method for male dogs and cats in the United States.

VASECTOMY

- Definition: Vasectomy is the bilateral removal or occlusion of a segment of the ductus deferens.
- Mechanism of action: Spermatozoa cannot move from the testes/epididymides through the vasa deferentia into the urethra for ejaculation.
- Pros:

  Testosterone production not altered; some people consider the effect of testosterone on the animal's behavior beneficial (teaser tom in cattery, for example)

  May be reversible
- Cons:

  Requires general anesthesia

May be irreversible (Azoospermia has been reported to develop within days after vasectomy, and reanastomosis of the ductus deferens is very difficult.)
No decrease in androgen-dependent behaviors or diseases

TESTICULAR/EPIDIDYMAL SCLEROSING AGENTS

- Definition: Testicular/epididymal sclerosing agents are compounds that are injected into the testes/epididymides. Compounds described include sodium alginate, lactic acid, zinc arginine, and chlorhexidine.
- Mechanism of action: Inflammation induces subsequent atrophy or fibrosis of spermatogenic tissue.
- Pros:
  Have been shown to have significant inhibitory effect on fertility
  Potentially reversible
- Cons:
  Require heavy sedation or general anesthesia
  Experimental; reported efficacy variable

Medical

Medical contraceptive methods for male dogs and cats are less invasive than surgical methods and do not require sedation or anesthesia. They also are potentially

reversible because they do not induce destruction of spermatogenic tissue.

GnRH AGONISTS

- Long-term administration of GnRH agonists, such as leuprolide acetate (0.1 or 1.0 mg/kg administered subcutaneously) or nafarelin acetate (0.5 or 2.0 μg/kg administered subcutaneously once daily for 44 days) leads to continued negative feedback on the pituitary, with decreased serum concentrations of LH and testosterone and subsequent aspermatogenesis. The duration of the effects depends on the source and release profile of GnRH. GnRH has been shown to be effective for up to 2 years.

OTHER HORMONAL THERAPIES

- Progestogens: Progesterone can be used as an inhibitor of libido and may have deleterious effects on spermatogenesis but also may induce diabetes mellitus and mammary nodules, hypertrophy (cats), or neoplasia. I recommend use of these compounds sparingly, if at all.
- Estrogens: Estradiol causes azoospermia and might be considered another contraceptive agent. Negative side effects include bone marrow suppression and squamous metaplasia and secretory stasis of the prostate. I recommend use of these compounds sparingly, if at all.

- Prolactin: Prolactin has been shown to cause azoospermia after sustained administration. If an effective delivery method could be found, prolactin might be an effective contraceptive candidate.

Immunologic

Immunologic strategies are targeted against systemic hormones or specific stages of spermatogenesis. Vaccines against GnRH have had varied degrees of success. The primary problem has been making the small GnRH molecule (decapeptide) antigenic enough to provide a sustained effect. Targeting primordial gametes or spermatozoal proteins for contraception has been the subject of significant research. This is an extremely important facet of human contraceptive research because these techniques generally do not affect libido. This is not a primary goal of canine or feline male contraception. Although no effective male immunocontraceptive vaccine is available, the possibilities for success are considerable.

Bibliography

Aitken RJ: New techniques in contraception: gossypol, vaccines and GnRH analogues. Proceedings of the Annual Symposium of the Eugenics Society, 1983.

England GC: Effect of progestogens and androgens upon spermatogenesis and steroidogenesis in dogs, *J Reprod Fertil Suppl* 51:123, 1997.

Goodpasture JC, Hiller MB, Lewis B et al: Same day appearance of orally administered, spermicidal 1-substituted imidazoles in dog ejaculates, *J Andro* 8:230, 1987.

Hammami-Hamza S, Doussau M, Bernard J et al: Cloning and sequencing of SOB3, a human gene coding for a sperm protein homologous to an antimicrobial protein and potentially involved in zona pellucida binding, *Mol Human Reprod* 7:625, 2001.

Lacoste D, Dube D, Trudel C et al: Normal gonadal functions and fertility after 23 months of treatment of prepubertal male and female dogs with the GnRH agonist [D-Trp6,des-Gly-NH2(10)]GnRH ethylamide, *J Andro* 10:456, 1989.

Miller LA, Johns BE, Elias DJ et al: Comparative efficacy of two immunocontraceptive vaccines, *Vaccine* 15:1858, 1997.

Naz RK, Leslie MH, Zhu X: Inhibition of murine spermoolemma binding by antibodies to an oocyte membrane (OM) antigen: implication in contraceptive vaccine development, *Am J Reprod Immunol* 45:52, 2001.

Naz RK, Zhu X: Molecular cloning and sequencing of a novel cDNA encoding for a protein involved in

human sperm function, *Biochem Biophys Res Commun* 284:911, 2001.

Naz RK, Zhu X, Kadam AL: Cloning and sequencing of cDNA encoding for a novel human testis-specific contraception vaccinogen: role in immunocontraception, *Mol Reprod Dev* 60:116, 2001.

Naz RK, Zhu X, Kadam AL: Identification of human sperm peptide sequence involved in egg binding for immunocontraception, *Biol Reprod* 62:318, 2000.

Paramo RM, Renton JP, Ferguson JM et al: Effects of medroxyprogesterone acetate or gonadotropin-releasing hormone agonist on suppression of spermatogenesis in the dog *(Canis familiaris)*, *J Reprod Fertil Suppl* 47:387, 1993.

Shafik A: Prolactin injection, a new contraceptive method: experimental study, *Contraception* 50:191, 1994.

Talwar GP, Singh O, Pal R et al: Vaccines for control of fertility and hormone dependent cancers, *Int J Immunopharm* 14:511, 1992.

Vickery BH: Comparisons of the potential utility of LHRH agonists and antagonists for fertility control, *J Steroid Biochem* 23:779, 1985.

16

Testicular and Epididymal Disorders

William B. Ley, G. Reed Holyoak, Wynne A. Digrassie, and Deborah Cartisano

AT A GLANCE

- Cryptorchidism
  - Cryptorchidism is lack of descent of one or both testes into the scrotum by 6 months of age. The condition is hereditary, with either a polygenic or

autosomal recessive inheritance hypothesized. Diagnosis is by physical examination. Retained testes are atrophic and generally are not visible on abdominal ultrasound. Animals with bilateral cryptorchidism are best differentiated from castrated animals based on measurement of serum testosterone after administration of gonadotropin-releasing hormone (GnRH). All cryptorchid animals should be neutered. Retained testes do not produce spermatozoa but do produce androgens, rendering affected animals subfertile or infertile yet exposing them to androgen-dependent disorders. Retained testes also are predisposed to neoplasia and torsion of the spermatic cord. Medical therapy causing testicular descent is not consistently successful and may be unethical.

- Orchitis/epididymitis
  - *Orchitis* and *epididymitis* refer to inflammation of the testis and epididymis, respectively. These conditions may occur separately or concurrently. Brucellosis is one infectious cause of epididymitis in dogs. Noninfectious causes include trauma and autoimmune disease. Diagnosis is by physical examination; affected testes/epididymides are swollen and painful. Further diagnostic tests that may be supportive include ultrasonography of the

scrotal contents, fine-needle aspiration (FNA) for cytology specimens and culture, and serologic testing for canine brucellosis. Orchiectomy is the treatment of choice.

- Torsion of the spermatic cord
  - Torsion of the spermatic cord is uncommon. The involved testis usually is neoplastic. Animals have clinical signs of an "acute abdomen." Definitive diagnosis requires exploratory laparotomy. Orchiectomy is the treatment of choice.
- Testicular neoplasia
  - Testicular neoplasia is common in dogs and rare in cats. The most common tumor types described are Sertoli cell tumor, seminoma, and interstitial (Leydig) cell tumor. All three are equally prevalent in descended testes. Sertoli cell tumor is more common in testes retained in the abdomen. None of the three tumor types commonly undergoes metastasis. Sertoli cell tumors may be associated with a paraneoplastic syndrome caused by estrogen production by the tumor, which is characterized by bilaterally symmetric alopecia, gynecomastia, and attraction of male dogs. Diagnosis of testicular neoplasia usually is by inspection. Small intratesticular tumors and retained neoplastic testes may be visible on ultrasound. Orchiectomy is the treatment of choice.

Cryptorchidism

Definition and Pathogenesis

Cryptorchidism is a congenital defect in which one or both of the testes do not descend into the scrotum at the appropriate time. Cryptorchidism may be unilateral or bilateral, with the testicles retained anywhere along the normal path of testicular descent. Retention usually is classified as subcutaneous when the testis is palpated between the scrotum and the inguinal ring or as inguinal or abdominal according to whether the testis is in the inguinal ring or in the abdomen.

It is important to point out that monorchidism (only one testis) and anorchidism (no testes at all) are extremely rare. For this reason, in all cases involving the absence of one or both testes, animals should be considered to have cryptorchidism until proven otherwise.

The incidence of cryptorchidism in cats has been reported to be 1.7% (23 of 1345 animals in one study). Two cats in that population had true monorchidism. The prevalence of cryptorchidism was significantly higher in Persian cats within that study population. There were more unilaterally than bilaterally affected cats. No significant difference was noted in location of unilaterally retained testes when comparing inguinal with abdominal and left with right sides. In general, bilaterally retained testes are located intraabdominally,

but bilateral, subcutaneous inguinal testicles have been reported to occur in the cat.

The incidence of cryptorchidism in dogs varies from 0.8%-9.8%. Heredity is almost certainly involved because cryptorchidism is more common in certain breeds, such as toy and miniature poodles, Yorkshire terriers, Chihuahuas, boxers, and miniature schnauzers, and in certain families. The degree of inbreeding appears to be greater in bilateral than in unilateral cases of cryptorchidism. Genetic factors do not explain all cases; some unilateral cryptorchid dogs have been used as breeding animals for years and produced many litters with no evidence of defects in their offspring. The exact mode of inheritance is unknown, but the possibility exists of either polygenic inheritance or a sex-limited autosomal recessive inheritance in which both male and female animals can carry the gene and can pass it on to their offspring. Cryptorchidism is associated with other hereditary traits, such as hip dysplasia, patellar dislocation, defects of the penis and prepuce, and umbilical hernia.

In puppies, testicular descent occurs soon after birth, normally from 3-10 days. In beagles and mixed-breed dogs, the testes always are palpable from 20-25 days of age. Generally, accurate palpation of the testes is possible at 2-4 weeks of age but normally takes place around the time of weaning, at 5-8 weeks. Testes should be palpable in all puppies at that time. If they are not

palpable within the scrotum by 8-10 weeks, a tentative diagnosis of cryptorchidism is made. To make a definitive diagnosis of cryptorchidism, it may be necessary in some animals to wait until the inguinal canals close at puberty, around 5-10 months of age. However, late testicular descent is not a desirable trait and should be selected against in dogs intended for breeding.

The exact factors involved in regulating testicular descent are unknown. However, normal hypothalamic-pituitary-gonadal axis, normal testicular hormone secretions, and perhaps normal müllerian-inhibiting factor (MIF) secretion and receptor function appear to be necessary for complete testicular descent.

Administration of GnRH and gonadotropins induces earlier than normal testicular descent in humans and animals. Anecdotal evidence suggests that GnRH or gonadotropin administration induces testicular descent in cryptorchid males. For these reasons, it has been theorized that cryptorchidism is caused by a deficiency in gonadotropin secretion. When changes in luteinizing hormone (LH) and testosterone concentrations were compared between normal and cryptorchid dogs, GnRH secretion and LH secretion in unilaterally cryptorchid dogs were decreased. The effectiveness of administration of either GnRH or human chorionic gonadotropin (hCG), which activates LH receptors in dogs and cats, in causing the descent of a retained testis has not been scientifically tested and has been tried with mixed success.

Normal levels of testicular hormones present during the right developmental periods are required for testicular descent. In the dog the outgrowth of the gubernaculum, which constitutes the intraabdominal phase of testicular descent, requires the presence of androgens. Testosterone also induces gubernacular regression during the final (intrainguinal or scrotal) phase of testicular descent. Androgens also have been implicated in the suppression of the growth of the cranial suspensory ligament (CSL) of the gonad. In males with fetal androgen deficiency, the CSL remains, causing a maldescent of the testis. In fetal androgenized females, the CSL is absent. Persistent bilateral CSL have been documented as occurring in a bilateral cryptorchid dog, indicating a potential role of these structures in inhibiting proper outgrowth of the gubernaculum and subsequent testicular descent.

In addition to androgens, there appears to be another testicular secretory factor associated with normal testicular descent. In freemartin pigs with cryptorchidism, gubernacular outgrowth does occur, leading to a well-developed cremaster muscle and vaginal process. In experimental situations, gubernacular outgrowth was not induced in females treated with androgens but was induced in females exposed to testicular secretions. This suggests that a nonandrogen testicular secretory product is involved in normal testicular descent. In an attempt to elucidate factors associated

with testicular descent in the dog, researchers performed orchiectomy with or without androgen replacement during gestation at the time of intraabdominal testicular migration (gestational day 49), immediately after parturition at the time of intrainguinal migration, and during the neonatal period (day 3 postpartum), at the final scrotal descent phase. It was found that the testis of the fetus and neonate induced gubernacular outgrowth and regression and was necessary for complete testicular descent. These processes were not completed in the orchiectomized puppies with androgen replacement therapy. It was concluded that gubernacular outgrowth and regression and subsequent testicular descent are regulated by an unidentified, nonandrogenic factor derived from Sertoli or germ cells. In a study involving cryptorchid and normal colts, testicular meiosis-activating sterol was shown to be associated with normal testicular descent. This factor has not been evaluated in the dog.

Leydig cells and Sertoli cells of the normal male fetal testis secrete testosterone and MIF, respectively, during embryogenesis. The normal sequence of reproductive tract development in the male requires the secretion of testosterone for mesonephric (wolffian) duct development into the epididymis, vas deferens, and seminal vesicles. Testosterone is converted to dihydrotestosterone via 5α-reductase, which then induces masculinization of the urogenital sinus and

external genitalia. The secretion of MIF induces a regression of the paramesonephric (müllerian) duct system. Defects in hormone production, timing of secretion, transport, or receptor upregulation or responsiveness lead to persistent müllerian duct syndrome. This syndrome often is accompanied by either unilateral or bilateral cryptorchidism, as described in humans and miniature schnauzers (see Chapter 1). The role, if any, of MIF in testicular descent in normal males has not been delineated.

Diagnostic Tests and Results

The diagnosis of cryptorchidism normally is made based on the findings of the initial physical examination performed at about 8 weeks of age. Careful examination of the scrotum and perineal area for surgical scars may indicate previous surgery in pubertal males. A thorough ultrasonographic examination of the abdomen and inguinal area may allow visualization of retained testes. Radiography usually is unrewarding because of the small size and nondistinct tissue density of the retained testis. Hormonal analysis is the most definitive diagnostic test for differentiation of bilaterally cryptorchid animals from castrated animals. Baseline serum testosterone concentrations of less than 0.02 ng/ml indicate a lack of retained testicular tissue. In dogs with abdominally retained testes, serum testosterone concentrations

usually are 0.1-2 ng/ml, whereas adult dogs with one or two scrotal testes usually have serum testosterone concentrations of 1-5 ng/ml. However, testosterone is secreted in pulses throughout the day, so challenge testing is more accurate than is evaluation of a single serum sample. A hormonal stimulation test with hCG or GnRH can be used to demonstrate the presence and production of testosterone. Several protocols exist.

PROTOCOL 1

- Draw a pretest blood sample.
- Administer either hCG (100 IU intramuscularly) or GnRH (50 μg subcutaneously).
- Draw posttest samples 12 and 24 hours later.
- A twofold to fourfold increase from the baseline value is considered significant.

PROTOCOL 2

- Administer GnRH (2 μg/kg intramuscularly).
- Draw blood 60 minutes later.
- A serum testosterone concentration of 3 ng/ml or greater is considered significant.

Treatment and Prognosis

Unilateral cryptorchids should be neutered because of the hereditary potential and poor semen quality observed in affected dogs. Although there is no current

definitive information that cryptorchidism is an inherited trait in cats, there was a higher reported prevalence in Persian cats, suggesting a hereditary basis. Testicular neoplasia is up to 14 times more likely in cryptorchid dogs than in normal dogs. Abdominal testes have an increased risk of developing Sertoli cell tumors and of undergoing torsion of the spermatic cord. Torsion of cryptorchid testes probably occurs because of neoplasia-related growth and the loose attachment of the testis to the dorsal aspect of the abdomen via the mesorchium. Inguinal testes also have an increased risk of becoming neoplastic, with seminoma being the tumor type that is most commonly reported. Abdominally retained testes are incapable of spermatogenesis but do produce testosterone, leaving the animal subfertile or infertile yet prone to androgen-dependent disorders. *Bilateral orchiectomy is recommended in all cases of cryptorchidism.* Breeding of the affected males, their parents, and their siblings should be strongly discouraged.

Several surgical approaches have been described for orchiectomy in both dogs and cats, depending on location of the retained testes. The use of laparoscopy has been reported in the diagnosis and treatment of cryptorchidism and testicular neoplasia in a dog. However, a ventral midline approach and laparotomy generally are used. If an inguinal testis is suspected, deep dissection to the inguinal fat pad allows visualization of the external inguinal ring and differentiation from the femoral

triangle. Most abdominally located testes are found near the urinary bladder. If not, the vasa deferentia may be located as they cross over the ureters and may be followed to and from the internal inguinal ring to locate the ipsilateral testis.

There have been no controlled studies on the efficacy of hCG or GnRH in inducing testicular descent in the dog. The administration of hCG has been reported to improve testicular descent in puppies younger than 16 weeks, but not in older dogs. There also is anecdotal evidence that GnRH may promote the descent of retained testes. However, the use of these gonadotropins is scientifically ambiguous. Their use will not help in cases of abdominally retained testes as animals age because the inguinal canal usually is closed by 6 months of age. Therefore they would potentially be effective only in cases of inguinally located testes. Furthermore, because of the heritability of cryptorchidism in the dog, the ethics of gonadotropin therapy are in question. Although there may be short-term benefits for an individual litter, the potential exists for propagating a genetic defect to the detriment of the breed.

If the diagnosis has been made and bilateral orchiectomy performed in dogs younger than 3 years, prognosis is excellent. Testicular neoplasia has been diagnosed in cryptorchid dogs as early as 3 years of age, therefore affecting the prognosis in dogs diagnosed with cryptorchidism later in life.

Orchitis/Epididymitis

Definition and Pathogenesis

Orchitis is inflammation of the testis. *Epididymitis* is inflammation of the epididymides. The two conditions may occur simultaneously or separately, depending on the inciting cause. There is no breed predilection. Young dogs are more commonly affected, although the reported age at time of diagnosis ranges from 11 months to 10 years. Infectious agents that may cause either orchitis or epididymitis include *Brucella canis, Escherichia coli, Proteus vulgaris, Pseudomonas mallei, Staphylococcus* species, *Streptococcus* species, *Mycoplasma* species, *Blastomyces* species, and canine distemper virus. Scrotal cestodiasis occurring in the dog has been reported to cause scrotal swelling and testicular pain. *Rhodotorula glutinis* was reported as a cause of bilateral epididymitis in a 4-year-old Great Dane. Trauma and autoimmune disease also may contribute to the development of orchitis and epididymitis. Routes of entry for infectious agents include retrograde movement up the reproductive tract from the prostrate or urethra, bacteremia, viremia, and direct entry by external trauma or puncture wound. Orchitis is rare in the tom but has been reported to occur after infections with *Mycobacterium* species, *Brucella* species, feline infectious peritonitis virus, and other aerobic bacteria introduced by traumatic injury, such as bite wounds.

Noninfectious lymphocytic orchitis also has been observed.

Autoimmune pathologic abnormalities may occur after infections or trauma or may be idiopathic in origin. Disruption of the blood-testis barrier created by Sertoli cell tight junctions allows access to the previously immunologically isolated spermatozoal antigens by the immune system. Cell-mediated and humoral defense mechanisms are set into action against the spermatogenic tissue with formation of systemic antisperm antibodies. Autoimmune orchitis also may be associated with a more widespread disorder, such as lymphocytic thyroiditis.

B. canis has a host range limited to domestic and wild canids. It is a gram-negative, nonmotile coccobacillus. Incidence in the United States is reported to range from 0.2%-9% of the population. The organisms are shed primarily in semen and urine but may also be spread through saliva and nasal secretions. Respiratory inhalation and ingestion (licking) are known to be the primary routes of entry to the susceptible dog. Venereal transmission can occur between infected stud dogs and bitches. Human exposure to dogs actively shedding organisms may lead to human infection (zoonosis).

Sperm granulomas consist of accumulations of spermatozoa and macrophages within tubules or the adjacent interstitium of efferent ductules or the epididymis. They may begin as spermatozoa are extravasated into

interstitial tissues or as spermatoceles with secondary macrophage infiltration. They may be induced by infection, trauma, or toxins but also may occur spontaneously.

Physical Examination Findings

Orchitis alone, epididymitis alone, or orchiepididymitis may be present. The condition can be unilateral or bilateral. The affected testes or epididymides are painful and swollen during the acute phase of orchitis in both dogs and cats. Fever may be present very early in the hematogenous phase of an infectious process. Hematologic changes may be noted during the acute phase as well but usually are not abnormal by the time the orchitis or epididymitis is clinically evident. When the epididymis is affected, it too will have focal to diffuse swelling that soon organizes and becomes localized. As the uncontrolled inflammatory process becomes chronic, the testis undergoes degeneration, fibrosis, and atrophy. Chronic epididymitis usually is associated with focal, firm swellings of the cauda, corpus, or caput epididymis. Visual appraisal and palpation can indicate slight to moderate swelling of the affected testes or epididymides. Pain is likely to be variably detected on palpation of the scrotal contents, and thus reluctance or refusal by the dog or cat to allow examination may be noted. Differential diagnoses

include inguinal and scrotal hernia, spermatic cord torsion, testicular neoplasia, varicocele or hydrocele, and sperm granuloma.

Dogs infected with *B. canis* may have a transient bacteremia or may have bacteremia for 6-60 months. Episodic fever may be detected by careful observation and monitoring but usually goes unobserved. Orchitis, epididymitis, scrotal dermatitis, oligozoospermia, and infertility all may be observed in affected dogs; epididymitis by itself is observed more commonly. Uveitis, glomerulonephritis, osteomyelitis, discospondylitis, meningitis, and pyogranulomatous dermatitis have all been reported to occur in dogs infected with *B. canis,* presumably secondary to bacteremia.

Diagnostic Tests and Results

Diagnostic tests beyond physical examination include ultrasonography of the scrotum, testes, and epididymides; semen collection for evaluation and culture; FNA of the affected testes or epididymides; and testing for canine brucellosis.

Orchitis does not have a characteristic pattern of echogenicity recognizable by ultrasonography, but patchy hypoechogenicity is described as an indication of testicular inflammation. Ultrasonography is most useful in ruling out other differentials, such as torsion of the spermatic cord.

Semen can be collected from male dogs by manual ejaculation for evaluation and submission for cytology and culture. Many dogs with orchitis or epididymitis experience pain and will not ejaculate.

If an ejaculate is obtained, seminal fluid can be submitted for aerobic, anaerobic, and mycoplasma cultures, both for diagnosis and to guide treatment. Growth of more than 100,000 organisms per milliliter of a single organism is significant. Semen culture does not localize infection to the testes or epididymides; dogs with positive cultures should also be evaluated for prostate disease at some point (see Chapter 17).

FNA of the affected testes or epididymides allows cytologic evaluation and submission of tissue for culture. Dogs with acute orchitis or epididymitis tolerate FNA without sedation. Presence of a large number of polymorphonuclear leukocytes (PMNs) is diagnostic. Tissue can be submitted for aerobic, anaerobic, and mycoplasma culture; any growth is significant. Sensitivity testing can be used to guide antibiotic treatment.

Treatment

Complete resolution or cure of orchitis or epididymitis is rare without removal of the affected tissue. Bilateral orchiectomy is the recommended treatment. Unilateral castration, sparing the nonaffected testis, may be the only hope of retaining fertility in affected male dogs that

have great breeding value, but it is not recommended. Antimicrobials alone rarely are effective in dogs but should not be overlooked as part of the treatment regimen. Aggressive broad-spectrum antibiotic therapy administered to cats for 2-3 weeks has been successful in resolving traumatic orchitis. After resolution of the acute phase, degenerative change of the seminiferous epithelium leading to testicular atrophy is to be expected in most cases. Nonsteroidal antiinflammatory agents should be considered part of the palliative treatment. Use of glucocorticoids in the treatment of autoimmune orchitis has been described in other species but not in the dog. Glucocorticoid therapy may itself suppress spermatogenesis.

Therapy with antimicrobials for *B. canis* infection has not proved to effect long-term resolution. Minocycline (25 mg/kg administered orally once daily for 14 days) in combination with dihydrostreptomycin (5 mg/kg administered intramuscularly twice a day for 7 days) or tetracycline (30 mg/kg administered orally twice a day for 21 days) in combination with streptomycin (20 mg/kg administered intramuscularly once daily for 14 days) have been reported for treatment of dogs infected with *B. canis* and may be useful for individually housed dogs as an adjunct to castration, to decrease shedding, and to ameliorate the bacteremic phase. In affected kennels, controlling the spread of the organism requires quarantine, identification of the

index cases, culling of those animals determined to be infected, strict surveillance for new cases, and serologic testing of all incoming animals before their introduction to the kennel.

Prognosis

Prognosis is good in dogs not infected with *B. canis* that undergo bilateral orchiectomy. In animals treated by unilateral orchiectomy or long-term antimicrobial therapy, response to treatment will not be observed until at least 60-62 days after resolution, the length of the spermatogenic cycle in dogs and cats. Careful palpation and ultrasonographic evaluation of the remaining testis should be performed to evaluate for atrophy or fibrosis. The expected response in the normal, nonaffected remaining testis is a compensatory hypertrophy. This may occur over the 3-6 months following complete resolution of the inflammatory condition.

Torsion of the Spermatic Cord

Definition and Pathogenesis

Torsion of the spermatic cord, often erroneously called *testicular torsion,* has been reported rarely in the dog. Rotation around the vertical axis of the spermatic cord generally involves a neoplastic, abdominally retained testicle. It has

been suggested that the intraabdominal site allows greater movement of a large, neoplastic testicle than would the scrotum. Sertoli cell tumors and seminomas often are diagnosed in these cases. Intrascrotal spermatic cord torsion also has been reported. Although congenital factors, physical or sexual aggression, trauma, and neoplasia have been implicated, pathogenesis in the dog remains unclear. Torsion of the spermatic cord must be considered in any male dog with an acute abdomen presentation, particularly in monorchid or cryptorchid patients.

Signalment

Spermatic cord torsion seems to be unrelated to size, age, or breed. Reported cases include dogs ranging in size from Pekingese to Great Danes and in age from 5 months to 10 years. One report involved two cryptorchid dogs from the same litter. The boxer, Pekingese, and Airedale terrier may be predisposed to spermatic cord torsion because of the high incidence of cryptorchidism in these breeds.

History and Clinical Signs

Clinical signs reported include lethargy, anorexia, vomiting, signs of distress, stiffness of gait, and abdominal discomfort. Signs that occasionally may be observed include tenesmus, diarrhea or constipation, dysuria,

anuria, hematuria, polyuria, polydipsia, vocalizations such as howling or yelping, and trembling.

Physical Examination Findings

General physical examination findings may include stiffness of gait or unwillingness to move, abdominal pain, either pyrexia or hypothermia, dehydration, and shock. With torsion of an abdominally retained neoplastic testis, a palpable abdominal mass, inguinal swelling and pain, symmetric alopecia, hyperpigmentation, gynecomastia, urethral bleeding, and pendulous prepuce may be present. With an intrascrotal spermatic cord torsion, an enlarged or painful testis, pitting edema of the scrotum, thickened spermatic cord, discoloration or decreased temperature of the scrotum, and pain associated with palpation of the testes may be observed.

Diagnostic Tests and Results

Definitive diagnosis of torsion of the spermatic cord requires exploratory laparotomy. Basic accompanying diagnostic tests should include a complete blood count (CBC), serum chemistry profile, and urinalysis because a definitive diagnosis of spermatic cord torsion necessitates surgery. Blood work results reported in dogs with torsion of the spermatic cord include hyperalbuminemia, leukocytosis with or without a left shift, anemia, increased

blood urea nitrogen, increased creatinine, and hyperphosphatemia. When Sertoli cell tumor is present, nonregenerative anemia, leukopenia, or thrombocytopenia related to estrogen toxicosis may occur. Urinalysis may reveal presence of red blood cells, increased white blood cells, proteinuria, and decreased urine specific gravity.

In cases of spermatic cord torsion in a cryptorchid animal, an abdominal mass may or may not be visible radiographically. Survey radiographs may reveal a caudal abdominal mass, enlarged iliac or sublumbar lymph nodes, and prostatic enlargement. Thoracic radiographs may be necessary to rule out metastatic lung disease when neoplasia is present.

Real-time (B-mode) and color-flow Doppler ultrasound are very useful in the definitive diagnosis of intrascrotal spermatic cord torsion. These imaging modalities, along with spectral Doppler ultrasound and radionuclide perfusion scanning, commonly are used to diagnose spermatic cord torsion and evaluate resulting vascular compromise in humans. Characteristic parenchymal and color Doppler signal changes in the dog have been described. Ultrasonographic changes that may be observed with torsion of an intrascrotal testis include decreased echogenicity of testicular parenchyma; hyperechoic, cranially displaced epididymis; enlarged spermatic cord; reduction of the testis-to-epididymis ratio; hypoechogenicity characteristic of edema around the epididymis and testis; abnormal course of spermatic cord and

testicular blood vessels; and loss of color-flow Doppler signal from the spermatic cord (Figure 16-1). Intrascrotal spermatic cord torsion must be differentiated from hematocele, hydrocele, inguinal hernia, infectious and noninfectious orchitis, epididymitis, varicocele, spermatocele, and testicular neoplasia. The collective ultrasonographic changes should aid the clinician in differentiating intrascrotal spermatic cord torsion from other scrotal and testicular abnormalities. Ultrasonography may be less useful in diagnosis of intraabdominal testis with torsion of the spermatic cord.

Exploratory laparotomy observations of an intraabdominal testis affected by torsion may include varying degrees of spermatic cord torsion; an enlarged, hemorrhagic, nodular, or neoplastic intraabdominal testis; or a small, discolored, necrotic testis. Histopathologic findings of affected testes that have been described include infarction, venous congestion, hemorrhage, hemosiderosis, fibrosis, coagulation necrosis of tubular structures, Sertoli cell tumor, seminoma, sarcoma, teratoma, embryonal carcinoma, and choriocarcinoma.

Treatment

Bilateral orchiectomy is the treatment of choice. Excision of the affected testicle alone may be considered in cases of intrascrotal spermatic cord torsion in a valuable breeding dog. Unilaterally cryptorchid dogs

Figure 16-1. Ultrasonographic image of the right testis of a 3-month-old German shepherd dog with torsion of the spermatic cord. The epididymis has increased echogenicity compared with the relatively hypoechoic testis. The anechoic areas that radiate from the mediastinum reflect testicular infarction. The white asterisks delineate the epididymis. (*From Pinto CR, Paccamonti DL, Partington B:* J Am Vet Med Assoc *219:1343, 2001.*)

should not be used for breeding. Surgical correction of intrascrotal spermatic cord torsion in a breeding dog generally is not recommended because spermatogenesis usually is irreversibly reduced after 1-2 hours of ischemia. Even with surgical correction and return of blood flow, hypoxia, infarction, and reperfusion injury often result in cell death and testicular fibrosis and atrophy. Duration and degree of rotation determine the extent of testicular damage. Therefore, when the owner wants the dog to maintain two testicles for aesthetic reasons, gross examination of the spermatic cord and testis must be performed to decide whether surgical correction is a viable option.

Shock, renal dysfunction, and severe anemia or thrombocytopenia indicate a need for fluid therapy, diuresis, or blood transfusion before surgery. Perioperative antibiotics, administration of antiinflammatory agents, and pain control should be considered. Prostatic abnormalities (see Chapter 17) and metastasis of testicular neoplasia may be discovered and addressed during exploratory laparotomy.

Prognosis

Prognosis is good after castration in cases of uncomplicated spermatic cord torsion in cryptorchid animals. Alopecia and other clinical signs related to a Sertoli cell tumor should resolve in 2-6 weeks after castration.

Prognosis for life also is good after early diagnosis and castration in dogs with intrascrotal spermatic cord torsion. Prognosis for future fertility in dogs treated with unilateral castration depends on the extent of involvement of the contralateral testis. In one case the affected testis was excised and sperm characteristics of the contralateral testis were found to be within normal limits 10 months after surgery. Prognosis is guarded for dogs in shock or renal failure at the time of presentation, those with malignant testicular neoplasia and metastasis, and those with abnormalities associated with the prostate, such as prostatic abscessation.

Testicular Neoplasia

Definition and Pathogenesis

Testicular neoplasia is common in dogs are rare in cats. Its rate of occurrence is second only to skin neoplasia in dogs. Neoplasia can occur in both descended and undescended testes, but the frequency is higher in cryptorchid testes. There are three main types of testicular neoplasia: Sertoli cell tumor, seminoma, and interstitial (Leydig) cell tumor. The frequency is equal among the tumor types in descended testes. However, in cryptorchid testes, the rate of occurrence of tumors is 60% for Sertoli cell tumors and 40% for seminomas. Interstitial cell tumors usually occur in descended testes. The mean age of dogs with testicular tumors is 10 years, and certain

breeds, including boxers, German shepherd dogs, poodles, and Shetland sheepdogs, are at higher risk.

The three main types of tumors all arise within the testicular parenchyma. However, other tumors, such as fibrosarcoma, hemangiosarcoma, and embryonic carcinoma, also can occur in the testis. These tumor types are rare. It is unknown why testicular tumors arise. Some have speculated that in cryptorchid testes, an alteration in thermoregulation predisposes the testis to neoplasia.

Sertoli cell tumors, as the name implies, arise from the Sertoli cell (nurse cell) of the seminiferous tubule. The normal function of this cell is to support the developing spermatogenic cells and secrete hormones for feedback to the hypothalamus and pituitary gland. Most Sertoli cell tumors are slow-growing tumors that are noninvasive; however, up to 20% may be malignant and spread to the local lymphatics and distant sites. These tumors usually are firm, nodular, and pale yellow to gray. Sertoli cell tumors either are hormonally nonproductive or secrete high concentrations of estrogen and inhibin, producing clinical signs of feminization.

Seminomas arise from spermatogenic cells. These are cells that normally would undergo development in the seminiferous tubule to form mature spermatozoa. These tumors also are slow growing and noninvasive. Up to 10% may metastasize to local or distant sites. Most of these tumors are hormonally silent and usually are soft on palpation, gray to white, and lobulated.

Interstitial (Leydig) cell tumors arise from the cells in the interstitial space surrounding the seminiferous tubules. These cells usually provide the hormonal support for the seminiferous tubules and feedback to the hypothalamus and pituitary gland. As with the other tumors, interstitial cell tumors are slow to grow and are noninvasive. Unlike the other two tumor types, interstitial cell tumors usually are an incidental finding at the time of necropsy and almost always are located in descended testes. They usually are hormonally silent, soft on palpation, and brown-orange on cross section.

Physical Examination Findings

The owner may be unaware of any problem, and the veterinarian may find the mass on routine yearly physical examination or on examination for another problem. In symptomatic animals the usual complaint is scrotal enlargement. Scrotal enlargement may be the only finding on physical examination. More thorough palpation of the scrotum may indicate that one testis is enlarged and lobulated and the other is either normal or atrophied. The atrophy of the contralateral testis usually is caused by pressure necrosis from the enlarged testis or altered hormonal feedback.

If the dog has cryptorchidism, there will be no external testicular enlargement. The dog may present for other systemic signs caused by a hormonally active tumor.

Seventy percent of the tumors in cryptorchid testes are hormonally active Sertoli cell tumors, which cause a male feminizing syndrome. This syndrome is the result either of increased estrogen production by the neoplastic Sertoli cells, increased conversion of testosterone to estrogen, or a decrease in androgen production with normal estrogen production. In healthy dogs, serum estrogen concentrations are less than 15 pg/ml. In dogs with functional Sertoli cell tumors, serum estrogen concentrations can range from 10-150 pg/ml. Clinical signs associated with this syndrome include bilateral, nonpruritic alopecia; gynecomastia; hyperpigmentation; squamous metaplasia of the prostate; and bone marrow suppression. There can be attraction of other males to the affected dog, and galactorrhea also may be observed. If bone marrow suppression is involved, the dog may present with complaints of lethargy, depression, inappetence, and vomiting. The dog may have pale mucous membranes and petechiae.

Diagnostic Tests and Results

Diagnostic tests include ultrasonography of the scrotum, testes, epididymides, and abdomen; thoracic and abdominal radiography to assess for evidence of metastases; a CBC and serum chemistry profile; and either aspiration or biopsy of the mass. Ultrasonography of the affected testis, using either a 5- or 7.5-mHz linear or sector probe, may reveal a hyperechoic density, a hypoechoic density, or

multiloculated cystic areas within the testis. Abdominal ultrasonography may be used to find a cryptorchid testicle and evaluate it for presence of a mass noted in its parenchyma. Once a mass is located, thoracic and abdominal radiographs should be obtained to search for areas of local and distant metastasis. If there is suspicion of bone marrow involvement, a CBC and chemistry panel should be performed. The CBC may reflect a nonregenerative anemia and leukopenia.

To determine the cause of the mass, either FNA, core biopsy, incisional biopsy, or excisional biopsy must be performed. Although FNA works well for determining whether there is active spermatogenesis, it is not the best method for diagnosing tumor types. Either a core biopsy or incisional biopsy is preferred for the valuable breeding dog. An excisional biopsy or orchiectomy is preferred if the dog has cryptorchidism or if breeding is not a future priority.

A core biopsy is performed with the animal under either heavy sedation or general anesthesia. The dog's prescrotal area should be prepared by using an aseptic technique and the dog placed in dorsal recumbency. An incision is made on the midline through the skin, and the testis is pushed to the incision. The vaginal tunic is incised over the testis, and the tunica albuginea is incised. The mass is located by palpation, and a Tru-Cut biopsy instrument (Cook Instruments, Bloomington, Ill.) is used to obtain a sample. Once the sample is

obtained, the tunics and the subcutaneous tissue are closed as for a routine orchiectomy.

An incisional biopsy is performed similar to the core biopsy. When the tunica albuginea is incised, the tissue that bulges out is shaved off by using a sterile double-edged razor. Routine closure is used. All biopsy samples should be fixed before they are sent to the laboratory. The preferred fixative to use is Bouin's fixative. It allows for good cellular differentiation of the testicular tissue and prevents seminiferous tubule shrinkage. The dilution should be the same as for formalin: 1 part tissue to 10 parts fixative.

One major drawback of performing these biopsies is iatrogenic testicular damage. The blood-testis barrier is disrupted when performing the biopsy, which may lead to an immune-mediated orchitis, resulting in further testicular destruction. If a blood vessel is punctured during the surgical biopsy procedure, a large hematoma may form, which can lead to pressure and thermal necrosis of one or both of the testes.

Treatment and Prognosis

Bilateral orchiectomy is the treatment of choice. If the dog is a valuable stud dog, unilateral orchiectomy can be considered. However, if the dog has cryptorchidism, both testes should be removed. If there is no evidence of metastasis and the tumor is removed, the prognosis is good.

Dogs that have concurrent male feminizing syndrome with bone marrow suppression may need further treatment, such as intravenous fluids, antibiotics, whole-blood transfusions, and glucocorticoids, until the signs resolve. A guarded prognosis is made for dogs with bone marrow suppression; regeneration of the bone marrow may take 3-6 weeks after surgery, if regeneration occurs at all. If metastases are found, the prognosis is also considered guarded to grave and either chemotherapy or irradiation therapy is instituted after surgery. Seminomas have been shown to be radiosensitive and may respond better to irradiation therapy than to chemotherapy.

Bibliography

Bird K, Rosenfield AT, Taylor KJ: Ultrasonography in testicular torsion, *Radiology* 147:527, 1983.

Bloom F: Retained testes in cats and dogs, *Mod Vet Pract* 43:60, 1962.

Bogh IB, Baltsen M, Byskov AG et al: Testicular concentration of meiosis-activating sterol is associated with normal testicular descent, *Theriogenology* 55:983, 2001.

Cox VS, Wallace LJ, Jessen CR: An anatomic and genetic study of canine cryptorchidism, *Teratology* 18:233, 1978.

Dunn ML, Foster WJ, Goddard KM: Cryptorchidism in the dog: a clinical survey, *J Am Anim Hosp Assoc* 4:180, 1968.

Eilts BE, Pechman RD, Hedlund CS et al: Use of ultrasonography to diagnose Sertoli cell neoplasia and cryptorchidism in a dog, *J Am Vet Med Assoc* 192:533, 1988.

Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction,* Philadelphia, 1996, WB Saunders.

Freshman JL, Amann RP, Bowen RA et al: Clinical evaluation of infertility in dogs, *Comp Cont Ed* 10:443, 1988.

Gebhardt K, Ellerbrock K, Pera I et al: Differential expression of novel abundant and highly regionalized mRNAs of the canine epididymis, *J Reprod Fertil* 116:391, 1999.

George F, Bassily N, Hess R: Intratubular spermatic granulomas of the canine efferent ductules, *Toxicol Pathol* 23:731, 1995.

George L, Duncan R, Carmichael LE: Semen examination in dogs with canine brucellosis, *Am J Vet Res* 40:1589, 1979.

Haynes HM, Pendergrass TW: Canine testicular tumors: epidemiologic features of 410 dogs, *Int J Cancer* 18:482, 1976.

Hricak H, Lue T, Filly RA et al: Experimental study of the sonographic diagnosis of testicular torsion, *J Ultrasound Med* 2:349, 1983.

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology,* Philadelphia, 2001, WB Saunders.

Kawakami E, Tsutsui T, Saito S et al: Changes in peripheral plasma luteinizing hormone and testosterone

concentrations and semen quality in normal and cryptorchid dogs during sexual maturation, *Lab Anim Sci* 45:258, 1995.

Kersten W, Molenaar GJ, Emmen JM et al: Bilateral cryptorchidism in a dog with persistent cranial testis suspensory ligaments and inverted gubernacula: report of a case with implications for understanding normal and aberrant testis descent, *J Anat* 189:171, 1996.

Larsen RE: Testicular biopsy in the dog, *Vet Clin North Am* 7:747, 1977.

Lopate C, Threlfall WR, Rosol TJ: Histopathologic and gross effects of testicular biopsy in the dog, *Theriogenology* 32:585, 1989.

Marshall LS, Oehlert ML, Haskins ME et al: Persistent müllerian duct syndrome in miniature schnauzers, *J Am Vet Med Assoc* 181:798, 1982.

McDonald RK, Walker M, Legendre AM et al: Radiotherapy of metastatic seminoma in the dog, *J Vet Int Med* 2:103, 1988.

McEntee K: *Reproductive pathology of domestic mammals,* New York, 1990, Academic Press.

Mevorach RA, Lerner RM, Greenspan BS et al: Color Doppler ultrasound compared to a radionuclide scanning of spermatic cord torsion in a canine model, *J Urol* 145:428, 1991.

Millis DL, Hauptman JG, Johnson CA: Cryptorchidism and monorchism in cats: 25 cases (1980-1989), *J Am Vet Med Assoc* 200:1128, 1992.

Pena FJ, Anel L, Dominguez JC et al: Laparoscopic surgery in a clinical case of seminoma in a cryptorchid dog, *Vet Rec* 142:671, 1998.

Peters MA, deJong FH, Teerds KJ et al: Aging, testicular tumors and the pituitary-testis axis in dogs, *J Endo* 166:153, 2000.

Pinto CR, Eilts BE, Paccamonti DL et al: Torsion of the spermatic cord in a stallion, *J Am Vet Med Assoc* 213:205, 1998.

Pinto CR, Paccamonti DL, Partington B: Torsion of the spermatic cord in a dog, *J Am Vet Med Assoc* 219:1343, 2001.

Rhodes JD, Foley CW: Cryptorchidism and intersexuality, *Vet Clin North Am* 7:789, 1977.

Romagnoli SE: Canine cryptorchidism, *Vet Clin North Am* 21:533, 1991.

Schoeb TR, Morton R: Scrotal and testicular changes in canine brucellosis: a case report, *J Am Vet Med Assoc* 172:598, 1978.

Tarhan R, Erbay ME, Erdogan E et al: Effects of unilateral testicular torsion on the blood flow of contralateral testis: an experimental study on dogs, *Scand J Urol Nephrol* 34:229, 2000.

Turner T: Torsion of the retained testicle in the dog, *J Small Anim Pract* 11:436, 1970.

Yates D, Heffernan M, Beynon R: Cryptorchidism in cats, *Vet Rec* 149:220, 2001.

17

Prostatic Disorders

Augustine T. Peter and William R. Widmer

AT A GLANCE

- Benign prostatic hypertrophy/hyperplasia (BPH)
  - BPH develops gradually, with prolonged exposure to testosterone and its metabolite, dihydrotestosterone (DHT). Virtually all middle-aged and older male dogs have some degree of BPH. Most dogs are asymptomatic. Clinical signs that may occur include

dripping of serosanguineous fluid from the penis unassociated with urination, rectal tenesmus and passage of ribbon-shaped feces, hemospermia, and hematuria. On rectal examination the prostate is symmetrically bilobed and the animal is not in pain. Radiographs and ultrasound reveal a uniformly enlarged prostate. Culture of seminal fluid is not significant. Castration is the treatment of choice. Medical therapy is available for valuable breeding dogs; the human drug, finasteride (Proscar, Merck, Rahway, NJ) can be given orally at a dosage of 0.1-0.5 mg/kg once daily for 1-4 months.

- Prostatitis
  - Prostate infection usually occurs secondary to another abnormality of the prostate, with BPH being the most common. Diagnosis is by culture of ejaculated semen, fluid collected by prostatic massage, or tissue collected by fine-needle aspiration (FNA). Growth of more than 100,000 organisms per milliliter of fluid or any growth from a tissue sample is considered significant. Antibiotics that penetrate the prostate well include the fluoroquinolones, trimethoprim/sulfonamide, and chloramphenicol. Concurrent therapy for the underlying disorder hastens the effect of antibiotics.
- Prostatic and paraprostatic cysts
  - Prostatic and paraprostatic cysts are benign cysts that may be within or outside of the prostate itself. Clinical signs usually are due to pressure of the large

cysts on the urethra or colon. Diagnosis is confirmed with abdominal ultrasonography. The treatment of choice is surgical resection with concurrent castration.

- Prostatic neoplasia
  - Prostatic neoplasia in dogs is invariably prostatic adenocarcinoma, which often has metastasized by the time of diagnosis of the primary tumor. Diagnosis is confirmed with FNA or biopsy, preferably guided by ultrasound. Treatment is palliative.

The prostate is an androgen-dependent retroperitoneal organ and is the only major accessory sex gland in the dog. It is bordered by the rectum dorsally and the symphysis pubis and abdominal wall ventrally. It completely encircles the proximal portion of the urethra at the trigone of the bladder. The bilobed prostate is surrounded by a fibromuscular capsule and is divided by a median raphe that is palpable on the dorsal surface per rectum. With advancing age, as the prostate enlarges, it assumes an abdominal position.

The prostate is made up of compound tubular alveolar glands that radiate from their urethral ductile openings. The alveolar portion of the prostate contains primary and secondary infoldings of secretory epithelium that project into the alveolar lumen, and the alveoli are separated by fibrous connective tissue. The prostate is divided into lobules by smooth muscle fascicles in its stroma. The prostatic artery and prostatic urethral veins

supply and drain blood from the prostate, respectively. Lymph is drained to the iliac lymph nodes. The hypogastric and pelvic nerves provide sympathetic and parasympathetic innervation, respectively.

The prostate secretes fluid under the control of hormonal factors (primarily DHT, a metabolite of testosterone) and stimulation by the parasympathetic nervous system. Expulsion of the prostatic fluid into the urethra is stimulated by the sympathetic nervous system. Prostatic fluid makes up the first and third fractions of the canine ejaculate. It reduces the consistency of the ejaculate and increases its volume and is also believed to aid in spermatozoal transport. Secretion is continuous in intact male dogs. It flows retrograde into the urinary bladder or antegrade to the external urethral orifice. The volume voided daily ranges from a few drops to several milliliters, depending on the size of the prostate. As seen with ultrasonographic examination, a normal prostate has a homogenous parenchyma (Figure 17-1) and usually is of greater echogenicity than most parenchymal organs.

A variety of prostatic disorders affect middle-aged and older dogs. The overall incidence of canine prostatic disease is 2.5% in male dogs (all ages) and 8% in male dogs older than 10 years. With the exception of prostatic neoplasia, which occurs with the same frequency in intact and neutered animals, prostatic disease generally

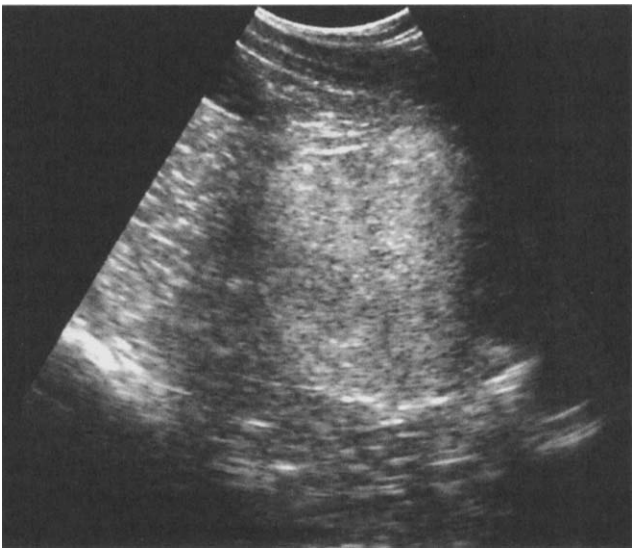


Figure 17-1. Sagittal-view sonogram of a normal middle-aged intact male dog. The prostatic parenchyma is homogenous and of greater echogenicity than most parenchymal abdominal organs. However, the echogenicity of the prostate varies according to the age and the influence of testosterone (intact or neutered). Centrally, the normal prostate contains a hyperechoic zone surrounding the intraprostatic urethra. The exact sonographic appearance depends on the quality of the sonograph, the ability of the sonographer, and the acoustic window.

affects intact male dogs. Benign hyperplasia, prostatitis and abscess, prostatic and paraprostatic cysts, and neoplasia are recognized as separate clinical entities. To initiate proper treatment, it is essential that along with the proper diagnosis of the condition, the pathogenesis of these entities be understood. Of all of these conditions, bacterial prostatitis should be a primary rule-out when dealing with an infertile stud dog. Prostatic disease in male cats is extremely uncommon.

Benign Prostatic Hypertrophy/Hyperplasia

Definition and Pathogenesis

In BPH there is an increase in prostatic epithelial cell size (hypertrophy) and number (hyperplasia), with the latter being more marked. The pathophysiology of BPH is not fully understood; however, the condition is believed to be hormonally modulated. It has been suggested that increased responsiveness of the prostate to testosterone and an altered androgen/estrogen ratio play a role. Testosterone serves as a prohormone for formation of DHT, an active metabolite that mediates intracellular processes of androgen action. This metabolism is under the influence of the inhibitable enzyme 5α-reductase. Two isoenzymes of 5α-reductase (types I and II) have been identified in dogs. Experimental data suggest two phases in the development of the condition: an

early phase that is characterized by glandular hyperplasia and a late phase that shows a more complex morphology dominated by cystic hyperplasia. During the early glandular phase, the concentration of DHT in serum is high. Later, the tissue concentrations of DHT decrease. The decreasing tissue concentrations seem to be counterbalanced by an increase in intranuclear androgen receptors, possibly mediated by elevated estrogen concentrations and other factors. The condition is not classified as a neoplasm in dogs, whereas in the human it is classified as a benign neoplasm.

Signalment

This disorder affects nearly every intact male dog, although not all dogs show clinical signs. This condition is common in older dogs, and the mean age at time of diagnosis is about 8 years. However, the condition can begin to develop as early as 2.5 years of age.

History and Clinical Signs

Most dogs with BPH do not have clinical signs, but some have tenesmus, serous or hemorrhagic urethral discharge, constipation, or hematuria. Occasionally, dysuria or stranguria may occur. The vascularity of the prostate is increased with hyperplasia, which leads to the clinical signs of hemorrhagic urethral discharge and

hematuria. Usually, systemic signs of illness are not present and the affected dogs are alert, active, and afebrile.

Physical Examination Findings

Rectal examination reveals mild to moderate, symmetric, and nonpainful prostatomegaly. The affected prostate has a smooth contour and is freely movable. There are no extrareproductive abnormalities.

Diagnostic Tests and Results

Either the results of urinalysis are normal or microscopic hematuria is present. If a urethral discharge is present, it is hemorrhagic or clear but not purulent. Similarly, semen and samples of prostatic fluid may be either normal or hemorrhagic. However, the volume of the semen may be reduced; this occurs primarily because of the reduced secretory function of the gland.

Results of a complete blood count (CBC) and serum chemistry profiles are unremarkable, as are cytologic and bacteriologic evaluation of prostatic fluid. A major protein in canine seminal plasma, canine secretory prostatic esterase, which is secreted by the prostate, is elevated fourfold in dogs with BPH. Assays for this protein are not available through all laboratories, nor is this test routinely performed for diagnosis of BPH in clinical practice.

Ultrasonographic examination of the prostate usually shows symmetric prostatomegaly, with variable appearance of the prostatic parenchyma. Generally, there is loss of the normal homogenous appearance (Figure 17-2). Overall increased echotexture may be present as a result of subgross cystic change, fibrosis, or increased vascularity. Occasionally, small intraprostatic cysts are identified. The ultrasonographic examination can be used to estimate prostatic volume. This information may be useful in determining the dosage of drugs used in medical management of the condition; however, further research is necessary to confirm this hypothesis.

Survey abdominal radiography reveals symmetric prostatomegaly and sometimes compression (displacement) of the colon dorsally and urinary bladder cranially. Retrograde urethrocystography may reveal urethroprostatic reflux and narrowing of the prostatic urethra. This procedure also is beneficial if the prostate is not readily visible on survey radiographs.

For cytologic examination, prostatic cells can be collected either via an ejaculate, prostatic massage, or needle aspiration or by obtaining a biopsy. The specimen collected can be used to diagnose the cellular pattern within the prostate and can be submitted for aerobic, anaerobic, and mycoplasma cultures. The diagnostic techniques described here are useful in diagnosing all diseases of the prostate, including BPH, prostatitis, and prostatic neoplasia.

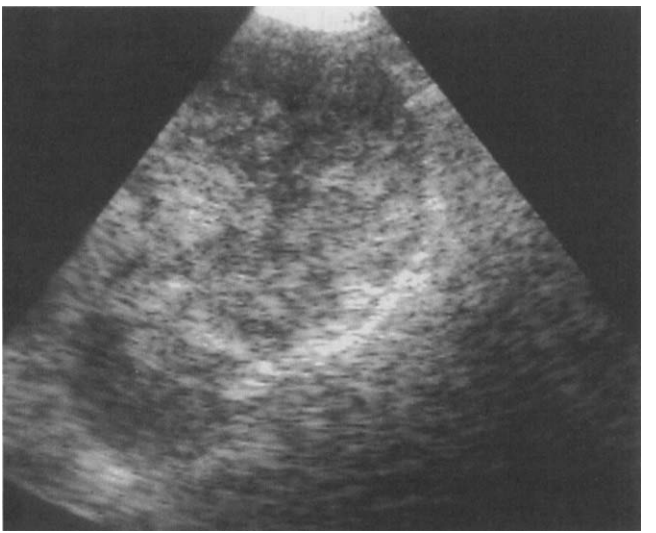


Figure 17-2. Benign prostatic hyperplasia. Sagittal-plane sonogram of the prostate of an 8-year-old intact male Scottish terrier with stranguria. Note the inhomogeneity of the prostatic parenchyma typical of benign prostatic hyperplasia. There is echotexture characterized by sonolucent and echoic regions without cavitation or nodule formation.

In dogs with BPH, although these methods may be necessary for a definitive diagnosis, a presumptive clinical diagnosis can be made in most cases based on the history and physical examination findings. Cytologic evaluation of prostatic aspirates may reveal normal epithelium, even in the presence of disease. A normal histologic specimen reveals dilated acini that are surrounded by smooth muscle and fibrous connective tissue with absence of inflammatory cells.

EJACULATE An ejaculate can be obtained by manual digital manipulation of the prepuce and penis of a dog, with or without the presence of a female dog in estrus. The semen sample should be collected in a sterile funnel or similar container (see Chapter 4). The semen undergoes cytologic examination and is submitted for aerobic, anaerobic, and mycoplasma cultures. Another less commonly used technique involves gathering a urine sample via cystocentesis and gathering a urethral sample before collecting the ejaculate. To gather a urethral sample, the clinician must retract the prepuce, exposing 2-4 cm of glans penis, and clean it with cotton sponges soaked in a 1:1000 dilution of aqueous Zephiran (Sanofi, New York, NY). The clinician then dries it with sterile sponges. A sterile calcium alginate urethral swab on a flexible aluminum shaft (Calgiswab; Remel, Lenexa, Kan.) is inserted into the urethra to a distance of approximately 5 cm, moved back and forth several times, and

withdrawn. The swab is separated from the shaft with sterile scissors and dropped into a test tube containing 3 ml of sterile lactated Ringer's solution.

PROSTATIC MASSAGE The dog needs to be sedated before beginning. The urinary bladder is catheterized, emptied, and flushed several times with sterile saline. The last flush, of 5-10 ml, is saved for comparison (preprostatic massage sample). The urinary catheter is retracted to the caudal pole of the prostate. The prostate is gently massaged per rectum for 1-2 minutes (Figure 17-3) and then another 5 ml of sterile saline is injected through the catheter. Material is repeatedly aspirated into the catheter while it is slowly advanced into the urinary bladder (postprostatic massage sample). Both the preprostatic and postprostatic massage samples are submitted for aerobic, anaerobic, and mycoplasma cultures. The results from the preprostatic and postprostatic massage samples are compared with urinalysis. This will help localize significant bacterial growth to the prostate, the urethra, or the urinary bladder. The veterinarian should be extremely careful not to massage too vigorously because doing so might result in rupture of an abscess and attendant complications (peritonitis and sepsis).

Another, less common approach is to use a urethral brush and obtain a sample directly from the prostatic urethra after massaging the prostate. This method limits contamination of the prostatic sample with material

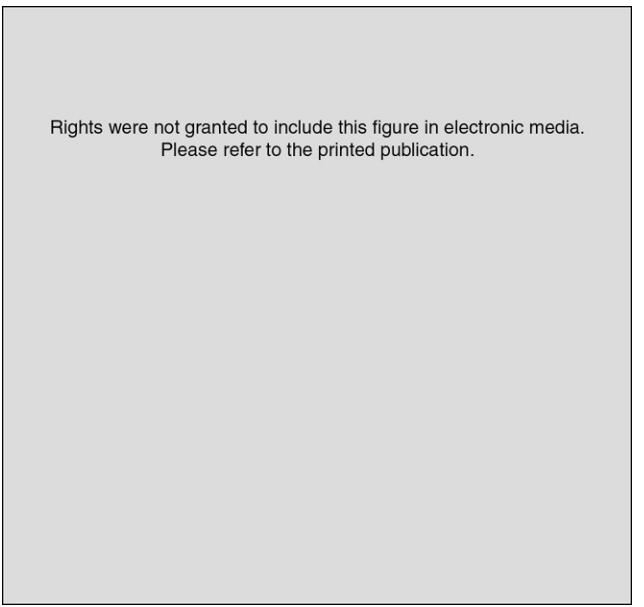


Figure 17-3. Schematic drawing depicting prostatic massage. The urinary catheter is passed to the level of the prostatic urethra. (*From Thrall MA, Olson PN, Freemyer FG:* J Am Anim Hosp Assoc *21:95, 1965.*)

from the urethra or bladder. Urine and urethral samples should be collected as described previously. The 1-mm-diameter brush is affixed to the end of a 90-mm wire stalk housed within a plugged, double-sheathed 6-Fr catheter. The tip of the catheter is positioned at the caudal pole of the prostate, as determined by rectal palpation. The prostate is massaged per rectum for 1 minute, and the inner catheter is advanced a distance of 1 cm into the prostatic urethra and is moved back and forth a few times. After the specimen is collected, the brush and the inner catheter are retracted into the outer catheter and the entire apparatus is withdrawn. The prostatic fluid and cells in the inner catheter and adhering to the brush, along with the urine and urethral specimens, are submitted for culture and cytologic examination.

FINE-NEEDLE ASPIRATION OF THE PROSTATE The prostate needs to be approached either percutaneously via a perirectal or transabdominal route or transrectally. The aspiration is performed by using an aseptic technique and a long needle with a stylet, such as a spinal needle. Instead of a spinal needle, a fine needle (22 gauge or smaller) may be used. A biopsy needle (Tru-Cut Biopsy Needle; Baxter Healthcare Corp., Valencia, Calif.) also may be used. The advantage of use of the biopsy needle is that more material can be obtained for histopathologic examination. The disadvantages are that the procedure bears a relatively higher

risk of seeding the abdominal cavity with abnormal cells or bacteria from a dog with an infected prostate and of damage to other abdominal structures.

With the perirectal approach, the needle is guided by rectal palpation. The dog needs to be sedated. The perineal area is prepared as for sterile surgery. A gloved finger is introduced into the rectum and the prostate palpated. The spinal needle is introduced with the other hand, alongside the rectum and into the prostate. Aspiration is performed several times with a syringe.

With the transabdominal approach, the needle can be placed by palpation or by ultrasound guidance. Ultrasound guidance is preferred to minimize injury to surrounding structures, including blood vessels, the trigone of the bladder, and the colon. The dog needs to be sedated. The prostate is visualized with ultrasound. The area of the abdomen over the prostate is prepared as for sterile surgery. A fine needle is introduced into the prostate and aspiration performed several times with a syringe.

Transrectal FNA of the prostate gland (Figure 17-4) is performed with the Franzen transrectal needle guide (Precision Dynamics Corp., Burbank, Calif.) and a 22-gauge or smaller needle. Transrectal FNA may be performed without sedation, unless the dog experiences pain as a result of abdominal or rectal palpation. A sterile surgical glove needs to be worn. The Franzen needle guide is placed on the index finger of the hand used for

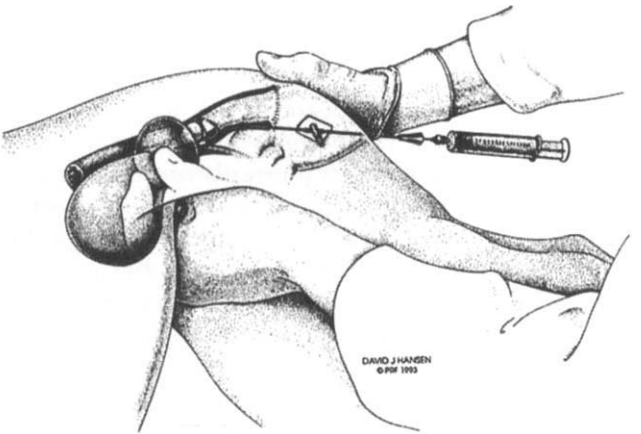


Figure 17-4. Transrectal fine-needle aspiration of the prostate using a Franzen needle guide. (*From Peter AT, Steiner JN, Adams LG:* Semin Vet Med Surg [Small Anim] *10:35, 1995.*)

palpation. Another sterile glove is placed over the needle guide. The area to be aspirated is palpated with the gloved index finger and the prostate stabilized through abdominal palpation with the other hand. An assistant should advance the needle through the needle guide and into the prostate, under guidance by the person performing rectal palpation. Suction is then applied to the fine needle, using a 12-ml syringe. The suction is released, and the needle is withdrawn. The aspirated

sample is submitted for cytologic examination and culture. The procedure may be repeated to aspirate additional areas of the prostate or to obtain additional samples for diagnostic testing.

SURGICAL BIOPSY Surgical biopsy is the most invasive means to obtain prostatic fluid and tissue samples. At times, it is the only procedure that can provide a definitive diagnosis. Because of the risks associated with percutaneous techniques, surgical biopsy via laparotomy is a preferred method for obtaining samples for histopathologic examination.

Treatment and Prognosis

Generally, treatment of BPH is indicated only for patients that have clinical signs of prostatic disease. *The treatment of choice for BPH is castration.* Prostatic involution begins soon after surgery, and within 7-10 days a decrease in prostatic size should be apparent with rectal palpation or radiography. A 50% reduction in size occurs in 3 weeks, and a 70% reduction in size occurs over 9 weeks. If the size of the prostatic does not decrease, the diagnosis should be reconsidered and the dog should be evaluated for other prostatic diseases. Prognosis for recovery is good in dogs after castration.

If the client is interested in using the stud dog for breeding, medical therapies exist. A variety of

pharmacologic agents have been used for medical treatment of BPH in dogs.

PROGESTATIONAL COMPOUNDS Progestational compounds are believed either to decrease prostatic androgen receptor number, to inhibit DHT intracellular receptors, or to inhibit 5α-reductase activity, reducing the formation of DHT from testosterone. Some of these compounds may exert their antiandrogenic influence by inhibiting pituitary LH secretion or by blocking androgen receptors. Therapy with megestrol acetate (Ovaban; Schering, Kenilworth, NJ; 0.5 mg/kg administered orally once daily for 4-8 weeks) or medroxyprogesterone acetate (3-4 mg/kg administered subcutaneously no more than every 10 weeks) has been described. Four weeks of treatment with megestrol acetate resulted in resolution of clinical signs attributable to hyperplasia with no decrease in production of spermatozoa. Use of progestational compounds for prostate diseases in male dogs is not approved in the United States. Some of these compounds also may have negative effects on pancreatic and adrenocortical function and may affect general metabolism and reproductive behavior.

ANTIANDROGENS Antiandrogens are compounds that can cause a dose-dependent decrease in prostate size, increased incidence and extent of prostatic atrophy, and decreased prostatic secretory function.

Treatment with azasteroid analogs, such as finasteride (Proscar; Merck, Rahway, NJ) at dosages of 0.1-0.5 mg/kg/day for 1-4 months, causes atrophy of both the glandular and stromal compartments of the prostate, with a subsequent decrease in prostatic weight and volume. Prostatic size decreases significantly by 4 weeks of treatment and reaches maximum atrophy, a 33%-50% reduction in pretreatment volume, by 6 weeks of treatment. This compound is a synthetic steroid type II 5α-reductase inhibitor and is used to treat BPH in humans. It is suggested that this agent can decrease intraprostatic DHT concentrations and may cause a decrease in serum DHT concentrations. Finasteride also can decrease the secretory function of the prostate. The decrease in size of the prostate in response to treatment is attributed to apoptosis rather than necrosis.

Treatment with finasteride does not affect testicular weight, histomorphology, or daily production of spermatozoa. Libido and semen quality are not affected. Treatment with finasteride has not been associated with any extrareproductive side effects. Despite its lack of side effects, collection of an ejaculate from the dog may be a problem at the peak of treatment as a result of reduced prostatic secretion. Because the compound does not have androgenic, estrogenic, and progestational properties, it has not been demonstrated to be associated with abnormal reproductive behavior. Because it has not been tested for teratogenic effect, use

in men who attempt to father children is not recommended. In dogs, no pups with birth defects have been reported born to sires receiving finasteride at the time of breeding. The drug is not approved for veterinary use and further, the cost may be prohibitive.

Flutamide (5 mg/kg administered orally daily for 7 weeks) has been successfully used experimentally for treatment of BPH in dogs. This compound is believed to inhibit androgen receptors. It is suggested that the decrease in size in response to the treatment is dose dependent. This compound may not interfere with libido or sperm production; however, its use is prohibited in the United States.

MISCELLANEOUS COMPOUNDS Other therapies described for the treatment of BPH in dogs include estrogen compounds, ketoconazole, certain nutritional supplements, smooth muscle relaxants, catecholamine receptors blockers, and gossypol. Results have been mixed, with complications reported in some cases. Therapy with the herb saw palmetto is not effective in dogs. Estrogens are purported to decrease prostatic size by reducing the cellular mass of the prostate. However, the clinician should be cognizant of the side effects, which include bone marrow suppression (e.g., anemia, thrombocytopenia, leukopenia), squamous metaplasia of prostate epithelium, and decreased secretory function of the prostate. Other procedures that have been

suggested for the treatment of BPH include transurethral collagenase injection, transurethral light treatment after photosensitization, transurethral high-intensity focused ultrasound, and rotoresect mechanical ablation plus high frequency tissue coagulation.

Prostatitis: Acute and Chronic Prostatitis, Prostatic Abscesses

Definition and Pathogenesis

Bacterial infection can lead to inflammatory disease of the prostate gland. Acute bacterial prostatitis is characterized by sudden inflammation of the prostate gland from bacterial infection. Acute bacterial prostatitis can progress to a chronic form. Abscessation is thought to result from severe or chronic infection of prostatic tissue. Abscessation also can result from infection of intraparenchymal or extraparenchymal prostatic cysts. Abscesses can become very large and may rupture, leading to peritonitis.

The prostate is protected against infection by intrinsic and extrinsic mechanisms. An antibacterial substance, prostatic antibacterial factor, is secreted by the prostate. The prostate is infected most commonly by bacteria ascending through the prostatic ducts and to a lesser extent via the hematogenous route. Disorders of the urethra (e.g., urolithiasis, strictures, neoplasia) or

prostate (e.g., cysts, squamous metaplasia, neoplasia) may predispose to the development of prostatitis. BPH often can lead to or predispose the gland to bacterial prostatitis. *Escherichia coli* is the most common infectious agent cultured from prostatic fluid or tissue of dogs with bacterial prostatitis, but other gram-negative and gram-positive bacteria have been incriminated. Infection with *Brucella canis* is rather uncommon, and fungal prostatitis caused by *Blastomyces* species or *Cryptococcus* species is rare. The role of mycoplasma in the pathogenesis of prostatitis is unknown.

Signalment

Infection of the prostate is common in dogs. The incidence is reported to be 18.6%. Acute prostatitis is identified much less frequently than chronic prostatitis. Sexually intact male dogs older than 9 years are most often affected. There is no known breed predisposition.

History and Clinical Signs

Dogs with acute prostatitis often have systemic clinical signs, such as anorexia, depression, vomiting, fever, gait abnormalities, and caudal abdominal pain. Dripping of fluid from the prepuce may be noted. Acute prostatitis may be associated with signs of systemic illness, decreased libido as a result of localized inflammation,

and fever-induced impairment of spermatogenesis. Concurrent occlusion of the ductus deferens, hemospermia, orchiepididymitis, and immune-mediated orchitis are other complications affecting the reproductive system.

Chronic prostatitis may be subclinical, and the case may present for a recurrent urinary tract infection, constant or intermittent urethral discharge, poor semen quality, or infertility. Although inflammation is present in chronic prostatitis, it is not severe enough to produce systemic symptoms.

If prostatic abscesses become very large, the dog may have tenesmus or dysuria. Incursion of the urethra can lead to partial urethral obstruction, with a chronically distended bladder, eventual detrusor dysfunction, and overflow urinary incontinence. Generalized peritonitis resulting from rupture of an abscess results in lethargy, fever, pain, and vomiting. Evidence of shock (e.g., tachycardia, pale mucous membranes, delayed capillary refill, weak pulse) may be noted in severe cases. In most cases abdominal pain is present, and in some cases icterus may be present as a result of sepsis or endotoxemia.

Physical Examination Findings

In dogs with acute prostatitis, findings on rectal palpation are variable because the acuteness of the condition

often does not allow time for changes in size and structure of the prostate. However, if there is a concurrent prostatic disease, such as BPH or prostatic abscessation, the prostate may be enlarged and the dogs react with pain when palpation of the prostate per rectum is performed.

In chronic bacterial prostatitis, findings on rectal palpation are variable depending on the degree of fibrosis secondary to chronic inflammation. Rectal palpation usually does not elicit signs of pain. The gland may vary in symmetry and consistency. Areas of infection may be focal, multifocal, or diffuse. In cases of prostatic abscessation, the prostate usually is enlarged and asymmetric and has a variable consistency.

Diagnostic Tests and Results

A clinical diagnosis usually is made based on the history, physical examination findings, laboratory evaluation, and response to treatment. Growth of significant numbers of bacteria (more than 100,000 bacteria/ml) from prostatic fluid or tissue must be present for a diagnosis of prostatic infection to be made.

In acute prostatitis, the CBC may show neutrophilia with a left shift. Serum chemistries may be normal or changes may be observed if the dog is dehydrated or has renal insufficiency or additional contemporary diseases. Hepatic disease may occur secondary to septicemia, and hepatic enzymes may be increased. Urinalysis often

reveals pyuria, bacteriuria, and proteinuria. Urine collected by cystocentesis is submitted for bacterial culture and sensitivity tests. Prostatic fluid may be collected by manual ejaculation, but the dog may be experiencing too much pain to ejaculate. Prostatic massage may be associated with severe complications, such as septicemia. Percutaneous perianal small-needle biopsy and unguided percutaneous small-needle aspiration are not recommended in dogs with suspected acute bacterial prostatitis. Ultrasound-guided FNA often is used to obtain a sample for culture and cytologic examination. Culture results of prostatic fluid and tissue obtained by this method accurately indicate the presence or absence of infection in the prostate. Despite its cost and accessibility, this technique has become the diagnostic method of choice for obtaining prostatic fluid and tissue specimens. Formation of a septic tract through the abdomen may be a complication.

Spermiograms reveal a decrease in percentage of morphologically normal spermatozoa, with increases in primary and secondary abnormalities. These changes are attributed to perturbation in the seminal fluid rather than changes at the tissue level of the testis. The influence of acute prostatitis on the secretory function of the gland is unknown, but it is believed that prostatitis is associated with decreased secretory function.

In dogs with chronic prostatitis, results of the CBC and serum chemistries usually are unremarkable.

Hematuria, pyuria, and bacteriuria often are revealed with urinalysis. Prostatic fluid and urine should be submitted for cytologic examination and culture. Culture of more than 100,000 organisms/ml of prostatic fluid obtained by ejaculation indicates prostatic infection. Results of imaging techniques are often nonspecific and may reflect only prostatomegaly. Ultrasonography may show asymmetry, focal or diffuse hyperechogenicity, or hypoechoic regions if abscessation is present.

With prostatic abscessation, a neutrophilic leukocytosis with or without a left shift often is observed, similar to that seen with acute prostatitis. Putative diagnosis of a prostatic abscess may be made by ultrasonography, but definitive diagnosis requires demonstration of purulent fluid within the cystic space visualized. This can be achieved with FNA or exploratory celiotomy. The latter procedure is preferred because it allows both diagnosis and treatment via surgical drainage of the abscess. It is recommended that a fluid from within the abscess and prostatic tissue be obtained during this procedure for cytologic examination and culture.

Treatment and Prognosis

In dogs with acute prostatitis, intravenous fluid administration often is necessary to correct hydration deficits at the time of presentation. Parenteral antimicrobial drugs should be administered for 48-72 hours. If the patient's

condition is stable, oral antimicrobial treatment is indicated. An antimicrobial agent, chosen based on culture and sensitivity testing, should be administered for 4-6 weeks. Because the blood-prostatic barrier usually is not intact in dogs with acute bacterial prostatitis, the choice of the antimicrobial agent should be guided by the results of the antibiotic sensitivity testing. Antibiotic agents with good prostatic penetration are preferred because of improved penetration when the blood-prostatic barrier is reestablished. The normal pH of the prostatic fluid is acidic and remains acidic in prostatic infection. For this reason, a basic antibiotic (high pKa) is preferred. This way the antibiotic can be ionized and trapped in the prostatic fluid. Furthermore, the antibiotic should have high lipid solubility and low binding to plasma proteins. Antibiotics that fit this description include trimethoprim/sulfonamide, chloramphenicol, erythromycin, clindamycin, and the fluoroquinolones. The first two antibiotics are adequate for both gram-negative and gram-positive organisms. The fluoroquinolones are also known for their zwitterionic properties. Because antibiotics with these properties have multiple pKas, they are able to diffuse into prostate regardless of intraprostatic and periprostatic pH. Adverse reactions must be considered when choosing an antibiotic for long-term therapy of recurring bacterial prostatitis. Castration may improve efficacy of treatment and should be considered, especially in dogs with recurrent signs of prostatitis.

Most dogs appear to respond rapidly to antimicrobial treatment; however, long-term prognosis is unknown. Chronic prostatitis that is difficult to eradicate may develop in some dogs. In other dogs, prostatic abscess may develop as a consequence of acute prostatitis. It is suggested that castration may prevent acute prostatitis from becoming chronic, but much work is needed to confirm such a hypothesis.

Dogs with chronic prostatitis must be treated with an antimicrobial drug that is capable of penetrating the intact prostatic capsule, such as those described earlier. Treatment for 4-6 weeks is recommended, with prostatic fluid or tissue culture performed 1 week and again 4 weeks after therapy is concluded to ensure resolution of infection. Castration may hasten resolution of chronic prostatitis, most likely by causing resolution of underlying BPH. Concurrent treatment with antibiotics and finasteride in breeding dogs may be beneficial (see Benign Prostatic Hypertrophy/Hyperplasia, Treatment and Prognosis).

Prostatic abscesses are difficult to treat. Prognosis is poor, and the survival rate is only 50%. Surgical drainage is currently the treatment of choice for prostatic abscesses. Needle aspiration, insertion of a drain (tube or Penrose drain), or marsupialization are some of the ways an abscess can be drained. Successful ultrasound-guided aspiration may be associated with a better prognosis. Postprocedural complications, such as septic

shock, can be serious problems. Two issues are important postsurgically: Suitable antibiotic treatment and castration of the animal are required to prevent the recurrence of the abscess, and culture specimens should be obtained 1 week and again 4 weeks after therapy is concluded to ensure resolution of infection.

Prostatic and Paraprostatic Cyst

Definition and Pathogenesis

Cysts are cavitating lesions that have a distinct wall (may be secretory in nature) and contain turbid fluid. Two types of prostatic cysts are recognized. Retention cysts are believed to result from changes to acini in response to estrogens. Pronounced cystic hyperplastic changes also may result in prostatic retention cysts. Paraprostatic cysts are fluid-filled structures located adjacent to the prostate. Many consider paraprostatic cysts dilated remnants of the uterus masculinus. However, the cause of paraprostatic cysts is debatable because histologic evaluation has not confirmed their origination from embryologic structures.

Signalment

Approximately 5% of dogs with prostatic disease have paraprostatic cysts. Paraprostatic cysts may be more

common in medium- to large-breed dogs older than 8 years. There seems to be no breed predisposition. Intact males may be affected more than castrated males.

History and Clinical Signs

Prostatic and paraprostatic cysts often do not cause clinical signs until their size is sufficient to impinge on the colon or urethra or until they undergo abscessation. Clinical signs commonly reported are tenesmus, lethargy, anorexia, and dysuria. In certain cases cysts can be connected to the urethra, in which case serosanguineous urethral discharge may be present and changes in prostatic fluid may be apparent.

Physical Examination Findings

Physical examination usually is unremarkable in these cases. In some cases a caudal abdominal mass may be palpable on rectal examination or abdominal palpation. If the cyst extends caudally, it may cause perineal swelling. In most cases prostatomegaly is the only finding on rectal palpation; a soft fluctuant mass also may be palpable.

Diagnostic Tests and Results

Laboratory tests may not help in diagnosing these conditions. Survey radiographs may show a second bladder-

like structure, and in very rare cases calcified walls (intramural mineralization) of the cyst may be observed. Contrast urethrocystography may reveal asymmetric enlargement of the prostate gland and a narrowed urethral lumen, and urethroprostatic reflux may be increased, although there is no communication with the cyst. In addition, contrast cystography helps differentiate the urinary bladder from a suspected paraprostatic cyst. Prostatic and paraprostatic cysts can be confused with prostatomegaly resulting from BPH, bacterial prostatitis, and neoplasia occurring at the time of survey radiography.

Ultrasound is very useful because of its ability to separate solid versus cystic lesions. Ultrasonography reveals an anechoic area with cystic change associated with the prostate. In certain cases a macroscopic cyst may occur along with BPH (Figure 17-5). Prostatic fluid may have a high protein concentration and low numbers of white blood cells and usually is sterile. Aspiration of suspected cysts could be dangerous because a prostatic abscess may have a similar clinical presentation.

Treatment and Prognosis

No medical therapy is available. Prostatic and paraprostatic cysts should be drained surgically by excision or marsupialization. Simple drainage with ultrasound guidance has been described but may allow recurrence.

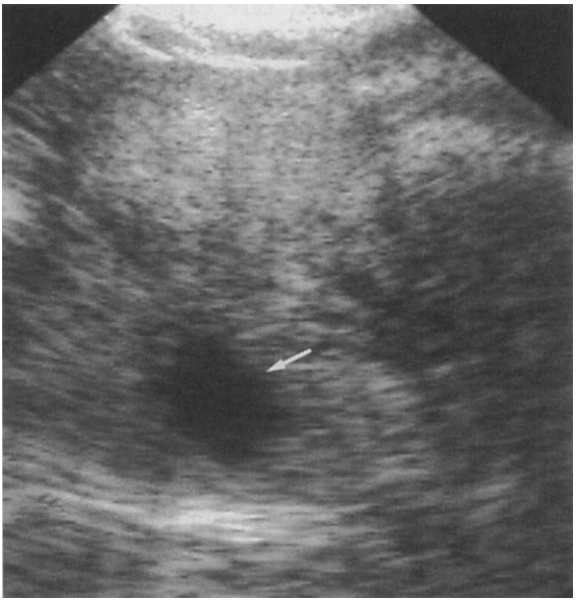


Figure 17-5. Benign prostatic hyperplasia and intraprostatic cyst. Transverse-plane sonogram of a 7-year-old intact mixed-breed male dog with palpable prostatic enlargement. The echotexture is homogeneous and a well-defined cyst *(arrow)* containing anechoic fluid can be seen in the right lobe of the prostate. Cystlike change also can be seen with prostatic abscess, but the fluid within the cavitation usually is echogenic, the walls are less well-defined, and the clinical presentation differs from that of benign prostatic hyperplasia.

Tacking of the residual cyst with omentum may help prevent recurrence. Castration also is recommended in dogs diagnosed as having cystic prostatic disease.

Prostatic Neoplasia

Definition and Pathogenesis

Prostatic neoplasia is uncontrolled, disorganized proliferation of prostatic tissue, most often of epithelial cells. The aging prostate gland is subject to neoplastic transformation, most commonly adenocarcinoma. It is believed that adenocarcinoma occurs after the initial neoplastic condition of prostatic intraepithelial neoplasia. Prostatic neoplasms other than adenocarcinoma that have been reported are transitional cell carcinoma, undifferentiated carcinoma, squamous cell carcinoma, and metastatic lesions from other primary tumors (lymphosarcoma, squamous cell carcinoma, and hemangiosarcoma). All of these tumors are malignant.

The cause of prostatic neoplasia is unknown. Although it may be hormonally related, it does not appear to exclusively involve testicular hormones. The tumors are locally invasive and may cause ureteral or urethral obstruction. Invasion through the prostatic capsule into surrounding pelvic musculature may occur. Pulmonary metastases may or may not be influenced by the level of circulating androgens (presence or absence of testicles).

Signalment

On the basis of necropsy studies, prevalence of prostatic neoplasia ranges from 0.29%-0.6% and accounts for 3.5%-15% of dogs with prostatic disease. It occurs both in intact and neutered dogs. Hence, castration does not have a sparing effect. Affected animals usually are older dogs (reported mean age, 10 years). There is no breed predilection, although most dogs with prostatic neoplasia are medium to large breeds.

History and Clinical Signs

The most common clinical signs in dogs with prostatic adenocarcinoma are weight loss, inappetence, rear limb lameness, and tenesmus. Lumbar pain, stranguria, polyuria/polydipsia, and dysuria/hematuria/pyuria are other commonly observed signs. Systemic signs may include depression, cachexia, and pyrexia. Dogs with metastases to bone have significantly greater occurrence of weight loss, emaciation, and lumbar pain. Secondary bacterial infection of the gland results in clinical signs of bacterial prostatitis as discussed earlier.

Physical Examination Findings

The prostate usually is asymmetrically enlarged, firm, and painful and often has nodular areas revealed on rectal palpation. Neutered dogs should have a palpably

small prostate gland, so a finding of even mild prostatic enlargement in a neutered dog should prompt suspicion of neoplasia. In some dogs with prostatic neoplasia, the prostate gland may be attached to surrounding tissues.

Diagnostic Tests and Results

History and physical examination findings in cases of neoplasia of the prostate can mimic either chronic prostatic infection or BPH. They can be differentiated from neoplasia by response to castration and presence of concurrent infections; BPH resolves after castration, whereas prostatic size is unchanged after castration in dogs with prostatic neoplasia and chronic prostatic infection often results in chronic or recurrent urinary tract infection, less commonly associated with prostatic adenocarcinoma. The marked prostatomegaly of prostatic abscessation and paraprostatic cysts also must be distinguished from prostatic neoplasia.

In dogs with prostatic adenocarcinoma, a CBC may reveal nonregenerative anemia, neutrophilic leukocytosis with or without a left shift, or monocytosis. Serum chemistries may show increased activity of hepatic enzymes, especially alkaline phosphatase, and hypoalbuminemia. Half of the affected population of dogs may have an increase in serum alkaline phosphatase. Obstruction of the urethra with or without bilateral

ureteral obstruction may result in azotemia. Prostatic fluid may contain neoplastic cells, but FNA or histopathologic examination of biopsy specimens often is necessary to confirm the diagnosis. Although specific tumor markers in serum are used for diagnosis of prostatic neoplasia in humans, they are not helpful in diagnosis of canine prostatic adenocarcinoma.

Radiographs often show only prostatomegaly, but they also may show an asymmetric or irregular prostate contour or intraprostatic mineralization (Figure 17-6). Focal or multifocal hyperechoic areas in the parenchyma with loss of normal architecture are discernible on ultrasonographs. In addition, ultrasonographic examination can detect asymmetry and irregularity of the prostatic contour. There may be poorly defined hyperechoic foci that may appear to coalesce. A few mineralized areas may be visible (Figure 17-7). Cavitary lesions may appear; these may represent infarction, necrosis, hemorrhage, or edema. Distention retrograde urethrocystography may reveal a distorted, narrow, and in certain instances, ruptured pelvic urethra. These changes are accompanied by periurethral asymmetry.

If a diagnosis of prostatic adenocarcinoma is made, the disease must be staged. Prostatic adenocarcinoma often metastasizes to the external and internal iliac lymph nodes, the lumbar vertebrae, and the lungs. Valuable staging tools include palpation of iliac lymph

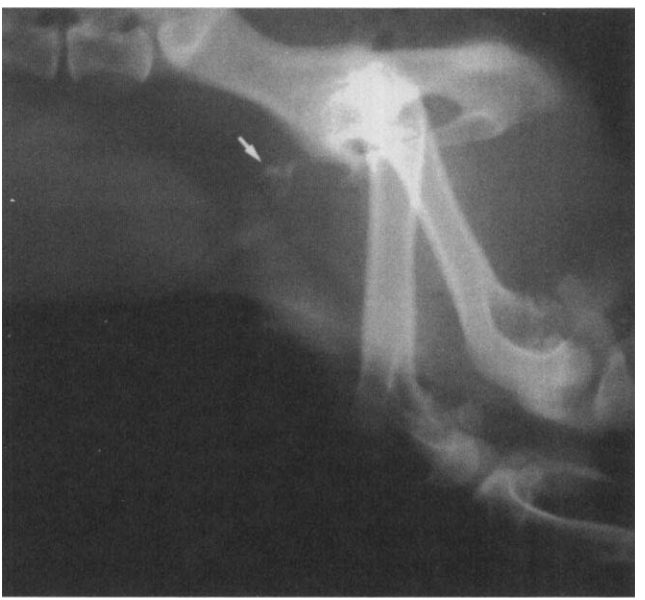


Figure 17-6. Prostatic carcinoma. Lateral radiographic projection of a 10-year-old intact male coonhound with hematuria. The enlarged prostate is ill defined, but there is recognizable mineralization *(arrow)* within the prostatic shadow.

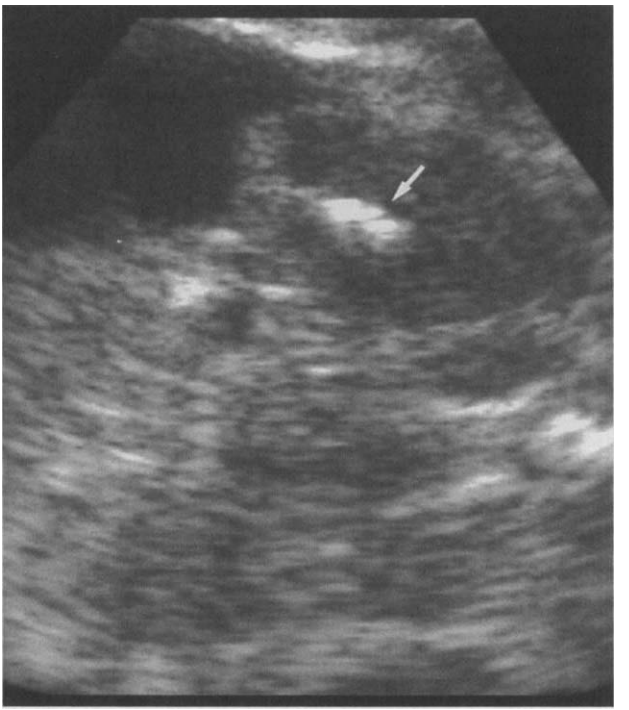


Figure 17-7. Prostatic carcinoma. Sagittal-view sonogram of the animal described in Figure 17-6 showing the prostate gland and part of the urinary bladder *(left)*. The prostatic echotexture is sonolucent (considered hypoechoic compared with normal prostate), and the mineralization *(arrow)* within the prostate is causing specular reflection and acoustic shadowing.

nodes on rectal examination, survey radiographs of the lumbar region, three-view thoracic radiographs (ventrodorsal [VD], left lateral, and right lateral), and nuclear bone scans. Even if thoracic radiographs show no evidence of metastasis, there is a 40% chance that metastases are present because micrometastatic lesions that go undetected in dogs with prostatic adenocarcinoma may be present.

Treatment and Prognosis

Treatment options for prostatic adenocarcinoma are limited. If there is no evidence of metastatic disease, local therapy (radiation) can be considered. Unfortunately, the neoplasm is not responsive to hormonal therapy. Because prostatectomy results in high rates of morbidity and failure, it is not recommended. Furthermore, urinary incontinence is a common complication after surgery. External-beam radiation and chemotherapy have limited success. In certain cases this treatment is reported to shrink the tumor with relief of urinary outflow and obstruction. The life span of the dog can be extended by a few months.

Prognosis for dogs with prostatic neoplasia is grave. Most dogs either die as a result of complications of the cancer or are euthanized shortly after diagnosis. Patients with no evidence of metastasis may have longer survival times.

Bibliography

Bamberg-Thalen B, Linde-Forsberg C: Treatment of canine benign prostatic hyperplasia with medroxyprogesterone acetate, *J Am Anim Hosp Assoc* 29:221, 1993.

Barsanti JA: Diseases of the prostate gland. Proceedings of the Society for Theriogenology Male Reproduction Symposium, Montreal, Quebec, Canada, 1997.

Barsanti JA, Finco DR: Canine bacterial prostatitis, *Vet Clin North Am* 9:679, 1979.

Barsanti JA, Finco DR: Canine prostatic diseases, *Vet Clin North Am* 16:587, 1986.

Barsanti JA, Finco DR, Mahaffey MM et al: Effects of an extract of *Serenoa repens* on dogs with hyperplasia of the prostate gland, *Am J Vet Res* 61:880, 2000.

Bell FW, Klausner JS, Hayden DW et al: Evaluation of serum and seminal plasma markers in the diagnosis of canine prostatic disorders, *J Vet Int Med* 9:149, 1995.

Feeney DA, Johnston GR, Klausner JS et al: Canine prostatic disease: comparison of ultrasonographic appearance with morphological and microbiological findings: 30 cases (1981-1985), *J Am Vet Med Assoc* 190:1027, 1987.

Feldman EC, Nelson RW: Prostatitis. In Feldman EC, Nelson RW (eds): *Canine and feline endocrinology and reproduction*, Philadelphia, 1996, WB Saunders.

Forrester SD, Purswell BJ: Diseases of the prostate. In Leib MS, Monroe WE (eds): *Practical small*

animal internal medicine, Philadelphia, 1997, WB Saunders.

Johnson SD, Kamolpatana K, Root Kustritz MV et al: Prostatic disorders in the dog, *Anim Reprod Sci* 60-61:405, 2000.

Johnston SD, Root Kustritz MV, Olson PN: *Canine and feline theriogenology,* Philadelphia, 2001, WB Saunders.

Kamolpatana K, Johnston SD: Effect of finasteride on serum dihydrotestosterone and testosterone in the dog. Proceedings of the Society for Theriogenology, Kansas City, Mo, 1996.

Kamolpatana K, Johnston GR, Johnston SD: Determination of canine prostatic volume using transabdominal ultrasonography, *Vet Radiol Ultrasound* 41:73, 2000.

Klausner JS, Osborne CA: Management of canine bacterial prostatitis, *J Am Vet Med Assoc* 182:292, 1983.

Ling GV: Diagnosis and medical management of prostate infections in dogs. Proceedings of the Society for Theriogenology, Toronto, Ontario, Canada, 1990.

Olson PE: Disorders of the canine prostate gland. Proceedings of the Society for Theriogenology, Denver, 1984.

Olson PN, Wright RH, Thrall MA: Disorders of the canine prostate gland: pathogenesis, diagnosis, and medical therapy, *Comp Cont Ed* 9:613, 1987.

Peter AT, Steiner JM, Adams LG: Diagnosis and medical management of prostate disease in the dog, *Semin Vet Med Surg (Small Anim)* 10:35, 1995.

Root Kustritz MV: Medical management of canine prostatic disease. Proceedings of the Society for Theriogenology, Baltimore, 1998.

Sirinarumitr K, Johnston SD, Root Kustritz MV: Effects of finasteride on size of prostate gland and semen quality in dogs with benign prostatic hypertrophy, *J Am Vet Med Assoc* 218:1275, 2001.

Thrall MA, Olson PN: Diagnosis of canine prostatic disease, *J Am Anim Hosp Assoc* 21:95, 1985.

18

Disorders of the Penis and Prepuce

Matthew Reeves and Philip Thomas

AT A GLANCE

- *Persistent penile frenulum* is lack of dissolution of tissue attaching the penile and preputial mucosa. Diagnosis is made based on visual inspection. Treatment is surgical transection.

- *Hypospadias* is failure of the urethral closure on the ventrum, anywhere along the length of the penile urethra. Diagnosis is made based on visual inspection. Treatment is surgical closure of the cleft or penile amputation and urethrostomy.
- *Penile trauma* may be associated with lacerations or fracture of the os penis. Diagnosis is made based on visual inspection or radiography. Treatment of lacerations is as for any skin wound, allowing for the fact that excessive hemorrhage may occur if the cavernous spaces of the penis are invaded. Fractures of the os penis may heal spontaneously but require stabilization if the fracture is displaced or if urethral damage or compression is present.
- *Priapism* is persistent erection. Causes include neoplasia or thromboemboli blocking venous outflow and neurologic disease. Diagnosis is made based on visual inspection. Treat the underlying cause, if possible. Penile amputation and urethrostomy may be necessary.
- *Balanoposthitis* is inflammation of the penile and preputial mucosa as a result of viral or bacterial infection or contact irritants, or it may occur as a component of atopic dermatitis. Diagnosis is made based on visual inspection and culture; moderate to heavy growth of a single organism is significant. Treat the underlying cause, if possible.

- *Paraphimosis* is inability of the animal to retract the nonerect penis into the prepuce. This may occur after breeding or after licking of the extended penis in intact or castrated male dogs. Diagnosis is made based on visual inspection. Treat by lubricating the penis and replacing it within the prepuce.
- *Urethral prolapse* is protrusion of urethral tissue from the external urethral orifice. The classic presentation is a reddened mass of tissue, as of a "red pea." Diagnosis is made based on visual inspection. Surgical resection is the treatment of choice.
- *Penile neoplasia* is uncommon. The most common tumor types reported are squamous cell carcinoma and transmissible venereal tumor. Surgical resection is the treatment of choice for squamous cell carcinoma. Transmissible venereal tumor is best treated with vincristine.

Persistent Penile Frenulum

Definition and Pathogenesis

Persistent penile frenulum is caused by a failure of separation of the preputial mucosa from the glans penis (the balanopreputial fold). This androgen-dependent separation normally occurs at birth or within the first few months of life. The penile

frenulum is an avascular, fibrous membrane of varying thickness that joins the ventral midline of the glans penis to the prepuce.

History and Clinical Signs

The frenulum prevents full extrusion of the penis and can lead to the following conditions.

- Urinary retention (and recurrent balanoposthitis)
- Urine scald dermatitis
- Refusal or inability to mate
- Pain or hemorrhage with attempts to extrude the penis
- Ventral or lateral deviation of the penis (phallocampsis)

Physical Examination and Diagnosis

Physical examination findings are diagnostic.

Treatment

Treatment requires surgical excision or sectioning of the frenulum. This can be performed with the animal under local anesthesia or under sedation and has a good prognosis. A heritable nature has been established.

Hypospadias

Definition and Pathogenesis

Hypospadias is a ventral opening in the penile urethra resulting from a defect in urethral closure. Urethral defects are classified as perineal, scrotal, penile, or glandular depending on their anatomic locations, and they commonly occur in the perineal region. Closure of the urethra normally occurs during fetal development, under the influence of dihydrotestosterone. Hypospadias has been induced experimentally in fetuses by administering exogenous progestogens or estrogens to the pregnant dam. Hypospadias also can be attributed to androgen receptor defects and may be associated with other urogenital tract anomalies and intersex (see Chapter 1).

History and Clinical Signs

On physical examination, hypospadias may be any of the following:

- An incidental finding
- Associated with urinary incontinence
- Accompanied by abnormal external genitalia or other congenital anomalies
- Accompanied by urinary tract infections and urine scald dermatitis

Physical Examination and Diagnosis

Physical examination findings are diagnostic.

Treatment

Surgical urethral reconstruction may be technically demanding but can be curative. Distal hypospadias (glandular) may respond to primary repair. Perineal urethrostomy and penile amputation are salvage procedures for rostrally located lesions.

Diphallus

Definition and Pathogenesis

Diphallus is duplication of the penis. It rarely occurs in the dog. The cause has not been determined.

Clinical Signs and Diagnosis

Clinical examination is diagnostic. The reported cases have been accompanied by other abnormalities of the urinary and genital tract, including other duplications.

Penile Hypoplasia

Definition and Pathogenesis

Penile hypoplasia is a smaller-than-normal penis. It is a rare condition occurring as part of a complex of

abnormalities in female pseudohermaphrodite dogs. An "infantile" penis has been described as a congenital condition but may be acquired in dogs castrated at 7 weeks of age.

History and Clinical Signs

Clinical signs may be absent, may reflect urine pooling in the prepuce, or may be accompanied by cryptorchidism.

Physical Examination and Diagnosis

Physical examination findings are diagnostic.

Treatment

Treatment may not be necessary. Preputial reconstruction has been described. Penile amputation and urethrostomy might be considered a salvage procedure in symptomatic animals.

Penile Trauma

Definition and Pathogenesis

Trauma to the penis occurs as a result of mating, fighting, and racing, as well as being involved in road traffic accidents. A fractured os penis may be immediately

apparent but more commonly is an incidental finding or is discovered as the primary cause of urethral obstruction secondary to callous formation at the fracture site.

History and Clinical Signs

Clinical signs vary depending on the nature and extent of the injury. Mild trauma can cause bruising, swelling, and lacerations to the penis, accompanied by hemorrhage and pain. Dogs with fractures of the os penis or urethral obstruction may present with stranguria, dysuria, and systemic signs. Long-standing injuries may be noted only as inability or reluctance to mate or penile deviation.

Physical Examination

A general and specific reproductive tract examination is indicated, including bladder palpation, neurologic examination, musculoskeletal examination, and a detailed examination of the prepuce and penis for physical injury.

Diagnostic Tests

If obstruction of the urinary outflow tract is suspected, serum biochemistry and urinalysis are indicated to assess for postrenal azotemia. Abdominal and penile

ultrasound (including dynamic ultrasound of the passage of a urethra catheter) is useful in assessing urethral integrity. Radiography is necessary to evaluate fractures of the os penis. Contrast radiography (retrograde urethrography) permits assessment of urethral integrity.

Treatment

Skin wounds should be cleaned and debrided. Absorbable suture material should be used to repair lacerations. Topical or systemic antimicrobials may be useful in preventing secondary infections.

During healing the penis should be extruded from the prepuce at least twice daily, both to assess wound healing and to prevent the formations of adhesions between the penis and prepuce. Sexual arousal should be avoided during the repair process because erection may lead to bleeding and wound dehiscence. Male dogs must be housed away from estrous bitches. If wounds fail to heal because of frequent erections, castration may be necessary.

Urinary outflow obstruction secondary to urethral damage necessitates bladder decompression either by cystocentesis, an indwelling catheter, or temporary urethrostomy. Urethral lacerations may require primary surgical closure.

Surgical stabilization is recommended for displaced fractures to the os penis or for sites at which urethral damage has occurred. This can be achieved using orthopedic

wires or a small bone plate. Nondisplaced fractures not associated with urethral damage generally heal well, with the penis providing support for fracture healing.

Prognosis

Wound healing may lead to callous formation and secondary urethral obstruction. Permanent urethrostomy or penile amputation may be necessary as a salvage procedure.

Priapism

Definition and Pathogenesis

Priapism is an erection that persists without sexual stimulation. Priapism can be the result of hemodynamic or neurologic disturbances that cause occlusion of the venous outflow tracts, such as thromboembolism and neoplasia. Sludging of erythrocytes and thrombosis leads to ischemia and necrosis. Priapism predisposes the penis to secondary damage, and exposed mucosal surfaces may become dry and fissured.

History and Clinical Signs

Clinical signs vary depending on cause, chronicity, and severity of secondary trauma. Ischemic damage to the

penis initially is painful before the penis becomes edematous, congested, and swollen. Self-mutilation may then occur.

Physical Examination and Diagnosis

A complete physical examination followed by a close examination of the entire penis and prepuce are diagnostic. Establishing a primary cause may be difficult. Hematology and biochemistry, coagulation profiles, complete examination of the genital and urinary tracts, abdominal ultrasound, spinal radiographs, and myelographs are useful in identifying specific causes.

Treatment

Treatment should be directed at both the primary cause and the lesion.

- If the primary cause can be determined, it should be addressed rapidly.
- The penis should be moistened, the penile surface cleaned, and specific penile injuries treated as necessary before replacement of the penis in the prepuce, if possible. Treatments proposed include removing clots from the cavernous spaces via incision into the cavernous spaces of the bulbus glandis and the pars longa glandis and manual expression. We have attempted to flush clots from the cavernous

spaces using heparin-containing solutions without success. Penile amputation may be necessary in refractory cases or when secondary damage to the penis is severe.

Prognosis

Irreversible changes occur rapidly. If penile amputation and urethrostomy must be performed, prognosis for the animal's life usually is good.

Balanitis, Posthitis, and Balanoposthitis

Definition and Pathogenesis

Balanitis and posthitis are, respectively, inflammation of the glans penis and the prepuce. Balanoposthitis is inflammation of the penis and prepuce. Organisms isolated generally are normal inhabitants of the prepuce. Other etiologic factors include preputial foreign bodies, neoplasia, urinary incontinence, and allergic dermatitis in atopic animals.

Microorganisms isolated from animals with balanoposthitis include the following:

- Bacteria, commonly including *Escherichia coli,* which are normal flora
- Mycoplasmas and ureaplasmas, which are common isolates of the prepuce in unaffected dogs, although

mycoplasmas are more commonly recovered from affected dogs than from healthy dogs
- Viruses, including herpesvirus and calicivirus, both of which are associated with vesicular lesions

Signalment

Balanoposthitis is a common finding in dogs and is rare in cats. Both prepuberal dogs and adult dogs are affected.

History and Clinical Signs

Dogs have excessive purulent, occasionally sanguineous, preputial discharge. The discharge varies in both volume and degree of purulence. Balanoposthitis usually causes no systemic signs but occasionally may be associated with excessive penile grooming. Examination of the penile skin and preputial mucosa may reveal scant to copious purulent material overlying mucosa that may be normal, erythematous, or ulcerated. Lymphoid follicles may be grossly visible in chronic disease, and vesicles may be present in dogs with viral-induced disease.

Healthy, intact male dogs secrete prostatic fluid continuously, which drains into the urinary bladder and down the urethra, forming a mass of mucoid, yellow-green discharge at the preputial orifice. This normal occurrence should not be associated with inflammation of penile and preputial mucosal surfaces.

Physical Examination and Diagnostic Tests

Physical examination of the penis and prepuce usually is diagnostic. It is important to rule out underlying causes, such as foreign bodies, neoplasia, ulcerative or inflammatory lesions, and congenital anomalies. Sedation or general anesthesia may be necessary for complete extrusion of the penis to be performed. Bacterial and mycoplasma cultures may be performed, but the results may not be meaningful. Herpesvirus induces a short-lived and poor humeral response, precluding serologic testing as a worthwhile diagnostic test; serologic results positive for herpesvirus are specific but not sensitive.

Treatment

Treatment is conservative, involving preputial lavage with sterile saline, weak antiseptic solutions (e.g., chlorhexidine, aqueous iodine), or topical antibiotics, as indicated by culture and sensitivity. Topical antibiotics should be used cautiously to avoid the risk of antibiotic resistance. The benefit of systemic antibiotics remains controversial, although enrofloxacin may be indicated if mycoplasmal infection is implicated as a primary cause of disease. Allergic dermatitis should be approached with an appropriate diagnostic and treatment plan for the skin.

Prognosis

Prognosis is good for cases in which an underlying cause is found and can be treated. If an underlying cause is not found, symptomatic therapy may be attempted but preputial discharge commonly recurs in days to weeks after treatment. Preputial discharge in prepuberal dogs generally resolves to some extent without treatment.

Phimosis

Definition and Pathogenesis

Phimosis is the failure of the penis to be exteriorized from the prepuce. This may occur because of stenosis of the preputial opening. Congenital and acquired causes have been identified. Acquired causes include trauma, cicatricial constriction, preputial inflammation, preputial infection, and neoplasia. Preputial hairs entangling the preputial opening are the most common cause of phimosis in longhaired cats. A congenital abnormality of the penis and prepuce has been reported to occur in the Boston terrier, resulting in phimosis. It may be accompanied by hypospadias or male pseudohermaphroditism.

History and Clinical Signs

Congenital causes of phimosis are detected in young animals and generally are associated with dribbling of urine

from the prepuce. In severe cases complete urine outflow obstruction is possible. Presentations in older animals generally are associated with an inability to copulate or pain during erection.

Physical Examination and Diagnostic Tests

Physical examination is diagnostic. The penis may be either painful or unable to be exteriorized.

Treatment and Prognosis

Corrective surgery involves increasing the diameter of the preputial orifice dorsally or ventrally. Corrective surgery generally is curative.

Paraphimosis

Definition and Pathogenesis

Paraphimosis is the condition in which the nonerect penis is unable to retract completely into the prepuce. Venous outflow from the penis is obstructed, and ischemic injury and secondary trauma follow. Thromboembolism of the cavernous spaces may occur. Causes include primary preputial disorders, primary penile diseases, and extrareproductive tract disease.

- *Penile:* entrapment after normal erection, trauma, neoplasia, or fractured os penis leading to penile swelling
- *Preputial:* after coitus or erection when the prepuce folds on itself narrowing the preputial orifice, preputial foreign body, preputial hair rings (especially in longhaired cats)
- *Extrareproductive:* neurologic disease affecting the parasympathetic nervous system or the hypogastric nerve

Signalment

Paraphimosis occurs commonly in dogs and is rare in cats.

History and Clinical Signs

Paraphimosis commonly occurs after periods of sexual excitement. Clinical signs depend on the duration of the injury because the likelihood of ischemic damage and secondary injury increases with time. The appearance of the penis varies from normal to congested and edematous. If the duration is prolonged, the penis becomes dry and excoriated. In severe cases the penis may become necrotic. Urethral integrity usually is maintained.

Physical Examination and Diagnosis

Visual inspection of the penis generally is diagnostic but may require sedation or general anesthesia.

Treatment

CONSERVATIVE Therapy is aimed at replacing the penis within the prepuce, thereby restoring its circulation. This usually is accomplished by manual reduction. The prepuce is gently retracted to facilitate unfolding of the prepuce to return it to its normal configuration. The prepuce can then be replaced to cover the surface of the penis with water-soluble lubricant. Gentle pressure applied to the penis may aid venous outflow. This procedure may require sedation or general anesthesia. If the penis has been damaged, its surface may need to be lavaged, wounds debrided, and antibiotics applied as necessary.

SURGICAL Surgery may be necessary to enlarge the preputial orifice, allowing the penis to be reduced. This can be achieved by making a ventral midline incision in the prepuce, replacing the penis, and closing the incision in separate layers. A temporary suture can be placed at the preputial orifice for 24 hours to reduce the likelihood of recurrence until swelling in the penis has subsided. Severe damage may necessitate complete or partial penile amputation and perineal urethrostomy.

Prognosis

Paraphimosis commonly recurs after periods of sexual excitement in intact animals. To prevent recurrence, efforts should be directed at reducing sexual activity or excitement. Castration is effective. Pharmacologic management with antiandrogenic compounds is less effective and may be associated with side effects.

Urethral Eversion/Prolapse

Definition and Pathogenesis

Prolapse of the urethra is characterized by appearance of a 1- to 5-mm mass of urethral mucosa, which has everted through the urethral meatus. The prolapse may appear or increase in size with erection.

Signalment

A familial inheritance is reported in English bulldogs and Boston terriers. Affected animals are commonly younger than 2 years.

History and Clinical Signs

Urethral eversion usually is associated with significant hemorrhage that occurs intermittently in association with sexual excitement.

Treatment

CONSERVATIVE Conservative treatment relies on reducing levels of sexual excitement and sexual activity. This may involve tranquilization and isolation of male dogs from bitches in heat or castration, which usually is associated with disappointing results.

SURGICAL Surgical resolution requires excision of the prolapse and suturing healthy urethral mucosa to penile skin. Care must be taken to avoid puncturing the corpus spongiosum. Castration should be considered at the time of surgery to reduce future sexual activity and excitement. Surgical treatment failure, with necessity of repeating surgical procedures, is not uncommon.

Prognosis

Relapse is common in intact dogs, although there are reports of successful breeding after surgical correction.

Penile Neoplasia: General Information

Definition and Pathogenesis

Neoplasia of the penis is rare in dogs and cats. Various tumor types occur. The most common neoplasms are transmissible venereal tumors (TVTs) and squamous cell carcinomas. Other neoplasms affecting the penis

include carcinomas, chondrosarcomas of the os penis, plasma cell tumors, and mast cell tumors.

History and Clinical Signs

Mass lesions may protrude from the preputial opening. Penile neoplasms may be associated with pain at mating, preputial discharge, and hemorrhage.

Treatment and Prognosis

Treatment and prognosis depend on the cell type and grade and evidence of metastasis. Surgical excisional biopsy and histopathologic examination are indicated.

Transmissible Venereal Tumors

Definition and Pathogenesis

TVTs originate from mucosal cells and occur as friable mass lesions on mucous membranes, particularly of the penis. The cause is unknown. The neoplastic cells do not appear to be canine in origin, containing only 59 ± 5 chromosomes, compared with the 78 chromosomes in healthy canine cells. A viral cause has been proposed but as yet is not identified. Transmission occurs by seeding of neoplastic cells during coitus. Sniffing or muzzling the neoplastic lesions may cause spread to other mucosal surfaces.

TVTs seem to have an incubation period of 5-6 weeks. TVTs have no direct effect on fertility but may physically impede copulation.

History and Clinical Signs

Dogs generally are presented because owners have noticed penile mass lesions protruding from the prepuce or a bloody preputial discharge.

Physical Examination

Examination of the penis generally reveals pink, nodular lesions, which vary in size and number. Large lesions often have a cauliflower appearance. The masses generally are friable, can have an ulcerated surface, and may bleed easily. Some appear pedunculated. In the absence of infection, the masses are not painful.

Diagnostic Tests

Diagnosis is based on physical examination findings and biopsy.

Treatment

Spontaneous regression may occur within months of diagnosis. Surgical excision, amputation, chemotherapy, and

radiotherapy have been used in the treatment of TVTs. Tumors are likely to recur in approximately 50% of cases regardless of treatment. Vincristine (0.5-0.7 mg/M^2 administered intravenously once every 14 days for three treatments or for at least one treatment beyond resolution of clinical signs) has perhaps shown most potential, inducing remission in more than 90% of cases without relapse.

Prognosis

Tumor metastasis is considered rare but has been reported to occur in abdominal and thoracic viscera, the brain, and the eye.

Other Acquired Disorders of the Penis

Definition and Pathogenesis

Vesicles, ulcers, and lymphoid follicle hyperplasia also occur on the penis. The cause of penile vesicles has not been determined. Herpesvirus has been implicated but not isolated from the vesicles. Ulcers are uncommon and generally associated with infection. Hyperplasia of lymphoid follicles at the base of the glans (where large numbers of small 1- to 2-mm nodules may appear) is of unknown significance and does not appear to be associated with clinical disease or to be transmissible. Viral causes have been proposed but not yet demonstrated.

History and Clinical Signs

Lesions may be associated with preputial discharge or excessive grooming of the penis or may be noticed as mass lesions protruding from the preputial orifice.

Diagnostic Tests

Definitive diagnosis is based on biopsy. Exfoliative cytology and bacterial and fungal cultures are sometimes necessary.

Treatment

Treatment depends on the lesion present. Wart lesions generally resolve spontaneously after biopsy.

Bibliography

England GC: *Allen's fertility and obstetrics in the dog,* Oxford, 1998, Blackwell Science.

Roberts SJ: *Veterinary obstetrics and genital diseases,* Woodstock, 1986, SJ Roberts.

Root Kustritz MV: Disorders of the canine penis, *Vet Clin North Am* 31:247, 2001.

Thomas P: Canine theriogenology. Presented at the Sunshine Coast AVA Annual Divisional Conference, Sunshine Coast, Australia, 1998.

Verstegen JP: Conditions of the male. In Simpson G, England G, Harvey M (eds): *Manual of small animal reproduction and neonatology,* Cheltenham, England, 1998, British Small Animal Veterinary Association.

19

Infertility in the Dog and Cat

Margaret V. Root Kustritz

AT A GLANCE

- Infertility in dogs
  - *Improper timing of breeding is the most common cause of apparent infertility in dogs.* Measure progesterone in serum to optimize breeding timing (see Chapter 2).
  - Subclinical uterine infection may occur. Collect a specimen for culture from the anterior vagina,

using a guarded swab, early in proestrus. Moderate to heavy growth of a single organism is significant. Treat with an appropriate antibiotic, based on culture and sensitivity testing, for 2-4 weeks.

- Brucellosis is associated with infertility in bitches and dogs. Regular serologic testing is recommended in all breeding animals (see Chapter 7).
- Hypothyroidism is associated with irregular cycles in the bitch and poor semen quality in the male dog. Concurrent measurement of free thyroxine (T_4) by dialysis and of canine thyroid-stimulating hormone (cTSH) in serum is the diagnostic scheme of choice. Appropriate supplementation of hypothyroid animals may or may not effect a change to normal fertility. Hypothyroidism may be hereditary.
- Abnormal semen quality may be associated with apprehension at the time of breeding or semen collection, prostate disease (see Chapter 17), testicular disorders (see Chapter 16), brucellosis (see Chapter 7), or hypothyroidism.

- Infertility in cats
  - Queens are seasonally polyestrous. If the environment is such that the queen is not exposed to at least 10 hours of light daily, she may not cycle.
  - A queen should be brought to the tom's environment. Copulation must take place at least four times during estrus to reliably induce ovulation. Serum

progesterone concentration greater than 2 ng ml after the queen goes out of behavioral estrus is indicative of ovulation having occurred.

- Viral causes of pregnancy loss in the queen include feline leukemia, feline infectious peritonitis, feline herpesvirus (rhinotracheitis), and panleukopenia. Toxoplasmosis and bacterial infections are uncommon causes of pregnancy loss in queens.
- Semen evaluation in tom cats is challenging; cystocentesis after copulation is a noninvasive technique that allows assessment for spermatogenesis but not evaluation of semen quality. Electroejaculation can be performed in anesthetized tom cats.

Infertility in the Dog

1. The bitch is cycling. (Proceed to 2.)

1′. The bitch has never cycled.

- Primary anestrus is lack of estrous activity in a bitch by 24 months of age.
- Causes include the following:

  Husbandry: Bitches that are malnourished or that are under stress because of heavy work or show schedules may not cycle. Housing with cycling bitches may stimulate estrous activity in anestrous bitches.

Previous ovariohysterectomy: Previous ovariohysterectomy may be diagnosed based on history, palpation or observation of an ovariohysterectomy scar at the ventral abdominal midline, or exploratory laparotomy. In spayed bitches, serum concentrations of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are elevated because of lack of negative feedback from the absent ovary to the anterior pituitary. Elevated LH concentration in blood may be identified in bitches with an in-house LH assay (Status-LH; Synbiotics, San Diego, Calif.). Measurement of serum estradiol 60-90 minutes after administration of gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) has been described; concentrations below the sensitivity of the assay used may be indicative of previous ovariohysterectomy in that bitch.

Silent heat: Silent heat is follicular development and ovulation in the absence of physical signs of heat, such as vulvar swelling and exudation of serosanguineous vaginal discharge. Diagnosis requires weekly assessment of vaginal cytology and monthly measurement of serum progesterone con-

centration, with serum progesterone concentration greater than 2 ng/ml indicating ovarian activity. Dogs bred appropriately during a silent heat are fertile.

Hermaphroditism (see Chapter 1): Abnormalities of sexual differentiation described in dogs include true hermaphroditism (presence of ovarian and testicular tissue), male pseudohermaphroditism (presence of testes and female or ambiguous external genitalia), and female pseudohermaphroditism (presence of ovaries and male or ambiguous external genitalia). Diagnosis of abnormalities of sexual differentiation usually requires exploratory laparotomy and histopathologic examination of excised gonadal tissue. Karyotyping also may be necessary.

Hypothyroidism: Hypothyroidism has been associated with primary anestrus, irregular interestrous intervals, low litter size, and general infertility. Poor semen quality has been described as occurring in hypothyroid male dogs, perhaps because of concurrent autoimmune thyroiditis and orchitis. Extrareproductive signs of hypothyroidism, such as lethargy, weight loss, and bilaterally symmetric alopecia of the trunk, may or may

not be present. Hypothyroidism is considered a familial disorder. Predisposed breeds include the golden retriever, Doberman pinscher, dachshund, Shetland sheepdog, Irish setter, Pomeranian, miniature schnauzer, and American cocker spaniel. The current preferred method of diagnosis is with concurrent measurement of serum cTSH and free T_4 by dialysis. In hypothyroid dogs, cTSH is elevated and free T_4 is decreased. Measurement of total T_4 in serum alone is not diagnostic because many extraneous factors cause artifactual decreases in total T_4. Dogs with hypothyroidism are treated by supplementation with T_4 at a dosage of 0.01-0.02 mg/kg administered orally twice a day. Serum thyroid hormones should be reevaluated every 6 months while dogs are receiving supplementary T_4. Although supplementation with T_4 may effect a return to normal reproductive function, the heritability of the disorder should be remembered when considering use of the animal for breeding.

Systemic disease: Systemic conditions, such as hyperadrenocorticism or diabetes mellitus, may affect reproductive function. A complete physical examination, complete blood

count, serum chemistry profile, and urinalysis should be evaluated in animals presented for infertility.

2. The interestrous interval is normal in length. (Proceed to 3.)

2′. The interestrous interval is abnormally long (longer than 12 months).

- *Breed:* Some breeds normally cycle less frequently than the average interestrous interval of every 7 months. These breeds include the basenji, dingo, and wolf-dog hybrids, all of which cycle yearly.
- *Hypothyroidism:* See 1′.

2″. The interestrous interval is abnormally short.

- *Breed:* Some breeds normally cycle more frequently than the average interestrous interval of every 7 months. These breeds include the German shepherd dog, rottweiler, and basset hound.
- *Split heat:* Split heat is defined as proestrous activity for 1-2 weeks, followed by an anestrous period averaging 1 month in duration before normal ovulatory estrus occurs. Diagnosis requires measurement of progesterone in serum during the intermediary anestrous period (serum progesterone concentration less than 2 ng/ml indicates that ovulation has not occurred) and after the second period of

estrous activity (serum progesterone concentration greater than 2 ng/ml indicates that ovulation has occurred). Split heat is not associated with infertility unless the bitch is forcibly bred during the anovulatory portion of the split heat.

- *Uterine disease* (see Chapter 12): Bitches with cystic endometrial hyperplasia (CEH) or subclinical uterine infection may more readily release prostaglandin from the endometrium, prematurely lysing the corpora lutea and increasing cycle frequency. CEH may be visible ultrasonographically as a fluffy thickening of the uterine wall. Because of the relative inaccessibility of the canine uterus, uterine infection usually is best diagnosed by anterior vaginal culture in estrous bitches.

2‴. The bitch is in persistent estrus.

- *Ovarian cyst* (see Chapter 11): Ovarian cystic disease in the bitch usually is caused by lack of ovulation of a cystic follicle. Persistent estrogen secretion is manifested clinically as persistent estrus. Although the presence of persistently elevated serum estrogen concentrations is diagnostic, estrogen assay is difficult and may not be available. Identification of cornified vaginal epithelial cells for 6 weeks or longer also is diagnostic of persistently

elevated serum estrogen concentrations. Ovarian follicular cysts may be visible with transabdominal ultrasound but often are difficult to see. Response to treatment with GnRH (50 μg administered intramuscularly) or hCG (1000 IU, half of which is administered intramuscularly and half of which is administered intravenously) may be diagnostic.

- *Granulosa cell tumor* (see Chapter 11): Functional granulosa cell tumors of the ovary may be manifested clinically as persistent estrus. A granulosa cell tumor may be palpable as a mass in the cranial abdomen or may be visible on survey radiographs or with transabdominal ultrasound. Ovariohysterectomy is the treatment of choice.

3. Normal copulation occurs. (Proceed to 5.)

3′. Normal copulation does not occur. (Proceed to 4.)

4. The male shows normal breeding behavior, but the bitch does not allow copulation.
   - *Behavior:* Dominant bitches may not allow male dogs to mount and achieve intromission. The problem is best circumvented by artificial insemination (see Chapter 3).
   - *Congenital vaginal abnormalities* (see Chapter 13): The congenital vaginal anomalies most often described as occurring in the dog are

vaginal septa of varying lengths and circumferential vaginal strictures. Most develop just cranial to the urethral papilla and are therefore palpable on digital vaginal examination and may be observed with almost any size vaginoscope. Depending on the extent of the anomaly, surgical repair may be attempted or the problem circumvented with artificial insemination and possible cesarean section; vaginal anomalies may relax sufficiently under the influence of the hormone relaxin late in gestation to permit spontaneous whelping.

- *Vaginal fold prolapse* (see Chapter 13): Vaginal fold prolapse is protrusion of edematous vaginal tissue through the vulvar lips. This is an estrogen-mediated phenomenon that subsides when serum estrogen concentrations decline and that often recurs during subsequent heat cycles. Bitches with vaginal prolapse usually must be bred by artificial insemination because male dogs mount but have difficulty achieving intromission of the penis in the presence of the prolapsed vaginal tissue. Vaginal prolapse may redevelop near the time of parturition and may or may not cause dystocia.

4′. The bitch shows normal breeding behavior, but the male does not mount or achieve intromission and ejaculation.

- *Behavior:* Submissive male dogs may be hesitant to breed a dominant bitch. Similarly, a male dog may be hesitant to approach a bitch that has been aggressive toward him, as may occur if the bitch is presented to the male too early in her season. Male dogs that consistently have been disciplined for showing breeding behavior at inappropriate times may inadvertently have been trained not to show such behavior.
- *Pain:* Male dogs with prostate disease (see Chapter 17) or pain of the spine or rear limbs may be unable or unwilling to mount the bitch and ejaculate. Complete physical and neurologic examinations and a workup for prostate disease, including semen cultures and imaging with possible aspirate or biopsy of the prostate, may be needed to localize the source of pain.
- *Poor libido:* Libido is variable in male dogs, and those with poor libido are difficult to manage clinically. Ejaculation may be effected by administration of GnRH (1-2 μg/kg subcutaneously 2-3 hours before attempted breeding). GnRH causes release of endogenous LH, which stimulates release of testosterone. This technique for enhancing reproductive performance should not be used routinely in

valuable stud dogs because persistent stimulation of testosterone release may lead to a decrease in the normal, pulsatile spontaneous release of testosterone, which is necessary for maintenance of spermatogenesis.

5. The bitch conceives after copulation.* (Proceed to 6.)

5′. The bitch does not conceive after copulation.* (Proceed to 7.)

6. Pregnancy is maintained.

6′. Pregnancy is not maintained.

- Infectious causes of pregnancy loss include the following (see Chapter 7):

  Herpesvirus: Canine herpesvirus causes fetal death and is manifested as pregnancy loss late in gestation, stillbirths, and early neonatal death. Dogs infected in the last 3 weeks of gestation or during the first 3 weeks of life are most likely to show clinical signs. Bitches infected before or after the last 3 weeks of pregnancy usually are asymptomatic. Most bitches lose at most one litter to canine herpesvirus, apparently creating enough

*Dogs do not produce any hormone unique to pregnancy, precluding pregnancy diagnosis early in gestation. Therefore lack of conception cannot be differentiated from early embryonic death. Conception is proved by positive pregnancy diagnosis (see Chapter 8).

memory cells to permit an appropriate immune response if reexposure occurs. The virus is weakly antigenic, so titers are likely to be elevated in bitches only at the time of pregnancy loss. No vaccine is commercially available.

Canine brucellosis: Brucella canis infection is characterized by late gestation pregnancy loss. Puppies born live to infected bitches may have low birth weight and poor weight gain. Infected bitches also may have chronic vaginal discharge. Male dogs infected with *B. canis* show epididymitis and a decline in semen quality. Both male and female dogs with brucellosis may be asymptomatic. Brucellosis testing usually is done with serology. Agglutination tests are good screening tests because they are sensitive, virtually never yielding a false-negative result. Both tube agglutination tests, which yield a titer, and slide or card agglutination tests, which yield a yes or no response, are available. Agglutination tests are not specific, however, so a positive result on an agglutination test always should be reevaluated with the agar gel immunodiffusion test available at Cornell University. *B. canis* is not curable in dogs, but titers can be decreased and

shedding of organisms reduced with antibiotic therapy and neutering of the infected animal.

Subclinical uterine infection: Aerobic bacteria and mycoplasma that are part of the normal vaginal flora have been implicated in pregnancy loss in dogs. Diagnosis requires culture of vaginal discharge from the bitch and stomach content or organ tissue from an aborted fetus.

- Noninfectious causes of pregnancy loss include the following:

Hypoluteoidism: Hypoluteoidism is inadequate progesterone secretion from luteal tissue during pregnancy. Insufficiency of corpora lutea may predispose the bitch to premature labor as serum progesterone concentrations fall too early in gestation. Serum progesterone concentration must be greater than 2 ng/ml to support pregnancy. Routine supplementation with progestogens is not recommended during pregnancy because excessive progesterone may masculinize female fetuses and prevent expulsion of nonviable fetuses. If serum progesterone concentrations are documented to decrease too early in gestation, supplementation may be instituted with progesterone in oil

(2 mg/kg administered intramuscularly every 3 days) or ally-trenbolone (Regumate; Hoechst-Roussell, Kansas City, Mo.; 0.088 mg/kg administered orally once daily) to no later than 61 days after ovulation. Serum progesterone concentrations must decline before labor is initiated in bitches; maintenance of pregnancy beyond 65 days after ovulation by artificial supplementation with progesterone causes fetal death.

7. Semen quality is normal. (Proceed to 8.)

7′. Semen quality is not normal. (Proceed to 9.)

8. In a bitch with no reproductive tract disease bred to a male with normal semen quality in which pregnancy does not occur, poor breeding management is the most likely problem. *Improper timing of breeding is the most common cause of apparent infertility in dogs.* Breeding management is optimized with measurement of progesterone in serum to pinpoint ovulation time within the bitch's estrous cycle. Serum progesterone concentration is 5-8 ng/ml on ovulation day, and the optimal breeding day in bitches is 2 days after ovulation (see Chapter 2).

9. Semen quality, although abnormal, is adequate to effect pregnancy. (Proceed to 10.)

   A minimum of 250 million normal spermatozoa per ejaculate must be introduced into the

bitch's reproductive tract to reliably effect pregnancy. Male dogs with abnormal motility or morphology of spermatozoa and a high total number of spermatozoa in the ejaculate may still be capable of effecting pregnancy. For example, a male with 1 billion spermatozoa in his ejaculate, only 50% of which are morphologically normal, still has 500×10^6 morphologically normal spermatozoa, which is well above the 250×10^6 required. Optimization of conception rate by breeding 2 days after ovulation, as assessed by serial measurement of serum progesterone concentrations, is strongly recommended when using a subfertile male dog.

9′. Semen quality is insufficient to effect pregnancy.

- *Normal semen quality* is defined as 300 million to 2 billion spermatozoa in the ejaculate, with more than 70% of the spermatozoa exhibiting progressive motility and more than 80% of the spermatozoa being normal in morphology.
- *Azoospermia* is absence of spermatozoa in the ejaculate. Causes may be pretesticular, testicular, or posttesticular. Pretesticular causes include apprehension at the time of semen collection or breeding; administration of drugs that impair spermatogenesis, such as high dosages of glucocorticoids or antineoplastic agents; hypothyroidism (see 1′); and

inguinal or scrotal hernia. Diagnosis of incomplete ejaculation as a result of apprehension may be made based on measurement of alkaline phosphatase in seminal fluid. Alkaline phosphatase in semen arises from the cauda epididymis; concentrations of less than 5000 IU/L in seminal fluid suggest that no fluid from the epididymis is present in the seminal fluid. Measurement of alkaline phosphatase in seminal fluid cannot differentiate incomplete ejaculation from obstructive causes of azoospermia. Testicular causes include intersex states; bilateral cryptorchidism; any thermal or physical insult to the testicular tissue, such as high fever or trauma occurring during the 2 months or more preceding lack of spermatozoa in the ejaculate; and testicular neoplasia (see Chapter 16). Posttesticular causes include sources of epididymal occlusion, such as spermatocele or sperm granulomas. Epididymal occlusion may be visualized during scrotal ultrasonography.

- *Oligozoospermia* is presence of fewer than 300 million spermatozoa in the ejaculate. Apparent oligozoospermia may occur in dogs with retrograde ejaculation, which is movement of spermatozoa into the urinary bladder during ejaculation instead of through the

penile urethra. The cause of retrograde ejaculation in dogs is unknown. Diagnosis is made based on a collection of urine by cystocentesis after ejaculation, with demonstration of an abnormally large number of spermatozoa in the urine sediment. Antegrade ejaculation can be effected by administration of the sympathomimetic drug, pseudoephedrine (4-5 mg/kg administered orally 1 and 3 hours before breeding). Teratozoospermia is presence of less than 80% morphologically normal spermatozoa in the ejaculate. Asthenozoospermia is presence of less than 70% progressively motile spermatozoa in the ejaculate. Oligozoospermia, teratozoospermia, and asthenozoospermia often occur concurrently. Causes include infection of the reproductive tract, localized either in the prostate (see Chapter 17) or testes (see Chapter 16) and including canine brucellosis (see 6′), testicular neoplasia (see Chapter 16), and hypothyroidism (see 1′).

10. A diagnosis of idiopathic infertility must be made.

Infertility in the Cat

1. The queen is cycling normally. (Proceed to 2.)

1′. The queen is not cycling.

Causes for lack of estrous cycling in queens include the following:

- *Sexual immaturity:* Queens go through puberty at 5-10 months of age, with longhaired breeds entering puberty later than shorthaired breeds. Cats will not enter puberty during the seasonal anestrus, from mid-October through December at temperate latitudes.
- *Previous ovariohysterectomy:* Previous ovariohysterectomy may be determined based on history, palpation or observation of an ovariohysterectomy scar at the ventral abdominal midline, or exploratory laparotomy. In spayed bitches, serum concentrations of the gonadotropins LH and FSH are elevated because of lack of negative feedback from the absent ovary to the anterior pituitary. There are no commercially available assays for feline LH and FSH.
- *Inadequate environmental stimuli:* Cats housed with 10 hours or more of continuous light daily cycle year round. Those housed with minimal lighting may enter a prolonged anestrus. Queens housed with or exposed to a tom may be more likely to show signs of estrus or may be more visible to the owner when in estrus because of the change in behavior of the tom.
- *Systemic illness of the queen:* Queens that have a systemic disease, such as diabetes mellitus, or a

chronic complaint, such as vomiting, may be less likely to cycle. Similarly, medications the cat has received in the past, such as those used for estrus suppression and pregnancy termination, may have affected her fertility (see Chapter 5). Lack of cycling has been reported to occur in cats with feline leukemia virus.

- *Silent heat:* Apparent lack of cycling may occur in queens with silent heat. Silent heat is defined as normal ovarian follicular development in the absence of estrous behaviors. Silent heat may be more common in cats low in the social hierarchy. Collection of weekly vaginal cytology specimens permits assessment of the cornification of vaginal epithelial cells associated with estrus. Queens diagnosed in estrus by cytology stand for breeding and are fertile.
- *Chromosome abnormalities:* True lack of cycling has been reported in cats with karyotypic abnormalities (38,XO) and in male pseudohermaphrodites (retained testes and female external genitalia) (see Chapter 1).
- *Miscellaneous causes:* Older queens may cycle less frequently. Lack of cycling after ovulation induction without conception (false pregnancy; see Chapter 2) and while nursing (lactational anestrus) is normal in cats.

2. Normal copulation is occurring. (Proceed to 3.)
2′. Normal copulation is not occurring. (Proceed to 4.)
3. The male mounts and achieves intromission. (Proceed to 5.)
3′. The male cannot mount and achieve intromission.

Causes for inability to mount and achieve intromission include the following:

- *Presence of a penile hair ring:* Hair is compacted at the base of the penis. This is easily diagnosed via inspection and usually is easily removed.
- *Apprehension:* Tom cats should be bred in their home territory and may benefit from socialization with the queen in the days before breeding.
- *Physical causes of poor libido and inability to mount:* Causes include pain of the spine or rear limbs, malnutrition, and obesity.

4. The queen allows the male to mount. (Proceed to 3.)
4′. The queen will not allow the male to mount.

A vaginal cytology specimen should be evaluated to determine whether the queen is in estrus, with cornification of vaginal epithelial cells indicative of elevated serum estrogen concentrations and presence of an ovarian follicle.

5. The queen is induced to ovulate (evidenced by elevation in serum progesterone concentration to greater than 2 ng/ml by 7 days after breeding). (Proceed to 6.)

5′. The queen is not induced to ovulate.

The amount of LH released depends on the number of copulations and the time during the estrous cycle when copulation occurs. Less than 50% of queens bred only once ovulate. More than four copulations during a given estrus are required to ensure LH release adequate for ovulation. Queens that cannot be induced to ovulate by copulation may be treated with GnRH (25 μg administered intramuscularly 12-24 hours after breeding). Physical stimulation of the vagina with a cotton-tipped swab or glass rod does not reliably induce ovulation in queens.

6. The queen conceives after copulation.* (Proceed to 7.)

6′. The queen does not conceive after copulation.* (Proceed to 8.)

*Cats do not produce any hormone unique to pregnancy, precluding pregnancy diagnosis early in gestation. Therefore lack of conception cannot be differentiated from early embryonic death. Conception is assumed to have occurred if the queen was bred an adequate number of times by a known fertile tom.

7. Pregnancy loss occurs after conception.
   - Loss of pregnancy near midgestation may not be clinically evident because the queen may resorb the fetal tissues. Abortion after midgestation usually is accompanied by discharge of fluid or fetal tissues from the vulva, although the occasional queen will resorb late in gestation with no clinical signs of pregnancy loss.
   - Causes of pregnancy loss in the cat include the following:

     Luteal insufficiency: Feline abortion may be caused by abnormally low serum progesterone concentrations. Insufficiency of the corpora lutea or possible placental sources of progesterone may predispose the queen to premature labor because serum progesterone concentrations fall too early in gestation. Serum progesterone concentrations must be greater than 2 ng/ml to support pregnancy. Routine supplementation with progestogens is not recommended during pregnancy because excessive progesterone may masculinize female fetuses, prevent expulsion of nonviable fetuses, and induce diabetes mellitus and mammary abnormalities. If serum progesterone concentrations are documented to decline too quickly during gestation, supplementation with

megestrol acetate (Ovaban; Schering-Plough, Kenilworth, NJ; 2.5 mg administered orally every other day through day 55 of pregnancy) can be attempted.

Bacterial infection: Bacterial infection of the uterus and subsequent pregnancy loss is uncommon in healthy cats housed in a clean environment. Cats aborting because of intrauterine infection with *Escherichia coli, Staphylococcus* species, and *Streptococcus* species usually have anorexia; depression; fever; straining; and fetid, yellow-brown vaginal discharge. A sample of the vaginal discharge should be collected for culture and sensitivity, and empirical treatment with amoxicillin (20 mg/kg administered orally twice a day) should be instituted pending culture results. Radiography or ultrasonography may be necessary to ensure complete expulsion of uterine contents. Bacterial abortion may occur in cats infected with feline leukemia virus (FeLV) secondary to viral immunosuppression.

Protozoal infection: Toxoplasmosis is a protozoal cause of abortion in the queen. Although most queens infected with toxoplasmosis are asymptomatic carriers, those that become systemically ill may abort and may show dys-

pnea, lethargy, diarrhea, lymphadenopathy, and central nervous system disturbance. Abortion occurs secondary to debilitating disease of the queen. Toxoplasmosis is an uncommon cause of feline abortion.

Viral infection: Viral infections that may cause abortion in queens include feline herpesvirus (FHV; rhinotracheitis), panleukopenia, feline infectious peritonitis (FIP), and FeLV. Abortion secondary to FHV usually occurs at 5-6 weeks of gestation and is most likely a nonspecific reaction to the upper respiratory infection induced because the virus has not been demonstrated in aborted placental or fetal tissues in cats inoculated with FHV. This virus is easily controlled with vaccination. Cats entering the cattery from outside should be vaccinated well before entry. The intranasal vaccine can safely be used in kittens as young as 3 weeks. Panleukopenia infection can cause abortion, stillbirths, and cerebellar hypoplasia in kittens infected in utero, with or without classic gastrointestinal signs in the queen. The organism is shed in all body secretions from infected animals but is easily killed by bleach. Vaccination of all animals in the cattery and any animals entering the

cattery should be required. Pregnant queens should not be vaccinated with the modified live virus vaccine. Abortion resulting from infection with FIP occurs late in gestation and often is associated with prolonged vaginal bleeding. The disease is difficult to diagnose with serologic testing because titers of the coronavirus causing FIP are indistinguishable from those of the nonpathogenic enteric coronavirus. Control involves good hygiene and avoiding use of communal feeding dishes. Inbreeding increases susceptibility to infection with FIP; removal of adults that sire offspring that become infected may be warranted in problem catteries. FeLV has been reported to cause abortion from 3 weeks of pregnancy to term. Because FeLV requires intimate contact between animals for spread, testing and removal of infected animals constitute an effective method of control.

8. The male has normal semen quality. (Proceed to 9.)

8′. The male does not have normal semen quality.

- Semen quality is difficult to assess in cats because of difficulty in obtaining a semen sample. Gross evaluation of semen quality may be attempted by collecting a vaginal cytology

specimen from the queen immediately after breeding. Retrograde ejaculation, movement of spermatozoa into the urinary bladder during ejaculation, occurs in normal toms. Collection of a urine sample by cystocentesis immediately after breeding, with centrifugation and examination of the sediment, also may allow gross evaluation of semen quality. Electroejaculation also may be performed; this requires general anesthesia and use of specialized equipment.

- Causes for abnormal semen quality in tom cats include the following:

  Karyotype abnormalities (calico and tortoiseshell male cats): Calico (black, white, and orange) and tortoiseshell (black and orange or gray and cream) male cats often are infertile. White coat color is carried on an autosome. The gene for black or orange coat color is carried on the X chromosome. Normal male cats, which have a single X chromosome, can exhibit either black or orange, but not both. Calico and tortoiseshell male cats must have two X chromosomes, suggesting either triploidy (39,XXY) or mosaicism/chimerism (XX/XY, XY/XY, XXY/XY). Azoospermia (absence of spermatozoa in the ejaculate) often accompanies this abnormal karyotype.

Testicular atrophy: Poor semen quality may be caused by malnutrition, hypervitaminosis A, elevated testicular or intrascrotal temperature secondary to fever or scrotal trauma, or advancing age.

9. A diagnosis of idiopathic infertility must be made.

Bibliography: Canine Infertility

Arbeiter K: Anovulatory ovarian cycles in dogs, *J Reprod Fertil Suppl* 47:453, 1993.

Bouchard GF, Solorzano N, Concannon PW et al: Determination of ovulation time in bitches based on teasing, vaginal cytology and ELISA for progesterone, *Theriogenology* 135:603, 1991.

Eilts BE: Pregnancy maintenance in the bitch using Regumate. Proceedings of the Society for Theriogenology, San Antonio, Tx, 1992.

Ellington JE: Diagnosis, treatment, and management of poor fertility in the stud dog, *Semin Vet Med Surg* 9:46, 1994.

England GC: Ultrasonographic diagnosis of nonpalpable Sertoli cell tumours in infertile dogs, *J Small Anim Pract* 36:476, 1995.

Evermann JF: Diagnosis of canine herpetic infections. In Kirk RW (ed): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Fontbonne A, Siliart B, Badinand F: Hormonal findings in dogs and bitches showing reproductive disorders, *J Reprod Fertil Suppl* 47:553, 1993.

Frenette G, Dube JY, Tremblay RR: Origin of alkaline phosphatase of canine seminal plasma, *Arch Androl* 16:235, 1986.

Freshman JL: Clinical approach to infertility in the cycling bitch, *Vet Clin North Am* 21:427, 1991.

Hashimoto A, Hirai K, Yamaguchi T et al: Experimental transplacental infection of pregnant dogs with canine herpesvirus, *Am J Vet Res* 43:844, 1982.

Jeffcoate IA: Gonadotrophin-releasing hormone challenge to test for the presence of ovaries in the bitch, *J Reprod Fertil Suppl* 47:536, 1993.

Jeffcoate IA: Identification of spayed bitches, *Vet Rec* 129:58, 1991.

Jeffcoate IA, McBride M, Harvey MJ et al: Measurement of plasma oestradiol after an injection of a gonadotrophin as a test for neutered bitches, *Vet Rec* 146:599, 2000.

Johnson CA: Reproductive manifestations of thyroid disease, *Vet Clin North Am* 24:509, 1994.

Johnson CA, Grace JA, Probst MR: Effects of maternal illness on perinatal health, *Vet Clin North Am* 16:555, 1987.

Johnson CA, Nachreiner RF, Mullaney TP et al: Reproductive manifestations of hypothyroidism, *Canine Pract* 22:29, 1997.

Johnson CA, Walker RD: Clinical signs and diagnosis of *Brucella canis* infection, *Comp Cont Ed* 14:763, 1992.

Johnston SD: Clinical approach to infertility in bitches with primary anestrus, *Vet Clin North Am* 21:421, 1991.

Johnston SD: Vaginal prolapse. In Kirk RW (ed): *Current veterinary therapy X,* Philadelphia, 1989, WB Saunders.

Johnston SD, Buoen LC, Weber AF et al: X trisomy in an Airedale bitch with ovarian dysplasia and primary anestrus, *Theriogenology* 24:597, 1985.

Johnston SD, Root MV: Serum progesterone timing of ovulation in the bitch. Proceedings of the Society for Theriogenology, San Antonio, Tx, 1995.

Kemppainen RJ: Effects of prednisone on thyroid and gonadal endocrine function in dogs, *J Endocrinol* 96:293, 1983.

Krawiec DR, Heflin D: Study of prostatic disease in dogs: 177 cases (1981-1986), *J Am Vet Med Assoc* 200:1119, 1992.

Kyles AE, Vaden S, Hardie EM et al: Vestibulovaginal stenosis in dogs: 18 cases (1987-1995), *J Am Vet Med Assoc* 209:1889, 1996.

Manning PJ: Thyroid gland and arterial lesions of beagles with familial hypothyroidism and hyperlipoproteinemia, *Am J Vet Res* 40:820, 1979.

Mickelsen WD, Memon MA, Anderson PB et al: The relationship of semen quality to pregnancy rate and

litter size following artificial insemination in the bitch, *Theriogenology* 39:553, 1993.

Nachreiner RF, Refsal KR: The Michigan State University thyroid function profile, *Canine Pract* 22:45, 1997.

Nesbitt GH, Izzo J, Peterson L et al: Canine hypothyroidism: a retrospective study of 108 cases, *J Am Vet Med Assoc* 177:1117, 1980.

Nichols R: Update: diagnostic testing for canine hypothyroidism. Proceedings of the American College of Veterinary Internal Medicine Forum, Lake Buena Vista, Fl, 1997.

Oettle EE, Soley JT: Sperm abnormalities in the dog: a light and electron microscopic study, *Vet Med Rev* 59:28, 1988.

Olson PN, Mulnix JA, Nett TM: Concentrations of luteinizing hormone and follicle-stimulating hormone in the serum or sexually intact and neutered dogs, *Am J Vet Res* 53:762, 1992.

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, 1992.

Olson PN, Seim HB, Park RD et al: Female pseudohermaphroditism in three sibling greyhounds, *J Am Vet Med Assoc* 194:1747, 1989.

Olson PN, Wrigley RH, Husted PW et al: Persistent estrus in the bitch. In Ettinger SJ (ed): *Textbook of veterinary internal medicine,* Philadelphia, 1989, WB Saunders.

Panciera DL: Hypothyroidism in dogs: 66 cases (1987-1992), *J Am Vet Med Assoc* 204:761, 1994.

Perkins NR, Thomas PG: Infertility in the bitch with normal oestrus cycles, *Aust Vet Pract* 23:77, 1993.

Peterson ME, Melian C, Nichols R: Measurement of serum total thyroxine, triiodothyronine, free thyroxine, and thyrotropin concentrations for diagnosis of hypothyroidism in dogs, *J Am Vet Med Assoc* 211:1396, 1997.

Post K, Barth AD, Kiefer UT et al: Retrograde ejaculation in a Shetland sheepdog, *Can Vet J* 33:53, 1992.

Purswell BJ: Pharmaceuticals used in canine reproduction, *Semin Vet Med Surg* 9:54, 1994.

Ramsey I, Herrtage M: Distinguishing normal, sick, and hypothyroid dogs using total thyroxine and thyrotropin concentrations, *Canine Pract* 22:43, 1997.

Reimers TJ: Endocrine testing for infertility in the bitch. In Kirk RW (ed): *Current veterinary therapy VIII,* Philadelphia, 1983, WB Saunders.

Rogers AL, Templeton JW, Stewart AP: Preliminary observations of estrous cycles in large, colony raised laboratory dogs, *Lab Anim Care* 26:1133, 1970.

Root MV, Johnston SD, Johnston GR: Vaginal septa in dogs: 15 cases (1983-1992), *J Am Vet Med Assoc* 206:56, 1995.

Root MV, Johnston SD, Olson PN: Concurrent retrograde ejaculation and hypothyroidism in a dog: case report, *Theriogenology* 41:593, 1994.

Rosychuk R: Management of hypothyroidism. In Kirk RW (ed): *Current veterinary therapy VIII,* Philadelphia, 1983, WB Saunders.

Sokolowski JH: The effects of ovariectomy on pregnancy maintenance in the bitch, *Lab Anim Sci* 21:696, 1971.

Sokolowski JH, Stover DG, VanRavenswaay F: Seasonal incidence of estrous and interestrous interval for bitches of seven breeds, *J Am Vet Med Assoc* 171:271, 1977.

Thacker EL: Etiology of adult-onset canine autoimmune hypothyroidism, *Canine Pract* 22:12, 1997.

Tsutsui T: Effects of ovariectomy and progesterone treatment on the maintenance of pregnancy in bitches, *Nippon Juigaku Zasshi* 45:47, 1983.

Wykes PM, Soderberg SF: Congenital abnormalities of the canine vagina and vulva, *J Am Anim Hosp Assoc* 19:995, 1983.

Bibliography: Feline Infertility

Boomsma RA, Mavrogianis PA, Verhage HG: Changes in endometrial and placental protein synthesis and morphology during pregnancy and pseudopregnancy in the cat, *Biol Reprod* 44:345, 1991.

Chakraborty PK, Wildt DE, Seager SW: Serum luteinizing hormone and ovulatory response to luteinizing hormone-releasing hormone in the estrous and anestrous domestic cat, *Lab Anim* 29:338, 1979.

Cline EM, Jennings LL, Sojka NJ: Analysis of the feline vaginal epithelial cycle, *Feline Pract* 10:47, 1980.

Concannon P, Hodgson B, Lein D: Reflex LH release in estrous cats following single and multiple copulation, *Biol Reprod* 23:111, 1980.

Dawson AB: Early estrus in the cat following increased illumination, *Endocrinology* 28:907, 1941.

Dieter JA, Stewart DR, Haggarty MA et al: Pregnancy failure in cats associated with long-term dietary taurine insufficiency, *J Reprod Fertil Suppl* 47:457, 1993.

Dow C: The cystic hyperplasia-pyometra complex in the cat, *Vet Rec* 74:141, 1962.

England GC: Pregnancy diagnosis, abnormalities of pregnancy and pregnancy termination. In Simpson GM, England GC, Harvey M (eds): *BSAVA manual of small animal reproduction and neonatology,* Cheltenham, England, 1998, British Small Animal Veterinary Association.

Goodrowe KL, Howard JG, Schmidt PM et al: Reproductive biology of the domestic cat with special reference to endocrinology, sperm function and in vitro fertilization, *J Reprod Fertil Suppl* 39:73, 1989.

Herron MA: Infertility from noninfectious causes. In Morrow DA (ed): *Current therapy in theriogenology,* Philadelphia, 1986, WB Saunders.

Hunter Banks D, Stabenfeldt G: Luteinizing hormone release in the cat in response to coitus on consecutive days of estrus, *Biol Reprod* 26:603, 1982.

Hurni H: Daylength and breeding in the domestic cat, *Lab Anim* 15:229, 1981.

Jemmett JE, Evans JM: A survey of sexual behavior and reproduction in female cats, *J Small Anim Pract* 18:31, 1977.

Johnson LM, Gay VL: Luteinizing hormone in the cat. II. Mating-induced secretion, *Endocrinology* 109:247, 1981.

Johnston SD: Premature gonadal failure in female dogs and cats, *J Reprod Fertil Suppl* 39:65, 1989.

Johnston SD, Root MV: Managing infertility in purebred catteries. In August JR (ed): *Consultations in feline internal medicine,* Philadelphia, 1997, WB Saunders.

Keenan LR: The infertile male. In Simpson GM, England GC, Harvey M (eds): *BSAVA manual of small animal reproduction and neonatology,* Cheltenham, England, 1998, British Small Animal Veterinary Association.

Lawler DF: Diagnosis and treatment of inflammatory uterine disease in cats, *Vet Med* August:750, 1998.

Lawler DF, Evans RH: Strategies for controlling viral infections in feline populations. In August JR (ed): *Consultations in feline internal medicine,* Philadelphia, 1997, WB Saunders.

Leyva H, Madley T, Stabenfeldt GH: Effect of light manipulation on ovarian activity and melatonin and prolactin secretion in the domestic cat, *J Reprod Fertil Suppl* 39:125, 1989.

McKinney HR: A study of toxoplasma infections in cats, *Vet Med Small Anim Clin* 68:493, 1973.

Michel B: Induction of oestrus in cats by photoperiodic manipulations and social stimuli, *Lab Anim* 27:278, 1993.

Petrak M, Carpenter J: Feline toxoplasmosis, *J Am Vet Med Assoc* 146:728, 1965.

Potter K, Hancock DH, Gallina AM: Clinical and pathologic features of endometrial hyperplasia, pyometra, and endometritis in cats: 79 cases (1980-1985), *J Am Vet Med Assoc* 198:1427, 1991.

Povey RC: Reproduction in the pedigree female cat: a survey of breeders, *Can Vet J* 19:207, 1978.

Root MV, Johnston SD, Olson PN: Estrous length, pregnancy rate, gestation and parturition lengths, litter size, and juvenile mortality in the domestic cat, *J Am Anim Hosp Assoc* 31:429, 1995.

Roth TL, Munson L, Swanson WF et al: Histological characteristics of the uterine endometrium and corpus luteum during early embryogenesis and the relationship to embryonic mortality in the domestic cat, *Biol Reprod* 53:1012, 1995.

Sanchez B, Pizarro M, Garcia P et al: Histological study of Leydig cells in the cat from birth to sexual maturity, *J Reprod Fertil Suppl* 47:349, 1993.

Sanchez B, Pizarro M, Garcia P et al: Postnatal development of seminiferous tubules in the cat, *J Reprod Fertil Suppl* 47:343, 1993.

Scott PP: Cats. In Hafez ES (ed): *Reproduction and breeding techniques for laboratory animals,* Philadelphia, 1970, Lea & Febiger.

Scott PP, Lloyd-Jacobs MA: Reduction in the anestrus period of laboratory cats by increasing illumination, *Nature* 184:2022, 1959.

Shille VM, Munro C, Walker Farmer S et al: Ovarian and endocrine responses in the cat after coitus, *J Reprod Fertil* 68:29, 1983.

Sturman JA: Effects of taurine on feline reproduction, *Vet Tech* 13:166, 1992.

Swanson WF, Roth TL, Brown JL et al: Relationship of circulating steroid hormones, luteal luteinizing hormone receptor and progesterone concentration, and embryonic mortality during early embryogenesis in the domestic cat, *Biol Reprod* 53:1022, 1995.

Thomas TN, Hall JA: Infertility in the bitch and queen, *Kal Kan Forum* Spring:4, 1986.

Troy GC, Herron MA: Infectious causes of infertility, abortion and stillbirths in cats. In Morrow DA (ed): *Current therapy in theriogenology,* Philadelphia, 1986, WB Saunders.

Umstead JA: Infertility in the male dog and cat, *Kal Kan Forum* Summer:4, 1986.

Verstegen JP: Pharmacological control of reproduction in the cat. In Simpson GM, England GC, Harvey M (eds): *BSAVA manual of small animal reproduction and*

neonatology, Cheltenham, England, 1998, British Small Animal Veterinary Association.

Verstegen JP, Silva LD, Onclin K et al: Echocardiographic study of heart rate in dog and cat fetuses in utero, *J Reprod Fertil Suppl* 47:175, 1993.

Wildt DE, Seager SW, Chakraborty PK: Effect of copulatory stimuli on incidence of ovulation and on serum luteinizing hormone in the cat, *Endocrinology* 107:1212, 1980.

Wright PJ, Watts JR: The infertile female. In Simpson GM, England GC, Harvey M (eds): *BSAVA manual of small animal reproduction and neonatology*, Cheltenham, England, 1998, British Small Animal Veterinary Association.

20

Emerging Technologies in Small Animal Theriogenology

Richard Fayrer-Hosken

AT A GLANCE

- *Xenotransplantation* is transfer of oocytes from one species into a unique location, such as beneath the kidney capsule, of another species. This technique may prove useful in endangered species. It has not yet

been performed in dogs; cat tissue has been transferred successfully into mice.

- *In vitro fertilization* (IVF) is formation of a zygote by coincubation of capacitated spermatozoa and a mature oocyte outside of the body, with successful penetration of one spermatozoon and development of a viable conceptus. In the dog IVF and subsequent embryo transfer have resulted in, at most, a 22-day pregnancy. In the cat live kittens have been born after IVF and embryo transfer.
- *Intracytoplasmic sperm injection* (ICSI) is introduction of one capacitated spermatozoon into the cytoplasm of a mature oocyte. No puppies have been produced with ICSI, but several litters of kittens have been born.
- *Embryo transfer* is placement of an embryo into a surrogate dam in which normal gestation occurs. Embryos can be recovered by flushing the uterus of a bred bitch or queen or by using the in vitro techniques described earlier. Limited success has been achieved with embryo transfer in dogs; success has been greater in cats.
- *Cloning* of cats has been successful, but cloning of dogs has not yet been reported.

The emerging technologies of assisted reproduction encompass a wide range of potential manipulations to produce kittens or puppies from reproductively compromised females or males. Although there are multiple reports of success with assisted reproductive

technologies in farm animal and laboratory animal species, gamete and embryo manipulation have been less successful in cats, with an even worse reported success rate in dogs. There are significant and inherent differences between the physiology of the canine and feline species that probably explain the differences. However, it is my personal feeling that attainment of mastery of the unrelenting portions of canine gamete physiology is only a matter of time.

The goal of any of these technologies is to provide an avenue for fertility salvage in the face of catastrophic reproductive insufficiencies or damage. The argument can be submitted that this is a valid and laudable endeavor. However, I think it is important that we never lose sight of the fact that a significant amount of small animal breeding involves strategies that lead to genetic convergence (inbreeding) rather than increased genetic vigor (outcrossing). As advocates of the new technologies, we must always strive to ensure the optimal health of the animals involved.

Oocyte Physiology

Oocyte Morphology

Canine and feline oocytes have a dense cytoplasm bound by a plasma membrane. The cytoplasm contains the nucleus, or condensed chromosomes, and various

organelles. While the oocyte is within the ovary, the Golgi apparatus plays an important role in yolk synthesis and formation of cortical granules. The cortical granules are a key factor in preventing polyspermy after fertilization. The presence of marginated cortical granules along the plasma membrane also is an indication of cytoplasmic maturity. Spindles of microtubules form during meiosis and mitosis to aid in the alignment of chromosomes. Surrounding cumulus cells provide nourishment to the oocyte and also interact with oviductal cilia while the egg is being transported through the uterine tube after ovulation.

Morphologic changes of the canine oocyte during different stages of the estrous cycle may vary with ability of the canine oocyte to communicate with cumulus cells. The gap junctions between the oocyte and cumulus cells are involved in the regulation of oocyte meiotic differentiation and maturation. Microinjection of 3% Lucifer Yellow into canine oocytes collected during different stages of the estrous cycle allowed evaluation of cumulus-oocyte gap junction communications. Of the oocytes collected during late proestrus, 89% (16 of 18 oocytes) exhibited functional cumulus-oocyte communications through gap junctions. This was in contrast to 0% (0 of 20 oocytes) for oocytes collected during anestrus.

Oocytes collected from ovaries are graded on a scale of 1 to 3, with grade 1 being darkly pigmented and

completely surrounded by one or more layers of cumulus cells (Figure 20-1); grade 2 being lightly pigmented with incomplete layers of cumulus cells; and grade 3 being degenerate with pale color, abnormal shape, and no attached cumulus cells.

Oocyte Maturation

IN VIVO MATURATION For the two cycles of the meiotic cell division of an oocyte, there are four phases: prophase, metaphase, anaphase, and telophase. Interphase is the time between cell cycles and is when DNA replication occurs. Prophase of the first meiotic division is divided into five stages: leptotene, zygotene, pachytene, diplotene, and diakinesis. In most species the oocyte reaches metaphase II (MII) before ovulation. However, this is not the case in the dog and fox. Canidae ovulate a primary oocyte, which contains an intact germinal vesicle and matures to a fertilizable secondary (MII) oocyte while in the uterine tube.

IN VITRO MATURATION In vitro maturation (IVM) of oocytes involves collection of immature oocytes from the ovary with subsequent culture to support maturation until the oocyte reaches a state at which it can be fertilized. A number of studies have been reported describing maturation of canine oocytes collected

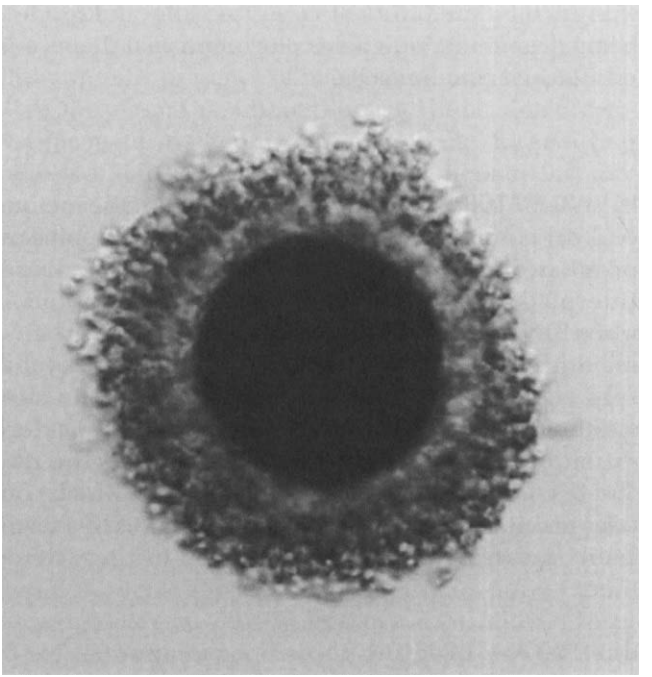


Figure 20-1. Grade 1 canine oocyte at 100× magnification, with a darkly pigmented cytoplasm completely surrounded by one or more layers of cumulus cells.

from ovaries at different stages of the estrous cycle. Media supplemented with estradiol, progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), equine chorionic gonadotropin, L-cysteine, and arachidonic acid did not significantly affect maturation rate of canine oocytes compared with controls. Maturation rates for oocytes cultured in any of several treatments with recombinant FSH were statistically significant when describing success of IVM, but very few MII oocytes were obtained (Figure 20-2). Superovulation, induction of ovulation of a larger number of eggs than is usual for the species, has been attempted in dogs and cats to optimize the number of oocytes retrieved. Neither superovulation nor IVM protocols have been very successful in dogs. In cats IVM of oocytes has been shown to be very successful, as have superovulation protocols.

Xenotransplantation

Xenotransplantation might provide some glimmer of hope in cases in which the female dog or cat is terminally ill and it is imperative that oocytes are conserved. This protocol involves the collection of ovarian cortex with transplantation to a unique location in another species. Research to date has involved implantation of oocytes from a reproductively desirable animal to beneath the kidney capsule of another animal, where

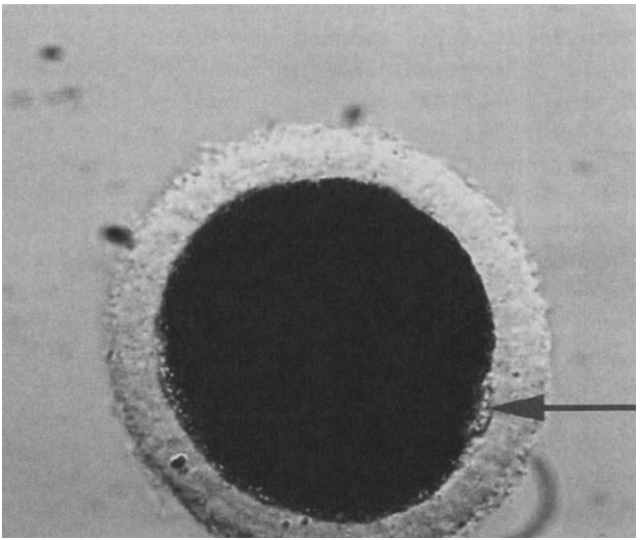


Figure 20-2. In vitro matured canine oocyte at the metaphase II stage, matured in TCM199 medium with recombinant follicle-stimulating hormone.

the oocytes would be protected and potentially viable after vascularization. No xenotransplantation of canine ovarian tissue has been performed, but it has been demonstrated that in the cat, xenografts to mice can lead to follicle development.

Innovative Technologies

In Vitro Fertilization

For performance of IVF, spermatozoa are capacitated in vitro and added to MII oocytes. Under the appropriate conditions a spermatozoon fertilizes an oocyte and produces a viable embryo. In the dog success with IVF has been limited. There is one report of a 22-day pregnancy; in that study bitches were induced to enter proestrus by treatment with cabergoline (see Chapter 2). Oocytes were retrieved from the dogs' ovaries by ovariohysterectomy at the time of ovulation and cultured in vitro in TCM199 medium containing 0.3% serum albumin for 24-78 hours. Capacitated spermatozoa were added to the oocytes at 12 hours, and the two cell types were cocultured for 48 hours. At that time, 2 of 90 oocytes had developed to the two-cell stage of embryogenesis. All embryos/oocytes were surgically transferred to the uterine lumen of a bitch during early diestrus. Ultrasonographic pregnancy evaluation was performed 20 days after embryo transfer; one conceptus was in the proximal region of the uterine body, and two concepti were in the left uterine horn. All concepti were considered to be undersized for their anticipated age. Ultrasonographic examination performed 2 days later showed that all the concepti were absent. In the cat IVF and subsequent embryo transfer have resulted in the birth of live kittens.

Intracytoplasmic Sperm Injection

ICSI is introduction of one capacitated spermatozoon into the cytoplasm of a mature oocyte. ICSI generally has been successful in species other than the dog and cat; it is a primary tool in human infertility treatment.

Although some success has been achieved with ICSI in the dog, no puppies have been produced by this technology. Ovaries are obtained via ovariohysterectomy, sectioning the ovarian cortex, incubating the tissue in an enzymatic solution containing collagenase (500 IU/ml) and DNase (75 IU/ml), and filtering to retrieve oocytes. The oocytes are graded, and only grade 1 oocytes are placed in culture. The oocytes are matured in TCM199 medium with penicillin, streptomycin, FSH, LH, and fetal calf serum at 38° C with 5% carbon dioxide (CO_2) and 100% humidity for 72 hours. In one study, 82 (38.5%) of 213 oocytes reached the MII stage. These oocytes were injected with spermatozoa; only 6 (7%) of 82 oocytes injected showed evidence of pronucleus formation, indicating successful fertilization.

ICSI in cats has proved to be far more successful, with the production of several litters. Oocytes were matured in vivo, retrieved from the uterus, fertilized as described earlier, and cultured to the morula (16 cell) stage before embryo transfer.

Embryo Transfer

Embryo transfer is placement of an embryo into a surrogate dam in which normal gestation occurs. Embryos can be recovered by flushing the uterus of a bred bitch or queen or by the in vitro techniques described earlier. Surrogate dams are synchronized with the administration of reproductive hormones, such that the embryos are placed within their uterus in early diestrus, as would occur naturally. Embryo transfer, using embryos created either in vivo or in vitro, has been successful in the dog, but with only a few live pups born to date. For the cat, as with the other technologies described, greater success has been reported. Kittens have been produced by embryo transfer with embryos created either in vivo or in vitro and by transfer either of fresh embryos or embryos that had been frozen and thawed. Embryo transfer is not yet a clinical reality for dogs and cats, but work extrapolated from these species has been used in endangered felid and canid species to good effect.

Cloning

To date, cloning of individuals has been successful in sheep, cattle, goats, mice, pigs, and cats. No live cloned puppies have been produced. In all cases cloning has been an extremely inefficient process, with a 1%-4% success rate despite a significant and focused volume of research.

Which techniques can be practically achieved and which techniques are not realistic for fertility salvage and offspring production? In the dog semen freezing (see Chapter 3) and standard embryo transfer have realistic expectations of success. However, IVF, xenotransplantation, freezing of oocytes, and cloning are not realistic technologies. In the cat semen freezing, embryo transfer, IVF, ICSI, and cloning are functional and realistic technologies.

Bibliography

Barber MR, Lee SM, Steffens WL et al: Immunolocalization of zona pellucida antigens in the ovarian follicle of dogs, cats, horses and elephants, *Theriogenology* 55:1705; 2001.

Bogliolo L, Leoni G, Ledda S et al: Intracytoplasmic sperm injection of in vitro matured oocytes of domestic cats with frozen-thawed epididymal spermatozoa, *Theriogenology* 56:955, 2001.

Bolamba D, Borden-Russ DK, Durrant BS: In vitro maturation of domestic dog oocytes cultured in advanced preantral and early antral follicles, *Theriogenology* 49:33, 1998.

Durrant BS, Pratt NC, Russ KD et al: Isolation and characterization of canine advanced preantral and early antral follicles, *Theriogenology* 49:917, 1998.

England GC, Verstegen JP, Hewitt DA: Pregnancy following in vitro fertilisation of canine oocytes, *Vet Rec* 148:20, 2001.

Farstad W: Reproduction in foxes: current research and future challenges, *Anim Reprod Sci* 53:35, 1998.

Fulton R, Durrant B, Keskintepe L et al: Assisted reproductive techniques for the treatment of canine infertility, *Soc Theriogenol Newsletter* 21:1, 1998.

Gomez MC, Pope CE, Harris R et al: Births of kittens produced by intracytoplasmic sperm injection of domestic cat oocytes matured in vitro, *Reprod Fertil Dev* 12:432, 2000.

Goodrowe KL, Howard JG, Wildt DE: Comparison of embryo recovery, embryo quality, oestradiol-17 beta and progesterone profiles in domestic cats *(Felis catus)* at natural or induced oestrus, *J Reprod Fertil* 82:553, 1988.

Goodrowe KL, Wall RJ, O'Brien SJ et al: Developmental competence of domestic cat follicular oocytes after fertilization in vitro, *Biol Reprod* 39:355, 1999.

Hewitt DA, England GC: The canine oocyte penetration assay: its use as an indicator of dog spermatozoal performance in vitro, *Anim Reprod Sci* 50:123, 1998.

Kinney GM, Pennycock JW, Schriver MD et al: Surgical collection and transfer of canine embryos, *Biol Reprod* 20(suppl 1):96A, 1979 (abstract).

Kraemer D, Kinney G, Schriver M: Embryo transfer in dogs and cats. Proceedings of the Embryo Transfer and in Vitro World Conference, 1989.

Luvoni GC, Luciano AM, Modina S et al: Influence of different stages of the oestrous cycle on cumulus-oocyte communications in canine oocytes: effects on the efficiency of in vitro maturation, *J Reprod Fertil Suppl* 57:141, 2001.

Otoi T, Murakami M, Fujii M et al: Development of canine oocytes matured and fertilised in vitro, *Vet Rec* 146:52, 2000.

Pope CE, Johnson CA, McRae MA et al: Development of embryos produced by intracytoplasmic sperm injection of cat oocytes, *Anim Reprod Sci* 53:221, 1998.

Shin T, Kraemer D, Pryor J et al: A cat cloned by nuclear transplantation, *Nature* 723:10, 2002.

Wilmut I, Schnieke AE, McWhir J et al: Viable offspring derived from fetal and adult mammalian cells, *Nature* 385:810, 1997.

Yamada S, Shimazu Y, Kawaji H et al: Maturation, fertilization, and development of dog oocytes in vitro, *Biol Reprod* 46:853, 1992.

Yamada S, Shimazu Y, Kawano Y et al: In vitro maturation and fertilization of preovulatory dog oocytes, *J Reprod Fertil Suppl* 47:227, 1993.

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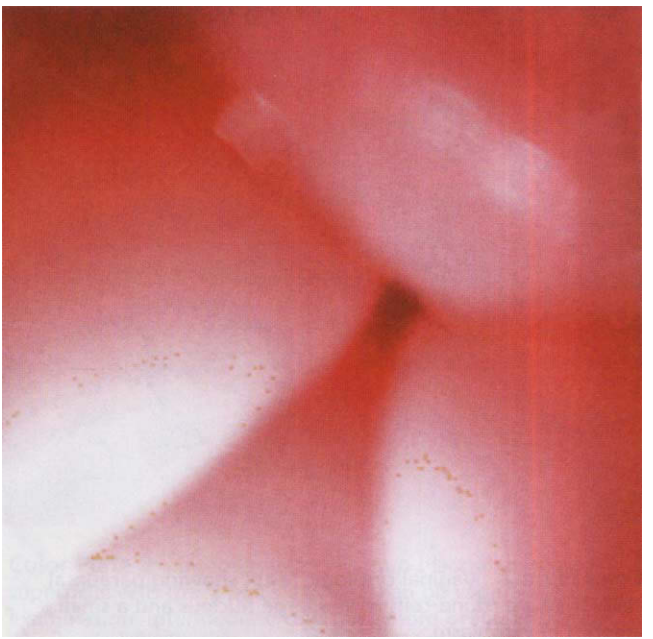
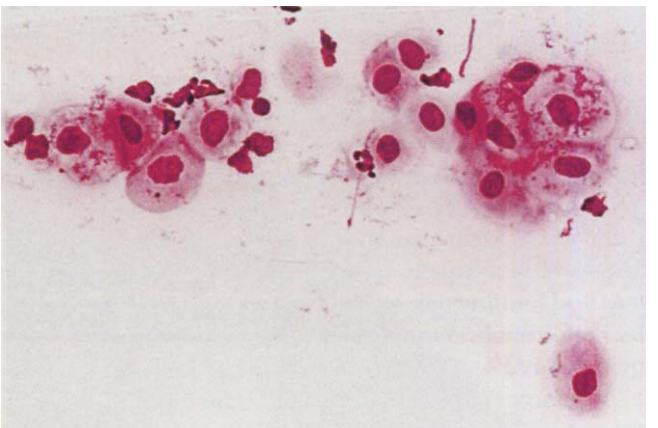
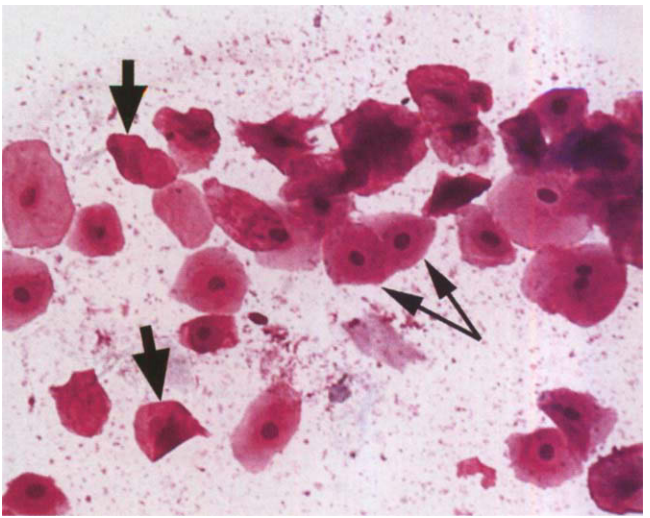


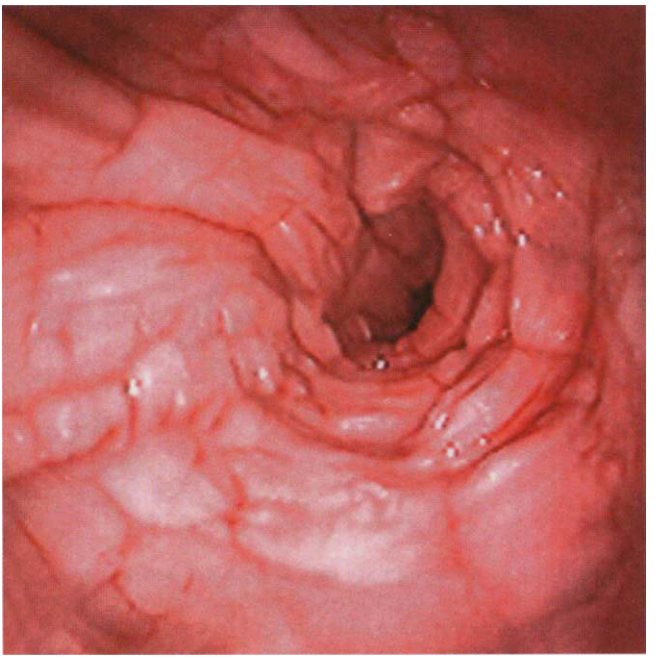
Color Plate 1 Endoscopic view of vaginal mucosal folds during proestrus. The folds are edematous, moist, and pink. The progesterone concentration of this bitch was less than 1 ng/ml.



Color Plate 2 Vaginal cytologic result showing parabasal cells. They are round cells with a large nucleus and a small amount of cytoplasm.



Color Plate 3 Intermediate cells *(thin black arrows)* and superficial cells *(thick black arrows)* seen on vaginal cytologic examination. Intermediate cells have more irregular borders than do parabasal cells, and they have a smaller nucleus and larger cytoplasm. Cornified superficial cells are dead vaginal epithelial cells. They have sharp, angular borders and contain a small, pyknotic nucleus or no visible nucleus.



Color Plate 4 Endoscopic view of the vagina of a bitch in estrus. The vaginal vault is crenulated. The serum progesterone of this bitch was 3.8 ng/ml.