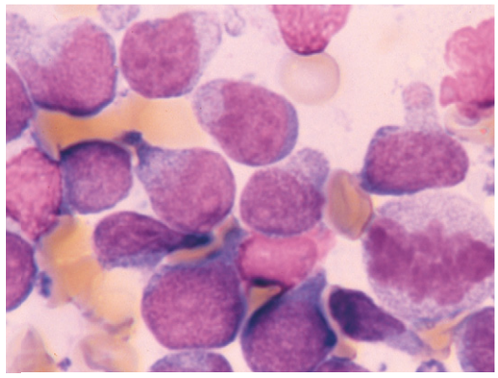
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.



CHAPTER 12

Reproductive System

Laia Solano-Gallego

FEMALE REPRODUCTIVE SYSTEM: MAMMARY GLANDS, OVARIES, UTERUS, AND VAGINA

Mammary Glands

Mammary gland lesions are common in female dogs and cats. Mammary gland enlargement may be related to a wide variety of disease processes, including cysts, inflammation, hyperplasia, and benign or malignant neoplasia. Important information in the investigation of mammary gland disease includes history, breed, and age; whether the gland was intact or older when the dog or cat was neutered; date of last estrus, pregnancy, or hormone therapy; size, number, and consistency of lesion(s); attachment to underlying tissue; rate of growth; presence of ulceration; and evidence of metastasis (Baker and Lumsden, 1999). Ancillary diagnostic tests used to evaluate mammary lesions include a thorough evaluation of health status involving a complete physical examination, complete blood count, serum biochemical profile, urinalysis and/or coagulation profile, imaging, cytology, and histopathology.

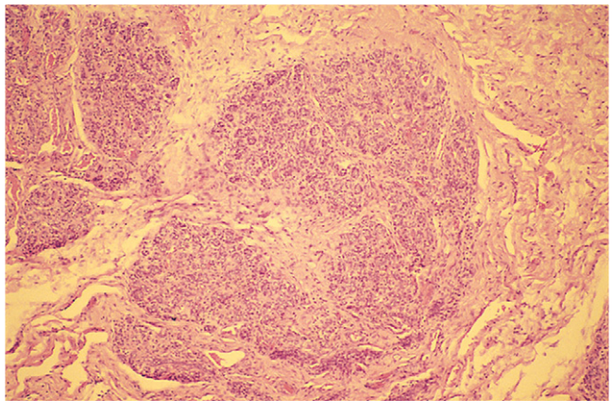
While histopathology and, more recently, cytology have been used to accurately classify mammary lesions as cysts, inflammation, or hyperplasia/neoplasia, determination of the malignant potential of mammary neoplasia can be difficult. Histopathology may often show poor correlation between histologic diagnosis of malignant neoplasia and biologic behavior. While a few studies have compared cytologic evaluation of mammary neoplasms with histologic analysis, no reports have related biologic behavior with cytologic diagnosis. However, the ease of obtaining cytologic specimens from mammary lesions, the low invasive nature, and relatively small expense make exfoliative cytology a useful diagnostic tool in the evaluation of mammary disease. When combined with history, signalment, and clinical findings, cytologic examination of mammary aspirates is particularly useful for differentiation between neoplastic disease, cystic lesions, or mastitis. Exfoliative cytology is also useful for evaluation of regional lymph nodes, distant metastatic sites, and neoplastic effusions associated with mammary malignancies. Unfortunately, use of cytology to evaluate mammary neoplasms can be difficult, and definitive diagnoses may not always be possible. Some of these difficulties are related to sample collection and others are simply inherent in the nature of mammary neoplasia. With an understanding of the potential difficulties of mammary cytology, the cytopathologist can provide useful diagnostic information concerning mammary gland disease.

Cytologic samples from mammary lesions may be obtained by expressing material from the gland, imprints or, more commonly, fine-needle aspiration (FNA) of the affected area. Proper sample collection is important for cytology to be useful in the evaluation of mammary tumors. Because of the considerable tissue heterogeneity that may be present within mammary tumors, sampling of multiple areas within a single tumor and similar samplings of additional tumors are very important. Care should also be taken to aspirate the periphery of a mammary mass as opposed to fluctuant areas within a solid lesion or the center of large tumors. These areas tend to yield fluid of low cellularity or necrosis resulting in a nondiagnostic sample.

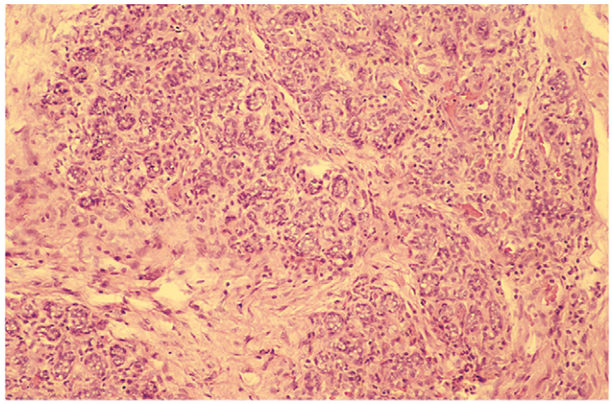
Normal Anatomy and Histology

Mammary glands are compound tubuloalveolar glands that are believed to be extensively modified sweat glands (Banks, 1986). In dogs and cats, five pairs of mammary glands are arranged as bilaterally symmetrical rows extending from the ventral thorax to the inguinal region. During pregnancy and lactation, the mammary glands undergo marked hypertrophy and hyperplasia to produce immunoglobulin-containing colostrum followed by milk.

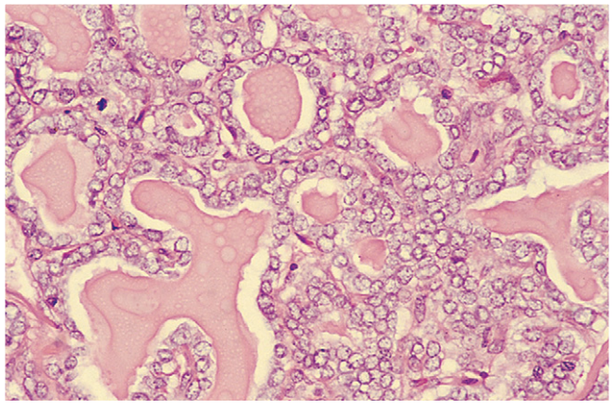
Histologically, mammary glands are composed of a secretory component consisting of alveolar secretory epithelial cells and the initial portion of the intralobular ducts (Banks, 1986) (Figs. 12-1 to 12-3). The secretory portion of the glands is drained by the ductular system, composed of nonsecretory columnar and cuboidal epithelium. Reticular connective tissue supports the alveoli and smaller ducts. Bundles of smooth muscle and elastic



■ **FIGURE 12-1. Normal, inactive mammary gland. Tissue section. Dog.** Lobules of glandular tissue are surrounded by abundant interlobular connective tissue. (H&E; LP.)



■ **FIGURE 12-2. Normal, inactive mammary gland. Tissue section. Dog.** The glandular portion of mammary tissue is composed of alveoli (acini) and intralobular ducts, which are lined by cuboidal to columnar epithelium. The interlobular ducts, composed of nonsecretory columnar and cuboidal epithelium, drain the alveoli. Reticular connective tissue supports the alveoli and smaller ducts. Bundles of smooth muscle and elastic fibers surround the large ducts. (H&E; IP.)



■ **FIGURE 12-3. Normal, lactational mammary gland. Tissue section. Dog.** The secretory portion of the gland is well developed and connective tissue elements are decreased. The alveolar lumens contain bright-pink secretory material. (H&E; HP oil.)

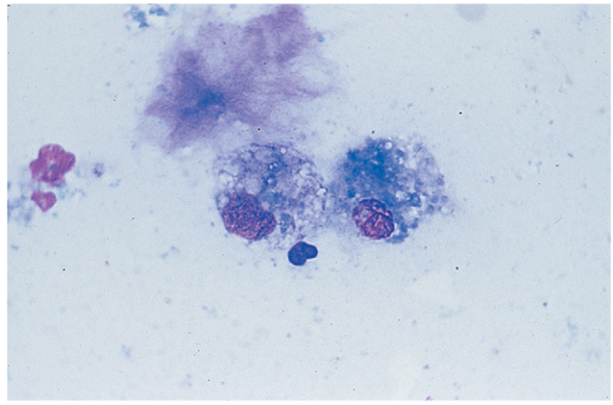
fibers surround the large ducts. Myoepithelial cells can be found between the alveolar epithelial cells and the underlying basement membrane.The normal histology of mammary gland from immature female dogs and sequential microscopic changes that occur during different stages of the estrous cycle are reviewed elsewhere (Rehm et al., 2007).

Normal Cytology

Normal mammary secretions are characterized cytologically by low numbers of sloughed secretory epithelial cells known as foam cells as well as macrophages and occasional neutrophils on an eosinophilic to basophilic proteinaceous background. Foam cells are large, individualized cells characterized by round to oval, eccentrically located nuclei and abundant amounts of vacuolated cytoplasm (Allison and Maddux, 2008). These cells may also contain amorphous, basophilic secretory material (Fig. 12-4). Foam cells resemble and can be difficult to distinguish from reactive macrophages. FNA cytology of normal mammary tissue usually reveals small amounts of blood with no to low numbers of nucleated cells and moderate to large amounts of basophilic, proteinaceous material, clear lipid droplets, and adipocytes (Allen et al., 1986). Small sheets and clusters of mammary secretory epithelial cells that are uniform in size and shape may be seen occasionally in aspirates of normal mammary tissue. Secretory epithelial cells exhibit round, dark nuclei and moderate amounts of basophilic cytoplasm. Acinar formations may be noted. Ductular epithelial cells are characterized by oval, basal nuclei with scant amounts of cytoplasm. Myoepithelial cells may be seen as darkly staining, oval free nuclei or as spindle-shaped cells (Allison and Maddux, 2008).

Mammary Cysts

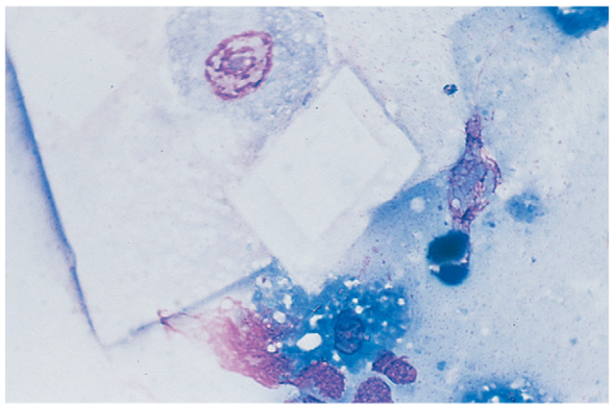
Mammary cysts or fibrocystic disease (FCD), also known as blue dome cyst or polycystic mastopathy, is a form of mammary dysplasia in which dilated ducts expand to



■ **FIGURE 12-4. Mammary gland aspirate. Foam cells. Cat.** The two foam cells have eccentrically located nuclei, low nuclear-to-cytoplasmic ratios, clear cytoplasmic vacuoles, and abundant amounts of basophilic secretory material. The background has a lightly basophilic, proteinaceous appearance consistent with normal mammary aspirates. (Wright-Giemsa; HP oil.)

form cavitary lesions (Brodey et al., 1983). FCD generally occurs in middle-aged to older animals, although the disease has been reported in dogs of 1 year of age. Formation of FCD may have a hormonal component as administration of medroxyprogesterone has been associated with development of FCD in dogs. In dogs, rapid growth during estrus and regression during metestrus has been noted. The rapid growth of cysts during estrus may be associated with rupture of the cysts. Ovariohysterectomy should be considered when mammary cysts grow and regress in association with the estrous cycle, particularly if multiple glands are involved. FCD is considered a benign lesion in dogs; however, the disease has been associated with development of mammary gland carcinoma.

Mammary cysts may present as a well-circumscribed, single cystic nodule or as a flat, rubbery, multinodular mass. The nodule(s) exhibit slow, expansile growth and the overlying skin may develop a blue color, hence the term *blue dome cyst* (Brodey et al., 1983). Mammary cysts may be classified as simple cysts characterized by a single layer of flattened lining epithelium or papillary cysts containing papillary outgrowths of the lining epithelial cells. Aspiration of mammary cysts typically yields a green-brown or blood-tinged fluid containing low numbers of foam cells and pigment-laden macrophages (Allison and Maddux, 2008). Neutrophils may be increased if inflammation is also present. Cholesterol crystals, which appear as large, rectangular crystalline structures often with a notched corner, may be present as a result of breakdown of cellular membranes within the cyst (Fig. 12-5). Epithelial cells derived from the cystic lining may be noted, particularly if the cyst has a papillary component. These cells tend to occur in dense sheets and clusters and may display some mild variation in nuclear size and shape. Mammary cysts may coexist with benign and/or malignant mammary tumors (Brodey et al., 1983). Therefore, aspiration or biopsy of solid areas of a mass associated with a cyst or other mammary masses should be performed to rule out the presence of concurrent mammary neoplasia.



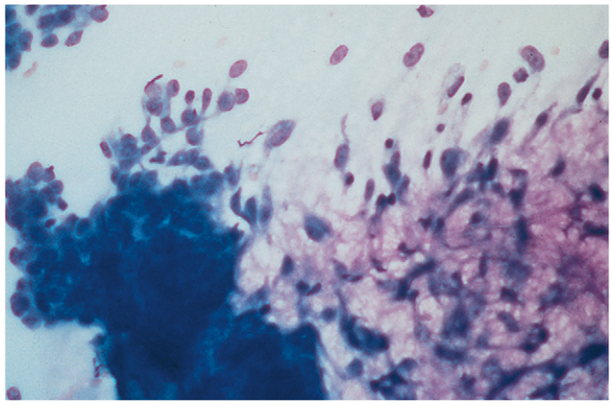
■ **FIGURE 12-5. Mammary cyst aspirate. Cholesterol crystals. Cat.** The clear, rectangular crystals are of varying size. Two foam cells are adjacent to the crystals. (Wright-Giemsa; HP oil.)

Mammary Gland Hyperplasia

Hyperplastic and dysplastic lesions of mammary glands include unilobular and multilobular hyperplasia, adenosis, and epitheliosis (Misdorp et al., 1999). These lesions occur in dogs and less commonly in cats (Yager et al., 1993). Mammary hyperplasia is characterized by proliferations of secretory or ductular epithelium or myoepithelial cells resembling the physiologic hyperplasia of pregnancy with some mild histologic atypia. Cytologically, these lesions may be difficult to distinguish from each other and from benign neoplasms such as adenomas or papillomas. Moderate to large numbers of epithelial cells arranged in sheets and clusters can be aspirated from hyperplastic mammary tissue. These cells, which are similar in appearance to normal mammary epithelial cells, display round nuclei with fine to lightly stippled chromatin of uniform size and shape and scant to moderate amounts of basophilic cytoplasm. Foam cells and macrophages may also be noted.

In cats, a form of mammary hyperplasia occurs that has been variously identified as fibroepithelial hyperplasia, feline mammary hypertrophy, mammary fibroadenomatous hyperplasia, or feline mammary hypertrophy/fibroadenomatous complex. Feline mammary fibroepithelial hyperplasia (MFH) is a clinically benign, fairly common condition affecting estrous-cycling or pregnant female cats usually less than 2 years of age (Mesher, 1997). MFH has also been reported in older intact and neutered cats of either gender receiving progesterone-containing compounds, such as megestrol acetate (Hayden et al., 1989) or depot medroxyprogesterone acetate (Loretti et al., 2005). MFH is considered a form of mammary dysplasia characterized by a rapid, abnormal growth of one or more mammary glands. In contrast to a neoplastic process, paired glands often exhibit a similar degree of enlargement (Lana et al., 2007). MFH is notable for a marked intralobular ductular proliferation identical to the ductular proliferation seen during the progesterone-influenced early stages of pregnancy (Misdorp et al., 1999). This typical histologic appearance, the occurrence in cycling females or cats administered progesterone, and the identification of progesterone receptors in MFH lesions from female and male cats have led to the belief that development of MFH involves endogenous or exogenous progesterone. MFH usually regresses over time without treatment, although secondary infections may require appropriate antibiotic therapy. Ovariohysterectomy, performed via a flank incision if the glands are greatly enlarged, will often result in regression of lesions and will prevent future recurrences (Lana et al., 2007). However, some cats do not respond to withdrawal of progestogens or ovariectomy and can be treated successfully with the progesterone receptor blocker aglépristone (Görlinger et al., 2002).

The cytologic appearance of MFH (Fig. 12-6) has been reported (Mesher, 1997). Aspirated material of a histologically confirmed MFH lesion consisted of a very uniform population of cuboidal epithelial cells arranged in thick clusters. The cuboidal epithelial cells were characterized by dense, round nuclei with small nucleoli and scant amounts of basophilic cytoplasm. A mesenchymal



■ **FIGURE 12-6. Mammary fibroepithelial hyperplasia. Tissue aspirate. Cat.** Sheet of epithelial cells and spindle cells in pink extracellular material. The epithelial cells are uniform in size and shape and the spindle cells display some mild anisokaryosis. (Wright; HP oil.) (From Mesher CI: What is your diagnosis? A 14-month-old domestic cat, *Vet Clin Pathol* 26:4, 13, 1997.)

population of spindle-shaped cells with narrow oval nuclei, one to two nucleoli, and tapering cytoplasm was also present. The mesenchymal cells displayed moderate variation in nuclear size (anisokaryosis) and cellular size (anisocytosis). Moderate amounts of pink extracellular matrix were associated with the mesenchymal cells. These cytologic findings correlated with the histologic findings of hyperplastic ductular epithelium (cuboidal epithelial population) and proliferation of edematous stroma (mesenchymal cells with extracellular matrix). The presence of abundant stromal elements helps to differentiate MFH from mammary neoplasia, which generally contains scant stromal material. Cytologic recognition of the characteristic cell types from mammary masses in a cat with appropriate signalment and clinical history can be considered highly suggestive of MFH, thus eliminating the need for mammary gland excision and allowing for appropriate medical and/or surgical management.

Mammary Gland Inflammation/Infection

Inflammation of the mammary glands is referred to as *mastitis* and may present as a focal lesion or may involve one or more glands. Mastitis may infrequently occur from hematogenous spread of organisms, nonlactation-associated trauma, fight wounds, or infected neoplasms. *Dirofilaria repens* infection of the mammary gland in the bitch (Manuali et al., 2005) and mastitis due to *Toxoplasma gondii* in a cat (Park et al., 2007) have been recently reported. Mastitis is most often associated with postparturient lactation. It can also occur during pseudopregnancy, as well after early weaning of puppies. It is thought to result from entry of infectious organisms through the teat orifice or damaged overlying skin (Gruffydd-Jones, 1980). Neonatal morbidity or mortality may be the first indication of mastitis. Clinical signs associated with mastitis include swollen, painful glands that result in discomfort while nursing. The glands may become abscessed or gangrenous with necrosis of overlying skin. The bitch or queen may also present with clinical signs of systemic illness such as anorexia, fever, vomiting, or diarrhea. A complete blood count may reveal an inflammatory leukogram characterized by either an increase in segmented and nonsegmented (band) neutrophils or a degenerative left shift with a predominance of immature neutrophils, especially if gangrenous mastitis is present (Ververidis et al., 2007).

Cytologic examination of secretions from inflamed and/or infected mammary glands is usually diagnostic; however, FNA may be needed for focal lesions. Large numbers of neutrophils are present, which may exhibit degenerative changes of karyolysis and karyorrhexis. Reactive macrophages, small lymphocytes, and plasma cells may also be seen, particularly with more chronic lesions. Infectious organisms may be visualized within neutrophils and, less commonly, macrophages, indicating a septic process. Various bacteria have been incriminated as etiologic agents of disease such as *Staphylococcus* spp., *Streptococcus* spp., and *E. coli*. Other types of bacteria and fungi can be isolated (Allison and Maddux, 2008). *Staphylococcus intermedius* is the most common cause of clinical and subclinical mastitis in the dog (Schafer-Somi et al., 2003). Culture and sensitivity of milk, inflamed mammary secretions, or aspirated material are warranted to determine appropriate antibiotic therapy.

The need for antibiotic therapy to treat bacterial mastitis depends on the severity of the lesions. Systemic antibiotic therapy is based upon culture and sensitivity results. Abscessed glands will need to be surgically débrided or drained. Warm, moist topical packs may be used for gangrenous mastitis, and the necrotic tissue can be excised or allowed to slough. Supportive care, including intravenous fluid therapy, may be necessary for the bitch or queen as well as nursing puppies or kittens. Also, puppies or kittens may require appropriate antibiotic therapy and should be weaned and reared by hand.

Some noninfectious inflammatory conditions of mammary glands have been described. Focal mastitic lesions may leave residual fibrotic nodules consisting of epithelial cell metaplasia, pigment-laden macrophages, nondegenerate neutrophils, small lymphocytes, and plasma cells (Allison and Maddux, 2008). Unlike mammary gland tumors, fibrotic nodules tend to occur in young dogs, do not increase in size, and are usually associated with a previous history of mastitis (Brodey et al., 1983).

Neoplasia

Canine Mammary Gland Tumors

Following skin tumors, mammary neoplasms are the second most common tumor in dogs and the most commonly seen tumor in bitches (Misdorp, 2002). Mammary gland tumors (MGT) rarely occur in male dogs with a reported annual incidence of 4 in 100,000 while the annual incidence is 207 in 100,000 in female dogs (Saba et al., 2007; Lana et al., 2007). Many of the MGT reported in male dogs have been associated with small tumor sizes, benign or well-differentiated malignant epithelial tumors, nondefinitive evidence of metastatic disease at diagnosis, and intense estrogen-receptor positivity. The median age for development of canine mammary gland

tumors is 10 to 11 years of age, with rare occurrence in bitches younger than 4 years old. Breed tendencies for MGT have been reported with a predisposition in several spaniels breeds, the Poodle, Dachshund, and other breeds (Sorenmo, 2003) and with a greater prevalence of malignant tumors in large breeds than in small breeds (Itoh et al., 2005). A heritable, familial tendency for development of mammary neoplasms in Beagles has been suggested (Benjamin et al., 1999). Development of MGT appears to have a hormonal component as evidenced by the sparing effect of ovariohysterectomy in first estrus cycles and by the increased length of survival time in dogs spayed less than 2 years before mammary carcinoma surgery when compared with dogs spayed longer than 2 years before tumor surgery or intact dogs (Sorenmo et al., 2000). Estrogen and progesterone receptors have been identified in normal, hyperplastic/dysplastic mammary tissue and a majority of mammary neoplasms (Lana et al., 2007; Millanta et al., 2005; de las Mulas et al., 2005). Interestingly, hormone receptor expression, which is a characteristic feature of mature mammary epithelial cells, tends to be decreased or absent in poorly differentiated tumors and metastatic lesions. It is well known that progesterone or synthetic progestins administration increases the incidence of MGT in dogs (Misdorp, 1991). Mechanisms involved in the progesterone-induced mammary gland tumors include an upregulation of growth hormone production by mammary epithelial cells (van Garderen and Schalken, 2002) and a rise in blood levels of insulin-like growth factor I (IGF-I) and IGF-II (Lana et al., 2007). Growth hormone and IGF may increase proliferation of susceptible or transformed mammary epithelial cells, resulting in neoplasia. Other risk factors to develop MGT are obesity at 1 year of age and low-fat/low-protein diet (Sorenmo, 2003). Other molecule targets have been investigated to elucidate prognosis or the pathways of tumorigenesis such as cyclooxygenase-2 (Millanta et al., 2006a), heat-shock proteins (Romanucci et al., 2006), VEGF (Millanta et al., 2006b), p53, BRCA1, c-erbB-2 , antiapoptotic and proapoptotic proteins (Lana et al., 2007), beta-catenin, E-cadherin and *Adenomatous Polyposis Coli* (APC) (Restucci et al., 2007) and connexin (Torres et al., 2005), as well as several proliferation markers such as proliferating cell nuclear antigen (PCNA) and Ki-67 (Lana et al., 2007). Immunocytochemical Ki-67 marker seems to be useful to identify malignant canine tumors and patient poor outcome (Zuccari et al., 2004).

Mammary tumors can present as single, firm, well-circumscribed masses to multiple, infiltrative nodules involving one or more glands. In animals with benign mammary tumors, the tumor is small, well circumscribed, and firm on palpation. Clinical findings associated with malignant neoplasms include a tumor diameter greater than 5 cm, recent rapid growth, ill-defined boundaries, infiltration of surrounding tissue, erythema, ulceration, inflammation, and edema. However, most benign and malignant canine mammary tumors exhibit none of these signs with the exception of dogs with advanced metastatic disease or inflammatory mammary carcinomas that typically have systemic signs of illness when they are diagnosed (Lana et al., 2007).

The majority of mammary neoplasms occur primarily in the caudal glands, presumably because of the larger amount of glandular tissue present (Sorenmo, 2003). Multiple mammary neoplasms are common, with 50% to 60% of dogs presenting with more than one mammary tumor. Multiple MGT in a dog are often not of the same histologic type and may exhibit differing biologic behaviors (Benjamin et al., 1999). Thus, a thorough search for additional tumors should be undertaken if a mammary mass is found, and separate cytologic and/or histologic analyses should be performed on each mammary tumor.

The ultimate goal of clinical, cytologic, and histologic evaluation of mammary gland neoplasms is to accurately predict the biologic behavior of the tumor. The World Health Organization International Histological Classification of Mammary Tumors of the dog and the cat combines histiogenic and descriptive morphologic classification, incorporating histologic prognostic features that have been associated with increasing malignancy (Misdorp et al., 1999). Most mammary gland tumors are of epithelial origin. Some tumors are composed of both epithelial and myoepithelial tissue, with areas of cartilage and bone, and a few tumors are of purely mesenchymal origin. About 50% of canine MGT have been classified as malignant based on histologic appearance (Brodey et al, 1983). While some classifications of mammary gland tumors, such as carcinosarcomas or sarcomas, have a consistently poor prognosis, histologic evidence of malignancy does not always imply a malignant course (Lana et al., 2007). In fact, only 50% of histologically diagnosed mammary carcinomas result in tumor-associated deaths (Brodey et al., 1983). Morphologic criteria of malignancy, such as cellular pleomorphism, mitotic activity, and individual grades of anaplasia, are not sufficient criteria for diagnosis of carcinomas. Instead, infiltration into skin and soft tissues and invasion of tumor cells into blood or lymphatic vessels have been identified as best histologic evidence of malignancy in mammary tumors (Misdorp, 2002). When stromal invasion is present, 80% of affected dogs will be dead within 2 years while, in the absence of stromal invasion, 80% of affected dogs will be alive after 2 years (Yager et al., 1993). Using stromal invasion as the primary criteria for malignancy, a lifespan study of over a thousand beagles was reported that correlated the various histologic classifications of epithelial mammary tumors with biologic behavior (Benjamin et al., 1999). Specifically, the study showed that ductular carcinomas accounted for 65.8% of all fatalities due to mammary neoplasia, even though these tumors composed only 18.7% of all mammary carcinomas. Of the malignant tumors, squamous cell carcinomas exhibited the lowest metastatic rate (20%), with carcinosarcomas exhibiting the highest rate of metastasis (100%). Ductular carcinomas metastasized more frequently than adenocarcinomas, 45% versus 35%, respectively.

Accurate and diagnostic exfoliative cytology of mammary tumors is associated with difficulties. Mesenchymal tumors or tumors with a fibrous or scirrhous component may not exfoliate well, leading to a poorly cellular sample inadequate for diagnosis. Tissue imprints or smears of tissue scrapings taken from biopsy samples may improve

cytologic diagnosis in these cases; however, imprints generally do not yield as good a sample for evaluation as aspirates (Baker and Lumsden, 1999). Also, mammary hyperplasia, dysplasia, benign tumors, and well-differentiated carcinomas tend to form a continuum of morphologic appearance, making cytologic differentiation of these lesions difficult (Benjamin et al., 1999). Lastly, the presence of stromal invasion, one of the most important criteria for determining the malignant potential of a mammary neoplasm, cannot be assessed by the cytologist. All of these factors can result in either false-positive or false-negative diagnosis of malignant mammary tumors using aspiration cytology. A few studies have examined the accuracy of cytology for detecting mammary malignancies as compared to histologic findings. Allen et al (1986) reported cytologic sensitivities for detecting malignancies of 25% and 17% and specificities of 62% and 49% for the two cytopathologists involved in the study. Positive (PPV) and negative (NPV) predictive values were generally similar between the two pathologists, with PPVs of 90% and 100% and NPVs of 75% and 59% (Allen et al., 1986). The diagnostic accuracy was reported as 79% and 66%. In another study, the sensitivity for cytologic detection of mammary malignancies was found to be 65% with a specificity of 94% (Hellman and Lindgren, 1989). The PPV was reported as 93% with an NPV of 67% and diagnostic accuracy of 79%. In a recent study, cytologic and histologic diagnostic agreement was 67.5%. However, when suspicious and insufficient/inadequate samples were excluded, a 92.9% agreement rate was obtained (Cassali et al., 2007). The same authors reported a sensitivity and specificity for the diagnosis of malignant tumors of 88.6% and 100%, respectively, and a sensitivity of 100% and specificity of 88.6% for the diagnosis of benign lesions. These studies did not correlate cytologic diagnosis with disease-free intervals or survival times, thus the use of cytologic criteria to accurately predict the biologic behavior of MGT is uncertain. Recent studies demonstrated that cells in malignant epithelial mammary tumors had significantly more irregular nuclear shapes that did control epithelial cells or cells in benign tumors based on differences in fractal dimension and on nuclear diameter and roundness. These morphometric parameters could help in the preoperative cytologic evaluation of canine mammary gland tumors (Simeonov, 2006a, Simeonov, 2006b).

Cytologic examination of most mammary tumors reveals a background containing variable amounts of blood, basophilic proteinaceous material, lipid, and foam cells. Aspirates of benign epithelial tumors (adenomas and papillomas) typically reveal moderate to large numbers of epithelial cells arranged in sheets and clusters (Fig. 12-7). These cells are uniform in appearance with smooth nuclear chromatin and occasionally prominent, single, small, round nucleoli (Allison and Maddux, 2008). Acinar structures may be seen in samples from adenomas and palisade; papillary and trabecular cell arrangement can be observed in other benign epithelial tumors (Masserdotti, 2006). Benign complex adenomas or papillomas, fibroadenomas, and benign mixed tumors may yield sheets and clusters of uniform-appearing epithelial cells and

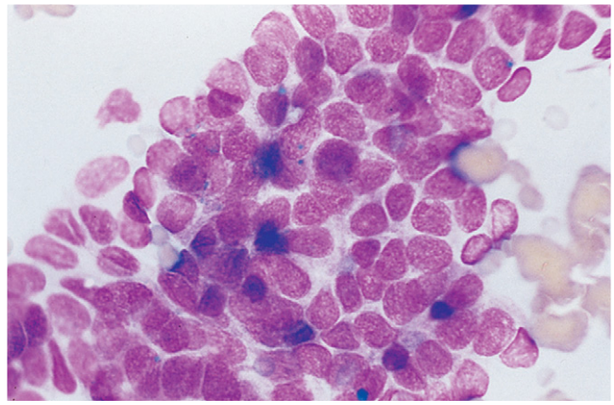


FIGURE 12-7. Mammary adenoma. Tissue aspirate. Cat. Sheet of epithelial cells with cells that are of uniform size and shape with a high nuclear-to-cytoplasmic ratio and fine nuclear chromatin. The cytoplasm is lightly basophilic and scant in amount. (Wright-Giemsa; HP oil.)

individualized or clumped spindle-shaped cells of myoepithelial (complex tumors) or mesenchymal (mixed tumors) origin. Myoepithelial cells may also appear as oval free nuclei (Allison and Maddux, 2008). Examination of mixed mammary tumors may reveal the presence of cartilage or bone elements such as osteoblasts, osteoclasts, hematopoietic cells, and/or bright-pink extracellular material representative of osteoid (Fernandes et al., 1998) (Figs. 12-8 and 12-9). Mixed mammary tumors can be difficult to diagnose using exfoliative cytology. For instance, the presence of spindle-shaped cells may not be sufficient for diagnosis of mixed or complex tumors. Allen et al. (1986) have noted that spindle cells were identified in the mammary tumors evaluated in their study, yet the presence of these cells did not correlate significantly with histologic classification of complex or mixed tumors. Aspirates of mixed tumors also may not reveal all of the cells composing the tumor. In a case report, aspiration of a mammary mass in a dog revealed the presence of osteoblasts displaying moderate anisokaryosis and

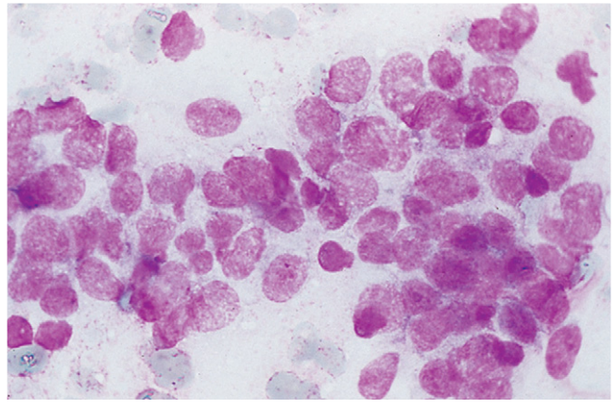
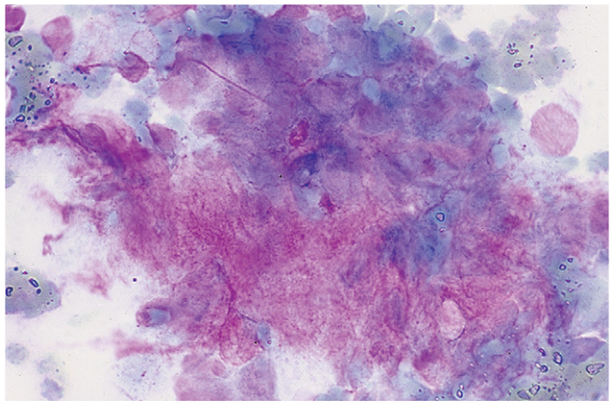
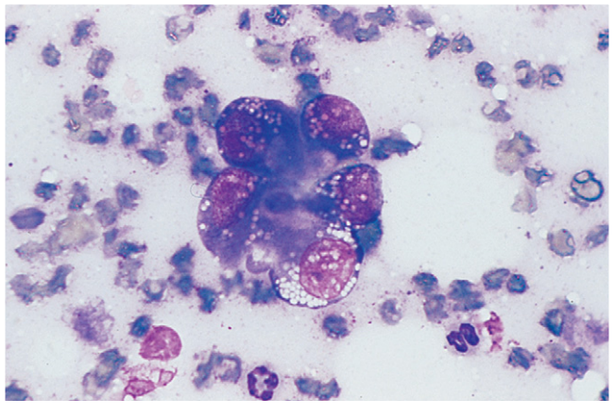


FIGURE 12-8. Mixed mammary tumor. Tissue aspirate. Dog. Epithelial cells display slightly coarse nuclear chromatin, high nuclear-to-cytoplasmic ratios, and mild to moderate anisokaryosis and anisocytosis. (Wright-Giemsa; HP oil.)



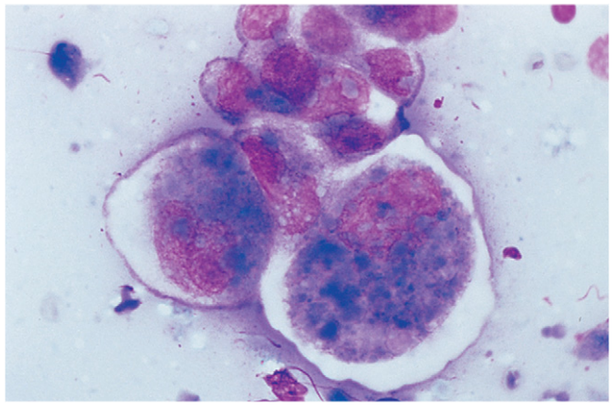
■ **FIGURE 12-9. Mixed mammary tumor. Tissue aspirate. Dog.** Clump of spindle-shaped cells associated with large amounts of extracellular pink material from the same aspirate shown in Figure 12-8. (Wright-Giemsa; HP oil.)



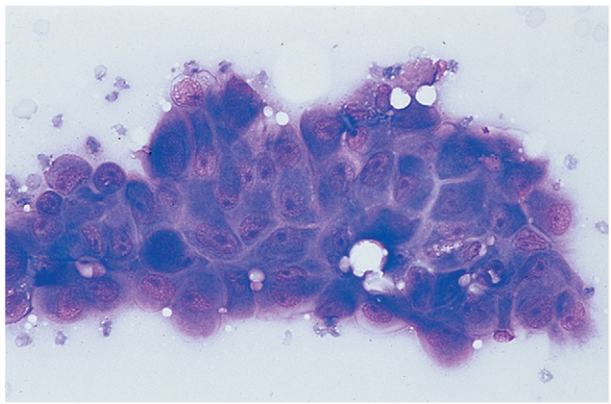
■ **FIGURE 12-11. Mammary adenocarcinoma. Tissue aspirate. Dog.** An acinar structure is shown. Note the presence of punctate cytoplasmic vacuoles as well as prominent nucleoli and moderate anisokaryosis. (Wright-Giemsa; HP oil.)

anisocytosis, osteoclasts, hematopoietic cells, and pink extracellular material (Fernandes et al., 1998). No epithelial cells were noted in the sample. Thus, the multiple differentials included benign or malignant mixed mammary tumor, osseous metaplasia, and osteosarcoma. Histopathology confirmed that the neoplasm was a benign mixed mammary tumor.

Malignant mammary tumors may be diagnosed based on the cytologic appearance of the cell types present and the observation of more than three criteria of malignancy. Adenocarcinomas are characterized by epithelial cells arranged in sheets (Fig. 12-10) and clusters, or sometimes individualized. Acinar arrangements (Fig. 12-11) may be observed (Masserdotti, 2006). The epithelial cells are typically round, with round to oval, eccentrically located nuclei and moderate amounts of basophilic cytoplasm that may contain amorphous basophilic secretory product and/or clear vacuoles (Allison and Maddux, 2008) (Fig. 12-12). Some of these vacuoles may appear as punctate vacuoles of variable number or as a diffuse clearing of the cytoplasm that



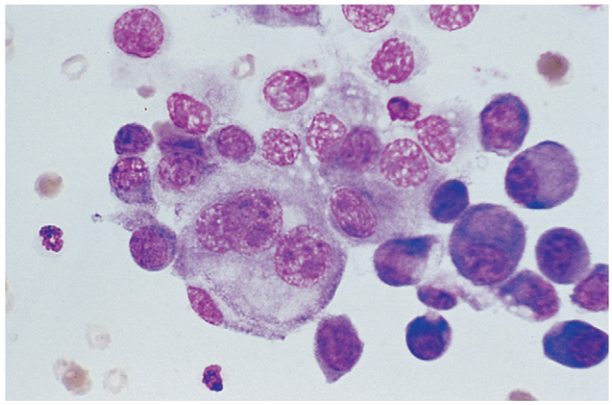
■ **FIGURE 12-12. Mammary adenocarcinoma. Tissue aspirate. Dog.** Marked anisokaryosis and anisocytosis of the epithelial cells are noted. These epithelial cells contain basophilic secretory material as well as diffuse peripheral cytoplasmic vacuolation. (Wright-Giemsa; HP oil.)



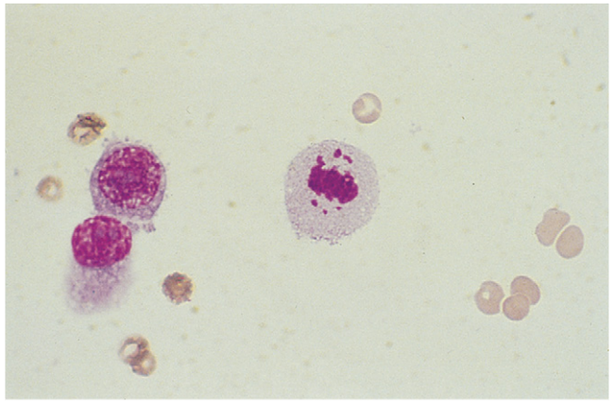
■ **FIGURE 12-10. Mammary adenocarcinoma. Tissue aspirate. Dog.** Sheet of epithelial cells displaying prominent cell-to-cell junctions. These cells also exhibit prominent, large nucleoli, moderate anisokaryosis, and deeply basophilic cytoplasm. (Wright-Giemsa; HP oil.)

distends the cell and displaces the nucleus peripherally. Criteria of malignancy that may be seen in these cells include increased nuclear-to-cytoplasmic ratio; moderate to marked variation in nuclear and cell size; nuclear molding; large, prominent, multiple, and/or abnormally shaped nucleoli; and binucleation and multinucleation. Increased mitotic activity and abnormal mitotic figures may be present (Figs. 12-13 and 12-14). Ductular carcinomas typically present with sheets and clusters of pleomorphic epithelial cells with high nuclear-to-cytoplasmic ratios and round, basal nuclei. These cells usually display more than three malignant criteria. Acinar structures, secretory product, and cytoplasmic vacuoles are not characteristic features of ductular carcinomas. Papillary and trabecular cell arrangements can be observed in malignant epithelial tumors (Masserdotti, 2006).

Anaplastic carcinomas may present with very large, extremely pleomorphic epithelial cells occurring singly and in small clusters (Allison and Maddux, 2008).These cells tend to have bizarre nuclear and nucleolar forms.



■ **FIGURE 12-13. Mammary carcinoma. Tissue aspirate. Dog.** Marked anisokaryosis, anisocytosis, prominent nucleoli, coarse nuclear chromatin, and binucleation are present in cells that also display poor cellular adhesion. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-14. Mammary carcinoma. Tissue aspirate. Dog.** Abnormal mitotic figure with lag chromatin from same specimen as shown in Figure 12-13. Lag chromatin results from abnormal formation of the mitotic spindle apparatus. Abnormal mitotic figures are considered one criterion of malignancy. (Wright-Giemsa; HP oil.)

Multinucleation and abnormal mitotic figures are frequently seen. Inflammatory carcinomas, which are a locally aggressive form of mammary carcinoma, also present with large, pleomorphic epithelial cells exhibiting various criteria of malignancy as well as large numbers of nondegenerate neutrophils and macrophages (Lana et al., 2007). The cytologic appearance of inflammatory carcinoma may resemble mastitis. However, history, signalment, and presence of very anaplastic epithelial cells should be helpful for differentiation of these two conditions.

Squamous cell carcinomas of the mammary gland appear cytologically similar to those found in other body sites. The malignant squamous cells tend to occur individually or in small sheets. The nuclei may vary from small and pyknotic to large, round, and immature with prominent nucleoli. The nuclear-to-cytoplasmic ratio is variable and binucleation may be noted. The cytoplasm of the tumor cells is moderately to deeply basophilic (nonkeratinized) or may have a blue-green color characteristic of keratinization. Mammary squamous cell carcinomas may ulcerate, leading to the presence of inflammatory cells and phagocytized bacteria in the cytologic sample (Allison and Maddux, 2008).

Aspirates of malignant mixed mammary tumors may reveal epithelial cells and spindle-shaped, individualized cells of mesenchymal origin with one of these populations displaying nuclear and cellular criteria of malignancy. However, the presence of either population or predominance of one cell type over the other may depend on the area of tumor aspirated (Allison and Maddux, 2008). In carcinosarcomas, both epithelial and mesenchymal populations should display malignant features. Mammary sarcomas, such as osteosarcoma, fibrosarcoma, and liposarcoma, are of similar cytologic appearance to those found in other body sites. Sarcomas tend to exfoliate poorly, often resulting in samples of low cellularity. Depending on the type of tumor, pink extracellular material or lipid may be present in the background. In general, sarcomas are characterized by spindle-shaped to irregular cells arranged individually and in small clumps. The cytoplasm of these cells is moderately to deeply basophilic and the cytoplasmic borders tend to be indistinct. The cells display cytologic features of malignancy similar to those described for epithelial neoplasms.

Feline Mammary Gland Tumors

Mammary tumors are the third most common tumor in the cat, after hematopoietic neoplasms and skin tumors (Misdorp, 2002; Hayes and Mooney, 1985). The median age for MGT development in the cat is 10 years of age or older. Almost all (99%) of feline MGT occur in intact females (Lana et al., 2007). Domestic short hair and Siamese cats appear to have higher incidence rates (Hayes et al., 1981).

Development of feline MGT is thought to have a hormonal component. Intact females have an almost seven-fold greater risk of developing mammary neoplasms as compared to neutered females, and ovariohysterectomy has been reported to decrease the risk of MGT to 0.6% compared to intact females (Hayes et al., 1981). Regular, but not irregular, administration of exogenous progesterone was associated with a significantly increased risk of benign mammary tumors and mammary carcinomas in cats (Misdorp, 1991). Hormone receptor analysis has shown that normal feline mammary tissue contains estrogen and progesterone receptors in levels similar to those found in the dog (Millanta et al., 2005). However, unlike canine MGT, the majority of feline mammary neoplasms express very low levels of estrogen and progesterone receptors, which may be related to the high rate of malignancy found with mammary neoplasia in the cat. Other molecule targets have been investigated to elucidate prognosis or the pathways of tumorigenesis such as cyclin A, Cox-2 (Millanta et al., 2006b), HER2, VEGF and E-cadherin (Lana et al., 2007).

In contrast to the dog, the majority of feline mammary tumors are malignant with some studies reporting a greater than 80% incidence of malignant neoplasms (Hayes et al., 1981). Adenocarcinomas are the most

prevalent malignant mammary tumor followed by carcinomas and sarcomas (MacEwen et al., 1984). Recently, secondary or postsurgical inflammatory carcinomas (Pérez-Alenza et al., 2004) and lipid-rich carcinoma (Kamstock et al., 2005) have been described, for the first time, in cats. Malignant MGT in cats tend to grow rapidly and metastasize to regional lymph nodes, lung, pleura, liver, diaphragm, adrenal glands, and kidneys (Lana et al, 2007). The single most important prognostic indicator for feline MGT is tumor size at time of diagnosis. Median survival times for cats with mammary tumors greater than 3 cm, between 2 and 3 cm, and less than 2 cm is 6 months, 2 years, and greater than 3 years, respectively (MacEwen et al., 1984). Thus, early diagnosis and treatment is very important for feline mammary malignancies.

The cytologic features of benign and malignant mammary neoplasms in the cat are similar to those described in the dog (see Figures 12-10, 12-13, 12-14).The reliability of cytologic criteria to differentiate between hyperplasia, benign tumors, and malignancies in the cat does not appear to have been reported (Baker and Lumsden, 1999). Given the high rate of mammary malignancy in cats, cytologic findings of a benign-appearing population of epithelial cells, particularly in an older cat with no history of progesterone administration, should be treated with some caution. In these cases, samples should be submitted for histopathologic examination to rule out the presence of a malignancy.

Treatment considerations will follow clinical and cytologic and/or histologic identification of a mammary neoplasm in a dog or a cat. If a malignancy is present, staging the extent of the disease should include three-view thoracic radiographs or CT scan of the lungs and any other potential metastatic sites as well as cytologic analysis of regional lymph nodes, metastatic lesions, and/or body cavity effusions. It has been proposed that treatment guidelines for malignant canine mammary gland tumors be based on tumor size, histopathologic type, and differentiation (Sorenmo, 2003). Surgical excision is the treatment of choice for both canine and feline mammary neoplasms. In dogs, it is recommended to perform ovariohysterectomy if intact in all malignant canine mammary gland tumors and to institute chemotherapy in an undifferentiated carcinoma in stage I (Sorenmo, 2003). There is limited information regarding efficacy of adjuvant therapy involving chemotherapeutics, radiation, or immune stimulation in canine and feline mammary malignancies. However, the combination of surgery and adjunctive doxorubicin chemotherapy resulted in improved long-term survival in cats with mammary gland adenocarcinoma (Novosad, 2003; Novosad et al., 2006). In addition, the combination of surgery and adjunctive 5-fluorouracil and cyclophosphamide chemotherapy demonstrated significant survival improvement in dogs with mammary gland carcinomas stage III/IV when compared with surgery alone (Karayannopoulo et al., 2001). In contrast, chemotherapy did not lead to an improved outcome in dogs with invasive malignant mammary gland tumors (Simon et al., 2006). The use of antiestrogens, such as tamoxifen, has been documented in a small number of clinical cases, with somewhat conflicting results in regard to tumor response. These drugs can be associated with undesirable estrogen-related side effects (Novosad, 2003).

Ovaries

Cytology is a valuable tool for diagnosis of ovarian tumors and ovarian cystic disease as recently demonstrated in a study with a diagnostic accuracy of cytology of 94.7% (Bertazzolo et al., 2004).

Special Collection Techniques

There is little information on ovarian cytology collection techniques. Ovarian biopsy is performed and surgical technique is well described elsewhere (Root Kustritz, 2006). Cytologic samples can be made by ultrasound-guided percutaneous fine-needle aspiration or intraoperatively during exploratory laparotomy.

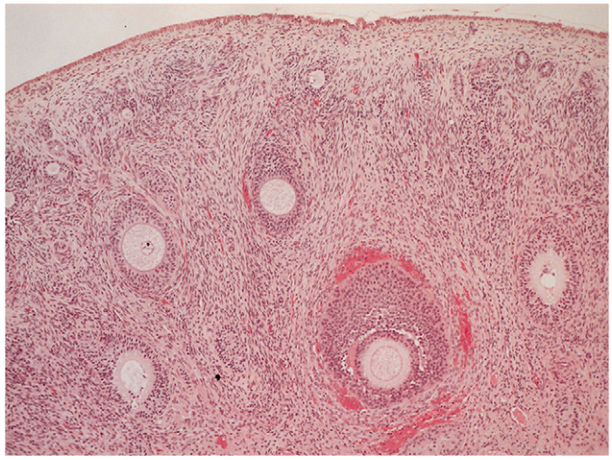
Normal Anatomy and Histology

The ovary is composed of three broad embryologic origins: 1) the epithelium, which includes the outer layer lining (surface) epithelium of the modified mesothelium, the rete ovarii (remnants of the mesonephric tubules), and in the bitch, the subsurface epithelial structures; 2) the germ cells; 3) the ovarian stroma including the sex cords, which together contribute the endocrine apparatus of the ovary (MacLachlan and Kennedy, 2002). Each ovary lies within an ovarian bursa, an extension of the mesosalpinx, which is a fold of the peritoneum. Cuboidal epithelium called germinal epithelium covers the cortex of the ovary, and a layer of dense connective tissue, the tunica albuginea, is present underneath the epithelium. The canine ovary has small ingrowths of the ovarian surface that are called subsurface epithelial structures. The cortex of the ovary contains follicles, stromal connective tissue, and blood vessels. The ova develop in follicles that are of four types: primordial, primary, secondary, and tertiary. Each developing follicle has the oocyte, multiple layers of granulosa cells, and more peripheral thecal connective tissue cells (Fig. 12-15). Ovulation occurs when the follicle ruptures, releasing the ovum and allowing the space to fill with blood and luteal cells to form the corpus hemorrhagicum and the corpus luteum, respectively. In bitches and queens, cords of epithelial cells called interstitial glands, which are cells of an endocrine type, occur throughout the stroma. A medulla consisting of richly vascularized loose connective tissue, lymphatics, and nerves lies internal to the ovarian cortex. Channels lined by cuboidal epithelium called rete ovarii are present in this region (Foster, 2007).

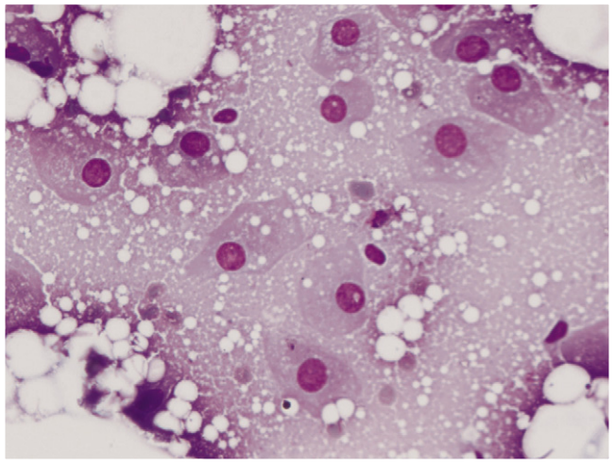
The normal histology of ovaries from immature female dogs and sequential microscopic changes that occur during different stages of the estrous cycle are reviewed elsewhere (Rehm et al., 2007).

Normal Cytology

Cytology of normal ovarian tissue usually reveals small amounts of blood with no to moderate numbers of nucleated cells and moderate to large amounts of basophilic, proteinaceous material and clear lipid droplets. Normal ovaries are characterized cytologically by low to



■ **FIGURE 12-15. Normal ovary. Tissue section. Dog.** Several developing follicles, each with an oocyte surrounded by a layer of granulosa cells, are present within the stroma of the ovarian cortex. The cortex is lined by a simple layer of cuboidal epithelium. (H&E; LP.) (Courtesy of Dr. Carlo Masserdotti.)

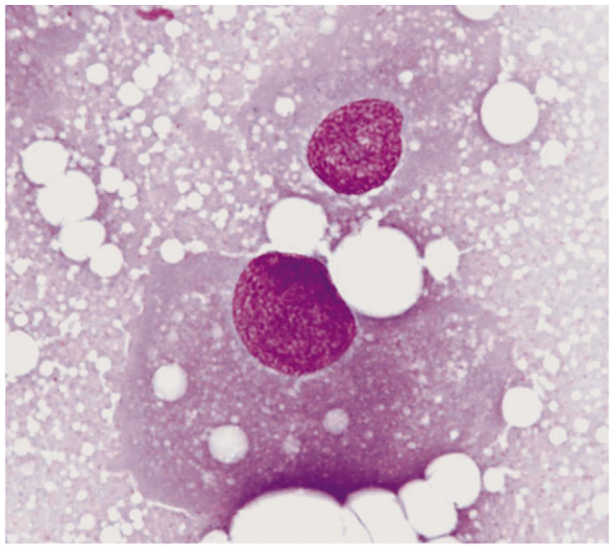


■ **FIGURE 12-17. Normal ovary. Cytologic preparation. Dog.** The basophilic background contains variable sized lipid droplets and red blood cells. Several singly luteal cells characterized by abundant pale basophilic cytoplasm with small clear discrete vacuoles and eccentric round to oval nuclei. (May-Grünwald-Giemsa; IP.)

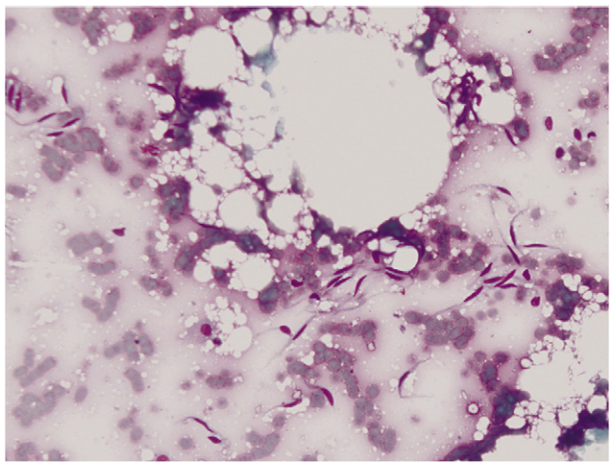
moderate numbers of one or more of the following cells based on the stage of the estrous cycle: adipocytes, individual fibrocytes/fibroblasts, small monolayered cohesive sheets of nonreactive mesothelial cells, granulosa cells that are uniform in size and shape and are arranged in acinar formations or in small loosely to cohesive aggregates, and singly luteal cells characterized by abundant pale basophilic cytoplasm with small, clear, discrete vacuoles and eccentric round to oval nuclei (Figs. 12-16, 12-17, 12-18, 12-19).

Cysts

Cysts in and around the ovary are a common finding during ovariohysterectomy in dogs and cats. There are two types of cysts: intraovarian and paraovarian. Intraovarian cysts include cystic rete ovarii, subsurface epithelial structure (only dog), vascular hematomas, and



■ **FIGURE 12-18. Normal ovary. Cytologic preparation. Dog.** The basophilic background contains variable sized lipid droplets. Two singly luteal cells characterized by abundant pale basophilic cytoplasm with small, clear, discrete vacuoles and eccentric round to oval nuclei. (May-Grünwald-Giemsa; HP oil.)

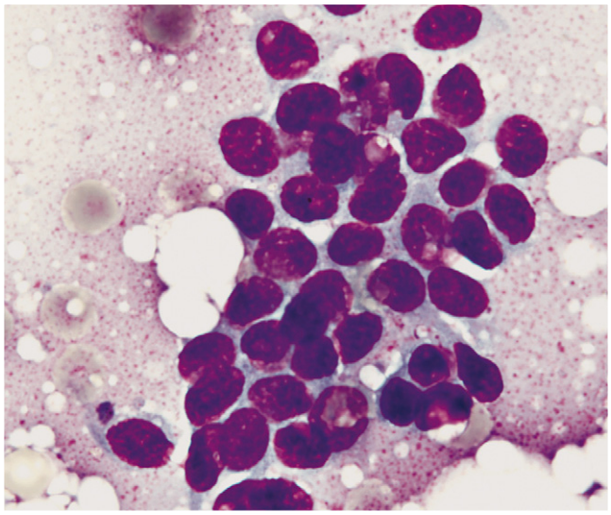


■ **FIGURE 12-16. Normal ovary. Cytologic preparation. Dog.** The basophilic background contains red blood cells, variable sized lipid droplets, and cellular debris. Numerous fibrocytes/fibroblasts are noted. (May-Grünwald-Giemsa; IP.)

adenomatous hyperplasia of the rete ovarii (Foster, 2007; Klein, 2007).

Inflammation

Oophoritis, or inflammation of the ovary, is rare in domestic animals. Bacterial oophoritis occasionally is found in cats and dogs (Foster, 2007). The inflammation is around the ovary and within the uterine tube, suggesting that the causative bacteria ascended from uterus (Van Israel et al., 2002). In cats, feline infectious peritonitis can cause oophoritis.



■ **FIGURE 12-19. Normal ovary. Cytologic preparation. Dog.** Cells that are uniform in size and shape and are arranged in small, loose aggregates are consistent with granulosa cells. (May-Grünwald-Giemsa; HP oil.)

Ovarian Neoplasia

Tumors of the ovary are uncommon in dogs and cats accounting for 0.5% to 6.3% of all canine tumors and 0.8% of all feline tumors (McEntee, 2002). The actual frequency of ovarian tumors may be underestimated as ovaries are not routinely sectioned at necropsy and are more commonly examined only if there is a gross lesion. In addition, the low frequency is affected by the fact that many companion animals are neutered at an early age. There are four main categories of ovarian tumors including epithelial, germ cell, sex cord–stromal, and mesenchymal. There are several other miscellaneous neoplastic diseases of the ovaries (mixed tumors and metastatic nonovarian malignant neoplasms). Clinical signs typically occur secondary to a space-occupying mass or to an effusion related to metastasis. Clinical signs in dogs with functional tumors secondary to excessive estrogen and/or progesterone production include signs of persistent estrus, pyometra, and bone marrow toxicity. Ovarian tumors can be an incidental finding at the time of ovariohysterectomy or necropsy (Klein, 2007).

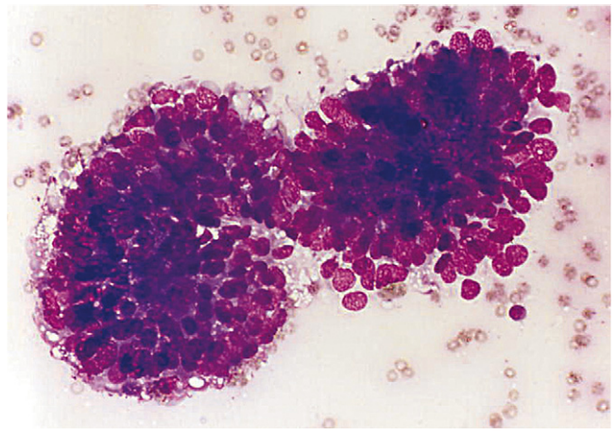
Epithelial Tumors

Epithelial tumors include papillary adenomas/cystadenomas, papillary adenocarcinoma, cystadenocarcinoma, rete adenomas, and undifferentiated carcinomas (MacLachlan and Kennedy, 2002) and account for 40% to 50% of canine ovarian tumors. Fifty percent of malignant epithelial tumors metastasize by implantation or lymphatic or vascular invasion. These tumors occur in older female dogs with a median age of 10 to 12 years (McEntee, 2002). Epithelial tumors are extremely rare in cats (Klein, 2007).

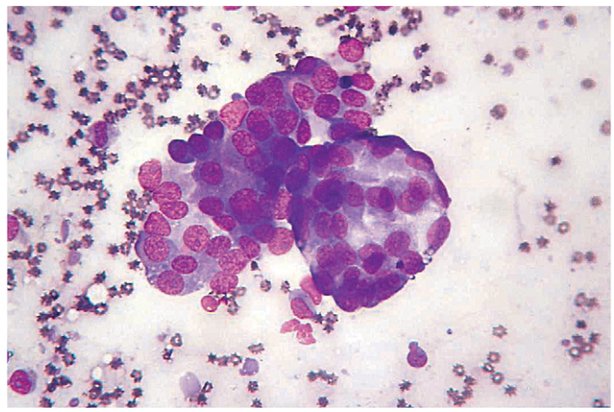
Papillary adenocarcinoma has been recently described cytologically. Cells are arranged in macro- to micropapillary forms (Masserdotti, 2006), acinar or tubular patterns, in cohesive clusters sometimes tridimensional, and occasionally as single cells (Figs. 12-20, 12-21, 12-22). Cells are round to polyhedral with a single oval nucleus. Nuclear chromatin is reticular to coarse. Nucleoli are indistinct to prominent single or multiple. Mild to marked anisokaryosis and anisocytosis are present. The cytoplasm is scarce to moderate and sometimes with finely discrete, clear vacuoles. Occasionally, large intracytoplasmatic vacuoles or signet ring cells are observed (Bertazzolo et al., 2004; Hori et al., 2006).

Sex-cordal Stromal Tumors

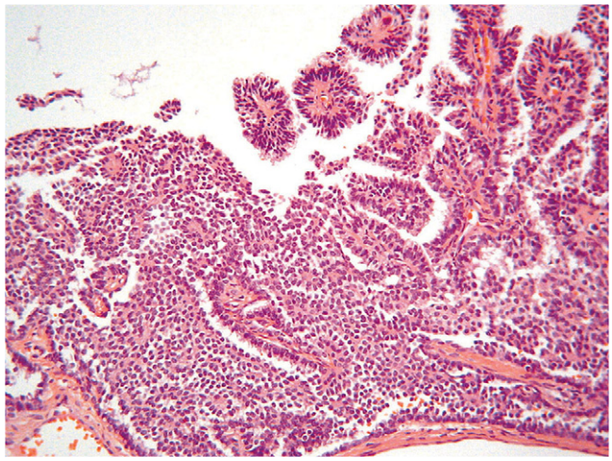
Sex-cordal stromal tumors include granulosa cell tumors, luteomas (also called interstitial gland, lipid, or interstitial cell tumors), and thecomas. In the dog, granulosa cell tumors account 50% of ovarian tumors and occur in elderly bitches with a median age of 10 to 12 years. Seventy-seven percent of granulosa cell tumors produce estrogens and/or progesterone and up to 20% are malignant. Granulosa cell tumor is the most common sex-cordal stromal tumor in older cats and more than 50% are malignant. Reported



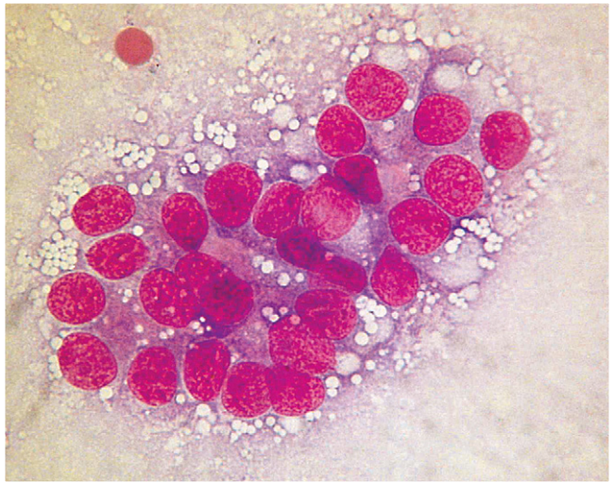
■ **FIGURE 12-20. Ovarian papillary adenocarcinoma. Cytologic preparation. Dog.** A cluster of cohesive neoplastic epithelial cells are arranged in a papillary pattern. (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)



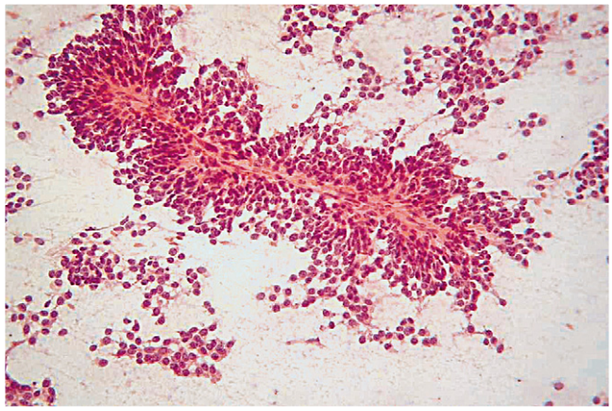
■ **FIGURE 12-21. Ovarian papillary adenocarcinoma. Cytologic preparation. Dog.** Shown is a round papillary cluster of cohesive neoplastic epithelial cells known as a "cell ball." (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)



■ **FIGURE 12-22. Ovarian papillary adenocarcinoma. Tissue section. Dog.** There is dense proliferation of hyperchromic epithelial cells, some of which display acinar formations. (H&E; LP.) (Courtesy of Dr. Walter Bertazzolo.)



■ **FIGURE 12-23. Granulosa cell tumor. Cytologic preparation. Dog.** A loosely monolayered aggregate of granulosa cells with a moderate amount of finely vacuolated cytoplasm is present. Cells are arranged in an acinar pattern. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)



■ **FIGURE 12-24. Granulosa cell tumor. Tissue section. Dog.** A large cluster of granulosa cells appears with a perivascular pattern. (H&E; LP.) (Courtesy of Dr. Walter Bertazzolo.)

metastatic sites include peritoneum, lumbar lymph nodes, omentum, diaphragm, kidney, pancreas, spleen, liver, and lungs (McEntee, 2002). Granulosa cell tumors may be confused sometimes with ovarian epithelial tumors even in histological preparations. Useful immunohistochemical markers to distinguish these two tumors are cytokeratin 7 and inhibin-α. Ovarian epithelial tumors cells are positive to cytokeratin 7 and negative to inhibin-α while granulosa tumor cells and thecomas are negative to cytokeratin 7 and positive to inhibin-α (Riccardi et al., 2007; Klein, 2007).

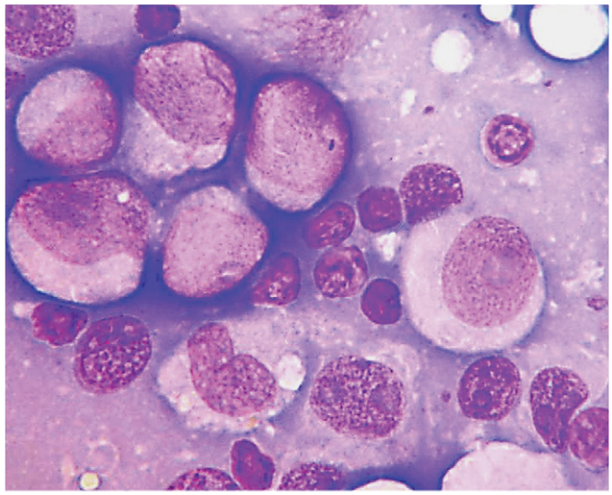
Cytologically, granulosa tumor cells are usually in monolayered, loosely cohesive clusters and often have acinar to tubular pattern (Fig. 12-23). Cells are arranged sometimes in an acinar pattern surrounding amorphous eosinophilic extracellular material called Call-Exner–like bodies. Capillary-like structures are occasionally evident inside large clusters of cells (Fig. 12-24). Single cells appear from round to polyhedral. Nuclei are round to oval with indistinct nucleoli and mild to moderate cellular atypia. The cytoplasm is scarce to moderate with variable amounts of vacuolated cytoplasm (Bertazzolo et al., 2004).

Feline luteomas have been recently cytologically described. Large round to oval cells arranged individually or in loose clusters are observed. Nuclei are central to eccentric with granular chromatin with prominent, small, central nucleoli. Anisokaryosis is mild to moderate. Cytoplasm is lightly basophilic with many variably sized clear vacuoles and occasionally small purple granules (Choi et al., 2005).

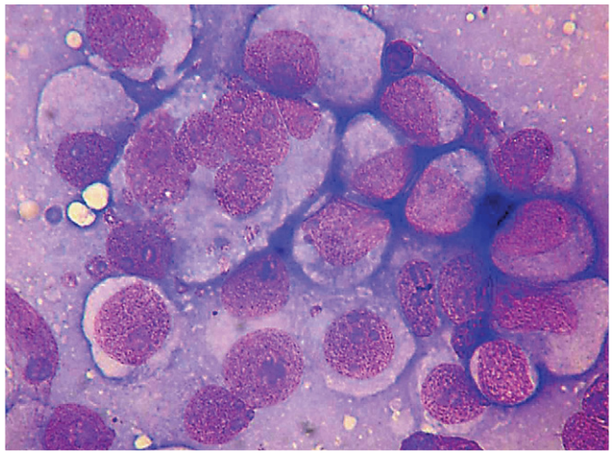
Germ Cell Tumors

Germ cell tumors include dysgerminoma (counterpart of the testicular seminoma), teratoma, and teratocarcinoma. Dysgerminoma represent a less-differentiated tumor than mature teratoma. Germ cell tumors comprise 6% to 20% of canine ovarian neoplasms and 15% to 27% of feline ovarian neoplasms. The median age of dogs with dysgerminoma is 10 to 13 years and with teratomas is 4 years. The age of cats that have been reported to have dysgerminomas ranges from 1 to 17 years with a median of 5 years. Metastasis is reported to develop in 10% to 20% of canine dysgerminomas with regional lymph nodes, liver, brain, and kidney as the primary sites. Young cats (5 to 8 months) and dogs have teratomas (McEntee, 2002; Klein, 2007).

Dysgerminomas are seen cytologically as a predominant population of markedly pleomorphic, large, round to polygonal cells arranged singly or in loose aggregates. Cells range from 20 to 70 μm of diameter. Nuclei are large and round to oval with chromatin stippled to reticular (Figs. 12-25 and 12-26). Nucleoli are prominent multiple and of variable shape and size. Aberrant mitotic figures and bi- or multinucleated cells are commonly noted. Anisocytosis and anisokaryosis are marked. The cytoplasm is scant clear to blue-gray with variably distinct margins. Occasionally eosinophilic, granular, intracytoplasmic material is



■ **FIGURE 12-25. Ovarian dysgerminoma. Cytologic preparation. Dog.** Large neoplastic cells are round and are arranged singly. Nuclei are pleomorphic in shape and located centrally or eccentrically with a stippled to coarse chromatin pattern and prominent nucleoli. Anisokaryosis and anisocytosis are moderate to marked. The cytoplasm is moderate to abundant and pale basophilic. Lysed cells and small lymphocytes are present. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)

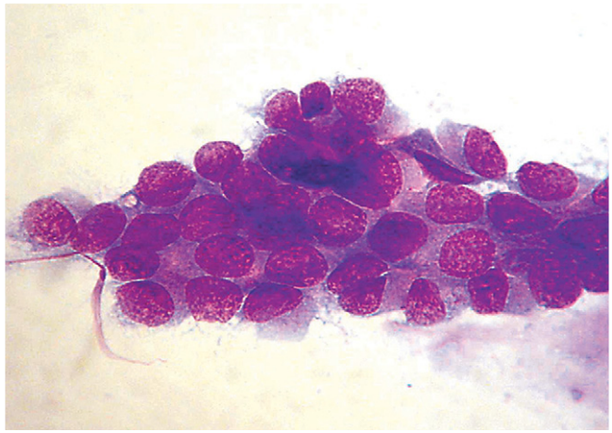


■ **FIGURE 12-26. Ovarian dysgerminoma. Cytologic preparation. Dog.** Note the multinucleated cell with marked anisokaryosis and anisocytosis. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)

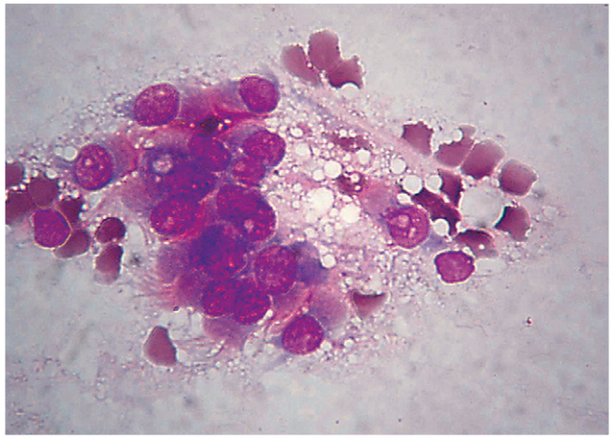
noted. Small lymphocytes can be observed (Bertazzolo et al., 2004; Brazzell and Borjesson, 2006).

Cytologically, teratomas are seen in a necrotic background, moderate neutrophilic-macrophagic inflammation, clusters of sebocytes or other mature epithelial cells, abundant keratin debris, and mature keratinocytes (Figs. 12-27, 12-28, 12-29, 12-30) (Bertazzolo et al., 2004).

Surgery remains the mainstay of treatment of ovarian tumors. A complete ovariohysterectomy is recommended. Careful examinations of all serosal surfaces and removal or biopsy of any lesions suspected of



■ **FIGURE 12-27. Teratoma. Cytologic preparation. Dog.** A cluster of cohesive epithelial basal-like cells is shown. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)



■ **FIGURE 12-28. Teratoma. Cytologic preparation. Dog.** Epithelial cells have a basally polarized round to oval nucleus with an evident eosinophilic brush border suggestive of differentiation towards respiratory epithelium. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)

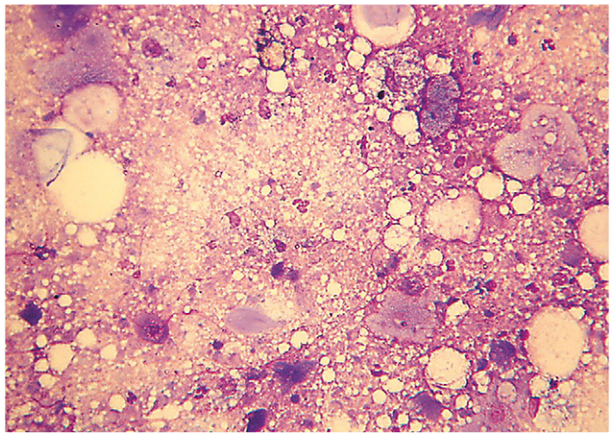
metastatic disease are recommended for staging purposes. Successful palliation with chemotherapy has been reported but no standard recommendations have been established (Klein, 2007).

Uterus

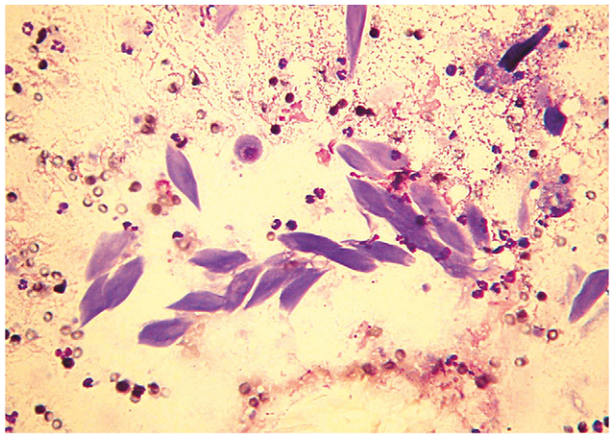
Indications for uterine cytology/biopsy include evaluation of degree of cystic endometrial hyperplasia, inflammation, neoplasia, and prognostic assessment for fertility (Root Kustritz et al., 2006).

Special Collection Techniques

Cells may be collected at the time of hysterotomy or be retrieved transcervically (Root Kustritz, 2006). This last technique involves visualizing the cervix with a rigid endoscope and passing a catheter through the cervix into the uterus. Samples for microbiology and cytology are



■ **FIGURE 12-29. Teratoma. Cytologic preparation. Dog.** Necrotic background, keratin debris, neutrophils and macrophages are evident. (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)



■ **FIGURE 12-30. Teratoma. Cytologic preparation. Dog.** Keratinocytes, keratin debris, neutrophils, and red blood cells are seen. (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)

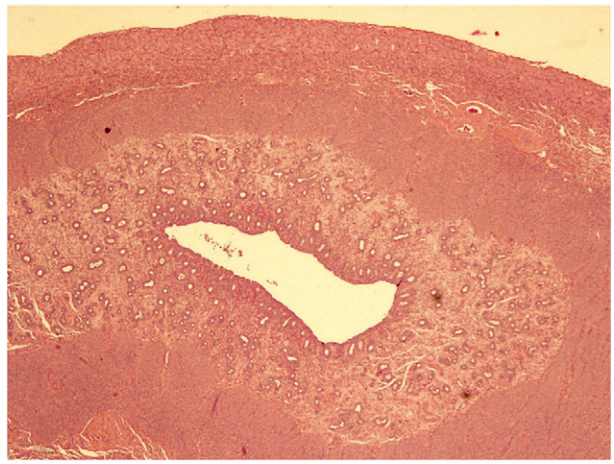
obtained by the infusion and aspiration of sterile normal saline. This technique allows uterine microbiology and cytology of the normal bitch throughout the reproductive cycle (Watts et al., 1997, 1998). Complications include vaginal inflammation, tearing, and endometritis mainly when samples are taken in anestrus (Watts et al., 1997). Another technique is hysteroscopy, performed in anesthetized bitches with a laparoscope and air insufflation of the uterus. Side effects are petechiae or ecchymosis on endometrium in 50% of bitches (Gerber and Nöthling, 2001).

Normal Anatomy and Histology

Cats and dogs have a bicornuate uterus with uterine horns and a uterine body. The uterine tubes have four regions: the infundibulum, ampulla, isthmus, and uterotubal junction. It is supported by a mesosalpinx. The mesosalpinx of the dog completely surrounds the ovary and has a large amount of fat; a small hole connects the bursa to the abdominal cavity. The infundibulum surrounds the ovary. The wall of the uterus has three layers: the outer perimetrium (serosa), middle myometrium, and inner endometrium (mucosa). The perimetrium is composed of loose connective tissue and covered by peritoneal mesothelium. The myometrium is divided into a thick, inner circular layer and a thin, outer longitudinal layer (Fig. 12-31). A richly vascularized and well-innervated stratum vasculare usually separates the muscle layers. The epithelium of the endometrium is simple cuboidal or columnar in the bitch and queen depending of the estrus cycle. Simple, branched endometrial glands extend into the lamina propria. The cervix is the structure that separates the external genitalia from uterus and is an effective barrier from the external environment. The cervix does not have transverse folds and tends to open dorsally (Foster, 2007). The normal histology of uterus from immature female dogs and sequential microscopic changes that occur during different stages of the estrous cycle are reviewed elsewhere (Rehm et al., 2007).

Normal Cytology

The normal endometrial epithelial cells vary morphologically throughout the reproductive cycle and have signs of epithelial degeneration defined as nuclear pyknosis, karyorrhexis or karyolysis, and/or cytoplasmic clear vacuoles during late diestrus and during early and mid-anestrus following diestrus and postpartum. The number of degenerating epithelial cells decreases with time until late anestrus, when all endometrial epithelial cells are cuboidal to low columnar and lack signs of degeneration. Endometrial epithelial cells are arranged in monolayered, cohesive clusters and acinar forms are



■ **FIGURE 12-31. Normal uterus. Tissue section. Dog.** The inner mucosa or endometrium is lined by cuboidal or columnar epithelium. The uterine glands present in the mucosa extend deep into the lamina propria as tubular formations. The dense area surrounding the endometrial glands is the myometrium, which consists of two encircling layers of smooth muscle. The outermost layer is the perimetrium or mesothelial-lined serosa. (H&E; LP.) (Courtesy of Dr. Carlo Masserdotti.)

commonly seen. Single cells are less frequently observed. The endometrial epithelial cells are low columnar during proestrus and estrus and columnar during early diestrus and pregnancy. During proestrus, estrus, early diestrus, and pregnancy, the cells have intact nuclei and uniformly staining cytoplasm. The nuclei of normal endometrial epithelial cells are usually round or oval with fine, stippled chromatin. The nuclei of degenerated endometrial cells are often of irregular shape and pyknotic. Neutrophils are the most common leucocytes observed during proestrus, estrus, diestrus, and early pregnancy, and lymphocytes and macrophages are frequently seen during anestrus. Erythrocytes are present in variable numbers at all stages of the reproductive cycle. Spermatozoa are observed in samples collected during estrus and early pregnancy in bitches that had their last mating 1 to 3 days previously. Bacteria are commonly observed during proestrus and estrus. Cornified cervical or vaginal cells are present during proestrus and estrus (Watts et al., 1998).

Microorganisms are frequently recovered from the uterus during proestrus and estrus, but rarely at other stages of the reproductive cycle. The uterine microflora often reflects the vaginal microflora during proestrus and estrus (Watts et al., 1997).

Inflammation

Cystic Endometrial Hyperplasia-Pyometra Complex and Metritis

Cytologic examination of vaginal discharges or uterus samples may be useful for diagnosis of inflammatory disease of the uterus in dogs and cats. Cystic endometrial hyperplasia-pyometra complex is a disease that is mainly characterized by progesterone-induced hyperplasia of the endometrium with cystic dilatation of the endometrial glands and inflammation of the uterus with purulent content in the uterine lumen (pyometra) leading to several clinical signs (Agudelo, 2005). The common presentation of pyometra involves older, unbred bitches presenting from 4 weeks to 4 months following estrus with mild to severe evidence of systemic illness (Smith, 2006). Clinical signs may include anorexia, depression, polyuria, and/or polydipsia and abdominal distention with or without vaginal discharge (open and closed-cervix pyometra, respectively). Typically, the bitch is afebrile and will often have leukocytosis, although leukopenia is also less commonly reported. Prerenal azotemia commonly accompanies the dehydration present. This systemic disease may result in death due to toxemia, renal disease, and peritonitis. There is an increased risk of pyometra in some breeds. Cystic endometrial hyperplasia-pyometra complex is considered to be less common in cats, probably because cats are induced ovulators, which limits uterine exposure to progesterone. The disorder is extensively reviewed elsewhere (Agudelo, 2005). *Escherichia coli* is the most frequently isolated microorganism in canine and feline pyometras (Hagman and Kühn, 2002). Anecdotally, *Tritrichomonas foetus* infection and cholesterol granuloma have been reported independently in the uterus of two different cats with pyometra (Dahlgren et al., 2007; Zanghì et al., 1999). Treatment of choice for pyometra is ovariohysterectomy with supportive therapy including appropriate antibiotic administration. The combination of a prolactin inhibitor, prostaglandin, and an antibiotic treatment in bitches with pyometra appears to have been effective in rapid clinical improvement, terminating the luteal phase and promoting uterine evacuation. This combination may be useful not only in bitches that are required for future breeding, but also in bitches that are a high anesthetic risk (England et al., 2007).

Metritis usually follows parturition and is characterized by a systemically ill animal with a malodorous uterine/vaginal discharge. The treatment of metritis is also ovariohysterectomy if the owner is not interested in further breeding or if severe systemic illness is present. Nursing puppies or kittens should be weaned and hand-raised.

Large numbers of neutrophils, many of which are degenerate (Olson et al., 1984b), characterize smears prepared from vaginal discharges resulting from open-cervix pyometra or metritis. Bacteria may be seen extracellularly and within the neutrophils. Muscle fibers from decomposing fetuses may rarely be visible in samples from metritis (Allison et al., 2008).

Uterine Neoplasia

Uterine tumors occur infrequently in dogs and cats accounting for 0.3% to 0.4% and 0.2% to 1.5% of all canine and feline tumors, respectively. Middle-aged to older animals are most commonly affected (Klein, 2007). In the dog, uterine leiomyomas are reported most common and leiomyosarcomas are comparatively rare. These tumors are of similar cytologic appearance to those found in other body sites. Uterine carcinomas are rare (McEntee, 2002). In cats, both leiomyoma and endometrial adenocarcinoma are reported with similar frequencies (Miller et al, 2003). A complete ovariohysterectomy is recommended, and attempts should be made to remove all tumors and metastatic foci (Klein, 2007).

Vagina

Examination of exfoliated vaginal cells for staging the estrous cycle is one of the most common uses of cytology in veterinary practice. This technique is easy to perform and, with some experience, can be successfully used by the clinician to optimize breeding of client animals. Cytologic examination of vaginal mucosal imprints and discharges is also useful for evaluation of vaginal inflammation and neoplasia of the female reproductive tract (Root Kustritz, 2006).

Special Collection Techniques

Several techniques have been described for obtaining vaginal cells for cytologic examination (Mills et al., 1979). Most commonly, a saline-moistened cotton swab or thin glass rod with a rounded tip is directed craniodorsally into the caudal vagina. The vestibule and clitoral fossa should be avoided since keratinized superficial squamous cells present in these sites may alter cytologic interpretations. Once craniad to the urethral orifice,

vaginal cells are obtained by gently passing the swab or glass rod over the epithelial lining (Root Kustritz, 2006). In an alternate method of sample collection, a small glass bulb pipette containing sterile saline is passed into the caudal vagina and cells are obtained by repeatedly flushing and aspirating the saline fluid (Olson et al., 1984a). Once collected, the exfoliated cells are gently transferred onto a clean microscope slide for staining. In addition, endoscopic vaginoscopy is a useful diagnostic procedure for evaluating the nature and the extent of disease in the vestibule and vagina and for obtaining adequate samples for microscopic evaluation. The technique is reviewed in depth elsewhere (Lulich, 2006). Although several types of stains have been used for cytologic evaluation of vaginal cells, Romanowsky-type stains or aqueous-based Romanowsky stains are most commonly used. These stains are easy to use in a clinical setting and provide good morphologic detail for determining the degree of maturation of the epithelial cells. Papanicolaou or trichrome stains have also been used for estrous cycle staging. These stains impart a distinctive orange staining to the keratin precursors abundant in superficial cells. The ratio of orange or eosinophilic cells to noneosinophilic cells, termed the *eosinophilic index,* can be used to assess the degree of maturation of the epithelial cells and subsequently stage the estrous cycle. However, these stains may yield variable staining results and the need for multiple solutions limits their practical use. However, an ultrafast modified Papanicolaou stain seems to be a useful technique in the study of vaginal cytology as a tool for assessing the estrous cycle in the bitch (Perez et al., 2005). Indications for vaginal culture include any disorder of the genitourinary tract associated with vulvar discharge and anterior vaginal culture in proestrus for diagnosis of uterine infection (Root Kustritz, 2006). The vagina is not sterile and larger numbers of normal flora are routinely cultured from the caudal vagina than the cranial vagina and during estrus than diestrus or anestrus. However, a larger number of organisms are retrieved from bitches with reproductive tract disease than from normal bitches. It is important to provide a quantitative culture result due to the fact that reproductive tract infection is caused by overgrowth of normal flora.

Normal Anatomy and Histology

The vagina is a musculomembranous canal extending from the uterus to the vulva. The vaginal wall is composed of an inner mucosal layer, a middle smooth muscle layer, and an external coat of connective tissue and peritoneum (cranially) (Banks, 1986). The mucosal layer consists of stratified squamous epithelium, which undergoes characteristic morphologic changes in association with the estrous cycle. Although the mucosa is typically nonglandular, intraepithelial glands have been observed during estrus in the dog. The vulva is anatomically similar to the caudal vagina. The vulva is composed of the vestibule containing the urethral orifice, the clitoral fossa, and the labia. The mucosa is lined by stratified squamous epithelium; some keratinized epithelial cells may be found in the vestibule and clitoral fossa (Allison et al., 2008) Vestibular glands within the submucosal layer of the vestibule are responsible for mucus production, which is most notable during estrus and at parturition (Banks, 1986). The normal histology of vagina from immature female dogs and sequential microscopic changes that occur during different stages of the estrous cycle are reviewed elsewhere (Rehm et al., 2007).

Normal Cytology

Four types of vaginal epithelial cells may be identified by exfoliative cytology. In order from the deepest and most immature cells to the most superficial and mature, these cells are basal, parabasal, intermediate, and superficial.

Basal cells are located along the basement membrane and give rise to the other epithelial cell types seen in a vaginal smear (Allison et al., 2008). Round nuclei and scant amounts of basophilic cytoplasm characterize these small cells. Because of their deep location, basal cells are rarely seen in vaginal preparations.

Parabasal cells are the smallest of the epithelial cells seen in routine vaginal cytologic samples. These cells have a high nuclear-to-cytoplasmic ratio, round nuclei of uniform size and shape, and basophilic cytoplasm. Parabasal cells or intermediate cells containing cytoplasmic vacuoles are called *foam cells;* the significance of the vacuoles is unknown (Olson et al., 1984a). These cells may be associated with diestrus and anestrus. Large numbers of parabasal cells may be seen in vaginal smears of prepubertal animals and should not be confused with neoplastic cells (Feldman and Nelson, 2004).

Intermediate cells may vary in size, but are generally twice the size of parabasal cells. The nuclear-to-cytoplasmic ratio is decreased with abundant amounts of blue to blue-green (keratinized) cytoplasm. The cytoplasmic borders are round to irregular and folded (Baker and Lumsden, 1999). Intermediate cells may also be called *superficial intermediate* or *transitional intermediate cells* (Allison et al., 2008).

Superficial cells are characterized by small round to pyknotic nuclei, abundant amounts of light blue to blue-green (keratinized) cytoplasm, and angular to folded cell borders. Some superficial cells contain dark-staining bodies of unknown significance (Olson et al., 1984a). As superficial cells age and become degenerate, the nuclei are lost and the cells become anucleated. Superficial cells with pyknotic nuclei and anucleated superficial cells have the same physiologic significance (Allison et al., 2008). Folded, angular cells with pyknotic or absent nuclei are called anuclear squames or anuclear superficial cells (Feldman and Nelson, 2004).

Metestrum cells are large, intermediate vaginal cells that appear to have one or more neutrophils contained within their cytoplasm. These cells are usually seen in diestrus or vaginitis and such cells are rarely observed in early proestrus (Feldman and Nelson, 2004).

Staging the Canine Estrous Cycle

Duration, cytologic appearance, and hormonal status of the different stages of canine estrous cycle are described in Table 12-1.

TABLE 12-1 Duration, Cytologic Appearance, and Hormonal Status of Stages of Canine Estrous Cycle

Stages and Duration of Estrous Cycle		CYTOLOGIC APPEARANCE					
		Epithelial Cells	Neutrophils	Red Blood Cells	Bacteria	Background	Hormonal Status
Proestrus (9 days; range 3–17 days)	**Early**	Mixture of parabasal, intermediate and few superficial cells	Present	May be abundant or absent. Usually present	Present	Granular or dirty appearance. Mucus can be present	Rising concentrations of estradiol and low concentrations of progesterone
	Late*	Mixture of superficial (>80%) and intermediate cells	Few or none	May be abundant or absent. Usually present	Present	Clear	
Estrus (9 days; range 3–21 days)		>80% superficial and anuclear squames cells. <5% parabasal or intermediate cells	Absent	Present or absent	Present	Clear	Declining estradiol concentrations and rising progesterone concentrations
Diestrus (2 months)		Abrupt 20% decrease in superficial cells and 15–20% increase in small, intermediate cells	Frequently present (few to many)	May be present but usually none	Present. Ingested bacteria within neutrophils may be seen	May contain large amounts of debris	High to low concentrations of progesterone
Anestrus (4.5 months)		Predominance of parabasal and intermediate cells. Superficial cells absent	Absent or low numbers	Absent	Absent or low numbers	Clear or granular	Low concentrations of progesterone

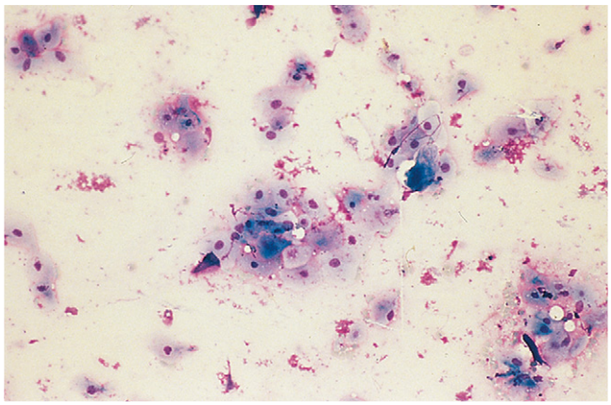
*It is not possible to distinguish late proestrus from estrus with vaginal cytology.

Proestrus

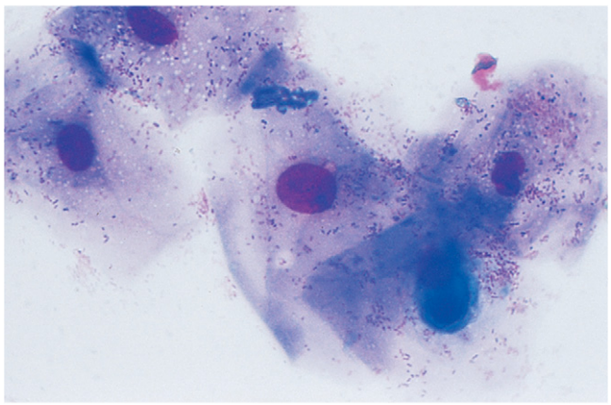
Proestrus (Figs. 12-32 and 12-33) is characterized by rising concentrations of estradiol and low progesterone concentrations (Freshman, 1991). As the estradiol concentrations increase, the vaginal epithelium proliferates and red blood cells move via diapedesis through uterine capillaries (Baker and Lumsden, 1999). In early to mid proestrus, neutrophils and a mixture of parabasal, intermediate, and superficial epithelial cells (Olson et al., 1984a) characterize the vaginal smear. As proestrus progresses, the neutrophils decrease in number and superficial epithelial cells begin to predominate.

Estrus

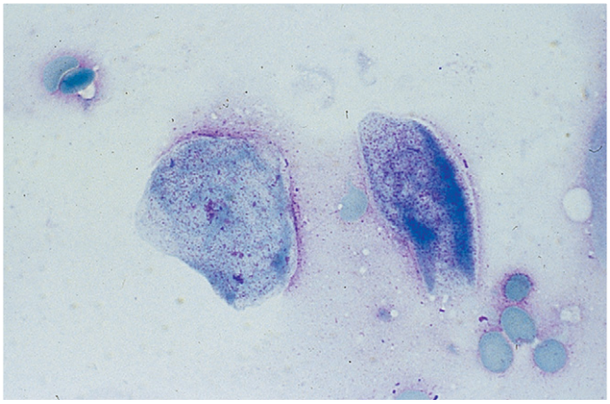
For optimal breeding efficiency, sperm should be present in the female reproductive tract as near to ovulation as possible. Although vaginal cytology has been shown to be a more accurate indicator of estrus (Fig. 12-34) and, subsequently, ovulation than behavioral signs, evidence of vaginal maturation or cornification is not closely associated with ovulation. Maximum cornification of vaginal superficial cells ranges from 6 days before the luteinizing hormone (LH) peak to 3 days after the LH peak (Olson et al., 1984a). Since ovulation usually occurs 1 to 2 days after the LH peak, vaginal cytology is not an accurate predictor of ovulation. Ova are viable for up to 2 days postovulation and sperm may remain viable for up to 4 days within the canine reproductive tract during estrus. Therefore, bitches should be bred every 2 to 3 days during cytologic estrus (greater than 90% superficial cells) for optimal breeding (Freshman, 1991). Use of plasma progesterone concentrations in combination with vaginal cytology more accurately indicates the time of ovulation, allowing for even greater breeding efficiency and more accurate estimation of the time of expected parturition (Wright, 1990).



■ **FIGURE 12-32. Proestrus. Vaginal smear. Dog.** There are intermediate epithelial cells with lower numbers of superficial cells. Red blood cells are present. The background has a basophilic appearance due to the presence of mucus. (Wright-Giemsa; HP oil.) (Sample provided by Rolf Larsen, University of Florida.)



■ **FIGURE 12-33. Late proestrus. Vaginal smear. Dog.** Intermediate and superficial cells appear with round to pyknotic nuclei and moderately basophilic cytoplasm with angular to folded borders. The cells are associated with large numbers of bacteria. (Wright-Giemsa; HP oil.) (Sample provided by Rolf Larsen, University of Florida.)



■ **FIGURE 12-34. Estrus. Vaginal smear. Dog.** Shown are anucleated (cornified) superficial epithelial cells along with the presence of red blood cells in the background. (Wright-Giemsa; HP oil.) (Sample provided by Rolf Larsen, University of Florida.)

Diestrus

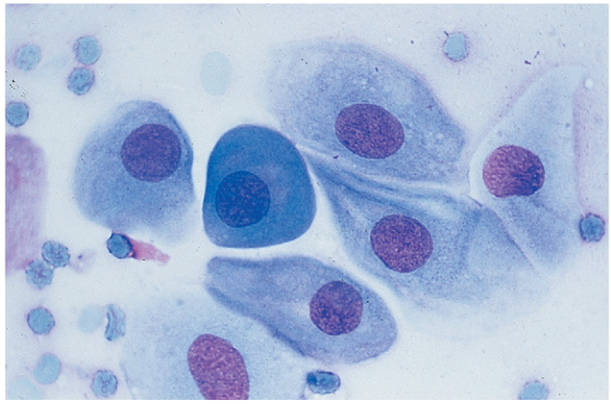
Diestrus (Figs. 12-35 and 12-36) is the luteal phase (Freshman, 1991). The decrease of superficial cells at the beginning of diestrus is usually more rapid than the increase of superficial cells occurring at estrus. Neutrophils frequently reappear during diestrus. Some neutrophils from normal bitches in diestrus contain ingested bacteria. The cytologic appearance of early proestrus and diestrus can be very similar; thus one vaginal smear is not adequate for differentiation of these two stages (Olson et al., 1984a). Once cytologic evidence of diestrus is apparent, breeding is unlikely to be successful.

Anestrus

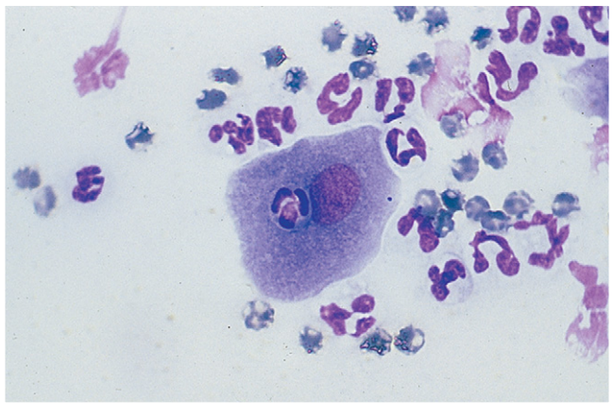
Anestrus, the period between the end of diestrus and the beginning of the next proestrus, is a time of uterine involution and endometrial repair (Freshman, 1991).

Staging the Feline Estrous Cycle

Cats are seasonally polyestrous. Coitus is necessary for ovulation, with successive estrous cycles occurring until ovulation takes place (Allison et al., 2008). The



■ **FIGURE 12-35. Diestrus. Vaginal smear. Dog.** Parabasal and intermediate epithelial cells are shown. The parabasal cells have round nuclei, moderate nuclear-to-cytoplasmic ratios, moderately to deeply basophilic cytoplasm, and round cell borders. The intermediate cells are larger with increased amounts of cytoplasm and angular borders. Red blood cells are present in the background. (Wright-Giemsa; HP oil.) (Sample provided by Rolf Larsen, University of Florida.)



■ **FIGURE 12-36. Diestrus. Vaginal smear. Dog.** Note the large number of neutrophils and red blood cells in the background. An intermediate epithelial cell containing a neutrophil (metestrum cell) is located in the center. These cells are not specific for diestrus and may be found whenever increased numbers of neutrophils are present. (Wright-Giemsa; HP oil.) (Sample provided by Rolf Larsen, University of Florida.)

average duration of estrus is 8 days (range 3 to 16 days) with an intermediate period of 9 days (range 4 to 22 days) if ovulation does not occur. In the presence of ovulation without pregnancy, the return to estrus may be delayed for about 45 days (Olson et al., 1984a). Vaginal cytology has been shown to accurately predict the various stages of the estrous cycle in the cat (Shille et al., 1979; Mills et al., 1979). Collection of smears for cytologic evaluation is similar to those described for the dog; collection of feline vaginal samples may rarely result in ovulation.

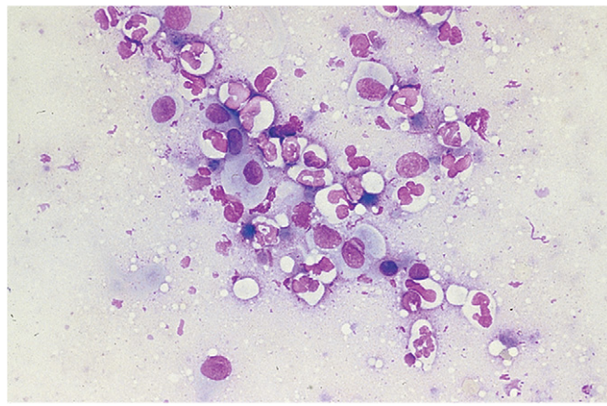
Changes in feline vaginal cytology during the estrous cycle are similar to those seen in the dog; however, some differences should be noted. Red blood cells are rarely seen in smears made at any stage of the cycle. Neutrophils are rare in smears from proestrus and are an inconsistent feature of diestrus. Superficial cells are the predominant cell type seen during estrus. In contrast to dogs, superficial cells comprise only 40% to 88% of the epithelial cells seen during feline estrus (Mills et al., 1979). Anucleated cells increase to about 10% of the epithelial population on the first day of estrus, with a maximum average of 40% anucleated cells by the fourth day of estrus. A prominent clearing of the vaginal smear background in association with estrus has been observed. This clearing occurred in 90% of feline estrus smears and was suggested to be a sensitive indicator of estrus in the cat (Shille et al., 1979).

Inflammation

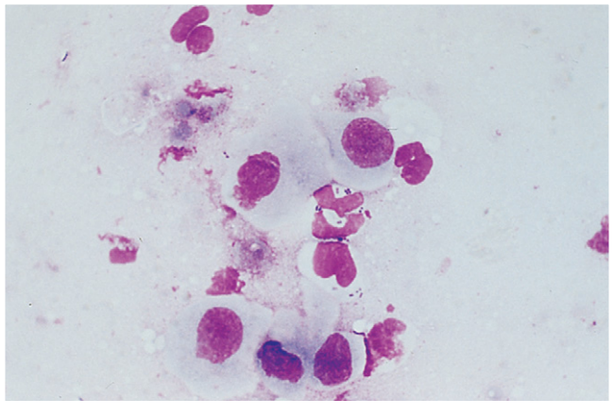
Vaginitis

Inflammatory disease of the vaginal mucosa is often related to noninfectious factors such as vaginal anomalies, clitoral hypertrophy, foreign bodies, neoplasia, or vaginal immaturity ("puppy vaginitis") (Olson et al., 1984b). Smears for cytologic evaluation of inflammation may be obtained from the vaginal mucosa, vaginal discharges, or FNA of vaginal/vulvar masses. Moderate to large numbers of neutrophils characterize acute vaginitis. In addition to neutrophils, lymphocytes and macrophages may be seen in more chronic inflammatory conditions (Allison et al., 2008). If an infectious component is involved in the inflammatory process, degenerate neutrophils and phagocytized bacteria may be seen (Figs. 12-37 and 12-38). Less commonly, hyphal elements related to fungal infection or pythiosis may be observed. Cytologic specimens may be submitted for silver stains to identify hyphae if fungal infection or pythiosis is suspected (Figs. 12-39 and 12-40).

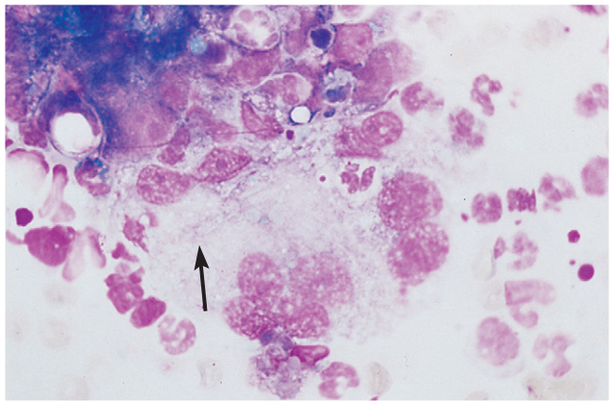
Treatment of vaginitis should involve identification and correction of any underlying conditions responsible for the inflammation. If sepsis is present, appropriate antibiotic therapy based on culture and sensitivity results should be instituted. Vaginitis can be associated with the



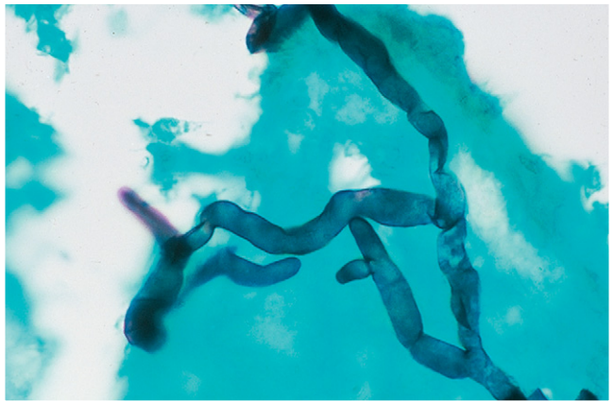
■ **FIGURE 12-37. Vaginitis. Tissue scraping. Dog.** Increased numbers of neutrophils from a vaginal papule. The neutrophils display degenerative nuclear changes of moderate to marked karyolysis. Degenerative changes are typically associated with bacterial infections. A few parabasal and intermediate epithelial cells are also present. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-38. Septic vaginitis. Tissue scraping. Dog.** Two degenerative neutrophils containing phagocytized bacteria from the vaginal scraping shown in Figure 12-37. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-39. Pyogranulomatous vaginitis. Tissue aspirate. Dog.** Pyogranulomatous inflammation is present in this specimen from a vulvar mass. Large numbers of neutrophils, lower numbers of eosinophils, and a multinucleated macrophage are present. Pale-staining linear structures suspicious for hyphae are seen associated with the macrophage *(arrow)*. (Wright-Giemsa; HP oil.)



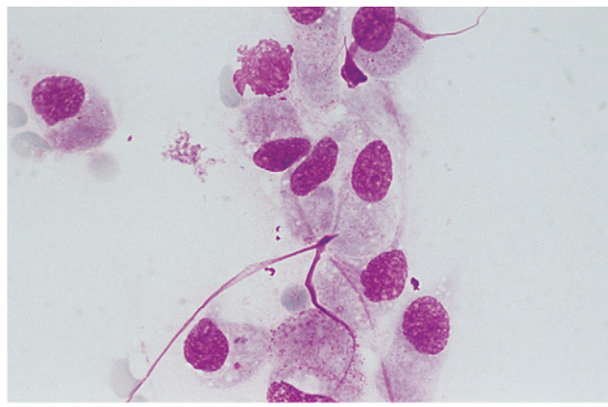
■ **FIGURE 12-40. Fungal vaginitis. Tissue aspirate. Dog.** Special stain of sample shown in Figure 12-39. Positive-staining, poorly septated, linear structures approximately 6 to 8 µm in width are present. Culture confirmed the presence of *Pythium* sp. (Gomori's methenamine silver; HP oil.)

presence of epithelial cells displaying atypical cellular features in response to the inflammatory process. In the absence of a tumor, therapy to alleviate the inflammation should eliminate the atypical cells. However, if an observable mass is present and/or atypical cells remain after appropriate treatment, further tests to rule out the presence of neoplasia should be considered.

Vaginal Neoplasia

Vaginal and vulvar tumors are uncommon and tend to occur in older animals (Olson et al., 1984b; McEntee, 2002). The presenting clinical sign generally is a slow-growing perineal mass. Clinical signs seen less frequently include vulvar bleeding or discharge, an enlarging vulvar mass, dysuria, hematuria, tenesmus, excessive vulvar licking, and dystocia (Klein, 2007). Leiomyomas, fibroleiomyomas, fibromas, and fibropapillomas (polyps) are the most common vaginal neoplasm in dogs and cats (Baker and Lumsden, 1999). These benign mesenchymal tumors are characterized by variable numbers of spindle-shaped cells of uniform size and shape arranged individually and in small clumps (Fig. 12-41). The nuclei are typically oval and scant to moderate amounts of wispy cytoplasm are present. The most common malignant tumor is leiomyosarcoma and distant metastasis has been reported. Other tumors with malignant potential include transmissible venereal tumors (TVT), adenocarcinoma, squamous cell carcinoma, urethral transitional cell carcinoma, osteosarcoma, mast cell tumor, and epidermoid carcinoma (Klein, 2007). The cytologic appearance of these tumors is similar to those found in other body sites. Treatment of vaginal tumors usually involves conservative surgical excision combined with ovariohysterectomy, which is usually is curative for benign tumors (Klein, 2007). In cases of malignant tumors, further evaluation to determine extent of local invasion or metastasis should be performed.

TVT may also be diagnosed using cytologic examination of vaginal smears or fine-needle aspirates. TVT are

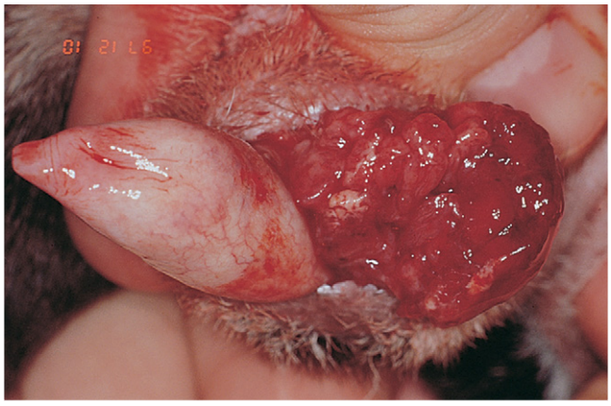


■ **FIGURE 12-41. Vaginal leiomyoma. Tissue imprint. Dog.** The cells are arranged individually or in small clumps and display round to oval nuclei with coarse nuclear chromatin, moderate nuclear-to-cytoplasmic ratios, and inconspicuous nucleoli. The cytoplasm is moderately basophilic and cell borders are indistinct. (Wright-Giemsa; HP oil.)

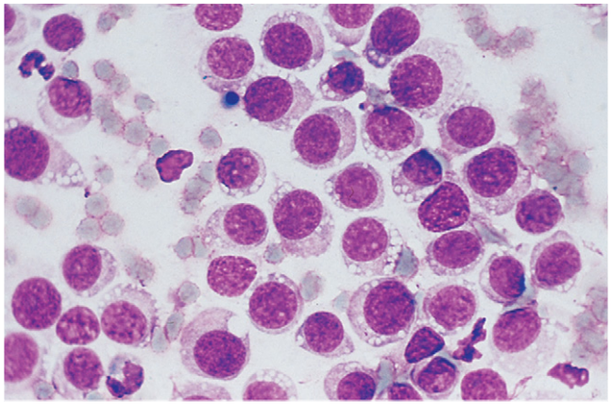
contagious, sexually transmitted tumors occurring in both genders. The tumors may be located in genital areas and extragenital sites such as the rectum, skin, oral and nasal cavities, and the eyes (Lorimier and Fan, 2007). They appear as firm, friable, tan, ulcerated, and nodular or polypoid masses (Fig. 12-42). In bitches, TVT may spread directly to the cervix, uterus, and oviducts. Although metastasis is uncommon, TVT can spread to regional lymph nodes, skin, and subcutaneous tissue. Other reported metastatic sites include lips, oral mucous membranes, eye, bone, musculature, abdominal viscera, lungs, and the central nervous system. TVT is suspected to be of histiocytic origin based on positive reactions to lysozyme, alpha-1-antitripsin, vimentin, and a macrophage-specific immunostain and negative reaction to immunostains specific for other cell types. Recently described is TVT with intracellular *Leishmania infantum* amastigotes that also suggests a histiocytic origin (Lorimier and Fan, 2007).

Aspirates of TVT generally yield large numbers of individualized, round cells (Figs. 12-43 and 12-44). The nuclei are round with clumped nuclear chromatin and single or multiple prominent nucleoli. The nuclei are located eccentrically. Moderate amounts of pale-blue cytoplasm frequently contain multiple punctate vacuoles. Mitotic activity is often high. Inflammation, as indicated by increased numbers of plasma cells, lymphocytes, macrophages, and neutrophils, may be present.

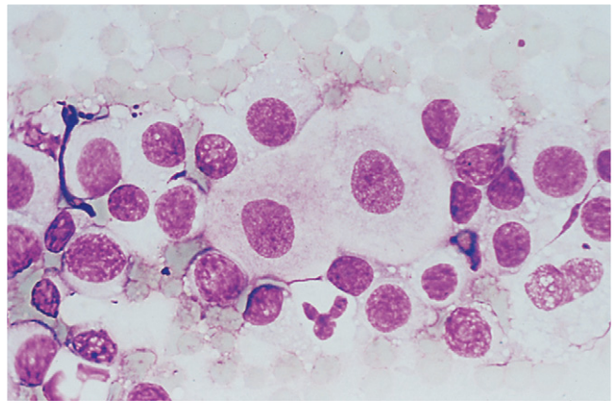
Marginal surgical resection is not considered effective treatment for TVT. The most effective treatments for TVT are chemotherapy and radiation. Single-agent therapy with vincristine has been shown to be very effective for TVT even in cases of metastatic disease. Doxorubicin is the drug of choice for TVT resistant to vincristine (de Lorimier and Fan, 2007).



■ **FIGURE 12-42. Transmissible venereal tumor. Genital mass. Dog.** The mass appears as a soft, friable, hemorrhagic mass on the prepuce.



■ **FIGURE 12-43. Transmissible venereal tumor. Vaginal mass imprint. Dog.** Large numbers of round cells are shown which have round nuclei, coarse nuclear chromatin, variably prominent nucleoli, and scant to moderate amounts of lightly basophilic cytoplasm. Many of the cells contain punctate cytoplasmic vacuoles, which is a characteristic feature of this tumor. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-44. Transmissible venereal tumor. Vaginal mass imprint. Dog.** Two intermediate epithelial cells (center) and individualized tumor cells from the same case as shown in Figure 12-43. Note the larger size and increased amounts of cytoplasm in the epithelial cells compared to the tumor cells. (Wright-Giemsa; HP oil.)

MALE REPRODUCTIVE SYSTEM: PROSTATE AND TESTES

Prostate Gland

Although the prostate gland is present in cats, the vast majority of prostatic disease is reported in the dog. Therefore, the following discussion of normal and abnormal findings associated with the prostate gland will be limited to the dog. Prostatic disorders are common in middle-aged and older male dogs and have been categorized as hyperplasia, cysts, inflammation, primary and metastatic neoplasia, and squamous metaplasia. More than one prostatic disorder may occur simultaneously (Baker and Lumsden, 1999; Johnston et al., 2000).

The primary presenting clinical findings associated with prostatic disease are signs of systemic febrile illness, lower urinary tract symptoms (hemorrhagic urethral discharge), abnormalities of defecation, and locomotion problems (Dorfman and Barsanti, 1995). Some cases of canine prostatic disease may be present without obvious clinical signs; therefore palpation of the prostate per rectum should be a part of all physical examination in mature intact and neutered male dogs. Normally, the

prostate should be smooth, symmetrical, and nonpainful. Abdominal palpation can be used to evaluate an enlarged prostate that has moved into the abdominal cavity. Ancillary diagnostic tests that may be used to evaluate suspected cases of prostatic disease include urinalysis, bacterial culture, radiography, and ultrasonography. Complete blood counts and serum biochemical profiles are usually normal in cases of prostatic illness; however, the presence of hemogram and biochemical abnormalities may help in diagnosis (Dorfman and Barsanti, 1995). Cytology, microbiology, and/or histopathology may be necessary for classification of the type of prostatic disease (Baker and Lumsden, 1999). Canine prostatic disease is commonly diagnosed using cytologic techniques, especially now that ultrasound guided FNA is widely available. The diagnostic accuracy of cytology in comparison with histopathologic diagnosis is 80% (Powe et al., 2004). In addition, cytology is a more sensitive method than histology for the detection of bacterial infection.

Special Collection Techniques

Urethral Discharge

Sampling of urethral discharge is a simple method for evaluation of prostatic abnormalities, but is the least effective technique (Baker and Lumsden, 1999). If present, urethral discharge is collected by retracting the prepuce, cleaning the glans, and collecting the discharge into a vial or onto a microscope slide for microscopic evaluation. Some samples may also be collected into sterile containers for bacterial culture and colony counts. Concurrent analysis of urine collected by catheterization or cystocentesis should be performed to differentiate between normal urethral flora and cystitis.

Semen Evaluation

A detailed description of canine and feline semen collection is not fully covered in this text but an in-depth review is available elsewhere (Freshman, 2002; Zambelli and Cunto, 2006). Ejaculate material for evaluation of prostatic disease can be obtained from intact dogs via manual stimulation; however, collection of semen may not be possible if the dog is inexperienced or in pain (Dorfman and Barsanti, 1995). A collection funnel may be used to separate the clear prostatic third fraction of the ejaculate from the sperm-rich first and second fractions (Olson et al., 1987). An aliquot for microbiologic analysis should be placed into a sterile culture tube with the remaining fluid retained for cytologic evaluation. If inflammation is suspected, the cytologic aliquot should be placed into a vial containing EDTA (Baker and Lumsden, 1999). Because of the presence of normal bacterial flora in the lower urethra, a quantitative culture should be performed on the ejaculate fluid. In the presence of inflammatory cells, high numbers (>100,000 cfu/mL) of gram-negative or gram-positive bacteria indicate an infectious process (Root Kustritz, 2006). If cytologic and microbiologic results are equivocal in regards to prostatic infection versus urethral contamination, a quantitative lower urethral culture to compare to the semen culture results may be useful (Dorfman and Barsanti, 1995).

Prostatic Massage

Prostatic massage is used primarily to collect prostatic fluid in dogs unable to ejaculate (Dorfman and Barsanti, 1995). The simplest method for prostatic massage involves passing a urinary catheter, guided by rectal palpation, to the caudal pole of the prostate. A syringe is attached to the catheter and fluid is aspirated as the prostate is gently massaged per rectum (Olson et al., 1987). A few milliliters of sterile saline may be flushed into the catheter and aspirated to facilitate collection of fluid for analysis. Urinary tract infection often accompanies infectious prostatitis, which may confound the results of prostatic massage. For these cases, an alternative massage procedure may be used to determine the source of the infection. The urinary bladder is catheterized, emptied of urine, and flushed with 5 mL of sterile physiologic saline. The fluid from this first flush is collected as the preprostatic massage fraction. The catheter is then retracted to the caudal pole of the prostate. Another 5 mL of sterile physiologic saline is injected through the catheter while the prostate is massaged per rectum. The catheter is then advanced back into the bladder and all the fluid in the bladder is collected. This fluid is the postprostatic massage fraction, which should be relatively free of urinary contamination (Root Kustritz, 2006). Bacterial colony counts and presence or absence of inflammatory cells from the pre- and postprostatic massage fractions can be compared to isolate the source of the infection. Ampicillin, which concentrates in urine but reaches lower concentrations in the prostate owing to its inability to cross the prostatic-lipid barrier, may be administered 1 day prior to prostatic massage to aid in isolation of the source of infection. In general, prostatic massage should be reserved for evaluation of prostatitis in dogs without urinary tract infection or in which the urinary tract infection is controlled. It should be noted that cytologic preparations obtained by catheterization typically yield a mixed population of urothelial cells (Thrall et al., 1985; Powe et al., 2004).

Fine-Needle Aspiration

FNA of the prostate gland has been shown to produce more reliable results and more prostatic cells than prostatic massage (Thrall et al., 1985). If the gland is enlarged, a transabdominal approach may be used. Transperineal and perirectal approaches have also been described (Olson et al., 1987). Ultrasound is particularly useful for guiding the aspiration needle, particularly if focal prostatic disease is present (Zinkl, 2008). The method of aspiration of the prostate gland is similar to that used for other tissues. A 22-gauge needle attached to a 12-mL syringe is directed into the gland and cells and/or fluid are aspirated. A drop of aspirate material or fluid is placed onto a slide. If necessary, any remaining material may then be submitted for culture.

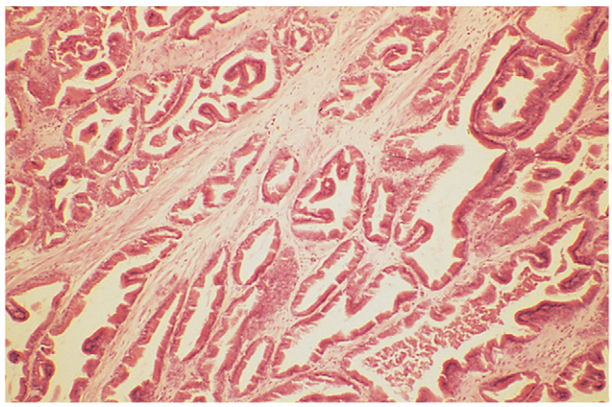
Use of FNA in cases of acute prostatitis or abscessation may be associated with a risk of peritonitis or seeding the infection along the needle tract (Dorfman and Barsanti, 1995). Dogs with suspected prostatic disease presenting with an inflammatory leukogram and fever should not undergo FNA. If purulent fluid is obtained

during aspiration of the prostate, aspiration should continue until all pressure is reduced to prevent leakage of the material (Baker and Lumsden, 1999). However, there are numerous reports in the veterinary literature documenting FNA for diagnosis or treatment of prostatic disease with no complications. Ultrasound-guided transabdominal FNA of the prostate is described elsewhere (Root Kustritz, 2006). FNA of the prostate gland has several advantages compared to other collection methods. Identification of squamous epithelial cells from a prostatic aspirate allows diagnosis of squamous metaplasia, whereas the presence of these cells in prostatic massage fluid could be misinterpreted as normal lower urinary tract squamous epithelial cells. Also, the greater cellular detail obtained via FNA increases the confidence of a diagnosis of neoplasia. The primary disadvantage of prostatic FNA is that focal lesions, such as neoplasia, may be missed (Thrall et al., 1985). However, use of ultrasound to guide the aspirate can lessen this possibility.

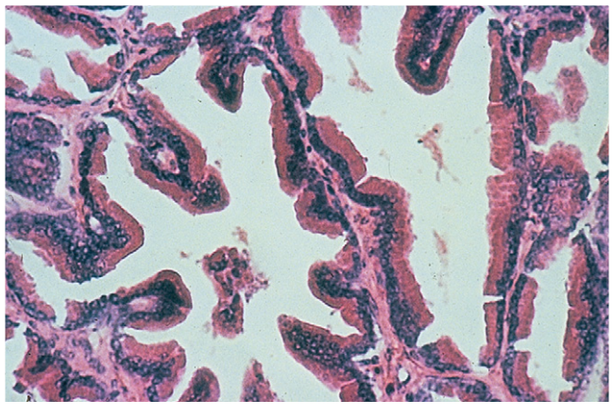
Normal Anatomy and Histology

The prostate gland secretes a fluid that promotes sperm survival and motility. Normal prostatic fluid is clear and represents the third fraction of the canine ejaculate, although some have suggested that the first fraction also originates from the prostate (Dorfman and Barsanti, 1995). The prostate gland is a glandular, muscular structure completely surrounding the proximal portion of the male urethra (Lowseth et al., 1990). Before 2 months of age, the prostate is located within the abdominal cavity. After breakdown of the urachal ligament until sexual maturity, the prostate lies in the pelvic canal. With increasing age, the prostate enlarges and moves over the pelvic brim into the abdomen. Bladder distension can also pull the prostate cranially into the abdomen.

The prostate gland is composed of compound tubuloalveolar glands radiating from the urethral opening (Figs. 12-45 and 12-46). The secretory alveoli contain primary and secondary enfoldings of epithelium that project into the alveolar lumen. A fibromuscular stroma surrounds the prostatic ducts, which are lined by cuboidal to columnar epithelium. Transitional epithelium lines the excretory ducts that open onto the urethra (Dorfman and Barsanti, 1995).



■ **FIGURE 12-45. Normal prostate gland. Tissue section. Dog.** The tubuloalveolar glands are surrounded by a fibromuscular stroma. Primary and secondary enfoldings of epithelium project into the alveolar lumen. (H&E; LP.) (Case material supplied by Roger Reep and Don Samuelson, University of Florida.)



■ **FIGURE 12-46. Normal prostate gland. Tissue section. Dog.** Higher magnification of specimen in Figure 12-45. Cuboidal and columnar epithelium line the prostatic lumens and ducts. Canine. (H&E; IP.) (Case material supplied by Roger Reep and Don Samuelson, University of Florida.)

Normal Cytology

The number and type of prostatic cells in cytologic samples from the prostate vary depending on the collection technique. Prostatic epithelial cells obtained via aspiration from normal dogs occur in frequent clusters and are cuboidal to columnar. These cells are uniform in size and shape and contain round to oval nuclei, which may be basilar in columnar cells. Nucleoli are usually small and inconspicuous. The cytoplasm is finely granular and basophilic (Thrall et al., 1985). Other cell types that may be seen, particularly from semen samples or prostatic massages, include spermatozoa, squamous epithelial cells, and transitional epithelial cells (urothelial cells) (Zinkl, 2008). Spermatozoa stain blue-green with Romanowsky and modified Romanowsky stains and may adhere to other cells. Squamous cells are large with abundant amounts of blue to blue-green (keratinized) cytoplasm. The nuclei of these cells may be round to pyknotic or absent. Cell borders are typically angular to folded. Transitional cells (urothelial cells) are larger than prostatic epithelial cells and have lighter-staining cytoplasm with a lower nuclear-to-cytoplasmic ratio. Normal ejaculate fluid may contain low numbers of neutrophils and red blood cells. Use of excessive amounts of ultrasound gel during ultrasound-guided FNA can result in large amounts of purple, variably sized, granular background debris that may obscure cellular detail (Zinkl, 2008). To prevent this artifact, excess gel should be removed before inserting the aspiration needle.

Prostatic Cysts

Prostatic cysts may occur as multiple, small cysts associated with benign hyperplasia, large prostatic retention, and periprostatic cysts that can be mineralized or result from osseus metaplasia, and cysts associated

with squamous metaplasia. Except for hyperplasia-associated cysts, prostatic cysts account for 2% to 5% of prostatic abnormalities (Dorfman and Barsanti, 1995). Another study reported a prevalence of 14% prostatic cysts in adult, large-breed dogs without genitourinary system problems, and bacterial cultures of prostatic cysts were positive in 42% of cases (Black et al., 1998). Small cysts may be palpated per rectum as small, fluctuant areas in an asymmetrically enlarged prostate. Large, discrete cysts may be palpated in the caudal abdomen or in the perineal area. Unless the cyst(s) become secondarily infected, clinical signs are uncommon (Olson et al., 1987). A bloody urethral discharge, dysuria, and tenesmus may be present owing to increased prostatic size. Recommended treatment is surgical resection, with or without concurrent castration (Johnston et al., 2000). Recently, ultrasound-guided, percutaneous drainage of prostatic cysts appears to be a useful alternative treatment (Boland et al., 2003).

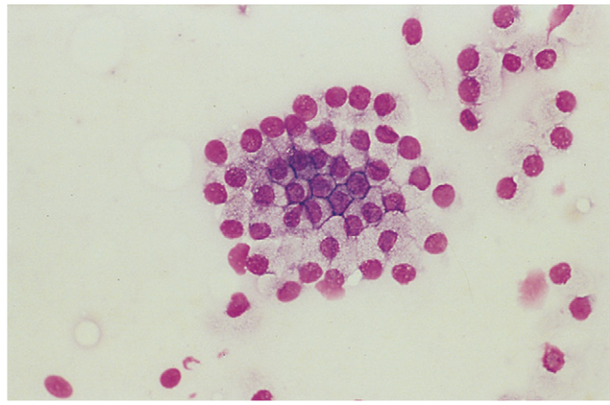
Aspiration of prostatic cysts typically yields variable amounts of serosanguineous to brown fluid (Baker and Lumsden, 1999). Cytologic examination of the fluid usually reveals absent or low numbers of normal-appearing epithelial cells with low to moderate numbers of neutrophils, macrophages, and small lymphocytes and erythrocytes on a red to brown background (Thrall et al., 1985; Boland et al., 2003).

Benign Prostatic Hyperplasia

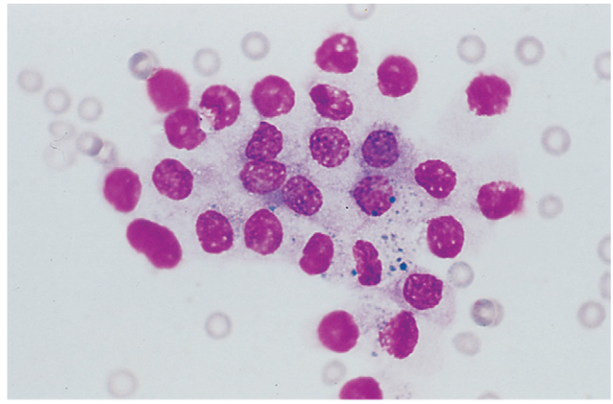
Benign prostatic hyperplasia (BPH) is a common finding in older intact male dogs. BPH is associated with increases in gland size and weight related to increases in interstitial tissue and gland lumens (Lowseth et al., 1990). Symmetrical cystic dilation of the glands results from increases in the interstitium and gland lumens. The pathogenesis of BPH is not completely understood. However, development of BPH is hormonally dependent and requires the presence of functioning testes (Dorfman and Barsanti, 1995). Dihydrotestosterone is accepted as a key hormone in stimulating enlargement of the canine prostate by enhancing growth in both stromal and glandular components (Johnston et al., 2000). Circulating levels of testosterone are often decreased in older male dogs; however, dihydrotestosterone concentrations are often increased in the hyperplastic tissue (Olson et al., 1987). Nuclear androgen receptor expression is increased in hyperplastic tissue of older Beagles, suggesting increased tissue sensitivity to circulating androgens. Additionally, estrogens appear to act synergistically with androgens in potentiating BPH and may also act directly on the prostate, resulting in stromal hypertrophy and squamous epithelial metaplasia. The treatment of choice for canine BPH is castration or finasteride treatment, as finasteride inhibits conversion of testosterone to dihydrotestosterone, causing prostatic involution via apoptosis (Sirinarumitr et al., 2001).

In men, the prostate is fixed anatomically such that enlargement causes urinary obstruction resulting in the most common presenting sign of dysuria (Lowseth et al., 1990). In dogs, the prostate gland is not fixed so that enlargement occurs in an outward direction resulting in constipation and tenesmus. Mild hemorrhagic urethral discharge can also be noted (Dorfman and Barsanti, 1995). However, clinical signs are often absent in canine BPH. Palpation of the prostate usually reveals a symmetrically enlarged, nonpainful gland; however, an irregular surface is occasionally felt (Johnston et al., 2000).

Epithelial cells obtained from a hyperplastic prostate gland are generally arranged in variably sized sheets and clusters in a honeycomb pattern (Masserdotti, 2006) (Figs. 12-47 and 12-48). The cells are uniform in appearance with round nuclei and small, round nucleoli. The nuclear-to-cytoplasmic ratio is low to moderate and the cytoplasm is basophilic. Mild increases in cell size and anisokaryosis may be noted (Baker and Lumsden, 1999). Cytologic samples yielding a normal-appearing population of prostatic epithelial cells from an enlarged prostate, particularly if the enlargement is symmetrical,



■ **FIGURE 12-47. Benign prostatic hyperplasia. Tissue aspirate. Dog.** Normal-appearing prostatic epithelial cells from an enlarged prostate. The cells are uniform in size and shape and are arranged in clusters and individually. The cluster of cells in the center display a characteristic "honeycomb" appearance. (Wright-Giemsa; HP oil.)

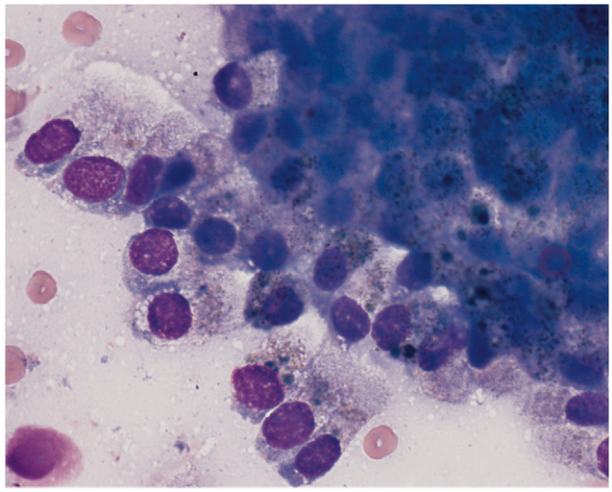


■ **FIGURE 12-48. Benign prostatic hyperplasia. Tissue aspirate. Dog.** Same case as in Figure 12-47. The prostatic epithelial cells display round nuclei, slightly coarse nuclear chromatin, and moderate amounts of lightly basophilic cytoplasm. A few cells contain small amounts of basophilic secretory product. (Wright-Giemsa; HP oil.)

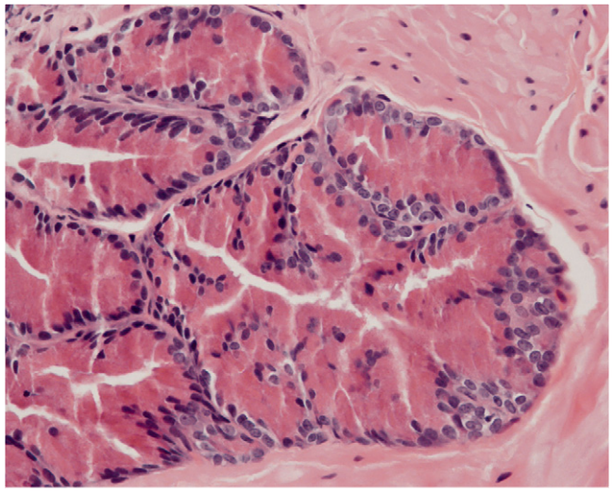
are consistent with a diagnosis of benign hyperplasia (Figs. 12-49 and 12-50).

Squamous Metaplasia

Increased circulating concentrations of estrogen can result in squamous metaplasia of the prostatic epithelium. During this process, the epithelial cells develop staining and morphologic characteristics of squamous epithelial cells. Estrogen receptors, which are present on ductal, stromal, and 10% of the prostatic epithelial cells, may mediate this responsiveness (Baker and Lumsden, 1999).



■ **FIGURE 12-49. Benign prostatic hyperplasia. Tissue aspirate. Dog.** Prostatic epithelial cells are columnar and uniform. Note the granulated appearance of the cells and accumulation of secretory pigment in the cell sheets and clusters. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)



■ **FIGURE 12-50. Benign prostatic hyperplasia, Tissue section. Dog.** Same case as in Figure 12-49. Hyperplastic epithelium is characterized by minimal anaplastic features. Nuclei have less nuclear chromatin density than normal but increased prominence of nucleoli. (H&E; IP.) (Courtesy of Rose Raskin, Purdue University.)

Although chronic irritation and inflammation can result in squamous metaplasia, the most common endogenous source of estrogen is Sertoli cell tumors (Powe et al., 2004). The prostate may be small as a result of decreased concentrations of testosterone or enlarged if cysts or abscessation is present. Clinical signs usually relate to hyperestrogenism. Treatment for squamous metaplasia is removal of the estrogen source.

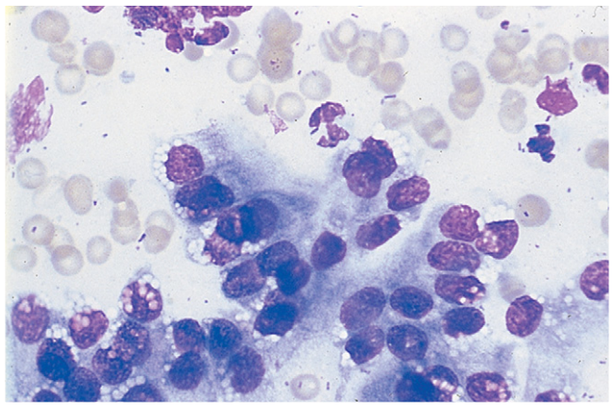
Prostatic Inflammation

Both acute and chronic infections occur in the canine prostate gland, usually as a result of ascent of normal aerobic urethral bacteria (including *Mycoplasma*) into prostate gland (Johnston et al., 2000). Hematogenous and local spread from other urogenital organs is also possible (Dorfman and Barsanti, 1995). *Escherichia coli* is the most commonly isolated organism from both acute and chronic cases of prostatitis followed by *Staphylococcus aureus*, *Klebsiella* spp., *Proteus mirabilis*, *Mycoplasma canis*, *Pseudomona aeruginosa*, *Enterobacter* spp., *Streptococcus* spp., *Pasteurella* spp., and *Haemophilus* spp. Anaerobic bacteria or fungal infections also have been observed via hematogenous spread, urethral ascent, or penetration through the scrotum with descending prostate infection from a testicular source. Alteration of normal architecture by diseases such as BPH, squamous metaplasia, and neoplasia can interfere with normal defense mechanisms or provide a medium (i.e., blood in cysts) for bacterial growth (Olson et al., 1987). Coalescing of focal areas of septic prostatitis or infection of prostatic cysts may result in prostatic abscessation (Baker and Lumsden, 1999).

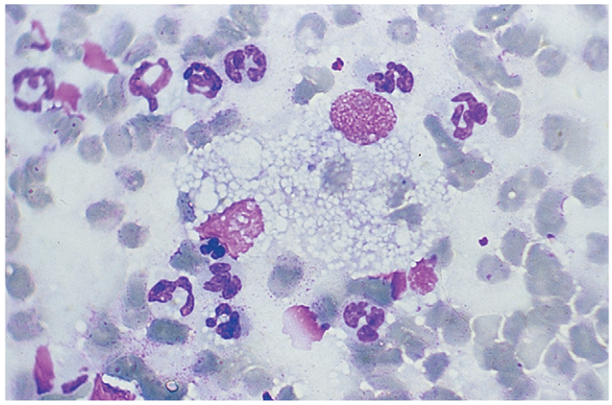
Acute prostatitis is usually associated with systemic signs of illness (fever, anorexia, and lethargy), straining to urinate or defecate, hematuria, edema of scrotum, prepuce, and hind limb or pain on rectal palpation of the prostate gland (Dorfman and Barsanti, 1995). The dog may also experience locomotor problems due to caudal lumbar or abdominal pain. An inflammatory leukogram with or without a left-shift is often present. Clinical signs in dogs with chronic prostatitis may be absent, or there may be recurrent urinary tract infection, poor semen quality with infertility, or sometimes decreased libido (Johnston et al., 2000). Intermittent or constant urethral discharge may also be noted. Prostatic abscesses may present with signs related to enlargement of the prostate (tenesmus, dysuria), constant or intermittent urethral discharge, and evidence of systemic illness related to endotoxemia or peritonitis. Treatment of prostatitis involves appropriate antibiotic therapy as determined by culture and sensitivity. In acute prostatitis, most antibiotics will reach the site of infection since the prostate-lipid barrier is disrupted (Olson et al., 1987). Antibiotics for treatment of chronic prostatitis should be selected for the ability to cross the lipid barrier, which is usually intact, and for the ability to concentrate in the prostate. In addition to appropriate antibiotic therapy, prostatic abscesses can be treated surgically with marsupialization of the gland, placement of a drain, or prostatectomy. All of these surgical procedures are associated with significant

complications. Castration should also be performed in dogs with prostatitis (Dorfman and Barsanti, 1995).

Cytologic evaluation of samples from bacterial prostatitis contains large numbers of neutrophils, many of which exhibit degenerative changes of karyolysis and karyorrhexis (Fig. 12-51). Macrophages may also be present, especially in chronic prostatitis (Fig. 12-52). In the absence of previous antibiotic therapy, intracellular and extracellular organisms may be seen (Boland et al., 2003). Epithelial cells that are present may appear normal or hyperplastic as evidenced by increased cytoplasmic basophilia, increased nuclear-to-cytoplasmic ratios, and mild anisokaryosis. Cellular atypia associated with prostatic epithelial cells in the presence of inflammation should be interpreted cautiously to avoid a false-positive diagnosis of neoplasia (Thrall et al., 1985).



■ **FIGURE 12-51. Septic neutrophilic prostatitis. Tissue aspirate. Dog.** Prostatic epithelial cells and neutrophils are present in this example of acute septic prostatitis. The neutrophils are degenerate as indicated by moderate karyolysis. Bacteria are present in the background and within the neutrophils. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-52. Mixed cell prostatitis. Tissue aspirate. Dog.** A mixed cell population is present in this case of chronic prostatitis. Increased numbers of neutrophils, the majority of which are nondegenerate, and two reactive macrophages are present. Infectious organisms were not seen in this sample. (Wright-Giemsa; HP oil.)

Prostatic Neoplasia

Prostatic malignant tumors in the dog are rare, with reported prevalences of 0.2% and 0.6% based on necropsy studies (Bell et al., 1991). Adenocarcinoma is the most commonly reported neoplasm of the prostate followed by transitional cell carcinoma arising from the prostatic urethra, but other epithelial neoplasms have been described such as undifferentiated carcinoma and squamous cell carcinoma (Dorfman and Barsanti, 1995; McEntee, 2002). Prostatic intraepithelial neoplasia, a precursor lesion of prostatic carcinoma, has been reported in both normal and neoplastic prostate glands. Other malignant neoplasms have been rarely described such as lymphoma and malignant mesenchymal tumors such as hemangiosarcoma and leiomyosarcoma (Hayden et al., 1999; Teske et al., 2002; Winter et al., 2006; Fan and de Lorimier, 2007).

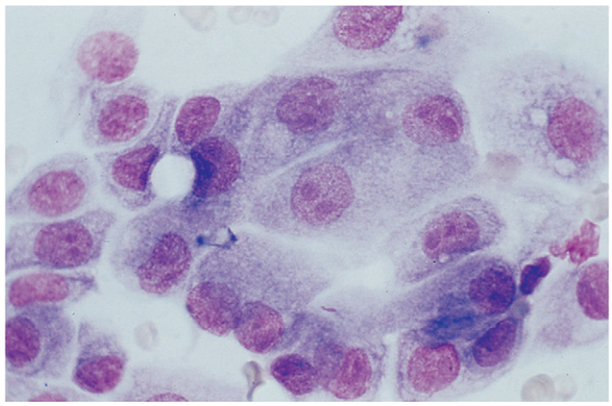
Prostatic carcinoma most frequently occurs in dogs 8 to 10 years of age (Dorfman and Barsanti, 1995), and neutered dogs are at higher risk (Bryan et al., 2007). Canine prostatic adenocarcinomas arise from ductal epithelium, which is predominantly androgen receptor negative suggesting that androgens may not be required for initiation or progression of this tumor (Fan and de Lorimier, 2007). Most canine prostatic carcinomas are locally invasive and metastatic. Metastases were present at necropsy in 80% to 89% of dogs with prostatic carcinoma and regional lymph nodes and lungs are the most common sites (Cornell et al., 2000). Other sites for metastasis are bone, urinary bladder, and mesentery. Bone metastases are most often located in the pelvis, lumbar vertebrae, and femur and can be lytic or proliferative (Dorfman and Barsanti, 1995). The disease carries a poor prognosis in untreated dogs with survival time of less than 2 months (Bell et al., 1991; Sorenmo et al., 2004).

Canine prostatic carcinoma is an insidious disease, with many dogs showing no evidence of clinical abnormalities until late in the course of the malignancy. The most frequently detected abnormality during physical examination is prostatomegaly, which is identified in 52% of the dogs with carcinoma. The enlargement is primarily asymmetrical (32%); however, sometimes symmetrical enlargement (6%) can be noted (Bell et al., 1991). Other physical abnormalities include depression, painful abdominal palpation, cachexia, pyrexia, dyspnea, dysuria, stranguria, hematuria, tenesmus, weight loss, gait abnormalities, and presence of an abdominal mass (Johnston et al., 2000). Complete obstruction of urinary flow may result in hydroureter, hydronephrosis, and subsequent renal failure (Fan and de Lorimier, 2007).

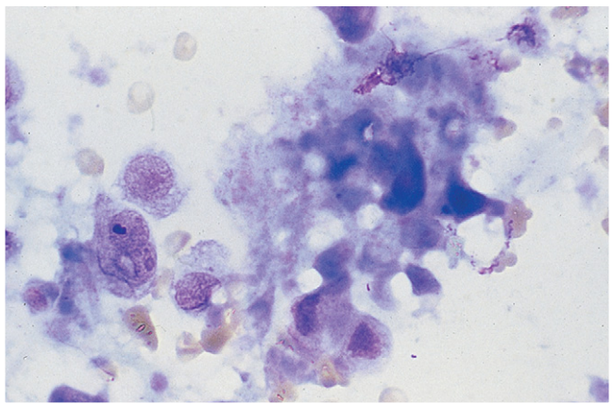
Therapy for prostatic carcinoma is usually palliative and may include prostatectomy or intraoperative radiation (Dorfman and Barsanti, 1995). However, in humans, epidemiologic and experimental evidence supports the use of nonsteroidal anti-inflammatory drugs (NSAIDs) for the prevention of cancer development. This chemopreventive effect may be partially mediated through the inhibition of cyclooxygenase-2 (COX-2) activity causing the blockade of endogenous prostaglandin E_2 production (Fan and de Lorimier, 2007). Canine normal prostatic tissues have failed to express COX-2 protein, but it was

detected in 75% to 88% of prostatic carcinomas (L'Eplattenier et al., 2007). In addition, a significant increase in survival time in dogs treated with COX-2 inhibitors occurred when compared with untreated dogs (Sorenmo, 2004).

FNA is useful for diagnosis of prostatic neoplasia. Cytologic evaluation of FNA samples from prostatic adenocarcinoma usually reveals large numbers of deeply basophilic epithelial cells arranged in variably sized clusters and sheets (Figs. 12-53 and 12-54). The nuclear-to-cytoplasmic ratio is often high and anisokaryosis and anisocytosis can be moderate to marked. Nuclei are round to pleomorphic and nucleoli are large, prominent, and often multiple. Binucleation may be noted. Adenocarcinoma and transitional cell carcinoma can be difficult to distinguish cytologically, and histopathology may be required for definitive diagnosis (Baker and Lumsden, 1999). Some acinar structures may be noted, which can help to differentiate the neoplasm from transitional cell carcinoma (Zinkl, 2008). Bell et al. (1991) reported that a diagnosis of neoplasia was established in 15 of 19 (79%) of samples submitted for cytologic analysis from dogs with histologically confirmed prostatic carcinoma. False-negative cytology results could have been related to small sample size, focal distribution of neoplastic lesions, or concurrent prostatitis and/or BPH. Serum and seminal plasma concentrations of acid phosphatase, prostate specific antigen and canine prostate specific esterase have not been shown to be useful in the definitive diagnosis of canine prostatic carcinoma (Gobello et al., 2002).



■ **FIGURE 12-53. Prostatic carcinoma. Cytologic preparation. Dog.** Neoplastic epithelial cells display prominent, large, multiple nucleoli, coarse nuclear chromatin, moderate anisokaryosis and anisocytosis, variable nuclear-to-cytoplasmic ratios, and binucleation. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-54. Prostatic carcinoma. Cytologic preparation. Dog.** Same case as in Figure 12-53. The amorphous basophilic material is compatible with necrosis, which can be found in aspirates of malignant tumors. Cellular features are indistinct. (Wright-Giemsa; HP oil.)

Testes

Unilateral or bilateral testicular enlargement is the primary indication for FNA and cytologic evaluation of the testes (Zinkl, 2008). Cytology is useful for differentiation between inflammatory or neoplastic conditions that cause testicular enlargement and to classify testicular canine neoplasia (Masserdotti et al., 2005). Testicular FNA has also been shown to be useful for evaluation of male infertility (Dahlbom et al., 1997). Testicular FNA is usually not associated with immediate or long-term adverse effects (Kustritz, 2005).

Special Collection Techniques

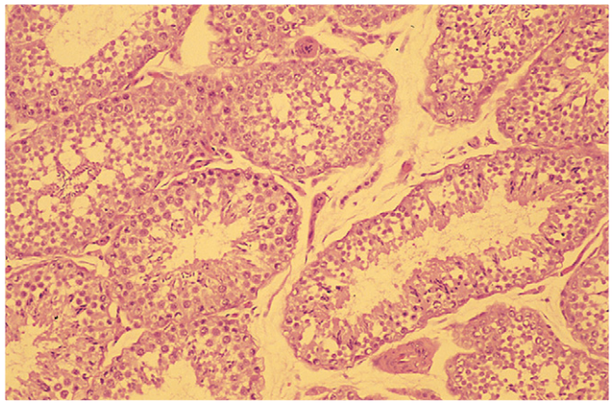
Routine FNA with a 20- to 25-gauge needle attached to 5- to 10-mL syringe is used for cytologic sampling of the testes (Kustritz, 2005). Because of the increased fragility of testicular cells, great care should be taken when preparing the slide of aspirated material and some authors recommend avoiding mechanical aspiration to obtain a better cytologic preparation (Masserdotti et al., 2005). The material should be very lightly smeared when preparing the cell monolayer. Alternatively, gentle touch imprints from available tissue may decrease cellular disruption (Baker and Lumsden, 1999). Imprints of testicular biopsies should be made rapidly after removal of the tissue to prevent degeneration of the cells.

Normal Anatomy and Histology

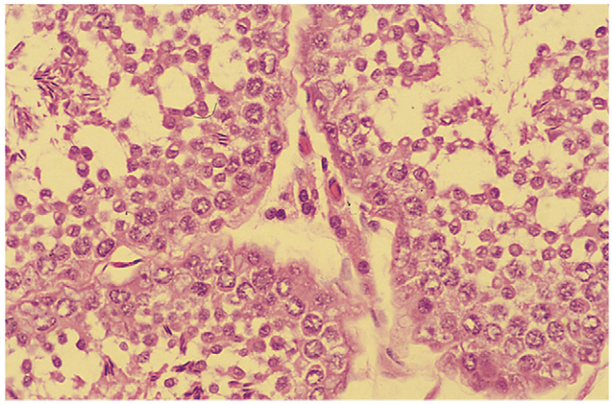
The testes are the site of spermatogenesis in the adult animal and exhibit both exocrine and endocrine function (Banks, 1986). The exocrine portion of the testes is a compound, coiled, tubular gland that produces spermatozoa as its secretory product. The germinal epithelium is actively involved in spermatogenesis. The endocrine portions of the testes are composed of the interstitial (Leydig) cells, which secrete testosterone, and Sertoli (sustentacular) cells, which provide support for the developing sperm (Figs. 12-55 and 12-56).

Normal Cytology

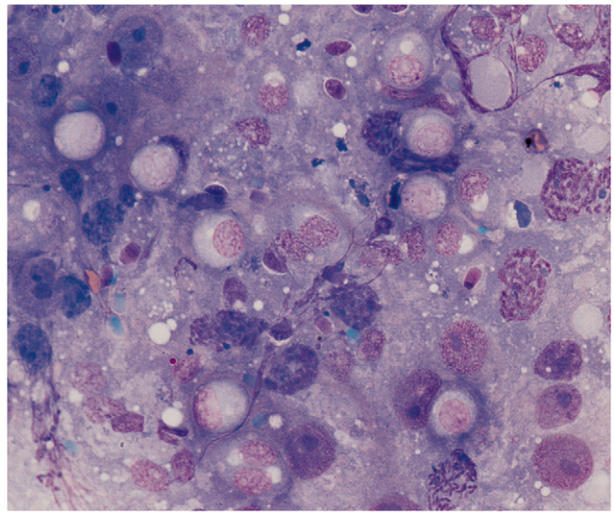
Normal testicular imprints are highly cellular with a predominance of ruptured cells and streaming nuclear material (Baker and Lumsden, 1999). When cells rupture, the nuclear chromatin becomes coarse and nucleoli are prominent. Testicular germinal cells are generally round, with coarse nuclear chromatin, a single large, prominent nucleolus, and moderate amounts of basophilic cytoplasm



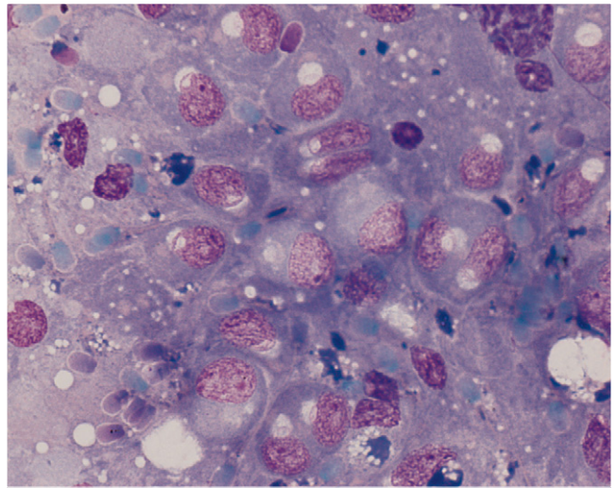
■ **FIGURE 12-55. Normal testes. Tissue section. Dog.** Multiple seminiferous tubules are present, which are surrounded by connective tissue containing low numbers of interstitial cells. (H&E; IP.)



■ **FIGURE 12-56. Normal testes. Tissue section. Dog.** Higher magnification of the seminiferous tubules from the same case as in Figure 12-55. Interstitial cells are seen in the center of the photomicrograph. Spermatocytes as well as early and late spermatids are seen within the tubules. Spermatocytes are characterized by round nuclei and coarse nuclear chromatin. During the maturation process, developing sperm move from the periphery of the tubule to the central lumen. Low numbers of Sertoli cells with smooth nuclear chromatin and single, prominent nucleoli are seen at the periphery of the tubules. (H&E; HP oil.)



■ **FIGURE 12-57. Normal testes. Tissue imprint. Dog.** Large germinal cells and round spermatocytes are present along with small, densely basophilic spermatids. The background contains few lightly basophilic, detached mature sperm heads. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)



■ **FIGURE 12-58. Normal testes. Tissue imprint. Dog.** Higher magnification of same specimen shown in Figure 12-57. Note large numbers of pale basophilic–staining mature sperm with thin, clear space around the heads. Frequent binucleation is present in the germinal cells that display reticulated chromatin and prominent nucleoli. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)

(Figs. 12-57 and 12-58). Mitotic activity is often high. More mature stages of developing sperm are characterized by oval, eosinophilic to pale-staining nuclei, and tails may be noted. Small groups of columnar cells, with indistinct cytoplasm and large round nuclei as single nucleoli, recognizable as Sertoli cells, can occasionally be evident. Scattered stellate or caudate Leydig cells, with microvacuolated cytoplasm and round nuclei are sometimes observed (Masserdotti et al., 2005).

Testicular Inflammation

In dogs, inflammatory disease of the testes (orchitis) or epididymis can be due to infection with *Brucella canis* (Wanke, 2004), *Pseudomonas* sp., *E. coli,* or *Proteus* sp. (Ladds, 1993). Orchitis and epididymitis are common in dogs with clinical patent leishmaniasis (Diniz et al., 2005). Intranuclear or intracytoplasmic inclusions may be seen in cases of distemper-associated orchitis (Ladds, 1993). Orchitis may also be associated with infection by the dimorphic yeast, *Blastomyces dermatitidis*. Orchitis has been recently reported in two dogs with Rocky Mountain spotted fever (Ober et al., 2004). Chronic purulent epididymitis associated with *Mycoplasma canis* infection has been described in one dog (L'Abee-Lund et al., 2003). In cats, orchitis or epididymitis is uncommon and orchitis

has been associated with coronavirus infection in one cat (Sigurdardottir et al., 2001) and the isolation of *Sporothrix schenckii* from a testis in another cat (Schubach et al., 2002). Acute orchitis is characterized by a predominance of neutrophils, some of which may exhibit nuclear degenerative changes. Macrophages, including multinucleated giant cells and lymphocytes, may be seen in chronic inflammatory disease or fungal (blastomycosis) or protozoal (such as *Leishmania infantum* [Diniz et al., 2005]) infections. Since the infectious organisms are usually not observed cytologically, culture should be performed to identify the pathogens (Baker and Lumsden, 1999).

Testicular Neoplasia

In the intact male dog, the testis is the second most common anatomic site for cancer development and testicular tumors account for approximately 90% of all cancers of male genitalia (Fan and de Lorimier, 2007). The three most common tumors are Leydig cell tumor/interstitial cell tumor (58%), seminoma (23%), and Sertoli cell tumor (19%) (Masserdotti et al., 2005), although cases of hemangiomas, granulosa cell tumors, teratomas, sarcomas, embryonal carcinomas, gonadoblastomas, lymphomas, and rete testis mucinous carcinomas have been rarely described. More than one type of testicular tumor is common in dogs (McEntee, 2002). Canine testicular tumorigenesis is not well known but it seems that critical cell cycle regulators such as cyclin D1 and E (Murakami et al., 2001) and insulin-like growth factor system (Peters et al., 2003) do not play a role while enhancement of angiogenic processes are observed in some tumors such as seminomas (Restucci et al., 2003). Testicular tumors occur frequently in aged male dogs. Cryptorchid testes have a higher incidence of Sertoli cell tumors and seminomas, with the right testis more frequently being retained and therefore predisposed to tumorigenesis (MacLachlan and Kennedy, 2002). Most primary testicular tumors are locally confined, with fewer than 15% having a metastatic phenotype. In dogs with localized disease, orchiectomy with scrotal ablation remains the treatment of choice and often is curative. Information about appropriate and effective management of metastatic disease is limited, although the use of radiation therapy and chemotherapy has been reported to increase survival time (Fan and de Lorimier, 2007).

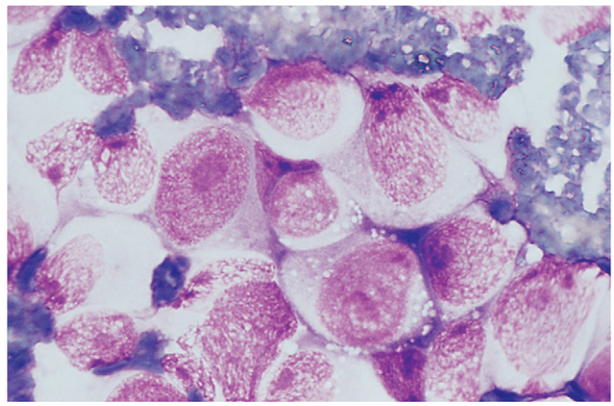
Testicular tumors are rare in cats. There are only isolated cases reports of testicular tumors in cats (McEntee, 2002) such as teratoma (Ferreira da Silva, 2002) and interstitial and Sertoli cell tumors (Miller et al., 2007).

High sensitivity (95% for seminoma, 88% for Sertoli cell tumor, and 96% for Leydig cell tumor), and specificity (100%) for the cytologic diagnosis of canine testicular tumors have been reported when compared with histopathologic evaluation. Cytologic evaluation permits accurate diagnosis and is useful in the management of the disease (Masserdotti et al., 2005).

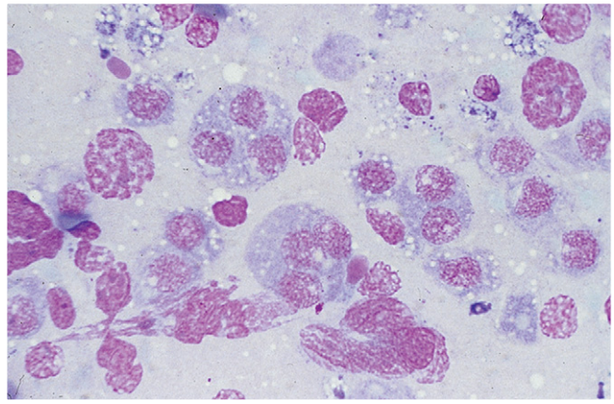
Seminoma

Seminomas arise from neoplastic transformation of the testicular germ cells. The mean age for development of seminoma is 10 years. Other than testicular enlargement, which may not be readily apparent if the tumor involves a cryptorchid testicle, clinical signs related to seminomas are rare. Six to 11% of canine seminomas metastasize, with primary metastatic sites including the inguinal, iliac, and sublumbar lymph nodes and the lungs or abdominal organs (MacLachlan and Kennedy, 2002; McEntee, 2002).

Cytologic differentiation of seminomas from other testicular tumors may be difficult. Cytologic preparations from seminomas often contain large numbers of lysed cells and free nuclei. These cells are large and round and arranged individually or occasionally in small aggregates. The nuclei are large and round, sometimes with irregular outlines. Nuclear chromatin is reticular to coarse and large, prominent nucleoli are commonly present (Fig. 12-59). Moderate anisokaryosis, anisocytosis, and binucleation and multinucleation may be present (Fig. 12-60). The cytoplasm is lightly to moderately basophilic with a moderate to high nuclear-to-cytoplasmic



■ **FIGURE 12-59. Seminoma. Tissue aspirate. Dog.** Neoplastic cells appear large with round nuclei, coarse nuclear chromatin, and prominent, large nucleoli. The cytoplasm is lightly basophilic and some cells contain small numbers of punctate cytoplasmic vacuoles. (Wright-Giemsa; HP oil.)



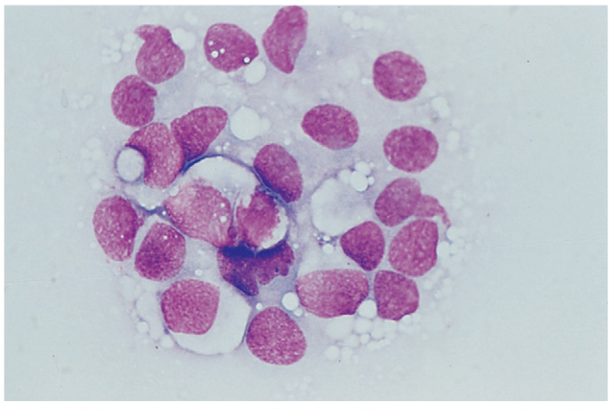
■ **FIGURE 12-60. Seminoma. Tissue aspirate. Dog.** Several multinucleated cells are noted along with the large number of lysed cells and free nuclei in the background. The tendency of testicular cells to rupture can make cytologic evaluation difficult. (Wright-Giemsa; HP oil.)

ratio. The presence of clear macrovacuoles in the cytoplasm is rarely noted. Numerous and aberrant mitoses are often observed. Small lymphocytes are frequently seen in seminomas. Lacy, granular eosinophilic material with the appearance of a tigroid or striped background is occasionally seen (Masserdotti et al., 2005).

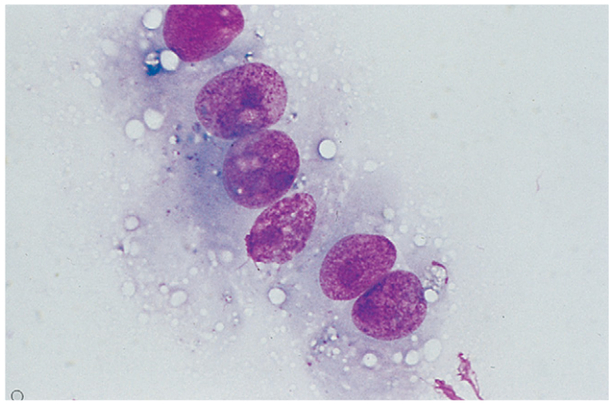
Sertoli Cell Tumor

Sertoli cell tumors are fairly common in retained testicles. Most dogs with Sertoli cell tumors are more than 6 years of age with a mean age of 9.5 years, although tumors in dogs as young as 3 years of age have been reported (McEntee, 2002; MacLachlan and Kennedy, 2002). About one third of canine Sertoli cell tumors are associated with excess production of estrogen, although both seminomas and interstitial cell tumors can cause hormonal imbalance. Reductions in the testosterone/estradiol ratio correlate better than absolute increase values of estradiol-17β with clinical signs of feminization including bilaterally symmetric alopecia and hyperpigmentation, a pendulous prepuce, gynecomastia, galactorrhea, atrophic penis, squamous metaplasia of the prostate, and/or bone marrow suppression (Mischke et al., 2002). Metastasis occurs in 10% to 14% of Sertoli cell tumors. Sites of metastasis are iliac lymph nodes primarily, but also other lymph nodes, spleen, liver, and kidney.

Cytologically, variable numbers of round to elongate pleomorphic cells characterize Sertoli cell tumors. These cells may occur individually or in small clusters with palisading formation (Masserdotti, 2006). Nuclei are generally round with fine nuclear chromatin and occasionally one to three prominent, large nucleoli are noted (Fig. 12-61). The lightly basophilic cytoplasm may vary from scant to abundant in amount, sometimes with indistinct margins. The presence of moderate-sized to large cytoplasmic vacuoles (Fig. 12-62) is typical (Masserdotti et al., 2005).



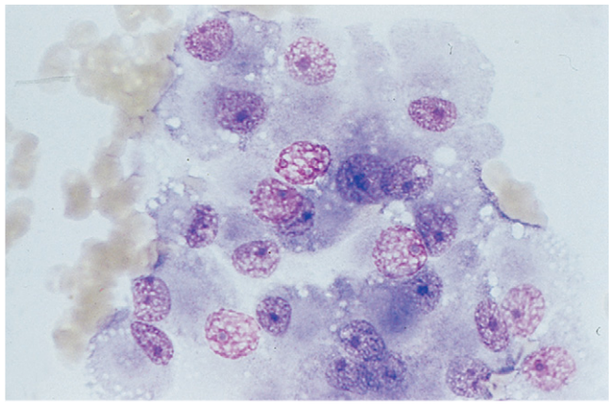
■ **FIGURE 12-61. Sertoli cell tumor. Tissue aspirate. Dog.** This animal presented with infertility, dermatitis with hyperpigmentation, and a testicular mass. The tumor cells have round to oval nuclei, slightly coarse nuclear chromatin, and moderate nuclear-to-cytoplasmic ratios. The nucleoli are small and variably prominent. The cytoplasm is lightly basophilic and cell borders are often indistinct. (Wright-Giemsa; HP oil.)



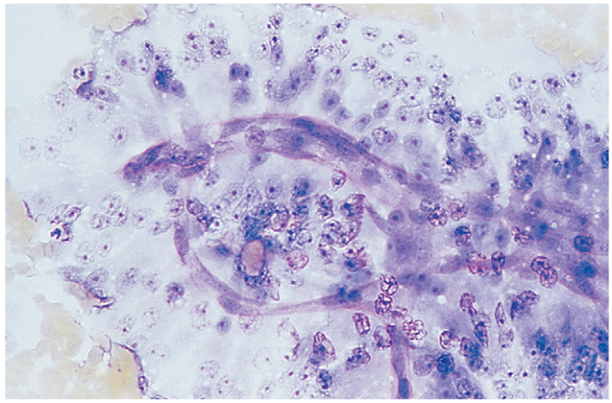
■ **FIGURE 12-62. Sertoli cell tumor. Tissue aspirate. Dog.** A row of tumor cells is shown from the same case as in Figure 12-61. Variably sized cytoplasmic vacuoles are seen in several of the cells. (Wright-Giemsa; HP oil.)

Interstitial Cell Tumors

Interstitial cell tumors are very common in the dog but only 16% of these tumors are associated with testicular enlargement; therefore, they are infrequently aspirated for cytologic analysis (MacLachlan and Kennedy, 2002; Baker and Lumsden, 1999). This tumor has been associated with increased production of testosterone and a high prevalence of prostatic disease and perianal gland neoplasms (McEntee, 2002). Interstitial cell tumors, but not Sertoli cell tumors or seminoma, produce inhibins and 3ß-hydroxsteroid dehydrogenases, which allows discrimination of interstitial cell tumors from other tumors of the canine testes (Taniyama et al., 2001). Cytologic samples from interstitial cell tumors are of variable cellularity. The cells are round or spindle-shaped and usually contain abundant amounts of lightly to moderately basophilic cytoplasm (Fig. 12-63). Perivascular arrangement (Fig. 12-64) is commonly seen



■ **FIGURE 12-63. Interstitial cell tumor. Tissue aspirate. Dog.** A cluster of tumor cells display coarse nuclear chromatin, prominent, single nucleoli, and large amounts of moderately basophilic cytoplasm. The nuclei are often located at the periphery of the cell. Punctate cytoplasmic vacuoles are present in the majority of the cells. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-64. Interstitial cell tumor. Tissue aspirate. Dog.** Lower magnification of the same case as shown in Figure 12-63. Palisading arrays of interstitial cells surround a central capillary. (Wright-Giemsa; HP oil.)

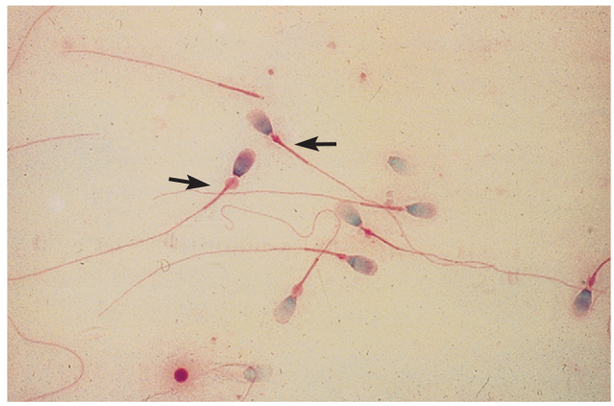
(Masserdotti, 2006). The nuclei are round to oval with fine, reticular chromatin and small, prominent nucleoli. The presence of nuclear pseudoinclusions is observed in half of the cases. Moderate to marked anisokaryosis and variable nuclear-to-cytoplasmic ratios are seen. Numerous small, uniform cytoplasmic clear vacuoles are common (see Fig. 12-63). Dark, irregularly shaped cytoplasmic granules may be present in some cells (Zinkl, 2008; Masserdotti et al., 2005).

Semen Abnormalities

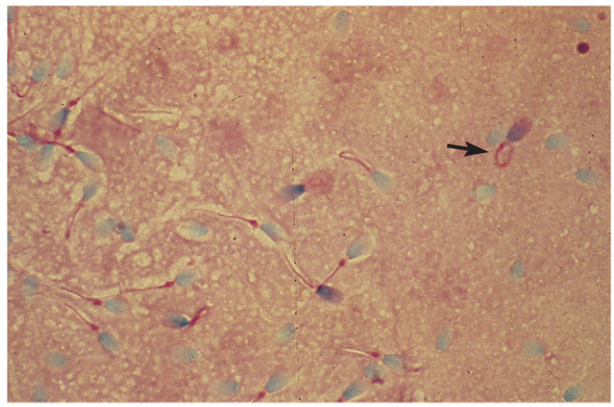
A detailed description of canine and feline semen collection and evaluation is not offered in this text but in-depth reviews are available elsewhere (Freshman, 2002; Rijsselaere et al., 2005; Zambelli and Cunto, 2006; Axner and Linde Forsberg, 2007; Root Kustritz, 2007). However, the cytologist is occasionally presented with seminal material from dogs or cats with infertility or suspected testicular or prostatic disease; thus the ability to recognize certain abnormalities is useful. Gross evaluation, pH, and light microscopy such as concentration, motility, and morphology are routinely used to evaluate the principal parameters of dog and cat semen. Concentration is usually determined using a counting chamber. Aqueous-based Wright stain or Romanowsky-type stain is often used to assess sperm morphology. In high-quality semen, nearly all of the sperm should be of similar morphology. Spermatozoal abnormalities are considered as primary or secondary and described in Table 12-2. Primary abnormalities occur mostly during defective spermatogenesis and are therefore more serious. Secondary abnormalities may occur during passage through the epididymis (defective maturation) or during collection and preparation of the slide (see Table 12-2). Severe abnormalities include abnormal size or shape of the sperm head or acrosomal cap, proximal or midpiece protoplasmic droplets, and coiled tails (Figs. 12-65 to 12-67). Less severe abnormalities include detached, normal-appearing heads and bent tails (Figs. 12-67 and 12-68). Normal semen samples should have less than 10% and 20% of primary and secondary abnormalities, respectively. Total canine and

TABLE 12-2 Canine Spermatozoal Abnormalities

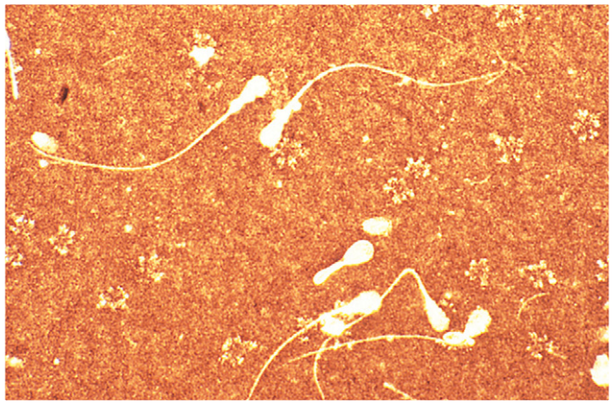
Location of Abnormality	Primary Abnormalities	Secondary Abnormalities
Head	Pyriform, tapered, narrow, small, giant, round, deformed, double heads	Detached head
Midpiece	Double and swollen midpiece and proximal droplet	Distal droplet
Tail	Tightly coiled and double tails	Bent, reverse, and distal coiled tails
Other		Released acrosome



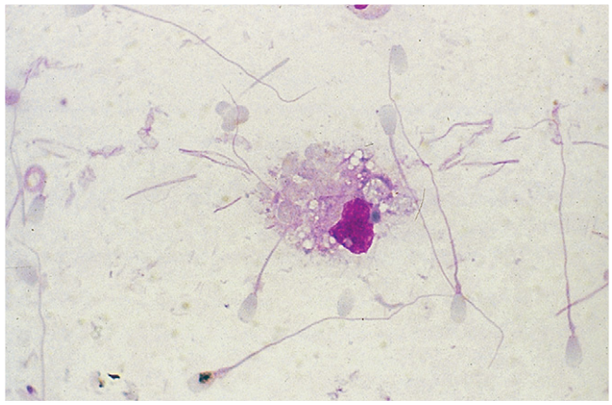
■ **FIGURE 12-65. Primary abnormalities. Semen smear. Dog.** Noninflammatory semen sample from a case of infertility. Sperm have prominent proximal protoplasmic droplets *(arrows)*. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)



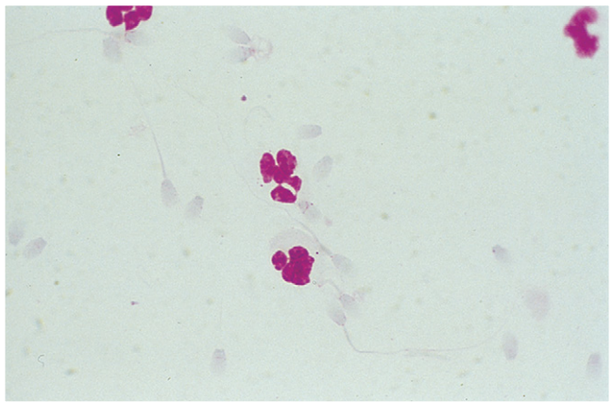
■ **FIGURE 12-66. Primary abnormalities. Semen smear. Dog.** Noninflammatory semen sample from a case of infertility. Against a heavy proteinaceous background, several sperm display tightly coiled tails *(arrow)* and proximal protoplasmic droplets. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)



■ **FIGURE 12-67. Primary and secondary abnormalities. Semen smear. Dog.** Noninflammatory semen sample from a case of infertility. Sperm abnormalities include proximal protoplasmic droplets, coiled tails, and bent tails. (India ink; ×250.) (Courtesy of Rose Raskin, Purdue University.)



■ **FIGURE 12-69. Inflammation. Semen sample. Dog.** A reactive macrophage and several relatively normal–appearing sperm from the same ejaculate demonstrated in Figure 12-68. (Wright-Giemsa; HP oil.)



■ **FIGURE 12-68. Inflammation and abnormal sperm. Semen sample. Dog.** An ejaculate sample from an animal with intermittent preputial bleeding. Mildly degenerate neutrophils are present. Several morphologic secondary abnormalities of the sperm (detached heads, bent tails, and coiled tails) are present. (Wright-Giemsa; HP oil.)

feline spermatozoal abnormalities should be less than 20% to 30% (Freshman, 2002).

Cytology of the sperm-rich and prostatic fractions should be evaluated separately by centrifuge or whole sample (less cellularity). Normal cytology of the sperm-rich fraction contains spermatozoa, white blood cells (WBC) (2/hpf to 4/hpf), epithelial cells, bacteria, and red blood cells. Increased or degenerate neutrophils or macrophages or intracellular bacteria indicate inflammation and/or infection (Figs. 12-68 and 12-69). If neutrophils exhibit degenerative changes, a search for infectious organisms should be performed. However, culture of the fluid may be necessary for identification of pathogens due to the fact that 55% of clinically meaningful aerobic, anaerobic, or myoplasmic bacterial growth has noninflammatory seminal fluid cytology (Root Kustritz et al, 2005). Normal cytology of prostatic fluid is characterized by small amounts of epithelial cells, bacteria, and WBC (2/hpf to 4/hpf) (Freshman, 2002). Lower urinary tract inflammation and/or prostatitis should also be considered when inflammatory cells are present in semen. The presence of abnormal prostatic epithelium in the semen sample warrants further evaluation of the prostate gland (Zinkl, 2008).

Other miscellaneous tests exist to evaluate semen such as live-dead staining with eosin-nigrosin stains, hyposmotic swelling test, and measurement of components of seminal fluid (Root Kustritz, 2007). The most often used seminal markers are alkaline phosphatase (ALP) and carnitine. Both components originate from the epididymis in the dog and have the same application as markers of patency of ductal azoospermia. In an azoospermic semen sample, measurement of ALP or carnitine activity is essential in determining if the azoospermia is due to problems with libido, testicular failure, or ductal blockage. A low ALP or carnitine activity indicates ductal blockage whereas a normal ALP or carnitine activity indicates testicular failure (Gobello et al., 2002; Freshman, 2002).

There are numerous limitations of light microscopical methods such as subjectivity and variability. Recently, several techniques have been described related to the capacity to reach, bind, penetrate, and fertilize an oocyte that may enable more accurate prediction of the fertilizing capacity of semen sample. Conventional light microscopic semen assessment is being replaced by fluorescent staining techniques, computer-assisted sperm analysis systems, and flow cytometry (Rijsselaere et al., 2005).

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