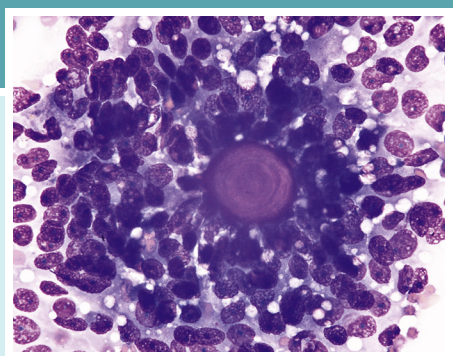
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.



CHAPTER 12

Reproductive System

Laia Solano-Gallego, Carlo Masserdotti

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.

Although 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 and prognosis (Matos et al., 2012). While a few studies (Cassali et al., 2007; Simon et al., 2009; Sontas et al., 2012) have compared cytologic evaluation of mammary neoplasms with histologic analysis with moderate to good accuracies, only one report has related biologic behavior with cytologic diagnosis (Simon et al., 2009). The cytologic diagnosis appears to have a good association with the duration of survival, recurrence-free interval, and metastasis-free interval (Simon et al., 2009). Therefore, the ease of obtaining cytologic specimens from mammary lesions, low invasive nature, moderate to good diagnostic accuracy, 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, fine-needle aspiration (FNA) or most useful fine-needle capillary sampling (FNCS) (Dey and Ray, 1993; Kate at al., 1988) 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, such as both solid and cystic areas, found within canine mammary tumors, sampling of multiple solid areas within a single tumor and similar samplings of additional tumors are essential. In contrast to dogs, feline tumors are uniformly distributed. Furthermore, areas of inflammation within the parenchyma of some neoplasms must be correctly interpreted. In large neoplasms the proliferative tissue can range from benign to malignant structures and, consequently, the interpretation of cytologic examination is directly related to the sampling area. 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 intact cellularity or necrosis, resulting in a nondiagnostic or poorly diagnostic 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 hyperplasia and hypertrophy 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 fibers surround the large ducts. Myoepithelial cells can be found between the alveolar epithelial cells and 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).

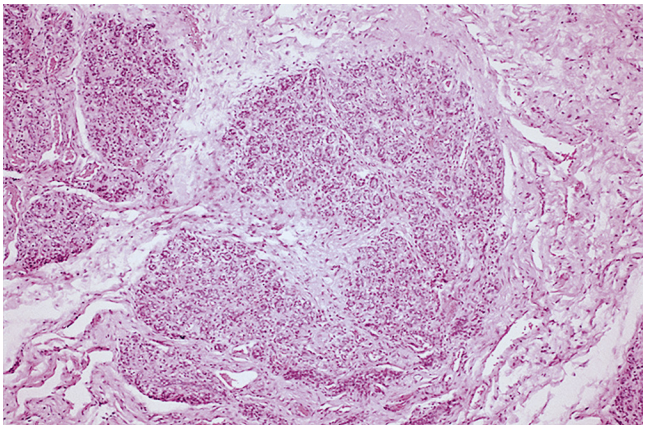


FIGURE 12-1 Normal. Inactive mammary gland. Tissue section. Dog. Lobules of glandular tissue are surrounded by abundant interlobular connective tissue. (H&E; LP.)

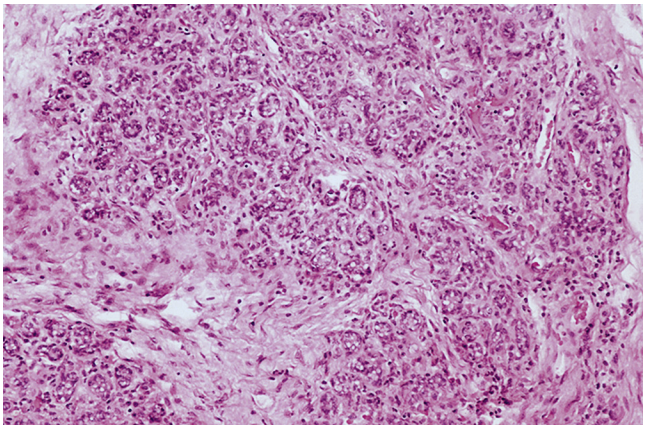


FIGURE 12-2 Normal. Inactive mammary gland. Tissue section. Dog. The glandular portion of mammary tissue is composed of alveoli (acini) and intralobular ducts, which are lined by cuboidal to columnar epithelium. The interlobular ducts, composed of nonsecretory columnar and cuboidal epithelium, drain 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.)

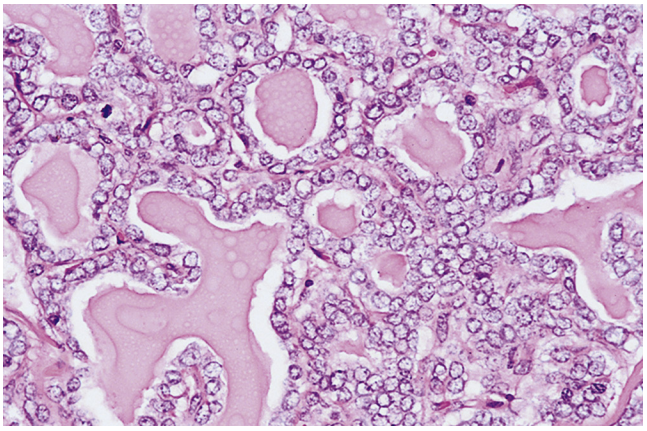


FIGURE 12-3 Normal. Lactational mammary gland. Tissue section. Dog. The secretory portion of the gland is well developed and connective tissue elements are decreased. The alveolar lumens contain bright-pink secretory material. (H&E; HP oil.)

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, 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 form cavitary lesions (Brodey et al., 1983). FCD generally occurs in middle-aged to older animals, although the disease has been reported in dogs 1 year of age. Formation of FCD may have a hormonal component because the administration of medroxyprogesterone has been associated with development of FCD in dogs. In dogs, rapid growth during estrus and regression during anestrus 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, it has been associated with development of mammary gland carcinoma. Mammary cysts

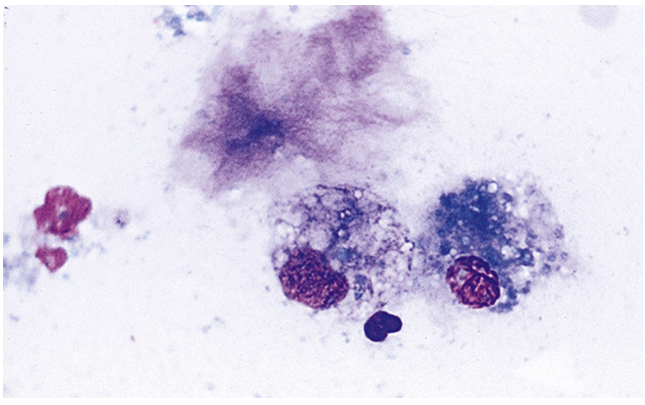


FIGURE 12-4 Mammary gland aspirate. Foam cells. Cat. The two foam cells have eccentrically located nuclei, low nuclear-to-cytoplasmic ratios, clear cytoplasmic vacuoles, and abundant amounts of basophilic secretory material. The background has a lightly basophilic, proteinaceous appearance consistent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

are rarely seen in cats due to the fact that neoplastic or nonneoplastic benign masses are less common in cats than in dogs (Giménez et al., 2010).

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.

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 duct 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, pregnant, or pseudopregnant female cats usually less than 2 years of age (Mesher, 1997). MFH can also occur subsequent to treatment with progesterone or synthetic progestogens (Giménez et al., 2010; Leidinger et al., 2011). MFH has also been reported in older intact and neutered cats of either gender (Giménez et al., 2010; Leidinger et al., 2011) mostly after receiving progesterone-containing compounds, such as megestrol acetate (Hayden et al., 1989) or depot medroxyprogesterone acetate (Loretti et al., 2005; Sontas et al., 2008). MFH is considered a form of mammary dysplasia characterized by a rapid, abnormal growth of one or more mammary glands without milk production. The mammary glands may be edematous, painful, ulcerative, and sometimes so large that animals have difficulty walking (Giménez et al., 2010). Systemic signs might include tachycardia, lethargy, and anorexia (Giménez et al., 2010). 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 that seen during the progesterone-influenced early stages of pregnancy (Misdorp et al., 1999). This typical histologic appearance, along with the occurrence in cycling females or cats administered progesterone and the identification of estrogen and progesterone receptors in MFH lesions from female and male cats (Martín de las Mulas et al., 2000; Ordás et al., 2004), suggests that both hormones are involved. 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 aglepristone (Görlinger et al., 2002).

The cytologic appearance of MFH (Fig. 12-6) has been reported (Leidinger et al., 2011; Mesher, 1997). Aspirated

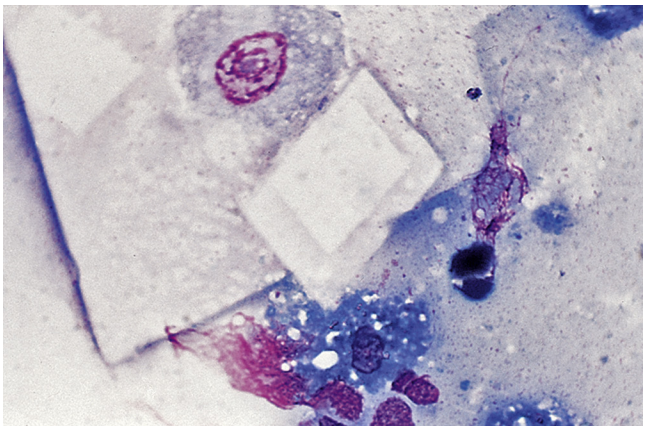


FIGURE 12-5 Mammary cyst aspirate. Cholesterol crystals. Cat. The clear, rectangular crystals are of varying size. Two foam cells are adjacent to the crystals. (Wright-Giemsa; HP oil.)

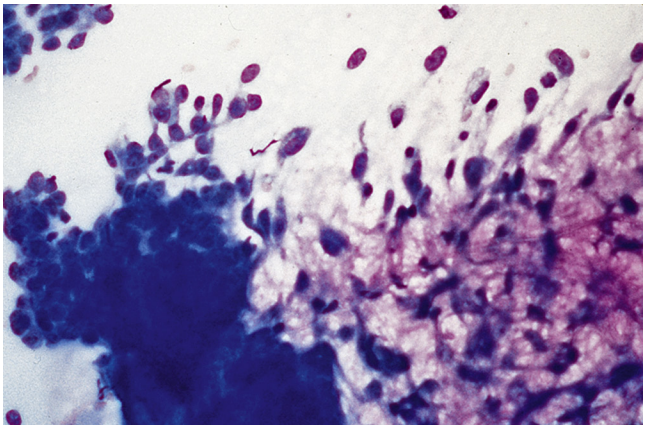


FIGURE 12-6 Mammary fibroepithelial hyperplasia. Tissue aspirate. Cat. Sheet of epithelial cells and spindle cells in pink extracellular material. The epithelial cells are uniform in size and shape and the spindle cells display some mild anisokaryosis. (Wright; HP oil.) (From Mesher CI: What is your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

material of a histologically confirmed MFH lesion consists of a very uniform population of cuboidal epithelial cells arranged in thick clusters. The cuboidal epithelial cells are characterized by dense, round nuclei with small nucleoli and scant amounts of basophilic cytoplasm and mild atypia. A mesenchymal population of spindle-shaped cells with narrow oval nuclei, one to two nucleoli, and tapering cytoplasm is also present. The mesenchymal cells display moderate variation in nuclear size (anisokaryosis) and cellular size (anisocytosis). Moderate amounts of pink extracellular matrix are associated with the mesenchymal cells. These cytologic findings correlate with the histologic findings of hyperplastic ductular epithelium (cuboidal epithelial population) and proliferation of edematous stroma (mesenchymal cells with extracellular matrix). Cytologic recognition of the characteristic cell types from mammary masses in a cat with appropriate signalment, clinical history, clinical presentation, and ultrasonographic findings can be considered highly suggestive of MFH, thus eliminating the need for mammary gland excision and allowing for appropriate medical and/or surgical management (Giménez et al., 2010; Leidinger et al., 2011). However, the cytologic features of MFH cannot be always distinguishable from true benign mammary neoplasms.

Mammary Gland Inflammation/Infection

Inflammation of the mammary glands is referred to as *mastitis* and may present as a focal lesion or 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), mycotic mastitis due to *Blastomyces dermatitidis* in three dogs (Ditmyer and Craig, 2011), and mastitis due to *Toxoplasma gondii* in a cat (Park et al., 2007) have been reported. Mastitis is most often associated with postparturient lactation. It can also occur during pseudopregnancy and after early weaning of puppies or kittens. 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 such as *Staphylococcus* spp., *Streptococcus* spp., and *Escherichia coli*. Other types of bacteria and fungi can also 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 debrided 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 (MGT), 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). MGT rarely occur in male dogs with a reported annual incidence of 4 in 100,000, whereas the annual incidence is 207 in 100,000 in female dogs (Lana et al., 2007; Saba et al., 2007). Many 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 MGT 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 Spaniel breeds, the Poodle, Dachshund, and other breeds (Sorenmo, 2003), 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 (de las Mulas et al., 2005; Lana et al., 2007; Millanta et al., 2005; Ribeiro et al., 2012). Other risk factors for MGT are obesity at 1 year of age and low-fat/low-protein diet (Sorenmo, 2003).

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 progestin administration increases the incidence of MGT in dogs (Misdorp, 1991). Mechanisms involved in the progesterone-induced MGT 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 (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.

Molecular targets have been investigated to elucidate prognosis or the pathways of tumorigenesis such as CA15.3 and LDH (Campos et al., 2012), Snail (Im et al., 2012), cyclooxygenase-2 (Millanta et al., 2006a), heat-shock proteins (Badowska-Kozakiewicz, 2012; Romanucci et al., 2006), VEGF (Millanta et al., 2006b), p53, *BRCA1*, *c-erbB-2* (Singer et al., 2012), antiapoptotic and proapoptotic proteins (Lana et al., 2007), β-catenin, E-cadherin and *adenomatous polyposis coli* protein (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 and erythropoietin receptor expression seem to be useful to identify malignant canine tumors and patient poor outcome (Zuccari et al., 2004; Sfacteria et al., 2005).

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 (IMC) 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 MGT is to accurately predict the biologic behavior of the tumor and prognosis in dogs and cats (Matos et al., 2012). The World Health Organization International Histological Classification of Mammary Tumors of the dog and the cat combines histogenic and descriptive morphologic classification, incorporating histologic prognostic features that have been associated with increasing malignancy (Misdorp et al., 1999). A new classification system of canine mammary tumors has been proposed with a histologic grading system. This has been reviewed (Goldschmidt et al., 2011), and it seems to be a useful prognostic tool (Peña et al., 2013). In addition, a consensus of diagnosis, prognosis, and treatment of canine mammary tumors has been reported and reviewed elsewhere (Cassali et al., 2011).

Most MGT 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 MGT, 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 the diagnosis of carcinomas. Instead, infiltration into skin and soft tissues and invasion of tumor cells into blood or lymphatic vessels have been identified as the 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, whereas when it is absent, 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 comprised only 18.7% of all mammary carcinomas. Of the malignant tumors, squamous cell carcinomas exhibited the lowest metastatic rate (20%) and carcinosarcomas exhibited the highest (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.

Some studies (Allen et al., 1986; Cassali et al., 2007; Hellman and Lindgren, 1989; Simon et al., 2009; Sontas et al., 2012) 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). PPV was reported as 93%, NPV as 67%, and diagnostic accuracy as 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. Similar results were found in other studies (Simon et al., 2009; Sontas et al., 2012). FNA cytology of canine mammary tumors appears to be a valuable diagnostic tool, although lower accuracy exists when inadequate samples are taken into consideration (Sontas et al., 2012). The majority of these studies did not correlate cytologic diagnosis with disease-free intervals or survival times. Only one study reported that cytologic diagnosis appears to have a good correlation with duration of survival, recurrence-free interval, and metastasis-free interval (Simon et al., 2009). Therefore, the use of cytologic criteria to accurately predict the biologic behavior of MGT needs further investigation. Some studies demonstrated that cells in malignant epithelial mammary tumors had significantly more irregular nuclear shapes than 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 MGT (Simeonov, 2006a, 2006b).

Cytologic examination of mammary tumors frequently reveals a background containing variable amounts of blood, basophilic proteinaceous material, lipid, and foam cells. Aspirates of benign epithelial tumors (adenomas and ductal 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 and palisading structures may be seen in samples from adenomas. Papillary and trabecular cell arrangement can be observed in other benign epithelial tumors (Masserdotti, 2006). Benign simple tumors may yield sheets and clusters of uniform-appearing epithelial cells, whereas benign complex tumors may yield a variable number of individualized or clumped spindle-shaped cells of myoepithelial origin is sometimes evident. Myoepithelial cells may also appear as oval free nuclei (Allison and Maddux, 2008). Examination of benign 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 or chondroid matrix (Fernandes et al., 1998) (Figs. 12-8 and 12-9). Benign mixed mammary tumors can be difficult to diagnose using exfoliative cytology. For instance, the presence of spindle-shaped cells may not be sufficient for the diagnosis of complex or mixed 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 comprising the tumor. In a case report, aspiration of a mammary mass in a dog revealed the presence of osteoblasts displaying moderate anisokaryosis and anisocytosis, osteoclasts, hematopoietic cells, and pink extracellular material (Fernandes et al., 1998). Another case featuring extramedullary hematopoiesis in a benign mixed mammary tumor in a dog contained cortical bone and marrow elements (Grandi et al., 2010). 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.

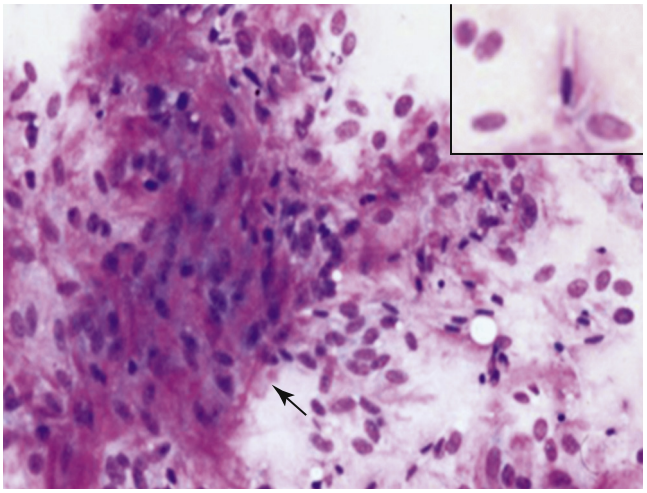


FIGURE 12-8 Mixed mammary tumor. Fine needle without aspiration. Female dog. Arrow indicates clump of myoepithelial cells associated with large amounts of extracellular pink material. *Inset:* A chondroid cell similar to others scattered among the epithelial cells. (Giemsa; IP.) (Courtesy of Noeme Sousa Rocha, FMVZ-UNESP Botucatu, Brazil.)

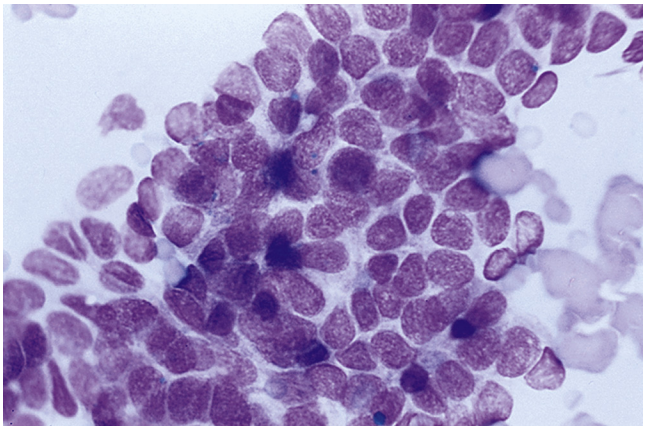


FIGURE 12-7 Mammary adenoma. Tissue aspirate. Cat. Sheet of epithelial cells with cells that are of uniform size and shape with a high nuclear-to-cytoplasmic ratio and fine nuclear chromatin. The cytoplasm is lightly basophilic and scant in amount. (Wright-Giemsa; HP oil.)

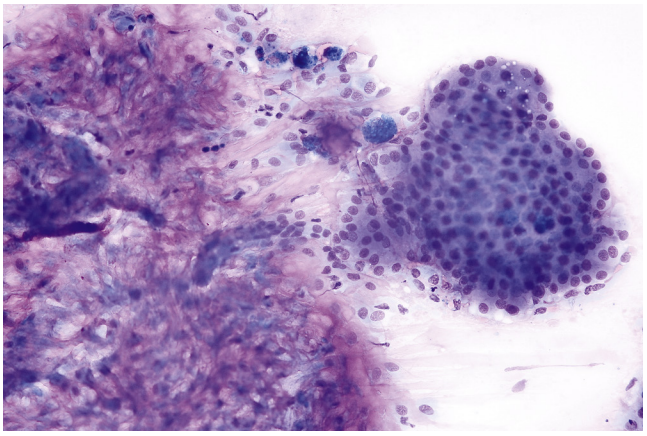


FIGURE 12-9 Mixed mammary tumor. Tissue aspirate. Dog. Clump of spindle-shaped myoepithelial cells with large amounts of extracellular pink material and a cluster of epithelial cells. (Wright-Giemsa; HP oil.)

Adenocarcinomas are characterized by epithelial cells arranged in sheets (Fig. 12-10) and clusters, or sometimes individualized (Figs. 12-11 to 12-13). 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 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 (Fig. 12-13A&C). Increased mitotic activity and abnormal mitotic figures may be present (Fig. 12-13B). 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).

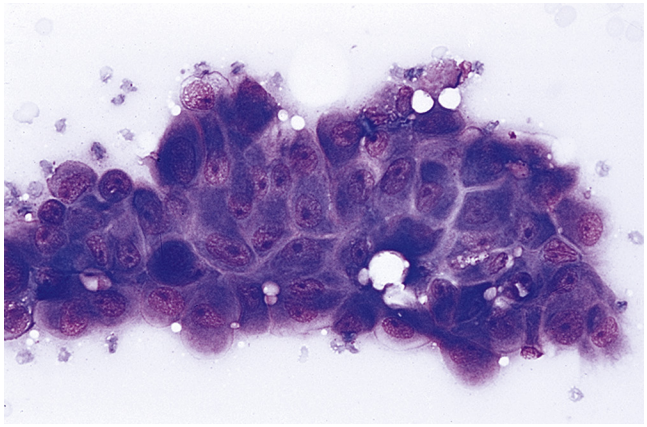


FIGURE 12-10 Mammary adenocarcinoma. Tissue aspirate. Dog. Sheet of epithelial cells displaying prominent cell-to-cell junctions. These cells also exhibit prominent, large nucleoli, moderate anisokaryosis, and deeply basophilic cytoplasm. (Wright-Giemsa; HP oil.)

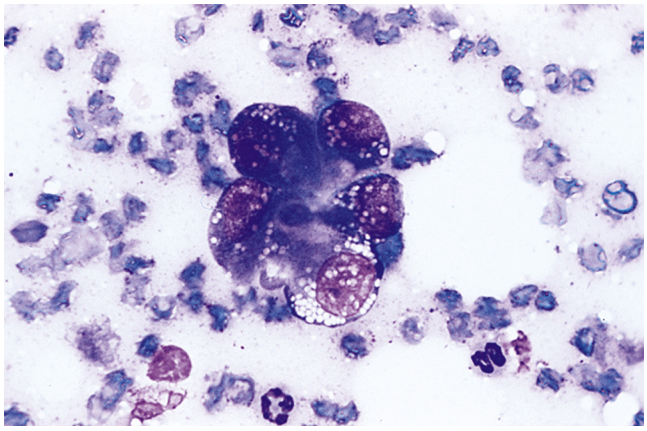


FIGURE 12-11 Mammary adenocarcinoma. Tissue aspirate. Dog. An acinar structure is shown. Note the presence of punctate cytoplasmic vacuoles as well as prominent nucleoli and moderate anisokaryosis. (Wright-Giemsa; HP oil.)

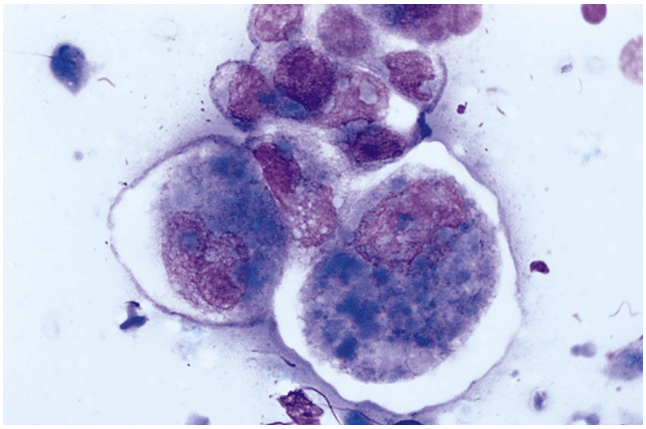


FIGURE 12-12 Mammary adenocarcinoma. Tissue aspirate. Dog. Marked anisokaryosis and anisocytosis of the epithelial cells are noted. These epithelial cells contain basophilic secretory material as well as diffuse peripheral cytoplasmic vacuolation. (Wright-Giemsa; HP oil.)

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. Multinucleation and abnormal mitotic figures are frequently seen. IMC, which are invasive and aggressive, fast growing, and a highly malignant form of mammary carcinoma, also present with large, pleomorphic epithelial cells exhibiting various criteria of malignancy (Lana et al., 2007). IMC affects humans and dogs and rarely cats (Pérez-Alenza et al., 2004) and is characterized by a fulminant clinical course, sudden presentation, edema, erythema, firmness, pain and warmth of the mammary glands, without primary IMC or with secondary IMC mammary nodules (Souza et al., 2009). Clinical signs may be present in one or both mammary chains. Associated clinical signs of inflammation may mimic mastitis and severe dermatitis. Histologically, several types of highly malignant mammary carcinomas have been described. The hallmark for the histologic confirmation of IMC is the invasion of dermal lymphatic vessels by neoplastic emboli. The blockage of the superficial lymphatics by tumor cells is responsible for the severe edema found in the region (Goldschmidt et al., 2011; Grandi et al., 2011; Souza et al., 2009). The most frequent cytologic findings are the presence of anaplastic epithelial cells, singly or in small clusters. Therefore the cytologic findings of highly malignant mammary epithelial cells in association with clinical signs of edema, warmth, pain, and erythema support a differential diagnosis of IMC (Solano-Gallego et al., 2011). This clinical entity has a guarded prognosis with a short survival time (Marconato et al., 2009), but medical treatment with piroxicam seems to improve clinical outcome and prolonged survival time (Souza et al., 2009).

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

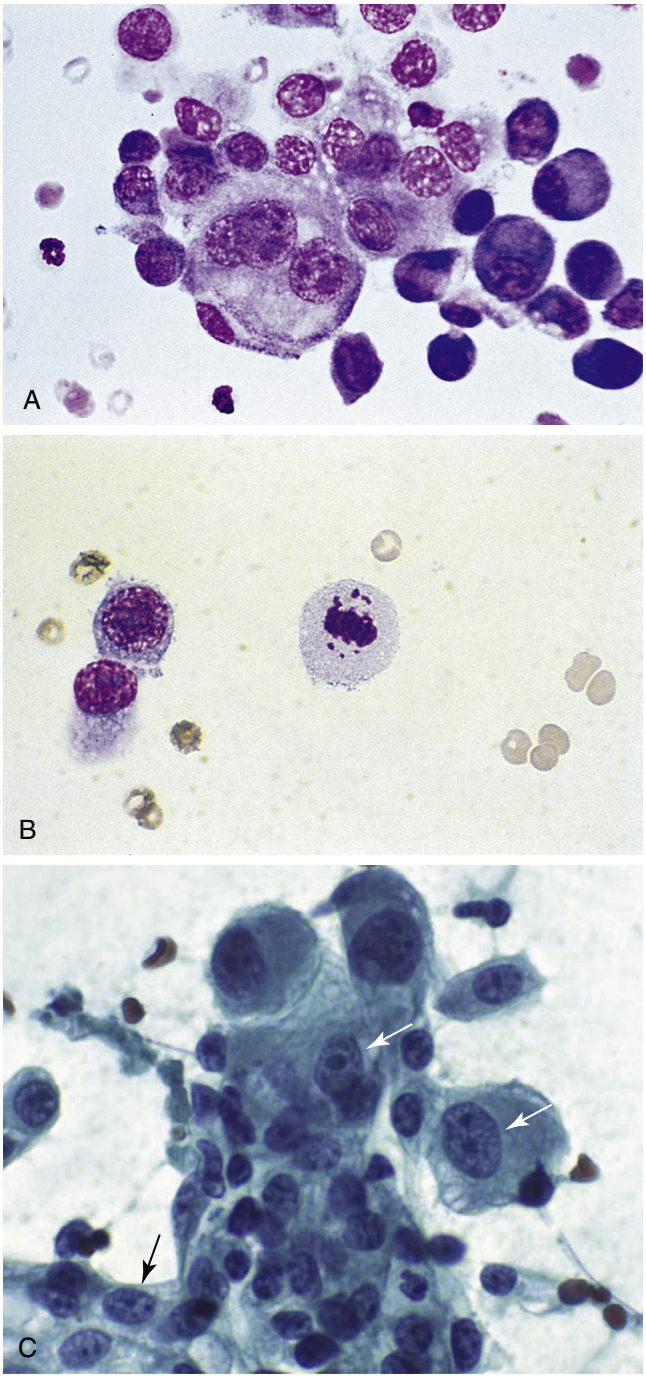


FIGURE 12-13 Mammary carcinoma. Tissue aspirate. Dog. Same case A-B. A, Marked anisokaryosis, anisocytosis, prominent nucleoli, coarse nuclear chromatin, and binucleation are present in cells that also display poor cellular adhesion. (Wright-Giemsa; HP oil.) **B, Abnormal mitotic figure with lag chromatin.** Lag chromatin results from abnormal formation of the mitotic spindle apparatus. Abnormal mitotic figures are considered one criterion of malignancy. (Wright-Giemsa; HP oil.) **C, Mammary carcinoma. Fine needle capillary sampling. Female dog.** Stippled nuclear chromatin with prominent nucleoli (*arrows*) are more readily visible in this type of stain. Green color denotes a young cell but an adult cell would be orange. (Papanicolaou; HP oil.) (C, Courtesy of Noeme Sousa Rocha, FMVZ-UNESP Botucatu, Brazil.)

cells and phagocytized bacteria in the cytologic sample (Allison and Maddux, 2008).

Aspirates of malignant mixed mammary tumors and of carcinosarcomas may reveal epithelial and spindle-shaped cells, individualized cells of myoepithelial or stromal origin. 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 malignant mixed mammary tumors, there are malignant epithelial cells and sometimes also benign-appearing myoepithelial cells with variable amount of osteoid or chondroid matrix, whereas 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 (Hayes and Mooney, 1985; Misdorp, 2002). The median age for MGT development in the cat is 10 years or older. Almost all (99%) of feline MGT occur in intact females (Lana et al., 2007), with rare instance in male cats (Fig. 12-14). 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 sevenfold 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 but similar to humans, most 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 (Burrai et al., 2010). Other molecular targets have been investigated to elucidate prognosis, the pathways of tumorigenesis or metastasis such as cyclin A, Cox-2 (Millanta et al., 2006b), HER2, VEGF, E-cadherin β-catenin (Lana et al., 2007; Zappulli et al., 2012), CXCR4 (Ferrari et al., 2012), matrix metalloproteinase (Akkoc et al., 2012), and AKT (Maniscalco et al., 2012).

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 (Giménez et al., 2010; Hayes et al., 1981; Misdorp, 1991). Moreover, the majority of feline mammary tumors are of simple type with rare myoepithelial component. Adenocarcinomas are the most prevalent malignant mammary tumor followed by carcinomas and sarcomas (MacEwen et al., 1984). Secondary or postsurgical IMC (Millanta et al., 2012; Pérez-Alenza et al., 2004) and lipid-rich carcinoma (Kamstock et al., 2005) have been described, for the

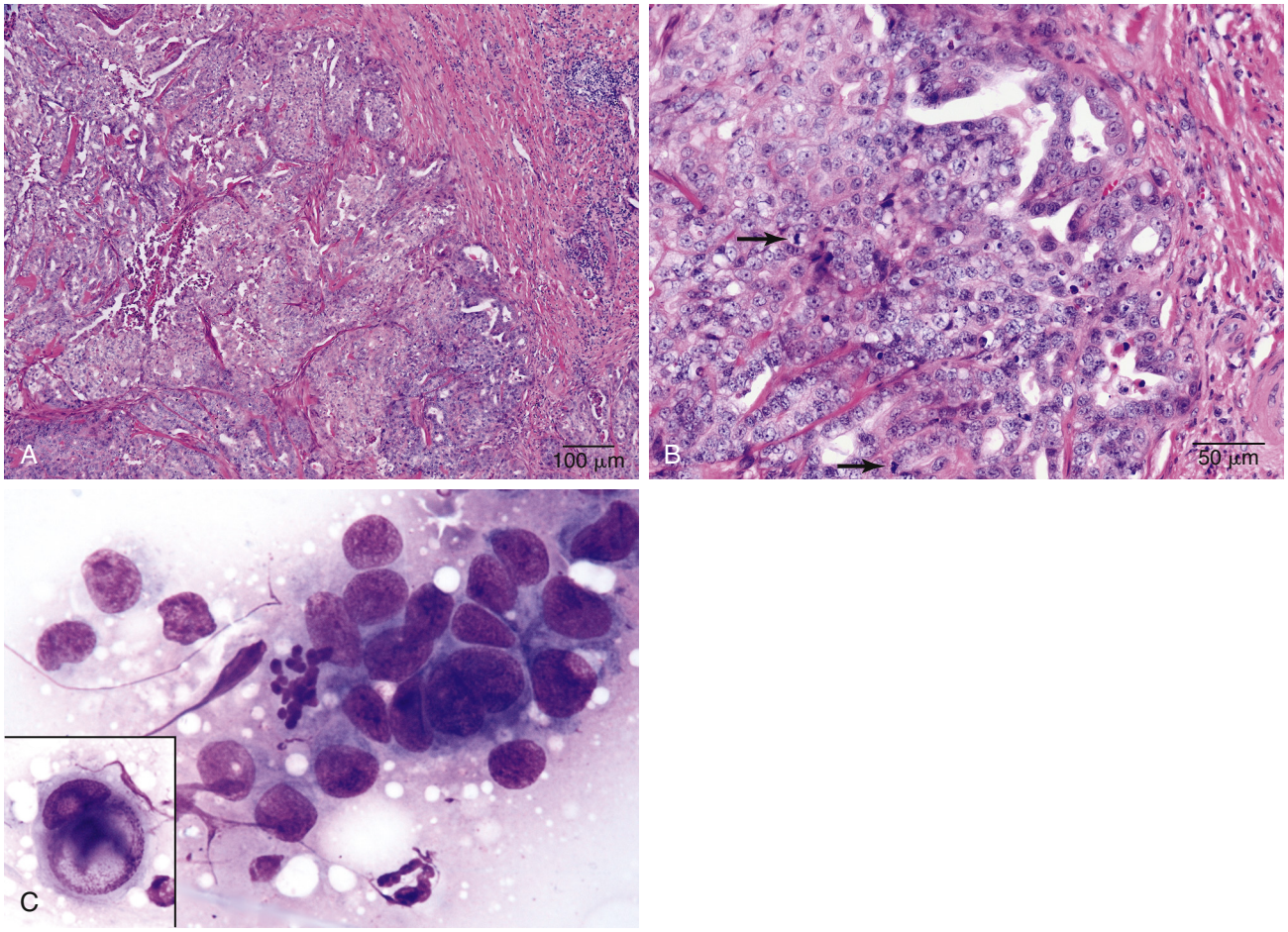


FIGURE 12-14 Mammary adenocarcinoma. Cat. Same case A-C. A, Loss of normal mammary gland architecture with solid and tubular neoplastic proliferation into surrounding connective tissue. The stroma also contains accumulations of small lymphocytes (upper right). (HE; LP.) **B,** Solid and tubular adenocarcinoma with infiltration into the surrounding connective tissue. Two mitotic figures are visible (*arrows*). Conspicuous nucleoli are noted in the carcinoma cells. (HE; IP.) **C,** Cytology material from male feline presenting with a mammary mass. Mitotic figure in the center adjacent to epithelial cells arranged in acinar formation. Carcinoma cells have high nucleocytoplasmic ratio, multiple and prominent nucleoli and anisokaryosis. (Romanowsky; HP oil.) *Inset:* Multinucleate cell displays nuclear molding and anisokaryosis. (Romanowsky; HP oil.) (A and B, Histopathology images courtesy of Prof. Jelinek, Veterinary Histopathological Laboratory, Prague, CZ. C, Images courtesy of Dr. Dita Novakova, Czech Republic.)

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 the time of diagnosis. Median survival time 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 (Fig. 12-14C) are similar to those described in the dog (Figs. 12-10 and 12-13). 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, suspected metastatic lesions, and/or body cavity effusions. It has been proposed that treatment guidelines for malignant canine MGT 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 (Giménez et al., 2010). In dogs, it is recommended to perform ovariohysterectomy if intact in all malignant canine MGT and to institute chemotherapy in an undifferentiated carcinoma in stage I (Sorenmo, 2003). There is limited information regarding the 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, but controls were not studied (Novosad, 2003; Novosad et al., 2006). In contrast, a cohort study did not find benefit of adjuvant doxorubricin-based chemotherapy (McNeill et al., 2009). In addition, the combination of surgery and adjunctive

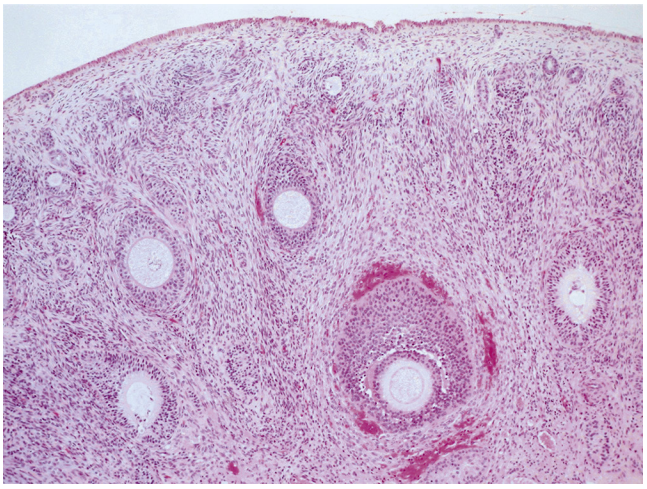


FIGURE 12-15 Normal ovary. Tissue section. Dog. Several developing follicles, each with an oocyte surrounded by a layer of granulosa cells, are present within the stroma of the ovarian cortex. The cortex is lined by a simple layer of cuboidal epithelium. (H&E; LP.)

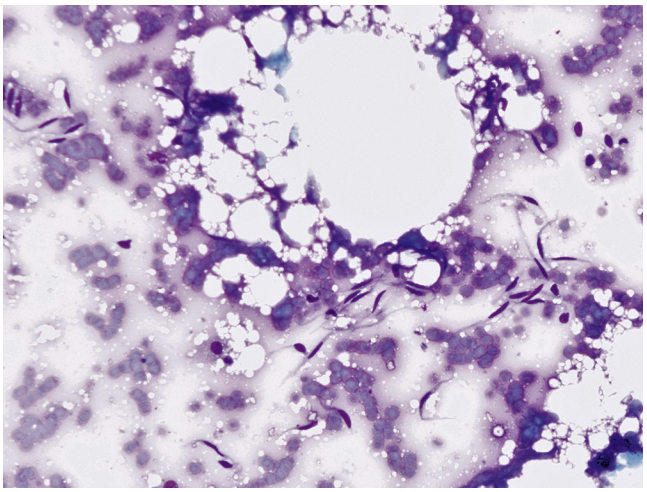


FIGURE 12-16 Normal ovary. Cytologic preparation. Stromal cells. Dog. The basophilic background contains red blood cells, variable sized lipid droplets, and cellular debris. Numerous fibrocytes/fibroblasts from the stromal area are noted. (May-Grünwald-Giemsa; IP.)

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 MGT (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 the diagnosis of ovarian tumors and ovarian cystic disease as demonstrated in a study with a diagnostic accuracy of cytology of 94.7% (Bertazzolo et al., 2004). In addition, although oophoritis and ovarian remnant syndrome (ORS) are rare in dogs and cats (Ball et al., 2010), cytologic evaluation might be useful in their diagnosis.

Special Collection Techniques

There is little information on ovarian cytology collection techniques. Ovarian tissue biopsy is performed, and surgical technique is well described elsewhere (Root Kustritz, 2006). Cytologic samples can be made by ultrasound-guided percutaneous FNA or intraoperatively during exploratory laparotomy, and could reduce the risk of a tissue biopsy procedure and exploratory laparotomy in some cases (Bertazzolo et al., 2004).

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

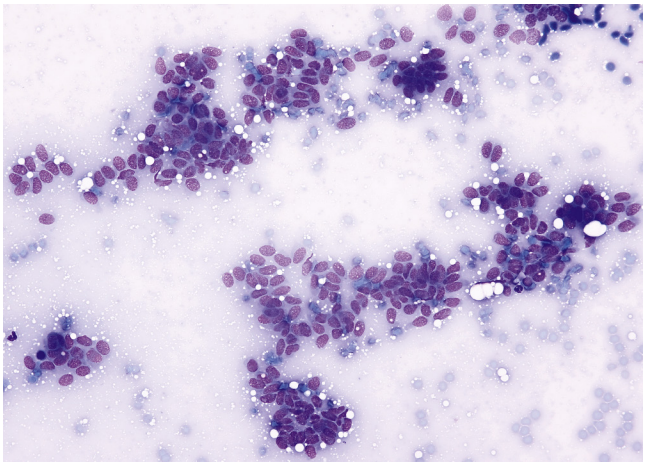


FIGURE 12-17 Normal ovary. Tissue aspirate. Granulosa cells. Dog. Cells that are uniform in size and shape and are arranged in small, loose aggregates. (Modified Wright; IP.) (Courtesy of Dr. Eleonora Piseddu.)

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).

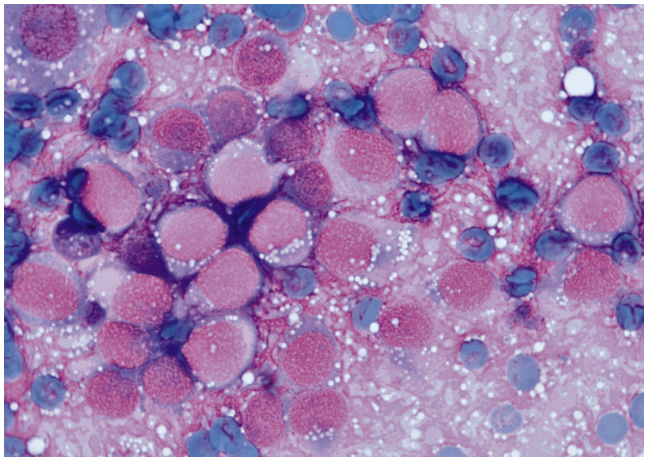


FIGURE 12-18 Normal ovary. Tissue aspirate. Immature cells. Dog. Note numerous 15-20 microns round cells with scant basophilic vacuolated cytoplasm and large round nuclei with reticular chromatin, and indistinct single or multiple small nucleoli. (Modified Wright; HP oil.) (Courtesy of Dr. Eleonora Piseddu.)

Normal Cytology

Knowledge of specific cytologic features of normal canine ovaries is important for identification of pathologic processes (Piseddu et al., 2012). A detailed cytologic description of aspirates from normal canine ovaries in different stages of estrus with comparison to histologic features is reported elsewhere (Piseddu et al., 2012).

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 moderate numbers of one or more of the following cells based on the stage of the estrous cycle: adipocytes, individual fibrocytes/fibroblasts, granulosa cells that are uniform in size and shape and are arranged in acinar formations or in small loosely to cohesive aggregates, round cells of unknown origin, rare leukocytes, and luteal cells (Figs. 12-16, 12-17, 12-18, 12-19).

Luteal cells are large cells with low nuclear-to-cytoplasmic ratios and variable degrees of anisocytosis and anisokaryosis. Cytoplasmic and nuclear features and shapes of luteal cells differed in early (Fig. 12-19) and late (Fig.12-20) diestrus. Anisocytosis and anisokaryosis are increased in early diestrus compared with late diestrus. Luteal cells exfoliated individually or in perivascular arrangements. In early diestrus, most luteal cells are round to polygonal or elongated and have distinct cell borders and mild to moderate anisocytosis and mild anisokaryosis. Nuclei are eccentric round to oval nuclei with finely stippled to reticular chromatin. A variable amount of cytoplasm appears finely granular and amphophilic to deeply basophilic with rarely small to medium-sized intracytoplasmic clear discrete vacuoles (Fig. 12-19) (Piseddu et al., 2012). In late diestrus, luteal cells are round and frequently have indistinct borders with blebbed margins. The cytoplasm is clear at the periphery and lightly basophilic in the center with numerous small clear discrete vacuoles. Nuclei have the same location and shapes reported for cells in early diestrus but have reticular to coarse chromatin with one to two prominent nucleoli (Fig. 12-20). Large pale pink dense structures (150 to 300 µm) are rarely observed extracellularly in late diestrus (Fig. 12-21) and are similar to corpora albicans

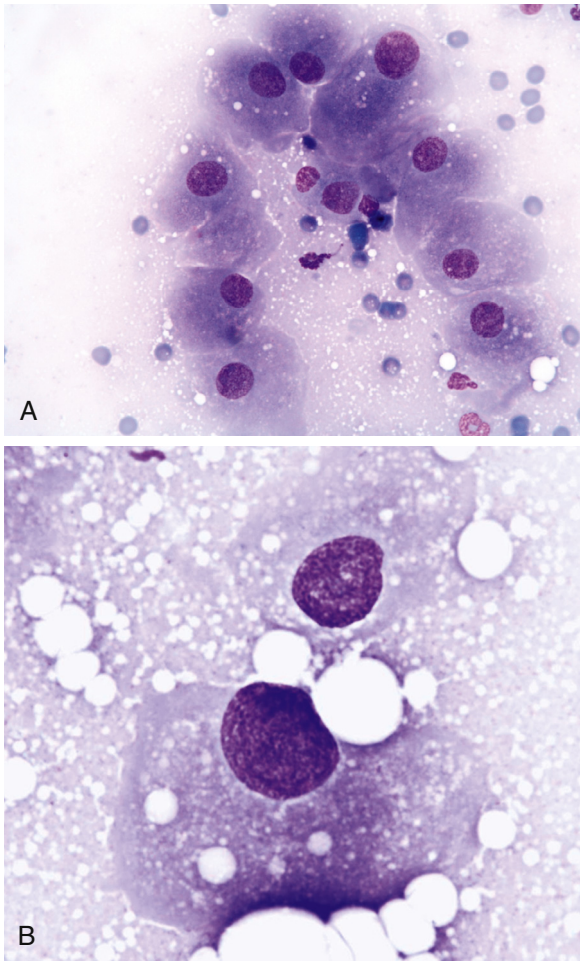


FIGURE 12-19 Normal ovary (early diestrus). Tissue aspirate. Luteal cells. Dog. A, The pale basophilic background contains variable sized lipid droplets and red blood cells. Several individual luteal cells characterized by abundant dense basophilic cytoplasm with low numbers of small clear discrete vacuoles and eccentric round to oval nuclei. Note mild to moderate anisokaryosis and mild anisocytosis. (Modified Wright; HP oil.) **B,** Two single luteal cells characterized by abundant pale basophilic cytoplasm with occasional small, clear, discrete vacuoles and eccentric round to oval nuclei. (May-Grünwald-Giemsa; HP oil.) (A, Courtesy of Dr. Eleonora Piseddu.)

found in histologic sections (Piseddu et al., 2012). In both early and late diestrus, binucleated cells and leuko-emperipolesis are noted frequently (Figs. 12-19A, 12-20) (Piseddu et al., 2012).

Spindle cells (Fig. 12-16) and granulosa cells are not associated with any particular estrous stage. Granulosa cells exfoliated in loose to cohesive aggregates, within which palisades and acinar-like arrangements are noted (Fig. 12-17). The cells are small, 10 to 15 µm in diameter, and round to elongate sometimes with short cytoplasmic tails. The cytoplasm is scant and basophilic with indistinct borders and, rarely, a few small clear vacuoles. Nuclei are oval to round with stippled to finely reticular chromatin with indistinct nucleoli. Granulosa cells frequently are associated with purple amorphous material (Fig. 12-22) (Piseddu et al., 2012). Round immature cells exfoliate individually and often are mixed with luteal or granulosa cells. They are medium-sized, 15 to 20 µm in diameter with distinct cell borders, high nuclear-to-cytoplasmic ratios, and scant to moderate amounts of lightly basophilic cytoplasm containing

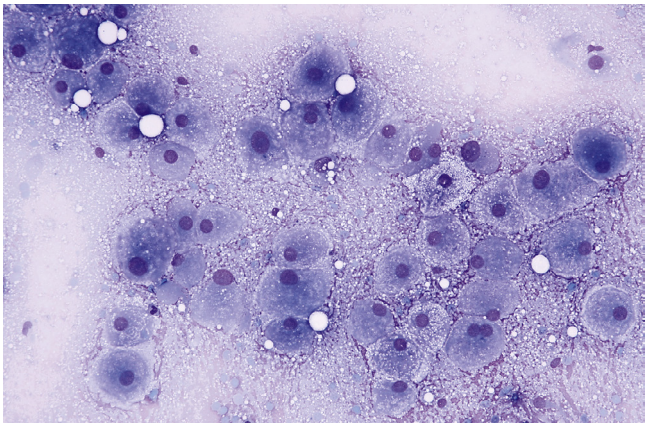


FIGURE 12-20 Normal ovary (late diestrus). Tissue aspirate. Luteal cells. Dog. Note many small vacuoles in the background and luteal cells with finely vacuolated cytoplasm and round eccentric nuclei with stippled chromatin. Binucleated cell is also noted. (Modified Wright; IP.) (Courtesy of Dr. Eleonora Piseddu.)

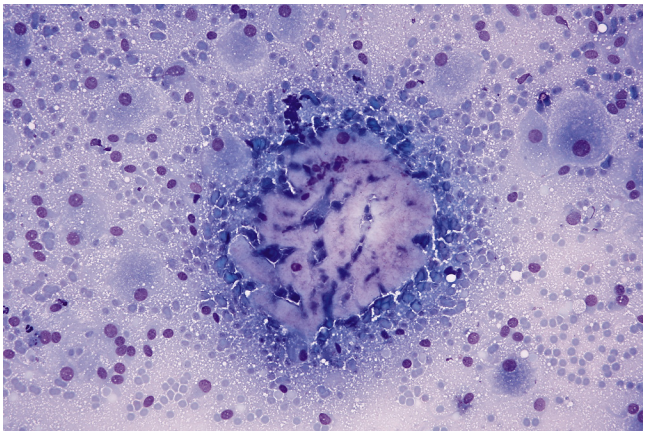


FIGURE 12-21 Normal ovary (late diestrus, corpus albicans). Tissue aspirate. Dog. The pale basophilic background contains variable sized lipid droplets and red blood cells. Large pale pink dense structure, suggestive of a corpus albicans, associated with luteal cells (late diestrus) is observed. (Modified Wright; IP.) (Courtesy of Dr. Eleonora Piseddu.)

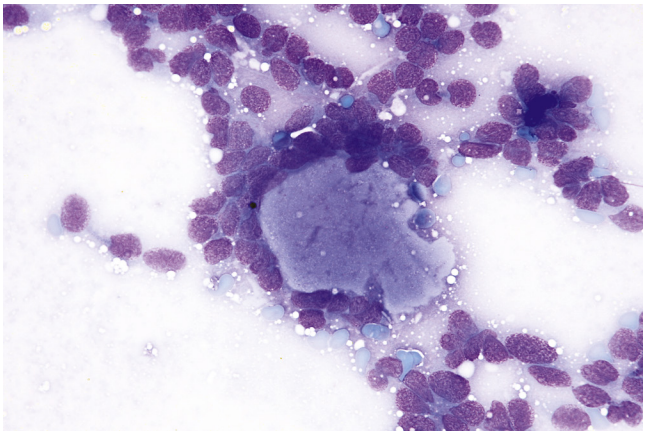


FIGURE 12-22 Normal ovary. Tissue aspirate. Dog. Blue-purple amorphous material is surrounded by loose aggregates of granulosa cells. This material is likely mucinous in nature. (Modified Wright; HP oil.) (Courtesy of Dr. Eleonora Piseddu.)

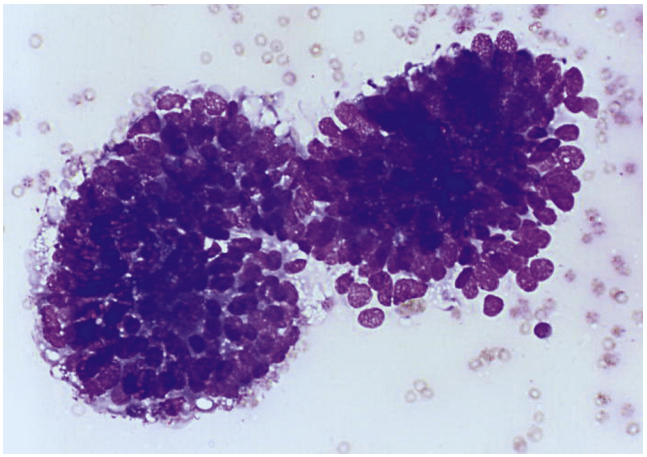


FIGURE 12-23 Ovarian papillary adenocarcinoma. Cytologic preparation. Dog. A cluster of cohesive neoplastic epithelial cells are arranged in a papillary pattern. (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)

small clear vacuoles. Nuclei are round and usually centrally placed with finely reticular chromatin and often small multiple indistinct nucleoli (Fig. 12-18). These cells are not observed on smears of ovaries in anestrus (Piseddu et al., 2012).

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 (dog only), vascular hematomas, and adenomatous hyperplasia of the rete ovarii (Foster, 2007; Klein, 2007). Cytologic findings are characterized by small amount of proteinaceous debris on occasionally hemodiluted background with dispersed vacuolated macrophages.

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.

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: epithelial, germ cell, sex cord–stromal, and mesenchymal. A primary ovarian rhabdomyosarcoma has been described for the first time (Boeloni et al., 2012). There are several other miscellaneous neoplastic diseases of the ovaries, including mixed tumors (Antuofermo et al., 2009) and metastatic nonovarian malignant neoplasms. Clinical signs typically occur secondary to a space-occupying mass or to an effusion related to metastasis (Bertazzolo et al., 2012). Clinical signs in dogs with functional tumors secondary

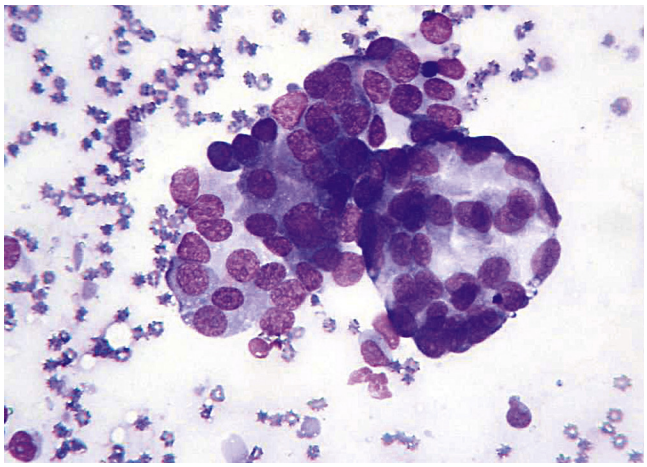


FIGURE 12-24 Ovarian papillary adenocarcinoma. Cytologic preparation. Dog. Shown is a round papillary cluster of cohesive neoplastic epithelial cells known as a "cell ball." (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)

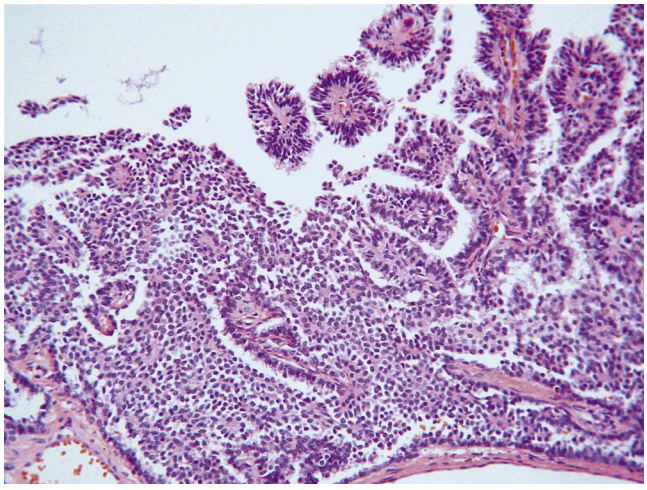


FIGURE 12-25 Ovarian papillary adenocarcinoma. Tissue section. Dog. There is dense proliferation of hyperchromic epithelial cells, some of which display acinar and papillary growth formations. (H&E; LP.) (Courtesy of Dr. Walter Bertazzolo.)

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 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. 12-23, 12-24, 12-25). Cells are round to polyhedral with a single oval nucleus. Nuclear chromatin is reticular to coarse. Nucleoli are indistinct to prominent and 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).

Ovarian carcinoma is often not diagnosed until peritoneal or pleural metastases develop, causing malignant neoplastic peritoneal and pleural effusion, with subsequent abdominal distension and dyspnea, respectively. Cytologic findings of malignant metastatic effusion of this tumor are similar to the findings observed in primary ovarian carcinoma aspirates (Masserdotti, 2006). Numerous large papillary aggregates of cells are present. Acinar arrangements are also seen. Neoplastic cells are monomorphic and show mild cytologic atypia (Bertazzolo et al., 2012; Salgado et al., 2012).

Sex Cord–Stromal Tumors. Sex cord–stromal tumors include granulosa cell tumors, luteomas (also called interstitial gland, lipid, or interstitial cell tumors), thecomas, and retiform Sertoli-Leydig cell tumor (Gomez-Laguna et al., 2008). In the dog, granulosa cell tumors account for 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 cord–stromal tumor in older cats, and more than 50% are malignant. Reported metastatic sites include the 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 histologic 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, whereas granulosa tumor cells and thecomas are negative to cytokeratin 7 and positive to inhibin-α (Klein, 2007; Riccardi et al., 2007). Another marker useful to differentiate granulosa cell tumors from ovarian epithelial tumors is Hector Battifora mesothelial epitope-1 (HBME-1). HBME-1 is one of the immunohistochemical markers employed in the diagnosis of ovarian epithelial tumors. Granulosa cells and related tumors are consistently negative for HBME-1 (Banco et al., 2011).

Cytologically, granulosa tumor cells are usually in monolayered, loosely cohesive clusters and often have acinar to tubular pattern (Fig. 12-26A). Cells are arranged sometimes in an acinar pattern surrounding amorphous eosinophilic extracellular mucin material called Call-Exner bodies (Figs. 12-26A&B). Capillary-like structures are occasionally evident inside large clusters of cells (Fig. 12-27). 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), embryonal

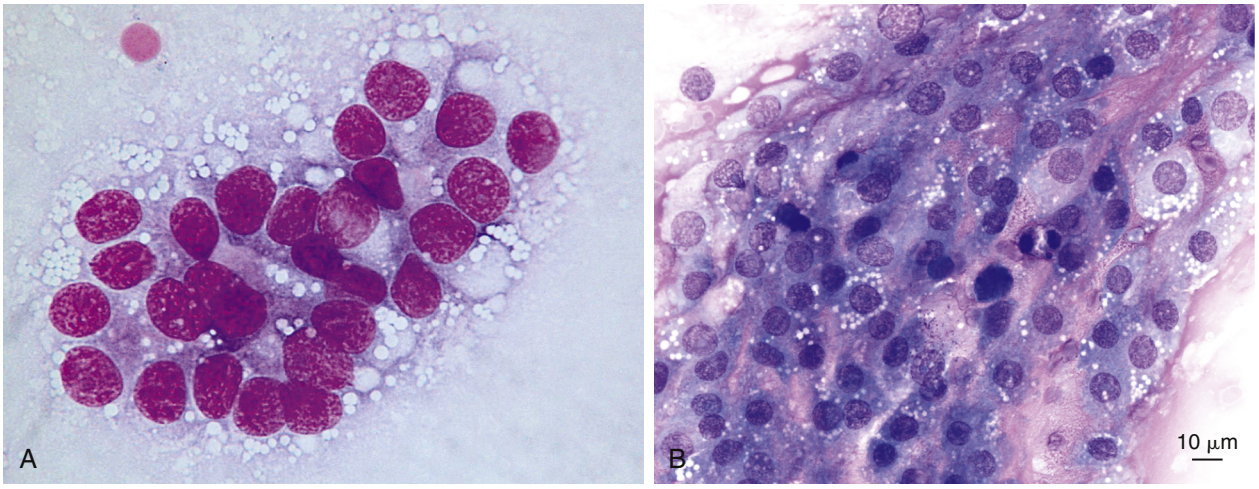


FIGURE 12-26 Granulosa cell tumor. Cytologic preparation. Dog. A, Loosely monolayered aggregate of granulosa cells with a moderate amount of finely vacuolated cytoplasm is present. Cells are arranged in an acinar pattern around a small amount of eosinophilic material, likely Call-Exner body. (May-Grünwald-Giemsa; HP oil.) **B,** Note the round to polygonal granulosa cells that were immunoreactive to inhibin-alpha and vimentin but negative for cytokeratin 7. Cytologically, these cells display moderate anisocytosis and anisokaryosis, finely to coarsely stippled chromatin, and variably-sized prominent nucleoli. Cells also have abundant basophilic cytoplasm with small clear vacuoles surrounded by abundant brightly eosinophilic material. This material was positive for PAS and Alcian blue, indicating mucin composition. (Romanowsky; HP oil.) (A, Courtesy of Dr. Walter Bertazzolo. B, Glass slide material courtesy of K. Banajee et al., Louisiana State University; presented at the 2012 ASVCP case review session.)

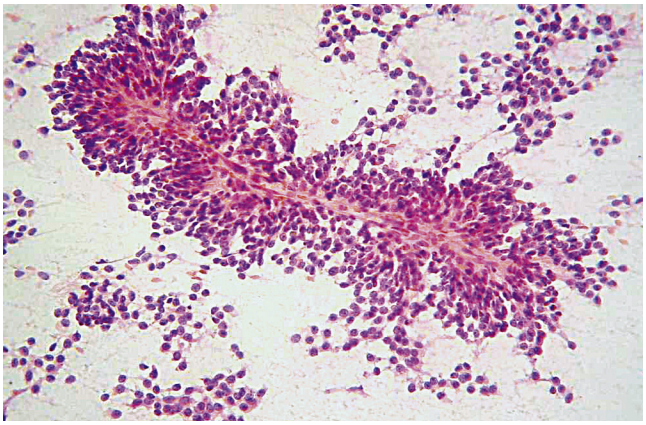


FIGURE 12-27 Granulosa cell tumor. Tissue section. Dog. A large cluster of granulosa cells appears with a perivascular pattern. (H&E; LP.) (Courtesy of Dr. Walter Bertazzolo.)

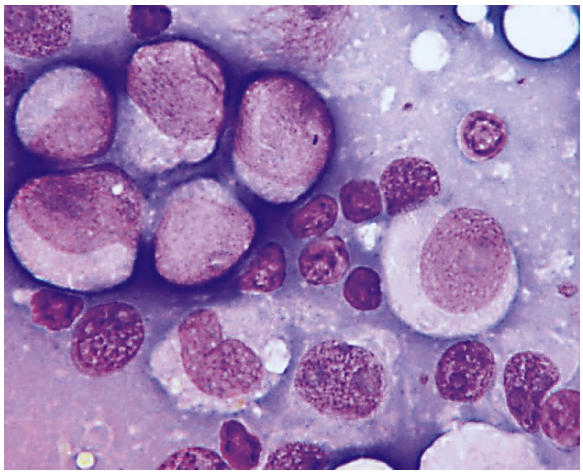


FIGURE 12-28 Ovarian dysgerminoma. Cytologic preparation. Dog. Large neoplastic cells are round and are arranged singly. Nuclei are pleomorphic in shape and located centrally or eccentrically with a stippled to coarse chromatin pattern and prominent nucleoli. Anisokaryosis and anisocytosis are moderate 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.)

carcinoma, teratoma, and teratocarcinoma (Gorman et al., 2010). Dysgerminoma represents 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 (Klein, 2007; McEntee, 2002).

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 in diameter. Nuclei are large and round to oval with chromatin stippled to reticular (Figs. 12-28 and 12-29). 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 noted. Small lymphocytes can be observed (Bertazzolo et al., 2004; Brazzell and Borjesson, 2006).

Cytologically, teratomas are characterized by a necrotic background, moderate neutrophilic-macrophagic inflammation, clusters of sebocytes or other mature epithelial cells, abundant keratin debris, and mature keratinocytes (Figs. 12-30, 12-31, 12-32, 12-33) (Bertazzolo et al., 2004). Malignant teratoma or teratocarcinoma is similar cytologically to teratoma. However, large pleomorphic cells with moderate to marked atypia and high mitotic activity are present in malignant teratoma (Gorman et al., 2010).

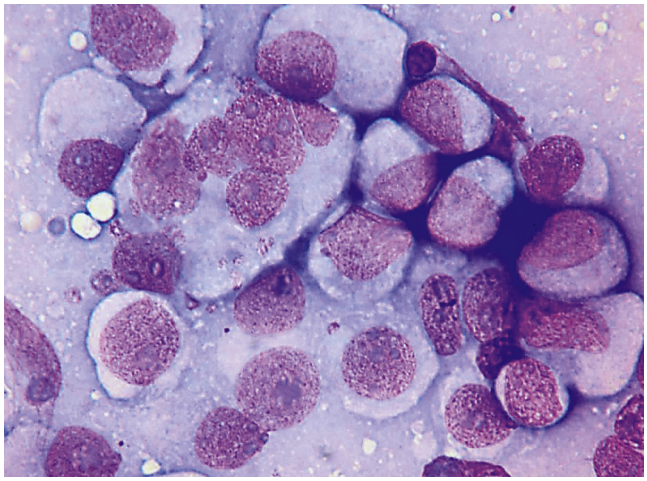


FIGURE 12-29 Ovarian dysgerminoma. Cytologic preparation. Dog. Note the multinucleated cell with marked anisokaryosis and anisocytosis. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)

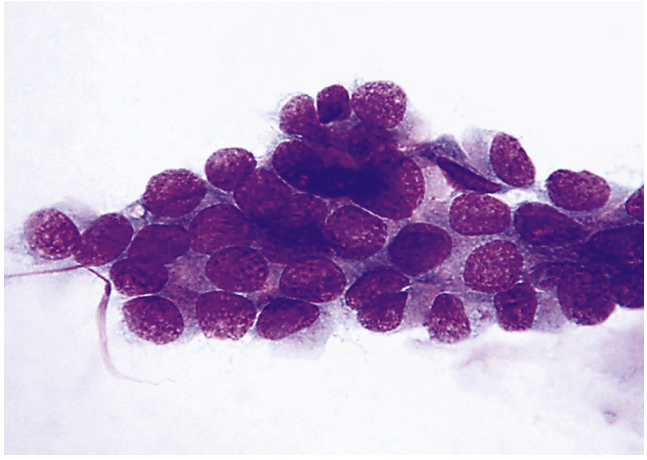


FIGURE 12-30 Teratoma. Cytologic preparation. Dog. A cluster of cohesive epithelial basal-like cells is shown. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)

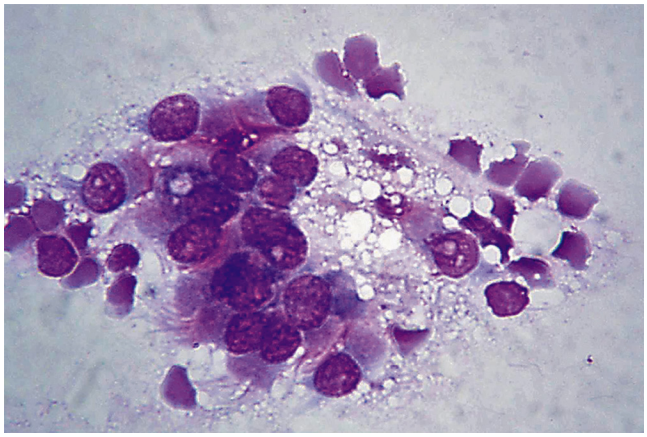


FIGURE 12-31 Teratoma. Cytologic preparation. Dog. Epithelial cells have a basally polarized round to oval nucleus with an evident eosinophilic brush border suggestive of differentiation towards respiratory epithelium. (May-Grünwald-Giemsa; HP oil.) (Courtesy of Dr. Walter Bertazzolo.)

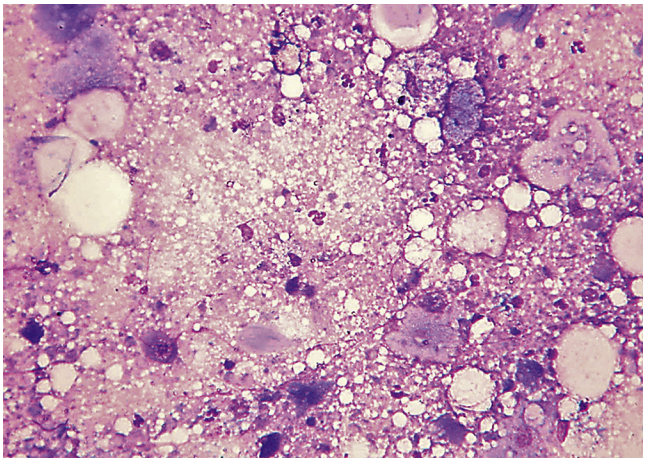


FIGURE 12-32 Teratoma. Cytologic preparation. Dog. Necrotic background, keratin debris, neutrophils and macrophages are evident. (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)

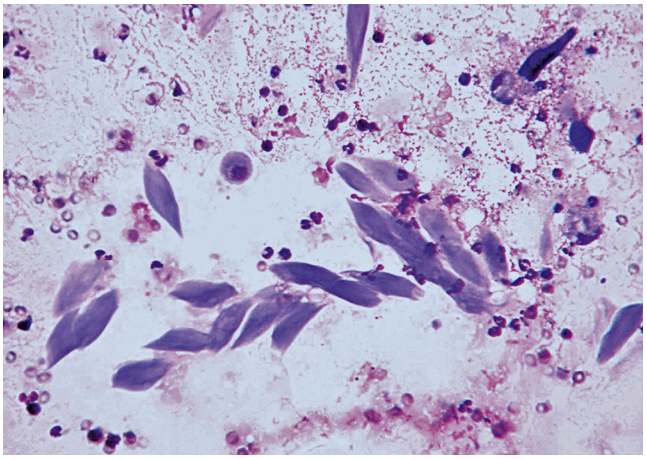


FIGURE 12-33 Teratoma. Cytologic preparation. Dog. Keratinocytes, keratin debris, neutrophils, and red blood cells are seen. (May-Grünwald-Giemsa; IP.) (Courtesy of Dr. Walter Bertazzolo.)

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 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, hematometra (Barrand, 2009), inflammatory endometrial polyp, uterine torsion (Chambers et al., 2011), 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 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: outer perimetrium (serosa), middle myometrium, and inner endometrium (mucosa) (Fig. 12-34A-C). 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. 12-34A). A richly vascularized and well-innervated stratum vasculare usually separates the muscle layers (Fig. 12-34C). 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 (Fig. 12-34B). The cervix is the structure that separates the external genitalia from the 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 the 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 commonly

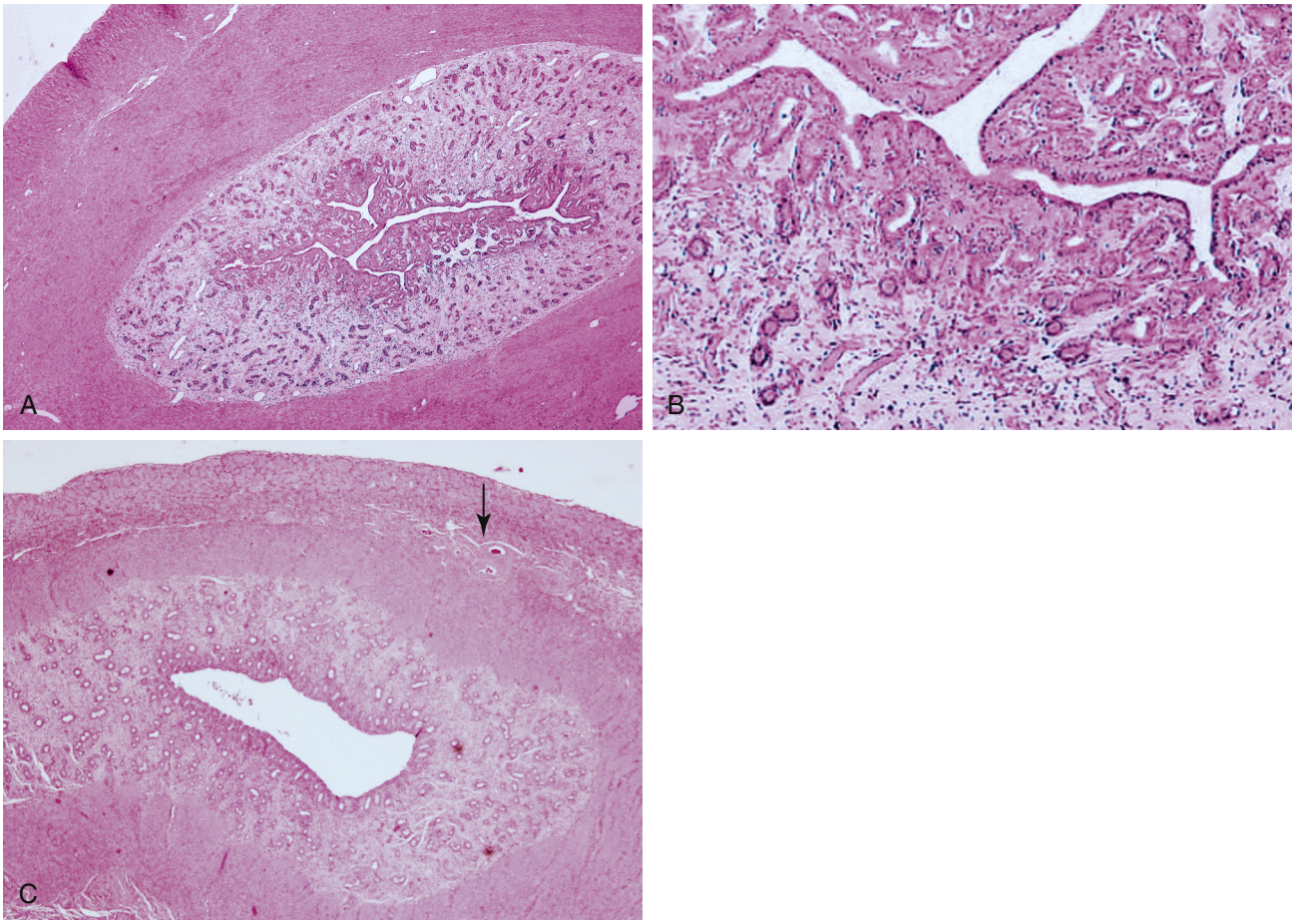


FIGURE 12-34 Normal uterus. Tissue section. Dog. Same section A and B. Proestrus. A, The dense area surrounding the endometrial glands is the myometrium, which consists of two layers of smooth muscle, inner circular and outer longitudinal. The outermost layer is the perimetrium or mesothelial-lined serosa. (H&E; LP.) **B,** Close magnification of luminal epithelium shows the extension of the uterine glands present of the mucosa into the lamina propria as tubular formations. The inner mucosa or endometrium is lined by cuboidal or columnar epithelium. (H&E; LP.) **C, Early estrus.** The mucosal glands as well as the deeper endometrial glands become more developed and extend across the lamina propria. The arrow indicates a region of prominent blood vessels. (H&E; LP.)

seen. Single cells are less frequently observed. The endometrial epithelial cells are low columnar during proestrus and estrus and are 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, whereas those of degenerated endometrial cells are often of irregular shape and pyknotic. Neutrophils are the most common leukocytes observed during proestrus, estrus, diestrus, and early pregnancy, and lymphocytes and macrophages are frequently seen during anestrus (Groppetti et al., 2010; Watts et al., 1998). Eosinophils are identified in samples collected during proestrus and estrus (Groppetti et al., 2010). Plasma cells are encountered during the late anestrus stage (Groppetti et al., 2010). 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 (Groppetti et al., 2010; 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/Metritis. Cytologic examination of vaginal discharges or uterus samples may be useful for the 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 dehydration. 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).

Cytologically, pyometra or uterine stump flushing samples are characterized by a low number of endometrial epithelial cells often with degenerative changes. Numerous nondegenerate and degenerate neutrophils together with many lymphocytes, macrophages, and plasma cells are also noticed. Intracellular and free bacteria are both abundant (Groppetti et al., 2010). 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 have a high anesthetic risk (England et al., 2007; Verstegen et al., 2008).

Metritis usually follows parturition and is characterized by a systemically ill animal with a malodorous uterine/vaginal discharge. Other causes of metritis can be bacterial (Fontaine et al., 2009), fungal such as aspergillosis, and mating-related (Walker et al., 2012). Bacterial or nonbacterial metritis is associated with infertility (Fontaine et al., 2009). 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 the 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 due to pregnancy (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 commonly and leiomyosarcomas are comparatively rare. These tumors are of similar cytologic appearance to those found in other body sites. Uterine carcinomas (McEntee, 2002) and hemangiosarcomas (Wenzlow et al., 2009) are rare. In cats, both leiomyoma and endometrial adenocarcinoma are reported with similar frequencies (Miller et al., 2003). Other tumors that are less commonly described include leiomyosarcoma, myxoid leiomyosarcoma (Cooper et al., 2006), endometrial stromal sarcoma (Sato et al., 2007), and mixed Mullerian tumor (adenosarcoma) in cats (Miller at 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, although some authors suggest that this method should be used with caution to determine the optimal mating period (Hiemstra et al., 2001; Moxon et al., 2010). Cytologic examination of vaginal mucosal imprints and discharges is also useful for the 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 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, ones such as methanolic or aqueous 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 that are 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 the 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 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, clitoral fossa, and 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

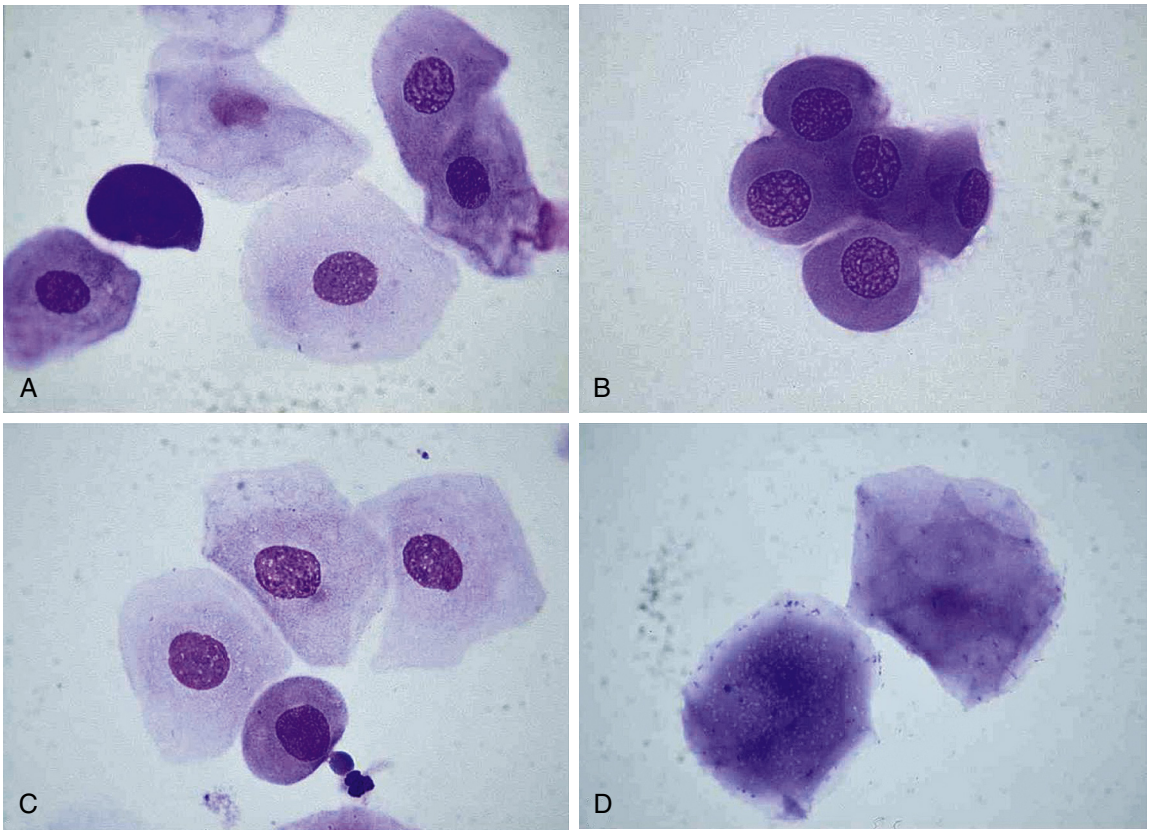


FIGURE 12-35 Vaginal epithelial cells. Vaginal smear. Dog. A, The small basophilic cell is a basal cell in between parabasal and intermediate squamous cells. **B,** Cluster of parabasal cells. **C,** Three intermediate cells and one parabasal cell. **D,** Squamous superficial epithelial cells. (Wright-Giemsa; HP oil.)

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 (Fig. 12-35).

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 nucleocytoplasmic 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). *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).

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).

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 and pictorially demonstrated in Fig. 12-36. The normal physiology and endocrinology of the estrous cycle of the bitch is reviewed elsewhere (Root Kustritz, 2012).

Proestrus. Proestrus (Fig. 12-37A&B) is characterized by rising concentrations of estradiol and low concentrations of progesterone (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. 12-38) 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).

Diestrus. Diestrus (Figs. 12-39 and 12-40) 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, frequently localized into the cytoplasm of epithelial cells (metaestrum cells). 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 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 (Mills et al., 1979; Shille 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).

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 3–21 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	Follicular development, rise in 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 (50%). <5% parabasal or intermediate cells	Absent	Present or absent	Present	Clear	Declining estradiol concentrations with subsequent increase of LH, ovulation, and rising preovulatory progesterone concentration
Diestrus (pregnant bitches 62– 64 d, nonpregnant bitches 49– 79 d)		Abrupt decrease in superficial cells and 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	Progesterone rises and then declines over this stage: rapid decline at end (pregnant dogs) or more gradually decline (non-pregnant dogs) Progesterone production is supported by LH and prolactin secretion
Anestrus (1–8 months)		Predominance of parabasal and intermediate cells. Superficial cells absent	Absent or low numbers	Absent	Absent or low numbers	Clear or granular	FSH elevated, LH concentrations increase late in stage after estrogen priming, low concentrations of progesterone

*It is not possible to distinguish late proestrus from estrus with vaginal cytology.
FSH, Follicle stimulating hormone; LH, Luteinizing hormone

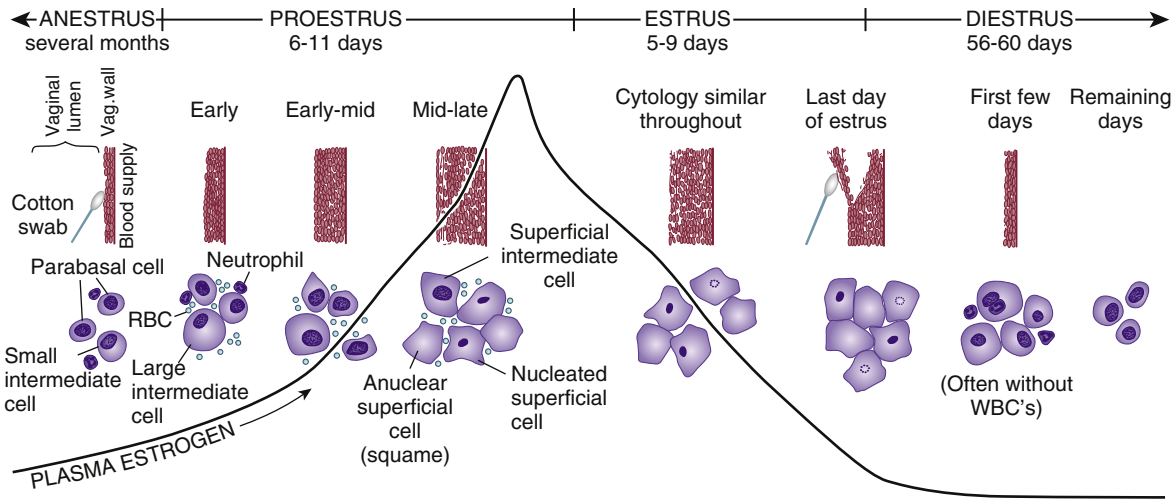


FIGURE 12-36 Changes in vaginal wall thickness, cell cytology, and estrous cycle relative to blood estrogen levels in the dog. (Modified from Feldman EC, Nelson RW: Ovarian cycle and vaginal cytology. In Feldman EC, Nelson RW (eds): Canine and feline endocrinology and reproduction, ed 3, Philadelphia, PA, 2004, Saunders, pp 755.)

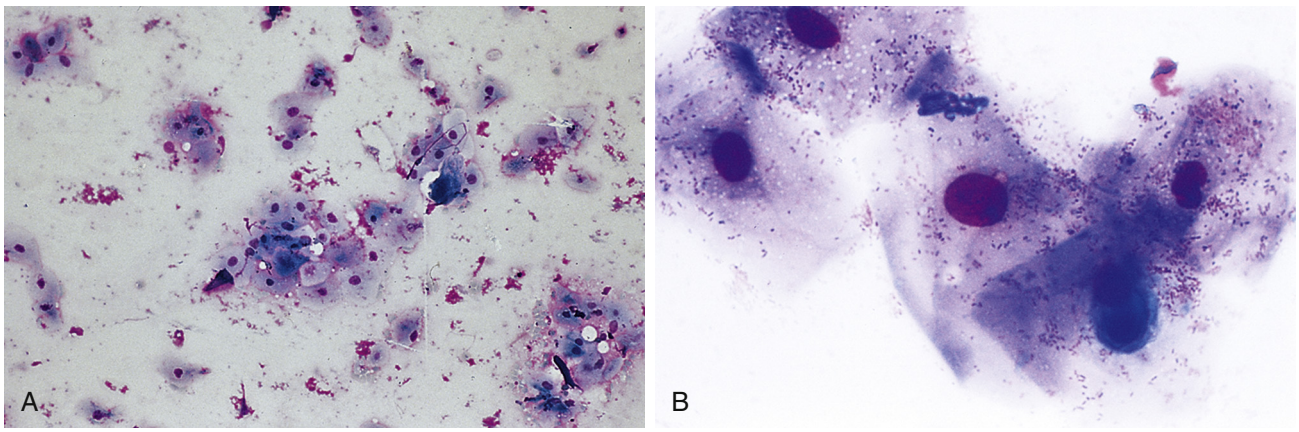


FIGURE 12-37 Proestrus. Vaginal smear. Dog. A, There are intermediate epithelial cells with lower numbers of superficial cells. Red blood cells are present. The background has a basophilic appearance due to the presence of mucus. (Wright-Giemsa; HP oil.) **B, Late proestrus.** Intermediate and superficial 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.) (A and B samples provided by Rolf Larsen, University of Florida.)

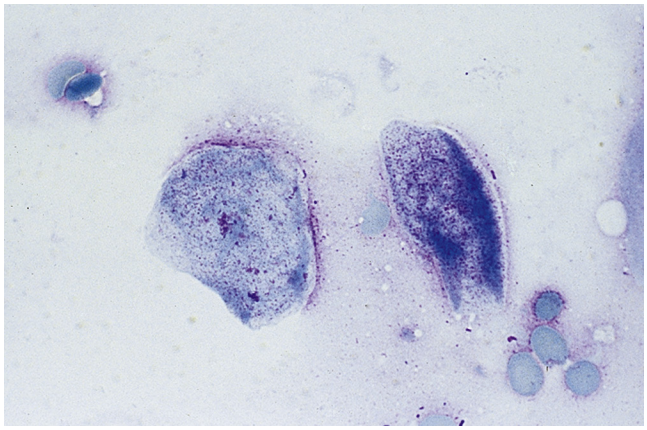


FIGURE 12-38 Estrus. Vaginal smear. Dog. Shown are anucleated (cornified) superficial epithelial cells along with the presence of red blood cells in the background. (Wright-Giemsa; HP oil.) (Sample provided by Rolf Larsen, University of Florida.)

Inflammation

Vaginitis. Inflammatory disease of the vaginal mucosa is often related to noninfectious factors such as vaginal anomalies, clitoral hypertrophy, retained fetal foreign bodies (Nicastro and Walshaw, 2007; Snead et al., 2010), 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. 12-41 and 12-42). Less commonly, yeast forms related to fungal infection such as *Malesszia sp*. or hyphal elements as in pythiosis (Fig. 12-43) may be observed. Cytologic specimens may be submitted for silver stains to identify hyphae if fungal infection or pythiosis is suspected (Fig. 12-44).

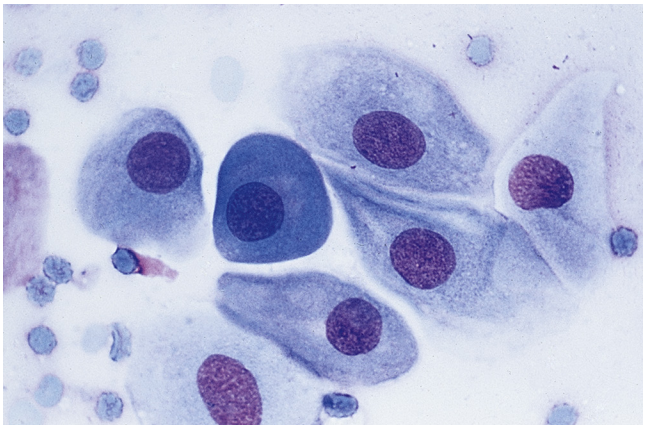


FIGURE 12-39 Diestrus. Vaginal smear. Dog. Parabasal and intermediate epithelial cells are shown. The parabasal cells have round nuclei, moderate nuclear-to-cytoplasmic ratios, moderately to deeply basophilic cytoplasm, and round cell borders. The intermediate cells are larger with increased amounts 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.)

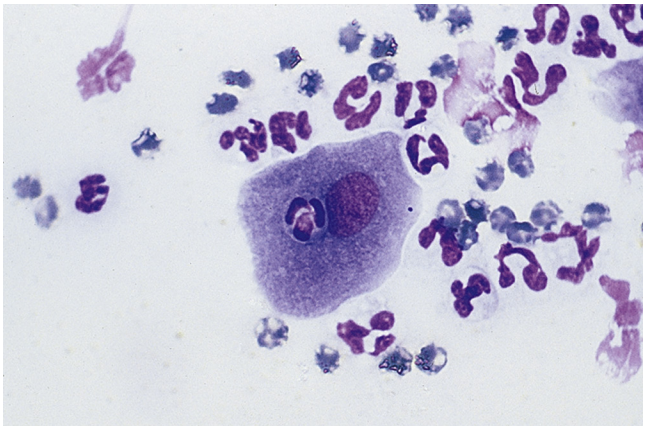


FIGURE 12-40 Diestrus. Vaginal smear. Dog. Note the large number of neutrophils and red blood cells in the background. An intermediate epithelial cell containing a neutrophil (metestrum cell) is located in the center. These cells are not specific for diestrus and may be found whenever increased numbers of neutrophils are present. (Wright-Giemsa; HP oil.) (Sample provided by Rolf Larsen, University of Florida.)

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 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 (McEntee, 2002; Olson et al., 1984b). 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,

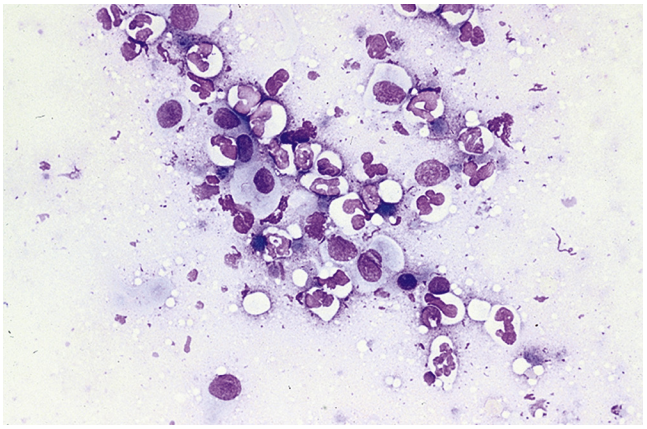


FIGURE 12-41 Suppurative vaginitis. Tissue scraping. Dog. Increased numbers of neutrophils from a vaginal papule. The neutrophils display degenerative nuclear changes of moderate to marked karyolysis. Degenerative changes are typically associated with bacterial infections. A few parabasal and intermediate epithelial cells are also present. (Wright-Giemsa; HP oil.)

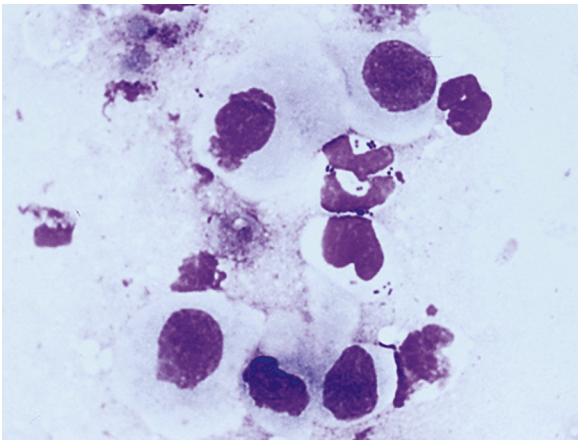


FIGURE 12-42 Bacterial vaginitis. Tissue scraping. Dog. Two degenerative neutrophils containing phagocytized bacteria from the vaginal scraping shown in Fig. 12-41. (Wright-Giemsa; HP oil.)

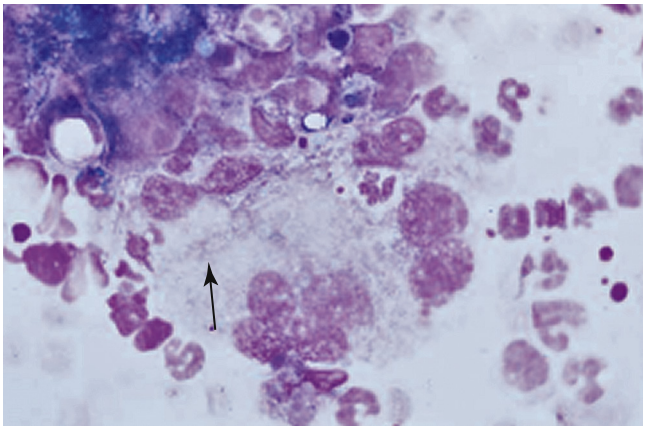


FIGURE 12-43 Pyogranulomatous vaginitis. Tissue aspirate. Dog. Pyogranulomatous inflammation is present in this specimen from a vulvar mass. Large numbers of neutrophils, lower numbers of eosinophils, and a multinucleated macrophage are present. Pale-staining linear structures suspicious for hyphae are seen associated with the macrophage (*arrow*). (Wright-Giemsa; HP oil.)

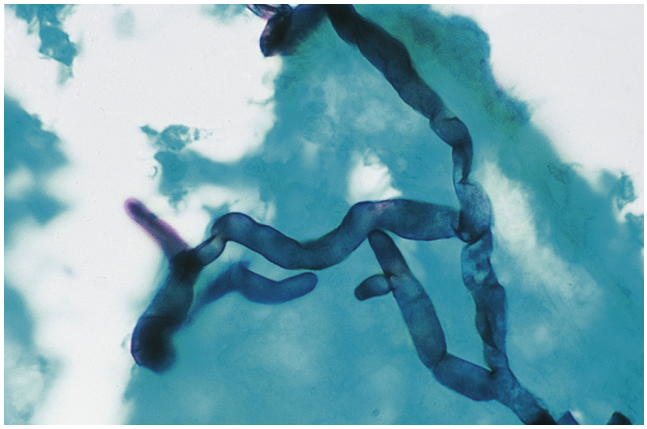


FIGURE 12-44 Fungal vaginitis. Tissue aspirate. Dog. Special stain of sample shown in Figure 12-43. Positive-staining, poorly septated, linear structures approximately 6 to 8 μm in width are present. Culture confirmed the presence of *Pythium* sp. (Gomori methenamine silver; HP oil.)

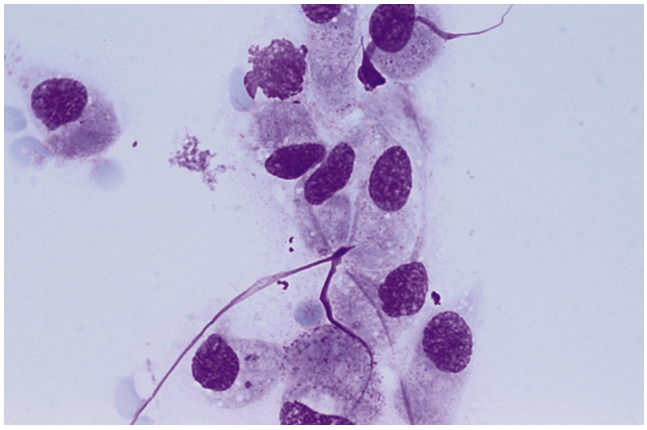


FIGURE 12-45 Vaginal leiomyoma. Tissue imprint. Dog. The cells are arranged individually or in small clumps and display round to oval nuclei with coarse nuclear chromatin, moderate nuclear-to-cytoplasmic ratios, and inconspicuous nucleoli. The cytoplasm is moderately basophilic and cell borders are indistinct. (Wright-Giemsa; HP oil.)

excessive vulvar licking, and dystocia (Klein, 2007). Leiomyomas, fibroleiomyomas, fibromas, and fibroepithelial polyps (Brown et al., 2012) are the most common vaginal neoplasm in dogs and cats (Baker and Lumsden, 1999). Lipoleiomyomas are also rarely reported (Sycamore and Julian, 2011). 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. 12-45). 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 (Brodey and Roszel, 1967). Other tumors with malignant potential include transmissible venereal tumors (TVT), adenocarcinoma, squamous cell carcinoma/epidermoid carcinoma, urethral transitional cell carcinoma, osteosarcoma, hemangiosarcoma, rhabdomyosarcoma, lymphoma, and mast cell tumor (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).

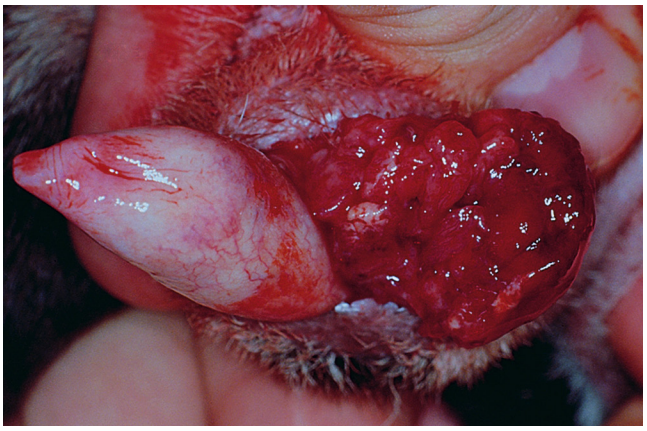


FIGURE 12-46 Transmissible venereal tumor. Genital mass. Dog. The mass appears as a soft, friable, hemorrhagic mass on the prepuce.

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 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 eyes (Lorimier and Fan, 2007). They appear as firm, friable, tan, ulcerated, and nodular or polypoid masses (Fig. 12-46). 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 central nervous system (Park et al., 2006). 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, which also suggests a histiocytic origin (Lorimier and Fan, 2007).

Aspirates of TVT generally yield large numbers of individualized, round cells (Figs. 12-47 and 12-48). 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).

MALE REPRODUCTIVE SYSTEM: PROSTATE AND TESTES

Prostate Gland

The cat has two accessory genital glands: the prostate and bulbourethral glands. Prostatic carcinoma, paraprostatic cysts, prostatic abscess, and prostatic squamous metaplasia are uncommonly reported in cats (Foster, 2012). Although the

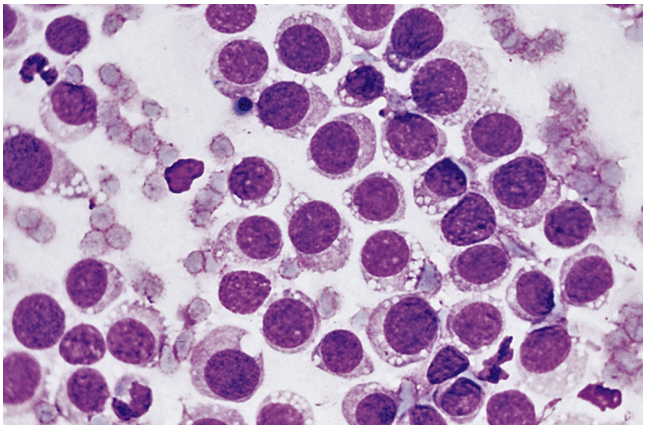


FIGURE 12-47 Transmissible venereal tumor. Vaginal mass imprint. Dog. Large numbers of round cells are shown that have round nuclei, coarse nuclear chromatin, variably prominent nucleoli, and scant to moderate amounts of lightly basophilic cytoplasm. Many of the cells contain punctate cytoplasmic vacuoles, which is a characteristic feature of this tumor. (Wright-Giemsa; HP oil.)

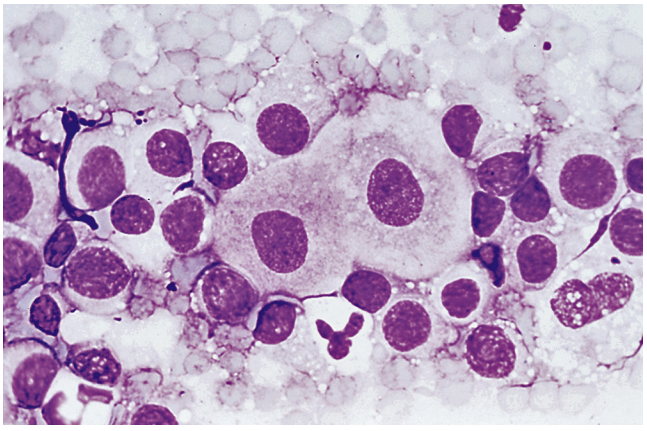


FIGURE 12-48 Transmissible venereal tumor. Vaginal mass imprint. Dog. Two intermediate epithelial cells (center) and individualized tumor cells from the same case as shown in Fig. 12-47. Note the larger size and increased amounts of cytoplasm in the epithelial cells compared to the tumor cells. (Wright-Giemsa; HP oil.)

prostate gland is present in cats, the vast majority of prostatic disease is reported in the dog. 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, squamous metaplasia, and primary or metastatic neoplasia. 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 signs (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 (Bradbury et al., 2009). 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 to classify the type of prostatic disease (Baker and Lumsden, 1999; Bradbury et al., 2009). 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 to evaluate 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 the 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 ethylenediaminetetraacetic acid (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/Wash. Prostatic massage is used primarily to collect prostatic fluid in dogs unable to ejaculate (Dorfman and Barsanti, 1995). The simplest method for prostatic massage or wash 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; Smith, 2008). 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 (Powe et al., 2004; Thrall et al., 1985).

Fine-Needle Aspiration. FNA or FNCS 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 reports in the veterinary literature documenting FNA for diagnosis or treatment of prostatic disease with no complications (Boland et al., 2003). Ultrasound-guided transabdominal FNA of the prostate is described elsewhere (Root Kustritz, 2006). FNA of the prostate has several advantages over other collection methods. Identification of squamous epithelial cells from a prostatic aspirate allows the 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. Prostatic biopsy is described elsewhere (Smith, 2008).

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, fibromuscular 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.

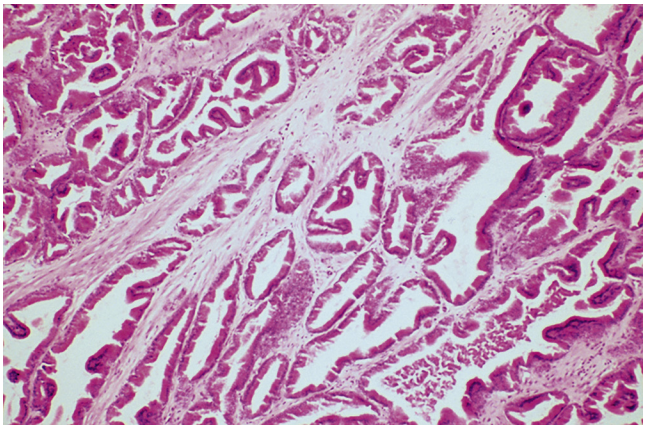


FIGURE 12-49 Normal prostate gland. Tissue section. Dog. The tubuloalveolar glands are surrounded by a fibromuscular stroma. Primary and secondary enfoldings of epithelium project into the alveolar lumen. (H&E; LP.) (Case material supplied by Roger Reep and Don Samuelson, University of Florida.)

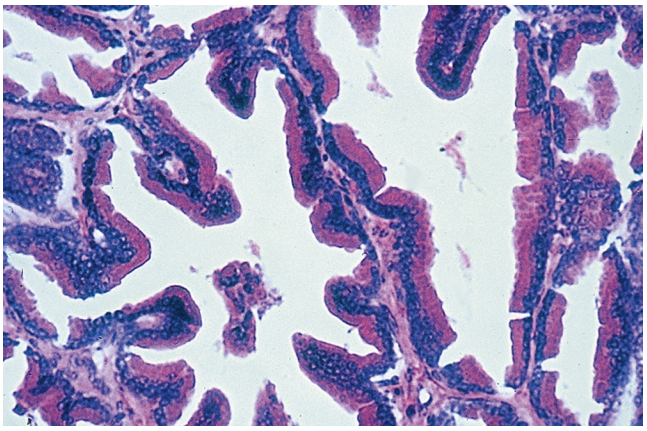


FIGURE 12-50 Normal prostate gland. Tissue section. Dog. Higher magnification of specimen in Figure 12-49. Cuboidal and columnar epithelium line the prostatic lumens and ducts. Canine. (H&E; IP.) (Case material supplied by Roger Reep and Don Samuelson, University of Florida.)

The prostate gland is composed of compound tubuloalveolar glands radiating from the urethral opening (Figs. 12-49 and 12-50). 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).

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 or microvacuolated 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

There are many cysts that develop within and around the prostate: paraprostatic cysts are those around the prostate, and prostatic cysts are those within the prostate (Foster, 2012). Prostatic cysts (see Fig. 2-6) may occur as multiple, small cysts associated with androgen-dependent benign hyperplasia as well as large retention cysts within the prostate tissue. Paraprostatic cysts may become mineralized or result from osseus 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). Prostatic cavitary lesions containing urine (urinary cysts) due to intraprostatic urethral fistulation have been reported (Bokemeyer et al., 2011). 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). 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 no or few normal-appearing epithelial cells; low to moderate numbers of neutrophils, macrophages, and small lymphocytes; and erythrocytes on a red to brown background (Boland et al., 2003; Thrall et al., 1985).

Benign Prostatic Hyperplasia

Benign prostatic hyperplasia (BPH) is a common finding in older intact male dogs. BPH is increase 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, its development 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 administration—finasteride inhibits conversion of testosterone to dihydrotestosterone, causing prostatic involution via apoptosis (Sirinarumitr et al., 2001). Other medical options exist for treating BPH and are reviewed elsewhere (Smith, 2008).

In humans, the prostate is fixed anatomically such that the 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. 12-51 and 12-52). 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 and occasionally vacuolated. 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, are consistent with a diagnosis of BPH (Fig. 12-53A&B).

Squamous Metaplasia

Increased circulating concentrations of estrogen can result in squamous metaplasia of the prostatic epithelium (Fig. 12-54A-C). 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). 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

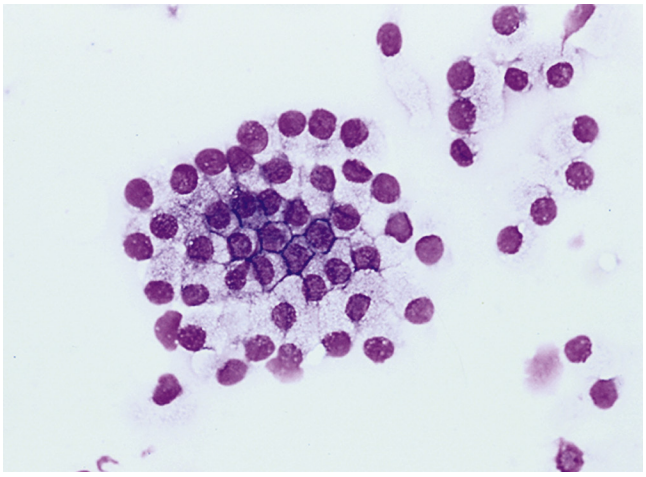


FIGURE 12-51 Benign prostatic hyperplasia. Tissue aspirate. Dog. Normal-appearing prostatic epithelial cells from an enlarged prostate. The cells are uniform in size and shape and are arranged in clusters and individually. The cluster of cells in the center display a characteristic "honeycomb" appearance. (Wright-Giemsa; HP oil.)

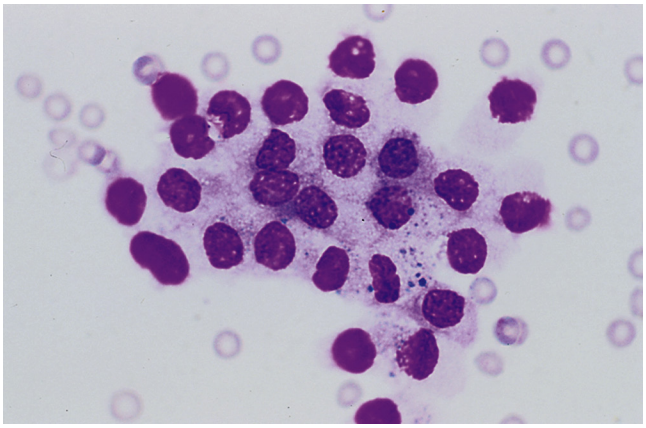


FIGURE 12-52 Benign prostatic hyperplasia. Tissue aspirate. Dog. Same case as in Fig. 12-51. The prostatic epithelial cells display round nuclei, slightly coarse nuclear chromatin, and moderate amounts of lightly basophilic cytoplasm. A few cells contain small amounts of basophilic secretory product. (Wright-Giemsa; HP oil.)

bacteria (including *Mycoplasma*) into the 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. (Johnston et al., 2000; Smith, 2008). *Brucella canis* may infect the canine prostate, but it is more commonly associated with epididymal and testicular infection and clinical signs referable to those organs (Johnston et al., 2000). Anaerobic bacteria or fungal infections (*Blastomyces dermatitidis* [Fig. 12-55] [Reed et al., 2010], *Cryptococcus neoformans*, or *Coccidioides immitis*) also have been observed via hematogenous spread, urethral ascent, or penetration through the scrotum with descending prostate infection from a testicular source (Johnston et al., 2000). Infertility and chronic prostatitis due to *Leishmania infantum* infection have been reported (Mir et al., 2012). 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 because the prostate-lipid barrier is disrupted (Olson et al., 1987). Antibiotics for the 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. 12-56). Macrophages may also be present, especially in chronic prostatitis (Fig. 12-57). 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).

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). Historically, adenocarcinoma was the most commonly reported neoplasm of the prostate followed by transitional cell carcinoma arising from the prostatic urethra. However, 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 neoplastic prostate glands (Leroy and Northrup, 2009; Matsuzaki et al., 2010). Other malignant neoplasms have been rarely described such as lymphoma and malignant mesenchymal tumors such as hemangiosarcoma and leiomyosarcoma (Fan and de Lorimier, 2007; Hayden et al., 1999; Teske et al., 2002;

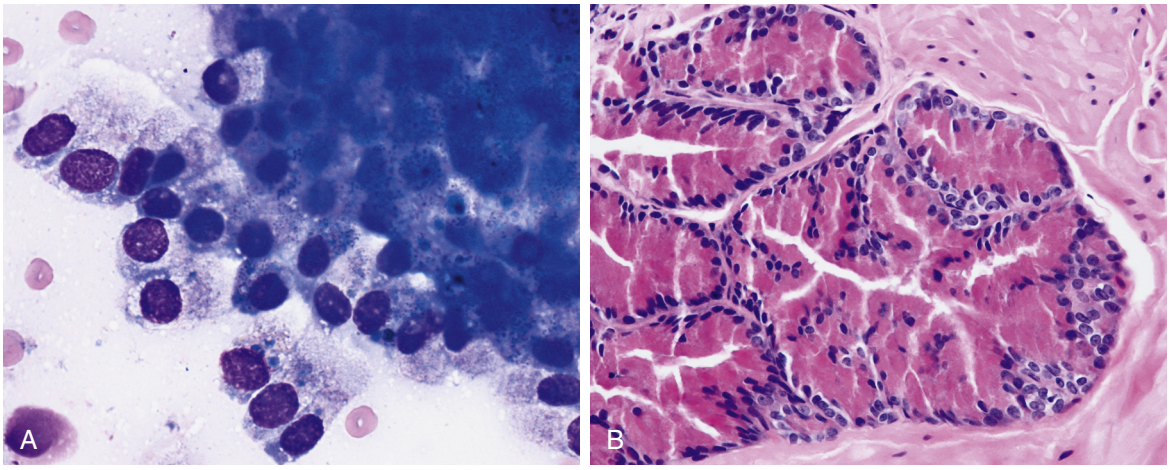


FIGURE 12-53 Benign prostatic hyperplasia. Dog. Same case A-B. A, Tissue aspirate. Prostatic epithelial cells are columnar and uniform. Note the granulated appearance of the cells and accumulation of secretory pigment in the cell sheets and clusters. (Wright; HP oil.) **B, Tissue section.** Hyperplastic epithelium is characterized by minimal anaplastic features. Nuclei have less nuclear chromatin density than normal but increased prominence of nucleoli. (H&E; IP.) (A and B, Courtesy of Rose Raskin, Purdue University.)

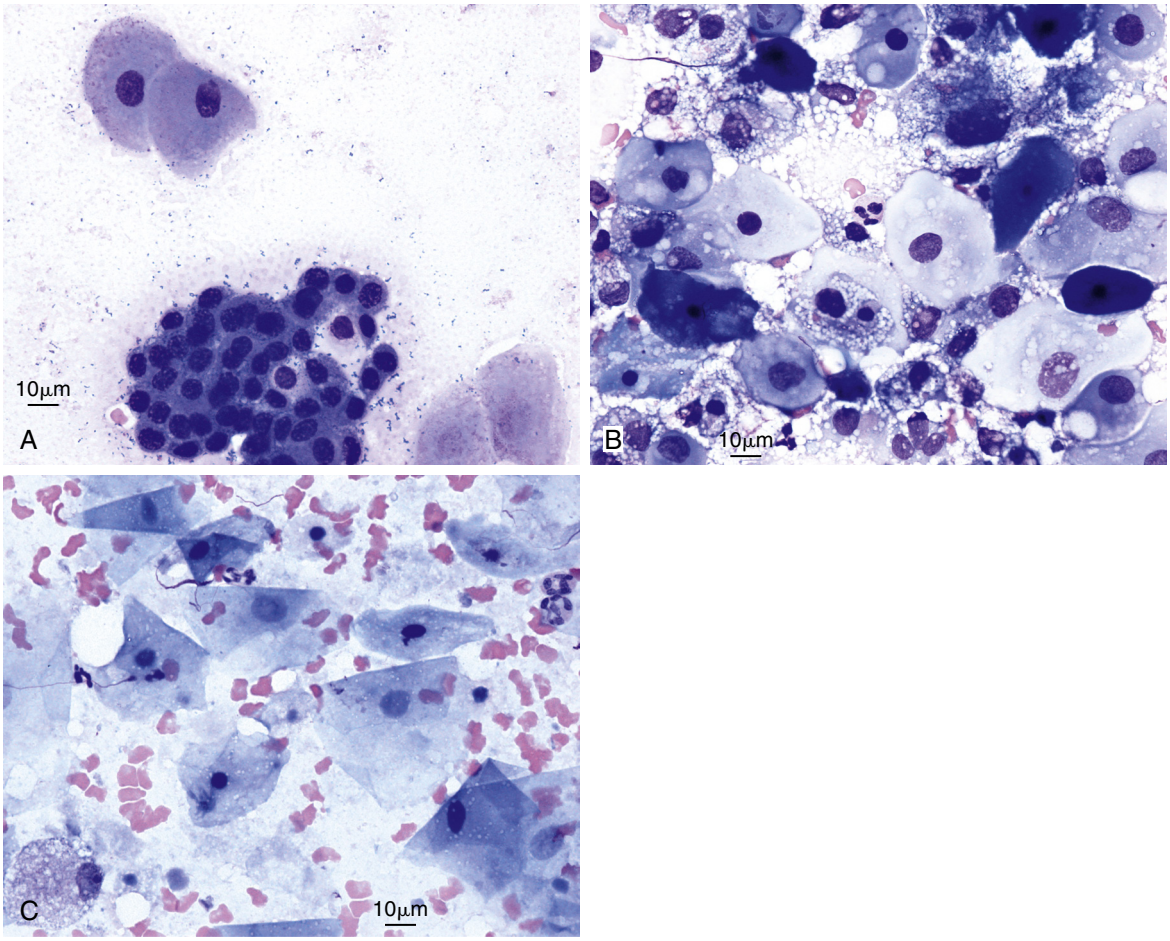


FIGURE 12-54 Squamous metaplasia. Prostate. Dog. A, Wash. Sheet of hyperplastic epithelium along with several squamous cells, two with small nuclei and two anucleate. The background is dotted by bacteria. Neutrophils (not shown) are mildly increased. (Modified Wright; HP oil.) **B, Tissue aspirate.** Highly cellular specimen with squamous epithelium in various stages of maturation and keratinization. (Modified Wright; HP oil.) **C, Tissue aspirate.** Mild neutrophilic and macrophagic inflammation accompany squamous cells present as mostly keratinized superficial epithelium. (Modified Wright; HP oil.) (A-C, Courtesy of Rose Raskin, Purdue University.)

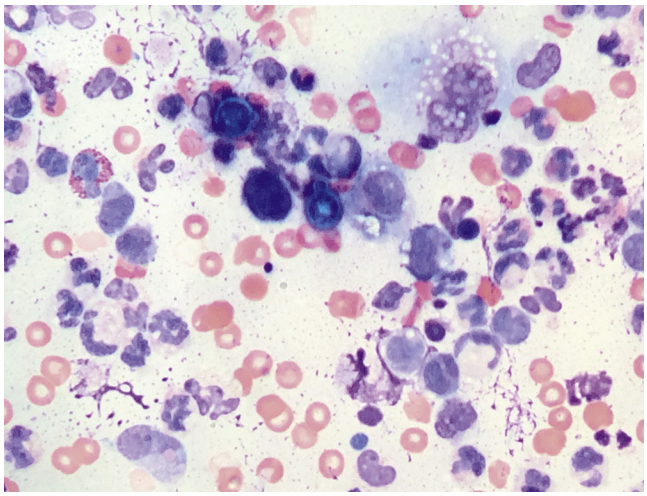


FIGURE 12-55 Fungal prostatitis. Tissue aspirate. Dog. History of sudden inability to urinate and on rectal palpation the prostate was markedly enlarged. Present are neutrophils, macrophages, and eosinophils along with two yeast forms, one of which displays broad-based budding. Previously treated for dermal blastomycosis 4 months earlier. (Modified Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)

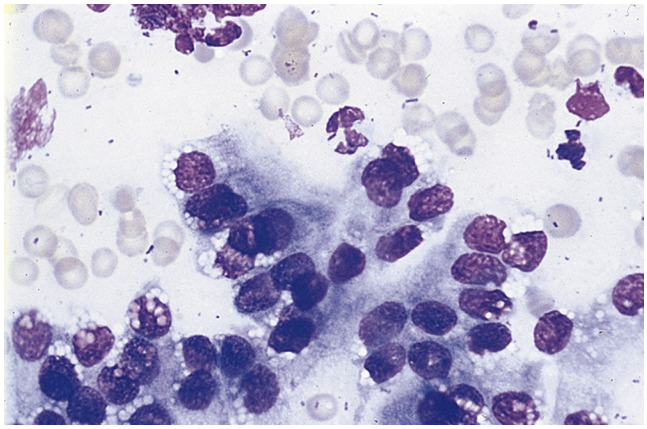


FIGURE 12-56 Septic neutrophilic prostatitis. Tissue aspirate. Dog. Prostatic epithelial cells and neutrophils are present in this example of acute septic prostatitis. The neutrophils are degenerate as indicated by moderate karyolysis. Bacteria are present in the background and within the neutrophils. (Wright-Giemsa; HP oil.)

Winter et al., 2006) and prostatic sarcomatoid carcinoma (Pinto da Cunha et al., 2007).

Currently, prostatic carcinoma is a term with several meanings in dogs. The convention is that carcinomas of the prostate are adenocarcinoma (from the prostate glandular tissue). However, there are several types of carcinoma in dogs, including adenocarcinoma (presumably from the glands), transitional cell carcinoma (presumably from the prostatic ducts), mixed carcinomas, and squamous cell carcinomas (Foster, 2012). There is disagreement as to which is the most common: glandular type *versus* transitional type since there is controversy regarding the cell of origin of canine prostate carcinoma (Leroy and Northrup, 2009).

Potentially, these neoplasms could originate from prostatic acinar epithelium, urothelium lining the prostatic urethra (e.g., transitional cell carcinoma), or ductal epithelium. Histologically,

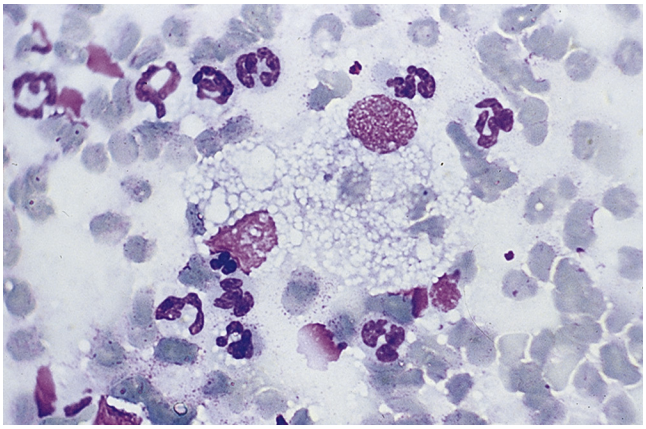


FIGURE 12-57 Mixed cell prostatitis. Tissue aspirate. Dog. A mixed cell population is present in this case of chronic prostatitis. Increased numbers of neutrophils, the majority of which are nondegenerate, and two reactive macrophages are present. Infectious organisms were not seen in this sample. (Wright-Giemsa; HP oil.)

most canine prostatic carcinomas are of an intraalveolar pattern but many also contain patterns similar to transitional cell carcinoma. In humans, prostate-specific antigen (PSA) distinguishes between prostatic carcinoma and urothelial (transitional cell) carcinoma. However, dog prostate cells do not produce PSA as in humans but rather a related kallikrein family enzyme, arginine esterase, for which there is no commercially available antibody. In addition, keratin 7 immunoreactivity and arginine esterase gene expression and enzyme activity do not distinguish canine transitional cell carcinomas from prostate carcinomas-glandular type (LeRoy et al., 2004). Currently, a ductal epithelium origin of prostatic carcinomas is supported, related to the fact that prostatic carcinomas often have acinar and urothelial morphologies involving the prostate gland and the bladder. In addition, embryonic urogenital sinus epitelium gives rise to the prostate gland and the bladder and, therefore, canine prostatic carcinoma might originate from a ductal epithelial cell capable of bimodal differentiation along both urothelial and prostatic acinar cell lines (Leroy and Northrup, 2009). Moreover, in another study, the expression of uroplakin III (UPIII), PSA, CK7, and CK18 indicates that canine prostatic carcinoma most likely originates from the collecting ducts rather than from the peripheral acini (Lai et al., 2008). Canine prostatic carcinomas are predominantly androgen receptor–negative, suggesting that androgens may not be required for initiation or progression of this tumor (Fan and de Lorimier, 2007). Comparative features of human and canine prostatic carcinoma are reviewed elsewhere (Leroy and Northrup, 2009).

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) and mostly develop the transitional type. Most canine prostatic carcinomas, mainly the transitional type, 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, 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 the diagnosis of prostatic neoplasia. Cytologic evaluation of FNA samples from prostatic carcinoma (glandular type) usually reveals large numbers of deeply basophilic, frequently vacuolated, epithelial cells arranged in variably sized clusters and sheets (Fig. 12-58A&B). 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. Glandular type prostatic carcinoma and transitional type can be difficult to distinguish cytologically and histopathologically (Baker and Lumsden, 1999). Some acinar structures may be noted in glandular type, which can help to differentiate this neoplasm from transitional type carcinoma (Zinkl, 2008). Moreover, the transitional cells show tailed shapes and only sporadically exhibit vacuolated cytoplasm, as consequences of glandular metaplasia. Neoplastic cells are arranged in discohesive, mostly bidimensional clusters. Nuclei are round to ovoid, with irregularly clumped chromatin, sometimes nucleolated and show moderate to marked anisokaryosis (Figs. 12-59, 12-60, 12-61, 12-62). Neoplastic canine prostate glands also frequently contain foci of BPH, cystic glandular dilatation, and significant suppurative and lymphoplasmacytic inflammation. 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, PSA, and canine prostate specific esterase have not been shown to be useful in the definitive diagnosis of canine prostatic carcinoma (Gobello et al., 2002).

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 a 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 such as atraumatic sampling (FNCS) (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 cell degeneration.

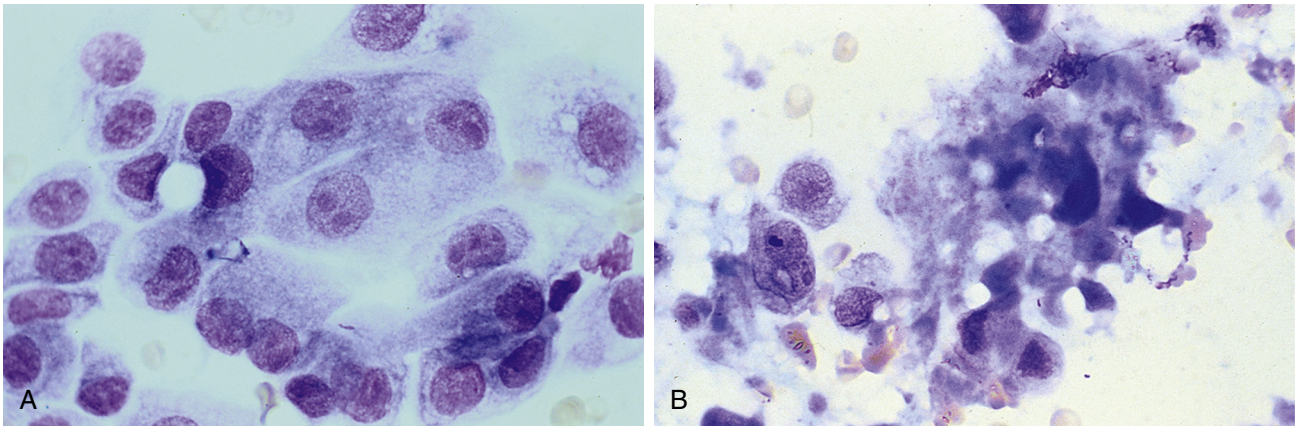


FIGURE 12-58 Prostatic carcinoma-glandular type. Cytologic preparation. Dog. Same case A-B. A, Neoplastic epithelial cells display prominent, large, multiple nucleoli, coarse nuclear chromatin, moderate anisokaryosis and anisocytosis, variable nuclear-to-cytoplasmic ratios, and binucleation. (Wright-Giemsa; HP oil.) **B,** 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.)

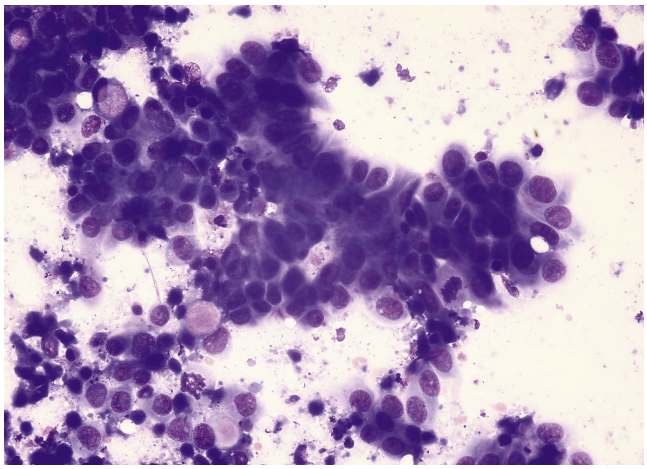


FIGURE 12-59 Prostatic carcinoma-transitional type. Cytologic preparation. Dog. A large cluster of epithelial cells arranged in peripherical palisades. Notice the presence of tailed cytoplasms and of some large cytoplasmic vacuoles. (Wright-Giemsa; HP oil.)

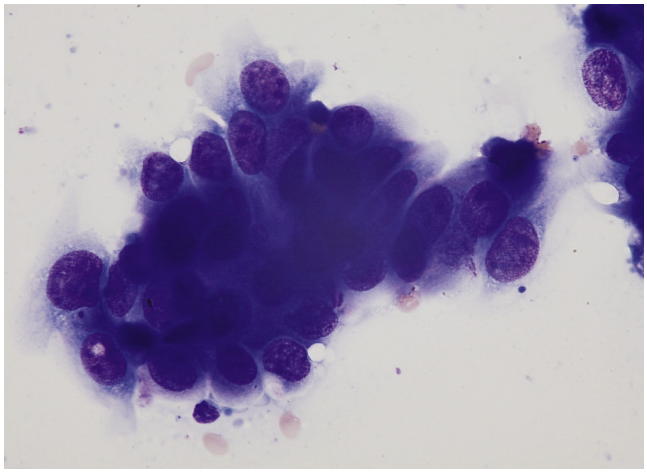


FIGURE 12-60 Prostatic carcinoma-transitional type. Cytologic preparation. Dog. The neoplastic cells show round to elongated cytoplasm, sometimes with a polar tail. (Wright-Giemsa; HP oil.)

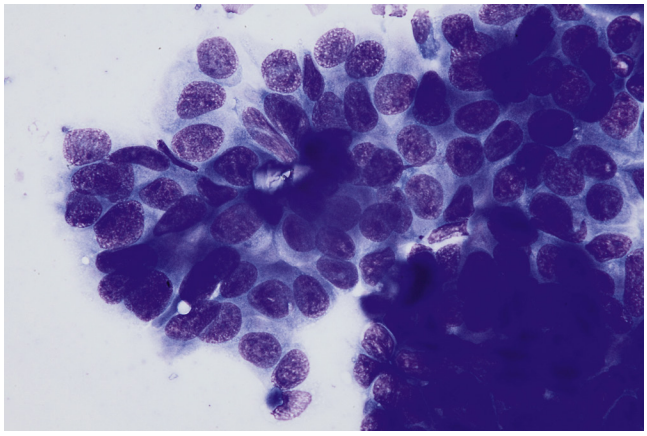


FIGURE 12-61 Prostatic carcinoma-transitional type. Cytologic preparation. Dog. The neoplastic cells have large round to ovoid nuclei; anisokaryosis and irregular clumped chromatin with prominent multiple nucleoli. (Wright-Giemsa; HP oil.)

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 convoluted seminiferous tubules are lined by some stratified layers of spermatogenic cells, which are actively involved in spermatogenesis, and Sertoli cells, which secrete estrogen and provide support for the developing sperm. The connective tissue between adjacent tubules contains interstitial (Leydig) cells, which secrete testosterone and are localized near the blood vessels (Figs. 12-63 and 12-64).

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 (Figs. 12-65 and 12-66). Presence of multinucleated cells is possible, as the result of *cytodieresis imperfecta,* a morphologic expression of incomplete separation of germinal cells during subdivision. Mitotic activity is often high. More mature stages of developing sperm are characterized

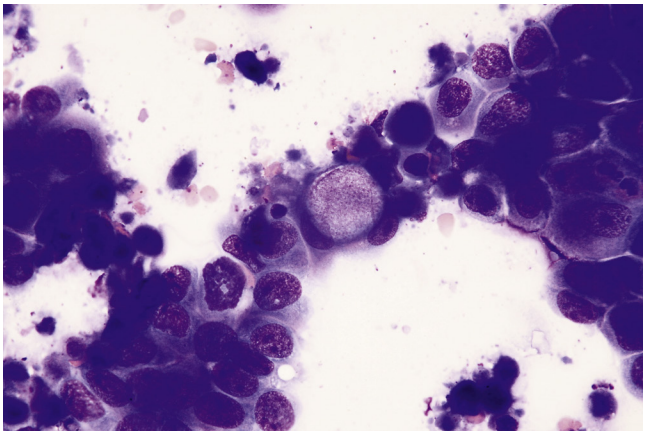


FIGURE 12-62 Prostatic carcinoma-transitional type. Cytologic preparation. Dog. The central cells show a large cytoplasmic vacuole, that displaces the nucleus in periphery. This feature is expression of glandular metaplasia. (Wright-Giemsa; HP oil.)

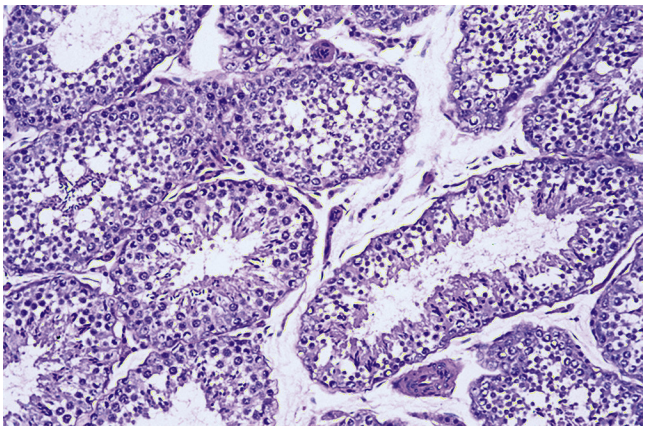


FIGURE 12-63 Normal testes. Tissue section. Dog. Multiple seminiferous tubules are present, which are surrounded by connective tissue containing low numbers of interstitial cells. (H&E; IP.)

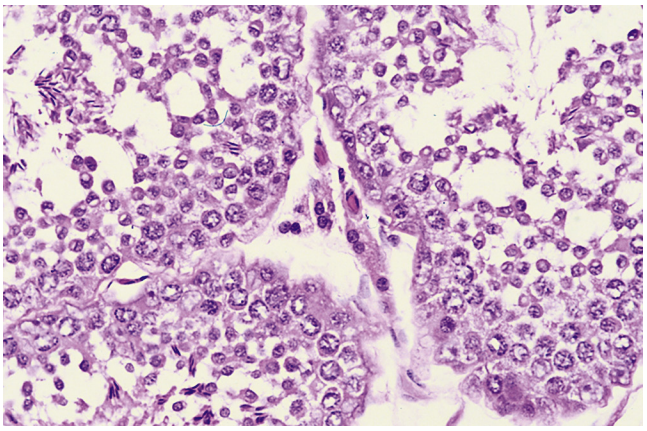


FIGURE 12-64 Normal testes. Tissue section. Dog. Higher magnification of the seminiferous tubules from the same case as in Fig. 12-63. Interstitial cells are seen in the center of the photomicrograph. Spermatocytes as well as early and late spermatids are seen within the tubules. Spermatocytes are characterized 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.)

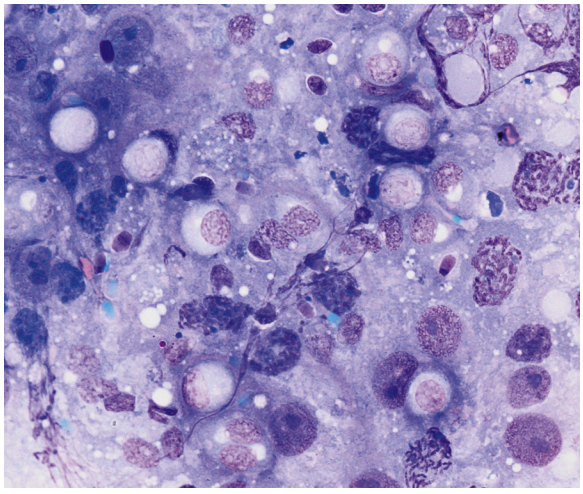


FIGURE 12-65 Normal testes. Tissue imprint. Dog. Large germinal cells and round spermatocytes are present along with small, densely basophilic spermatids. The background contains few lightly basophilic, detached mature sperm heads. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)

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 be evident. Scattered stellate or caudate Leydig cells, with microvacuolated cytoplasm, sometimes with bluish granules of lipofuscin pigment 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; Manna et al., 2012). Intranuclear or intracytoplasmic inclusions may be seen in cases of

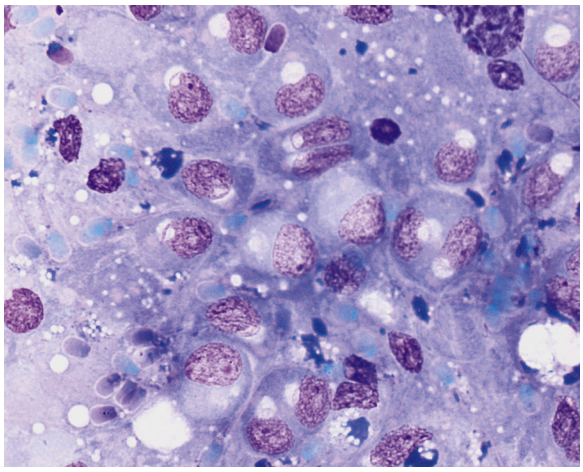


FIGURE 12-66 Normal testes. Tissue imprint. Dog. Higher magnification of same specimen shown in Figure 12-65. Note large numbers of pale basophilic–staining mature sperm with thin, clear space around the heads. Frequent binucleation is present in the germinal cells that display reticulated chromatin and prominent nucleoli. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)

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.

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 (Radi, 2004). 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 IGF (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, discrete 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. 12-67). Moderate anisokaryosis, anisocytosis, and binucleation and multinucleation may be present. The cytoplasm is lightly to moderately basophilic with a moderate to high nuclear-to-cytoplasmic ratio. The presence of clear macrovacuoles in the cytoplasm is rarely noted. Numerous and aberrant mitoses are often observed (Fig. 12-67). Small lymphocytes are frequently seen in seminomas (Fig. 12-68). 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 (MacLachlan and Kennedy, 2002; McEntee, 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 17β-estradiol 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 primarily iliac lymph nodes and also other lymph nodes, spleen, liver, and kidney.

Cytologically, variable numbers of round to elongate pleomorphic cells with indistinct cytoplasm are common features of Sertoli cell tumors (Fig. 12-69). These cells may occur individually or in small clusters, occasionally with palisading

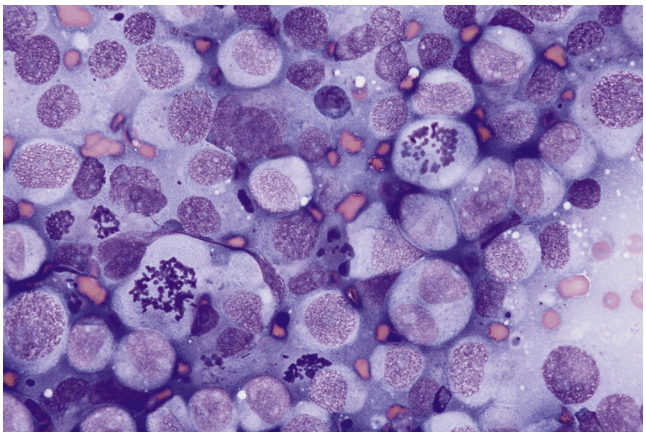


FIGURE 12-67 Seminoma. Tissue aspirate. Dog. Neoplastic cells appear large with round nuclei, coarse nuclear chromatin, and prominent, large nucleoli. The cytoplasm is lightly basophilic and some cells contain small numbers of punctate cytoplasmic vacuoles. Two mitotic figures are also evident. (Wright-Giemsa; HP oil.)

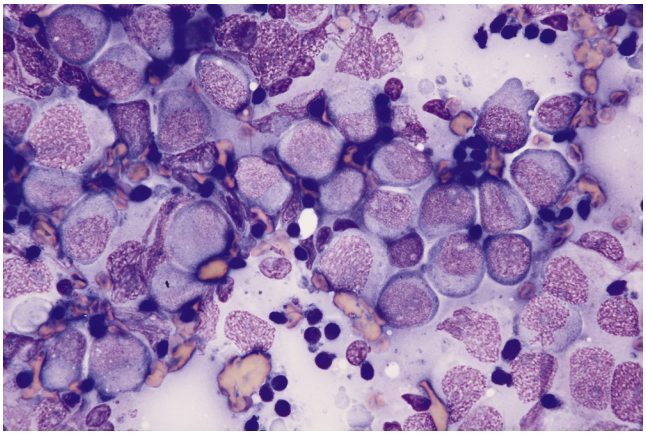


FIGURE 12-68 Seminoma. Tissue aspirate. Dog. Several round neoplastic, focally disrupted, cells are associated with many small mature lymphocytes. (Wright-Giemsa; HP oil.)

formation (Fig. 12-69) (Masserdotti, 2006). Nuclei are generally round with fine nuclear chromatin and occasionally one to three prominent, large nucleoli are noted. 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. 12-70) is typical (Masserdotti et al., 2005). Sertoli cell tumor can produce uncommonly rosette-like structures characterized by elongated cells centered around amorphous eosinophilic extracellular dense material, the so called "Call-Exner bodies" (Fig. 12-71) as commonly observed in granulosa cell tumor, its ovarian counterpart (Masserdotti et al., 2008).

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 3β-hydroxsteroid

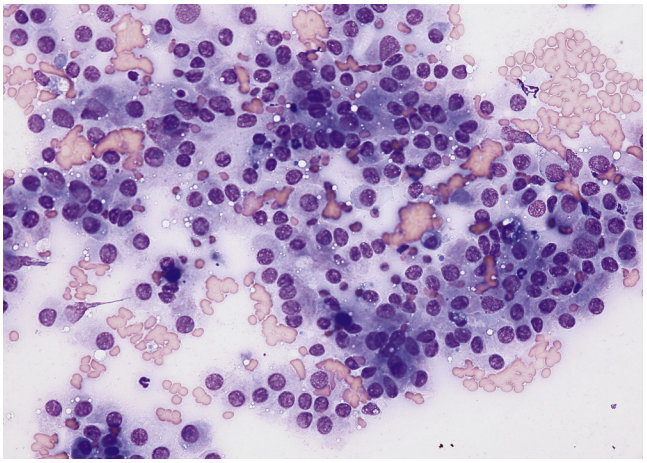


FIGURE 12-69 Sertoli cell tumor. Tissue aspirate. Dog. The tumor cells are arranged in sheets. The cytoplasm is lightly basophilic and cell borders are often indistinct. Nuclei are round to oval, with slightly coarse nuclear chromatin, and moderate nuclear-to-cytoplasmic ratios. (Wright-Giemsa; HP oil.)

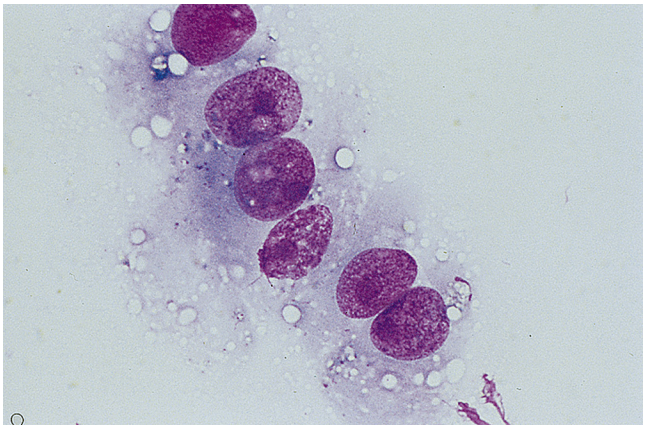


FIGURE 12-70 Sertoli cell tumor. Tissue aspirate. Dog. A row of tumor cells is shown. Variably sized cytoplasmic vacuoles are seen in several of the cells. (Wright-Giemsa; HP oil.)

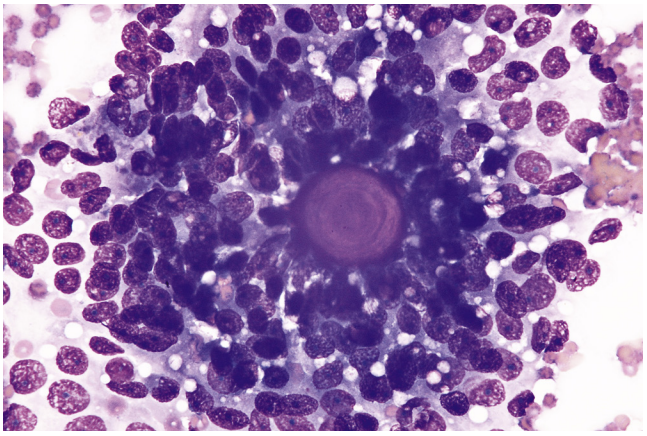


FIGURE 12-71 Sertoli cell tumor. Tissue aspirate. Dog. A Call-Exner body, a rosette-like structure characterized by elongated cells centered around amorphous eosinophilic extracellular dense material. (Wright-Giemsa; HP oil.)

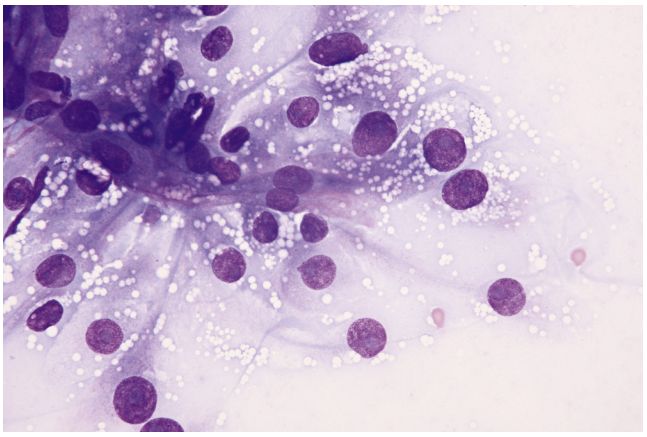


FIGURE 12-72 Interstitial cell tumor. Tissue aspirate. Dog. A cluster of tumor cells display coarse nuclear chromatin, prominent, single nucleoli, and large amounts of moderately basophilic cytoplasm. The nuclei are often located at the periphery of the cell. Punctate cytoplasmic vacuoles are present in the majority of the cells. (Wright-Giemsa; HP oil.)

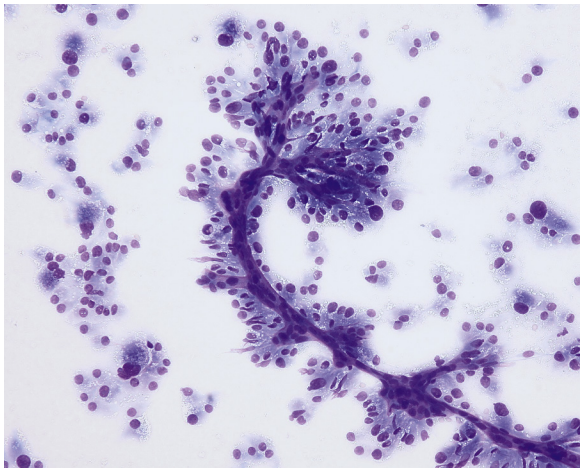


FIGURE 12-73 Interstitial cell tumor. Tissue aspirate. Dog. Lower magnification of the same case as shown in Figure 12-72. Palisading arrays of interstitial cells surround a central capillary. (Wright-Giemsa; IP.)

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. 12-72). Perivascular arrangement (Fig. 12-73) is commonly seen (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 (Fig. 12-72). 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

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

are available elsewhere (Axner and Linde Forsberg, 2007; Freshman, 2002; Rijsselaere et al., 2005; Root Kustritz, 2007; Zambelli and Cunto, 2006). 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 Romanowsky or methanolic-Romanowsky stains are 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 (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. 12-74 to 12-76). Less severe abnormalities include detached, normal-appearing heads and bent tails (Figs. 12-76 and 12-77). Normal semen samples should have less than 10% and 20% of primary and secondary abnormalities, respectively. Total canine and 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 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. 12-77 and 12-78). 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 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).

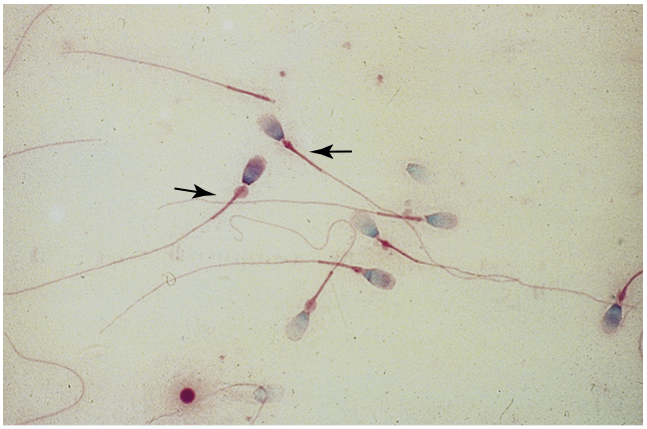


FIGURE 12-74 Primary abnormalities. Semen smear. Dog. Noninflammatory semen sample from a case of infertility. Sperm have prominent proximal protoplasmic droplets (*arrows*). (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)

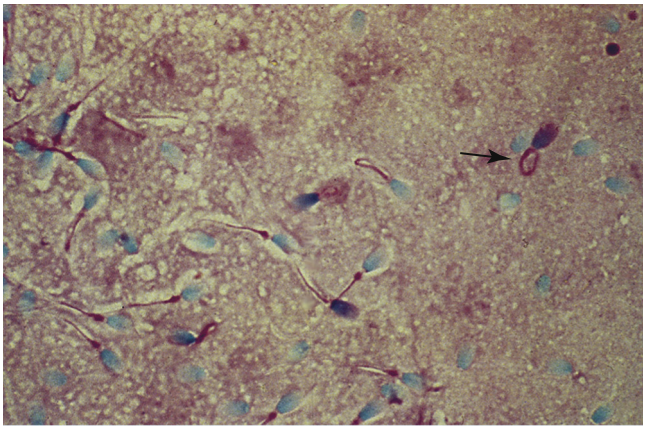


FIGURE 12-75 Primary abnormalities. Semen smear. Dog. Noninflammatory semen sample from a case of infertility. Against a heavy proteinaceous background, several sperm display tightly coiled tails (*arrow*) and proximal protoplasmic droplets. (Wright; HP oil.) (Courtesy of Rose Raskin, Purdue University.)

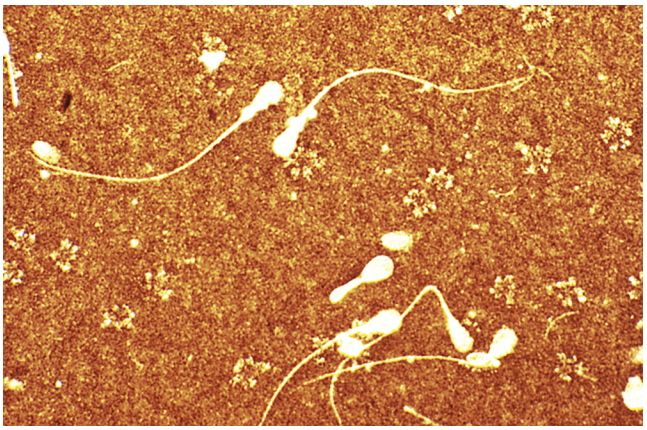


FIGURE 12-76 Primary and secondary abnormalities. Semen smear. Dog. Noninflammatory semen sample from a case of infertility. Sperm abnormalities include proximal protoplasmic droplets, coiled tails, and bent tails. (India ink; 250×.) (Courtesy of Rose Raskin, Purdue University.)

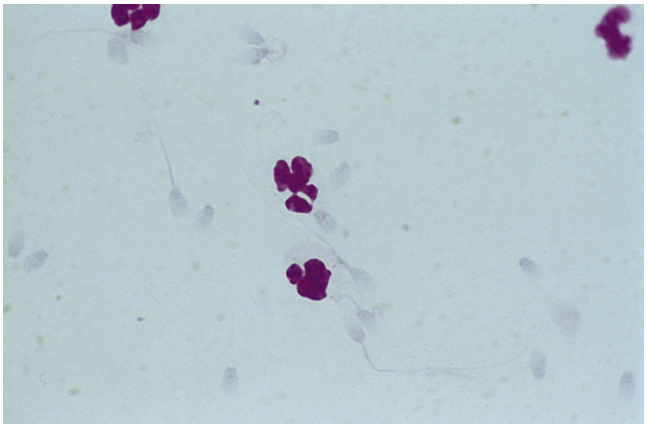


FIGURE 12-77 Inflammation and abnormal sperm. Semen sample. Dog. An ejaculate sample from an animal with intermittent preputial bleeding. Mildly degenerate neutrophils are present. Several morphologic secondary abnormalities of the sperm (detached heads, bent tails, and coiled tails) are present. (Wright-Giemsa; HP oil.)

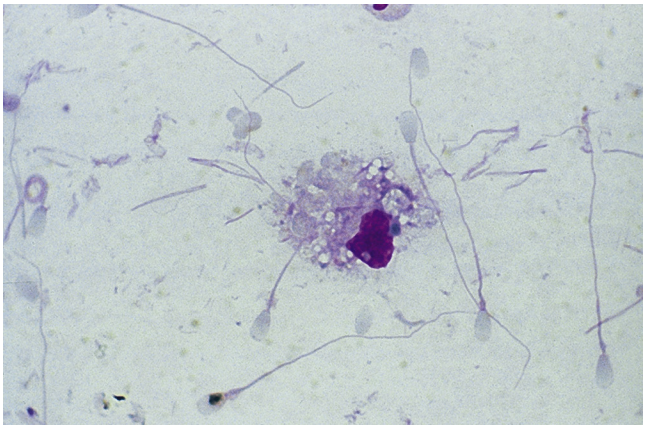


FIGURE 12-78 Inflammation. Semen sample. Dog. A reactive macrophage and several relatively normal-appearing sperm from the same ejaculate demonstrated in Fig. 12-68. (Wright-Giemsa; HP oil.)

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).

REFERENCES

Agudelo CF: Cystic endometrial hyperplasia-pyometra complex in cats: a review, *Vet Q* 27:173–182, 2005.

Akkoc A, Inan S, Sonmez G: Matrix metalloproteinase (MMP-2 and MMP-9) and steroid receptor expressions in feline mammary tumors, *Biotech Histochem* 87:312–319, 2012.

Allen SW, Prasse KW, Mahaffey EA: Cytologic differentiation of benign from malignant canine mammary tumors, *Vet Pathol* 23:649–655, 1986.

Allison RW, Maddux JM: Subcutaneous glandular tissue: mammary, salivary, thyroid, and parathyroid. In Cowell RL, Tyler RD, Meinkoth JM, DeNicola DB (eds): *Diagnostic cytology and hematology of the dog and cat*, ed 3, St. Louis, 2008, Mosby, pp 112–117.

Allison RW, Thrall MA: Olson, PN: Vaginal cytology. In Cowell RL, Tyler RD, Meinkoth JM, DeNicola DB (eds): *Diagnostic cytology and hematology of the dog and cat*, ed 3, St. Louis, 2008, Mosby, pp 378–389.

Antuofermo E, Cocco R, Borzacchiello G, et al: Bilateral ovarian malignant mixed Mullerian tumor in a dog, *Vet Pathol* 46:453–456, 2009.

Axner E, Linde Forsberg C: Sperm morphology in the domestic cat, and its relation with fertility: a retrospective study, *Reprod Domest Anim* 42:282–291, 2007.

Badowska-Kozakiewicz AM, Malicka E: Immunohistochemical evaluation of expression of heat shock proteins HSP70 and HSP90 in mammary gland neoplasms in bitches, *Pol J Vet Sci* 15:209–214, 2012.

Baker RH, Lumsden JH (eds): *Color atlas of cytology of the dog and cat*, St. Louis, 1999, Mosby, pp 235–251, 253-262.

Ball RL, Birchard SJ, May LR, et al: Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007), *J Am Vet Med Assoc* 236:548–553, 2010.

Banco B, Antuofermo E, Borzacchiello G, et al: Canine ovarian tumors: an immunohistochemical study with HBME-1 antibody, *J Vet Diagn Invest* 23:977–981, 2011.

Banks WJ (ed): *Applied veterinary histology*, Baltimore, 1986, Williams & Wilkins, pp 348-378, 489-504, 506–523.

Barrand KR: Unilateral uterine torsion associated with haematometra and cystic endometrial hyperplasia in a bitch, *Vet Rec* 164:19–20, 2009.

Bell FW, Klausner JS, Hayden DW, et al: Clinical and pathologic features of prostatic adenocarcinoma in sexually intact and castrated dogs: 31 cases (1970-1987), *J Am Vet Med Assoc* 199:1623–1630, 1991.

Benjamin SA, Lee AC, Saunders WJ: Classification and behavior of canine mammary epithelial neoplasms based on life-span observations in beagles, *Vet Pathol* 36:423–436, 1999.

Bertazzolo W, Dell'Orco M, Bonfanti U, et al: Cytological features of canine ovarian tumours: a retrospective study of 19 cases, *J Small Anim Pract* 45:539–545, 2004.

Bertazzolo W, Bonfanti U, Mazzotti S, et al: Cytologic features and diagnostic accuracy of analysis of effusions for detection of ovarian carcinoma in dogs, *Vet Clin Pathol* 41:127–132, 2012.

Black GM, Ling GV, Nyland TG, et al: Prevalence of prostatic cysts in adult, large-breed dogs, *J Am Anim Hosp Assoc* 34:177–180, 1998.

Boeloni JN, Reis AM, Nascimento EF, et al: Primary ovarian rhabdomyosarcoma in a dog, *J Comp Pathol* 147:455–459, 2012.

Bokemeyer J, Peppler C, Thiel C, et al: Prostatic cavitary lesions containing urine in dogs, *J Small Anim Pract* 52:132–138, 2011.

Boland LE, Hardie RJ, Gregory SP, et al: Ultrasound-guided percutaneous drainage as the primary treatment for prostatic abscesses and cysts in dogs, *J Am Anim Hosp Assoc* 39:151–159, 2003.

Bradbury CA, Westropp JL, Pollard RE: Relationship between prostatomegaly, prostatic mineralization, and cytologic diagnosis, *Vet Radiol Ultrasound* 50:167–171, 2009.

Brazzell JL, Borjesson DL: Intra-abdominal mass aspirate from an alopecic dog, *Vet Clin Pathol* 35:259–262, 2006.
Brodey RS, Goldschmidt MH, Roszel JR: Canine mammary gland neoplasms, *J Am Anim Hosp Assoc* 19:61–90, 1983.
Brodey RS, Roszel JF: Neoplasms of the canine uterus, vagina, and vulva: a clinicopathologic survey of 90 cases, *J Am Vet Med Assoc* 151:1294–1307, 1967.
Brown PJ, Evans HK, Deen S, et al: Fibroepithelial polyps of the vagina in bitches: a histological and immunohistochemical study, *J Comp Pathol* 47:181–185, 2012.
Bryan JN, Keeler MR, Henry CJ, et al: A population study of neutering status as a risk factor for canine prostate cancer, *Prostate* 67:1174–1181, 2007.
Burrai GP, Mohammed SI, Miller MA, et al: Spontaneous feline mammary intraepithelial lesions as a model for human estrogen receptor- and progesterone receptor-negative breast lesions, *BMC Cancer* 10:156, 2010.
Campos LC, Lavalle GE, Estrela-Lima A, et al: CA15.3, CEA and LDH in dogs with malignant mammary tumors, *J Vet Intern Med* 26:1383–1388, 2012.
Cassali GD, Gobbi H, Malm C, et al: Evaluation of accuracy of fine needle aspiration cytology for mammary tumours: comparative features with human tumours, *Cytopathology* 18:191–196, 2007.
Cassali GD, Lavalle GE, De Nardi AB, et al: Consensus for the diagnosis, prognosis and treatment in canine mammary tumors, *Braz J Vet Pathol* 4:153–180, 2011.
Chambers B, Laksito M, Long F, et al: Unilateral uterine torsion secondary to an inflammatory endometrial polyp in the bitch, *Aust Vet J* 89:380–384, 2011.
Choi US, Seo KW, Oh SY, et al: Intra-abdominal mass aspirate from a cat in heat, *Vet Clin Pathol* 34:275–277, 2005.
Cooper TK, Ronnett BM, Ruben DS, et al: Uterine myxoid leiomyosarcoma with widespread metastases in a cat, *Vet Pathol* 43:552–556, 2006.
Cornell KK, Bostwick DG, Cooley DM: Clinical and pathological aspects of spontaneous canine prostatic carcinoma: a retrospective analysis of 76 cases, *Prostate* 45:173–183, 2000.
Dahlbom M, Makinen A, Suominen J: Testicular fine needle aspiration cytology as a diagnostic tool on dog infertility, *J Small Anim Pract* 38:506–512, 1997.
Dahlgren SS, Gjerde B, Pettersen HY: First record of natural *Tritrichomonas foetus* infection of the feline uterus, *J Small Anim Pract* 48:654–657, 2007.
de las Mulas JM, Millán Y, Dios R: A prospective analysis of immunohistochemically determined estrogen receptor alpha and progesterone receptor expression and host and tumor factors as predictors of disease-free period in mammary tumors of the dog, *Vet Pathol* 42:200–212, 2005.
de Lorimier LP, Fan TM: Canine transmissible venereal tumor. In Withrow SJ, Vail DM (eds): *Withrow & MacEwen's small animal clinical oncology*, St. Louis, 2007, Saunders, pp 799–804.
de M Souza CH, Toledo-Piza E, Amorin R, et al: Inflammatory mammary carcinoma in 12 dogs: clinical features, cyclooxygenase-2 expression, and response to piroxicam treatment, *Can Vet J* 50:506–510, 2009.
Dey P, Ray R: Comparison of fine needle sampling by capillary action and fine needle aspiration, *Cytopathol* 4:299–303, 1993.
Diniz SA, Melo MS, Borges AM, et al: Genital lesions associated with visceral leishmaniasis and shedding of *Leishmania* sp. in the semen of naturally infected dogs, *Vet Pathol* 42:650–658, 2005.
Ditmyer H, Craig L: Mycotic mastitis in three dogs due to, *Blastomyces dermatitidis, J Am Anim Hosp Assoc* 47:356–358, 2011.
Dorfman M, Barsanti J: Diseases of the canine prostate gland, *Comp Cont Ed Pract* 17:791–810, 1995.
England GC, Freeman SL, Russo M: Treatment of spontaneous pyometra in 22 bitches with a combination of cabergoline and cloprostenol, *Vet Rec* 160:293–296, 2007.
Fan TM, de Lorimier LP: Tumors of the male reproductive system. In Withrow SJ, Vail DM (eds): *Withrow & MacEwen's small animal clinical oncology*, St. Louis, 2007, Saunders, pp 637–648.
Feldman EC, Nelson RW: Ovarian cycle and vaginal cytology. In Feldman EC, Nelson RW (eds): *Canine and feline endocrinology and reproduction*, St. Louis, 2004, Saunders, pp 752–775.
Fernandes PJ, Guyer C, Modiano JF: What is your diagnosis? Mammary mass aspirate from a Yorkshire terrier, *Vet Clin Pathol* 27(79):91, 1998.
Ferreira da Silva J: Teratoma in a feline unilateral cryptochid testis, *Vet Pathol* 39:516, 2002.
Fontaine E, Levy X, Grellet A, et al: Diagnosis of endometritis in the bitch: a new approach, *Reprod Domest Anim* 44(Suppl 2):196–199, 2009.
Foster RA: Common lesions in the male reproductive tract of cats and dogs, *Vet Clin North Am Small Anim Pract* 42:527–545, 2012.
Foster RA: Female reproductive system. In McGavin MD, Zachary JF (eds): *Pathologic basis of veterinary disease*, St. Louis, 2007, Mosby, pp 1263–1315.
Freshman JL: Clinical approach to infertility in the cycling bitch, *Vet Clin North Am Small Anim Pract* 21:427–435, 1991.
Freshman JL: Semen collection and evaluation, *Clin Tech Small Anim Pract* 17:104–107, 2002.
Gerber D, Nöthling JO: Hysteroscopy in bitches, *J Reprod Fertil* 57:415–417, 2001. Suppl.
Giménez F, Hecht S, Craig LE, et al: Early detection, aggressive therapy: optimizing the management of feline mammary masses, *J Feline Med Surg* 12:214–224, 2010.
Gobello C, Castex G, Corrada Y: Serum and seminal markers in the diagnosis of disorders of the genital tract of the dog: a mini-review, *Theriogenology* 57:1285–1291, 2002.
Goldschmidt M, Peña L, Rasotto R, et al: Classification and grading of canine mammary tumors, *Vet Pathol* 48:117–131, 2011.
Gómez-Laguna J, Millán Y, Reymundo C, et al: Bilateral retiform Sertoli-Leydig cell tumour in a bitch. Alpha-inhibin and epithelial membrane antigen as useful tools for differential diagnosis, *J Comp Pathol* 139:137–140, 2008.
Görlinger S, Kooistra HS, van den Broek, et al: Treatment of fibroadenomatous hyperplasia in cats with aglépristone, *J Vet Intern Med* 16:710–713, 2002.
Gorman ME, Bildfell R, Seguin B: What is your diagnosis? Peritoneal fluid from a 1-year-old female German Shepherd dog, *Vet Clin Pathol* 39:393–394, 2010.
Grandi F, Colodel MM, Monteiro LN, et al: Extramedullary hematopoiesis in a case of benign mixed mammary tumor in a female dog: cytological and histopathological assessment, *BMC Vet Res* 6:45, 2010.
Grandi F, Salgado BS, Monteiro LN, et al: Cytologic diagnosis of mammary neoplastic and non neoplastic diseases in dogs, Letter to the editor, *Vet Clin Path* 40:411–413, 2011.
Groppetti D, Pecile A, Arrighi S, et al: Endometrial cytology and computerized morphometric analysis of epithelial nuclei: a useful tool for reproductive diagnosis in the bitch, *Theriogenol* 73:927–941, 2010.
Gruffydd-Jones TJ: Acute mastitis in a cat, *Feline Pract* 10:41–42, 1980.
Hagman R, Kühn I: *Escherichia coli* strains isolated from the uterus and urinary bladder of bitches suffering from pyometra: comparison by restriction enzyme digestion and pulsed-field gel electrophoresis, *Vet Microbiol* 84:143–153, 2002.
Hayden DW, Barnes DM, Johnson KH: Morphologic changes in the mammary gland of megestrol acetate-treated and untreated cats: a retrospective study, *Vet Pathol* 26:104–113, 1989.
Hayden DW, Klausner JS, Waters DJ: Prostatic leiomyosarcoma in a dog, *J Vet Diagn Invest* 11:283–286, 1999.
Hayes AA, Mooney S: Feline mammary tumors, *Vet Clin North Am Small Anim Pract* 15:513–520, 1985.
Hayes HM, Milne KL, Mandell CP: Epidemiological features of feline mammary carcinoma, *Vet Rec* 108:476–479, 1981.
Hellman E, Lindgren A: The accuracy of cytology in diagnosis and DNA analysis of canine mammary tumors, *J Comp Pathol* 101:443–450, 1989.
Hiemstra M, Schaefers-Okkens AC, Teske E, et al: The reliability of vaginal cytology in determining the optimal mating time in the bitch, *Tijdschr Diergeneeskd* 126:685–689, 2001.
Hori Y, Uechi M, Kanakubo K, et al: Canine ovarian serous papillary adenocarcinoma with neoplastic hypercalcemia, *J Vet Med Sci* 68:979–982, 2006.
Im KS, Kim JH, Kim NH, et al: Possible role of Snail expression as a prognostic factor in canine mammary neoplasia, *J Comp Pathol* 147:121–128, 2012.
Itoh T, Uchida K, Ishikawa K, et al: Clinicopathological survey of 101 canine mammary gland tumors: differences between small-breed dogs and others, *J Vet Med Sci* 67:345–347, 2005.
Johnston SD, Kamolpatana K, Root Kustritz MV, et al: Prostatic disorders in the dog, *Anim Reprod Sci* 60-61:405–415, 2000.

Kamstock DA, Fredrickson R, Ehrhart EJ: Lipid-rich carcinoma of the mammary gland in a cat, *Vet Pathol* 42:360–362, 2005.

Karayannopoulo M, Kaldrymidou E, Constantinidis TC: Adjuvant postoperative chemotherapy in bitches with mammary cancer, *J Vet Med* 48:85–96, 2001. Series A.

Kate MS, Kamal MM, Bobhate SK, et al: Evaluation of fine needle capillary sampling in superficial and deep-seated lesions. An analysis of 670 cases, *Acta Cytol* 42:679–684, 1998.

Klein MK: Tumors of the female reproductive system. In Withrow SJ, Vail DM (eds): *Withrow & MacEwen's small animal clinical oncology*, St. Louis, 2007, Saunders, pp 610–618.

Kustritz MV, Johnston SD, Olson PN, et al: Relationship between inflammatory cytology of canine seminal fluid and significant aerobic bacterial, anaerobic bacterial or mycoplasma cultures of canine seminal fluid: 95 cases (1987-2000), *Theriogenology* 64:1333–1339, 2005.

L'Abee-Lund TM, Heiene R, Friis NF, et al: *Mycoplasma canis* and urogenital disease in dogs in Norway, *Vet Rec* 153:231–235, 2003.

Ladds PW: The male genital system: the testes. In Jubb KVF, Kennedy PC, Palmer N (eds): *Pathology of domestic animals*, ed 4, San Diego, 1993, Academic Press, pp 485–512.

Lana SE, Rutteman GR, Withrow SJ: Tumors of the mammary gland. In Withrow SJ, Vail DM (eds): *Withrow & MacEwen's small animal clinical oncology*, St. Louis, 2007, Saunders, pp 619–636.

Lai CL, van den Ham R, van Leenders G, et al: Histopathological and immunohistochemical characterization of canine prostate cancer, *Prostate* 68:477–488, 2008.

Leidinger E, Hooijberg E, Sick K, et al: Fibroepithelial hyperplasia in an entire male cat: cytologic and histopathological features, *Tierarztl Prax Ausg K Kleintiere Heimtiere* 39:198–202, 2011.

L'Eplattenier HF, Lai CL, van den Ham R, et al: Regulation of COX-2 expression in canine prostate carcinoma: increased COX-2 expression is not related to inflammation, *J Vet Intern Med* 21:776–782, 2007.

Leroy BE, Northrup N: Prostate cancer in dogs: comparative and clinical aspects, *Vet J* 180:149–162, 2009.

LeRoy BE, Nadella MV, Toribio RE, et al: Canine prostate carcinomas express markers of urothelial and prostatic differentiation, *Vet Pathol* 41:131–140, 2004.

Loretti AP, Ilha MR, Ordas J, et al: Clinical, pathological and immunohistochemical study of feline mammary fibroepithelial hyperplasia following a single injection of depot medroxyprogesterone acetate, *J Feline Med Surg* 7:43–52, 2005.

Lowseth LA, Gerlach RF, Gillett NA, et al: Age-related changes in the prostate and testes of the beagle dog, *Vet Pathol* 27:347–353, 1990.

Lulich JP: Endoscopic vaginoscopy in the dog, *Theriogenology* 66:588–591, 2006.

MacEwen EG, Hayes AA, Harvey J, et al: Prognostic factors for feline mammary tumors, *J Am Vet Med Assoc* 185:201–204, 1984.

MacLachlan NJ, Kennedy PC: Tumors of the genital systems. In Meuten DJ (ed): *Tumors in domestic animals*, ed 4, Ames, 2002, Iowa State Press, pp 547–573.

Maniscalco L, Iussich S, de Las Mulas JM, et al: Activation of AKT in feline mammary carcinoma: a new prognostic factor for feline mammary tumours, *Vet J* 191:65–71, 2012.

Manna L, Paciello O, Morte RD, et al: Detection of *Leishmania* parasites in the testis of a dog affected by orchitis: case report, *Parasit Vectors* 5:216, 2012.

Manuali E, Eleni C, Giovannini P, et al: Unusual finding in a nipple discharge of a female dog: dirofilariasis of the breast, *Diagn Cytopathol* 32:108–109, 2005.

Marconato L, Romanelli G, Stefanello D, et al: Prognostic factors for dogs with mammary inflammatory carcinoma: 43 cases (2003-2008), *J Am Vet Med Assoc* 235:967–972, 2009.

Martín De Las Mulas J, Millán Y, Bautista MJ, et al: Oestrogen and progesterone receptors in feline fibroadenomatous change: an immunohistochemical study, *Res Vet Sci* 68:15–21, 2000.

Masserdotti C: Architectural patterns in cytology: correlation with histology, *Vet Clin Pathol* 35:388–396, 2006.

Masserdotti C, Bonfanti U, De Lorenzi D, et al: Cytologic features of testicular tumours in dog, *J Vet Med A Physiol Pathol Clin Med* 52:339–346, 2005.

Masserdotti C, De Lorenzi D, Gasparotto L: Cytologic detection of Call-Exner bodies in Sertoli cell tumors from 2 dogs, *Vet Clin Pathol* 37:112–114, 2008.

Matos AJ, Baptista CS, Gärtner MF, et al: Prognostic studies of canine and feline mammary tumours: the need for standardized procedures, *Vet J* 193: 24–31, 2012.

Matsuzaki P, Cogliati B, Sanches DS: Immunohistochemical characterization of canine prostatic intraepithelial neoplasia, *J Comp Pathol* 142:84–88, 2010.

McEntee MC: Reproductive oncology, *Clin Tech Small Anim Pract* 17:133–149, 2002.

McNeill CJ, Sorenmo KU, Shofer FS, et al: Evaluation of adjuvant doxorubicin-based chemotherapy for the treatment of feline mammary carcinoma, *J Vet Intern Med* 23:123–129, 2009.

Mesher CI: What is your diagnosis? A 14-month old domestic cat, *Vet Clin Pathol* 26:4, 1997.

Millanta F, Calandrella M, Bari G, et al: Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues, *Res Vet Sci* 79:225–232, 2005.

Millanta F, Citi S, Della Santa D, et al: COX-2 expression in canine and feline invasive mammary carcinomas: correlation with clinicopathological features and prognostic molecular markers, *Breast Cancer Res Treat* 98:115–120, 2006a.

Millanta F, Silvestri G, Vaselli C, et al: The role of vascular endothelial growth factor and its receptor Flk-1/KDR in promoting tumour angiogenesis in feline and canine mammary carcinomas: a preliminary study of autocrine and paracrine loops, *Res Vet Sci* 81:350–357, 2006b.

Millanta F, Verin R, Asproni P, et al: A case of feline primary inflammatory mammary carcinoma: clinicopathological and immunohistochemical findings, *J Feline Med Surg* 14:420–423, 2012.

Miller MA, Hartnett SE, Ramos-Vara JA: Interstitial cell tumor and Sertoli cell tumor in the testis of a cat, *Vet Pathol* 44:394–397, 2007.

Miller MA, Ramos-Vara JA, Dickerson MF, et al: Uterine neoplasia in 13 cats, *J Vet Diagn Invest* 15:515–522, 2003.

Mills JM, Valli VE, Lumsden JH: Cyclical changes of vaginal cytology in the cat, *Can Vet J* 20:95–101, 1979.

Mir F, Fontaine E, Reyes-Gomez E: Subclinical leishmaniasis associated with infertility and chronic prostatitis in a dog, *J Small Anim Pract* 53:419–422, 2012.

Mischke R, Meurer D, Hoppen HO: Blood plasma concentrations of oestradiol-17beta, testosterone and testosterone/oestradiol ratio in dogs with neoplastic and degenerative testicular diseases, *Res Vet Sci* 73: 267–272, 2002.

Misdorp W: Progestagens and mammary tumours in dogs and cats, *Acta Endocrinol* (Copenh) 125:27–31, 1991.

Misdorp W: Tumors of the mammary gland. In Meuten DJ (ed): *Tumors in domestic animals*, ed 4, Iowa State Press, 2002, Ames, pp 577–606.

Misdorp W, Else RW, Hellmén E, et al: Histological classification of mammary tumors of the dog and the cat. In *World Health Organization international histological classification of tumors of domestic animals,* Second Series, Vol VII. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, 1999.

Moxon R, Copley D, England GC: Quality assurance of canine vaginal cytology: a preliminary study, *Theriogenology* 74:479–485, 2010.

Murakami Y, Tateyama S, Uchida K: Immunohistochemical analysis of cyclins in canine normal testes and testicular tumors, *J Vet Med Sci* 63:909–912, 2001.

Nicastro A, Walshaw R: Chronic vaginitis associated with vaginal foreign bodies in a cat, *J Am Anim Hosp Assoc* 43:352–355, 2007.

Novosad CA: Principles of treatment for mammary gland tumors, *Clin Tech Small Anim Prac* 18:107–109, 2003.

Novosad CA, Bergman PJ, O'Brien MG: Retrospective evaluation of adjunctive doxorubicin for the treatment of feline mammary gland adenocarcinoma: 67 cases, *J Am Anim Hosp Assoc* 42:110–120, 2006.

Ober CP, Spaulding K, Breitschwerdt EB, et al: Orchitis in two dogs with Rocky Mountain spotted fever, *Vet Radiol Ultrasound* 45:458–465, 2004.

Olson PN, Thrall MA, Wykes PM, et al: Vaginal cytology: Part I, A useful tool for staging the canine estrous cycle, *Compend Contin Educ Pract* 6:288–297, 1984a.

Olson PN, Thrall MA, Wykes PM, et al: Vaginal cytology: Part II, Its use in diagnosing canine reproductive disorders, *Compend Contin Educ Pract* 6:385–390, 1984b.
Olson PN, Wrigley RH, Thrall MA, et al: Disorders of the canine prostate gland: pathogenesis, diagnosis, and medical therapy, *Compend Contin Educ Pract* 9:613–623, 1987.
Ordás J, Millán Y, de los Monteros AE, et al: Immunohistochemical expression of progesterone receptors, growth hormone and insulin growth factor-I in feline fibroadenomatous change, *Res Vet Sci* 76:227–233, 2004.
Park CH, Ikadai H, Yoshida E, et al: Cutaneous toxoplasmosis in a female Japanese cat, *Vet Pathol* 44:683–687, 2007.
Park MS, Kim Y, Kang MS, et al: Disseminated transmissible venereal tumor in a dog, *J Vet Diagn Invest* 18:130–133, 2006.
Peña L, De Andrés PJ, Clemente M, et al: Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up: relationship with clinical and histological characteristics, *Vet Pathol* 50:94–105, 2013.
Perez CC, Rodriguez I, Dorado J, et al: Use of ultrafast Papanicolaou stain for exfoliative vaginal cytology in bitches, *Vet Rec* 156:648–650, 2005.
Pérez-Alenza MD, Jiménez A, Nieto AI, et al: First description of feline inflammatory mammary carcinoma: clinicopathological and immunohistochemical characteristics of three cases, *Breast Cancer Res* 6:300–307, 2004.
Peters MA, Mol JA, van Wolferen ME: Expression of the insulin-like growth factor (IGF) system and steroidogenic enzymes in canine testis tumors, *Reprod Biol Endocrinol* 1:22–29, 2003.
Pinto da Cunha N, Ghisleni G, Romussi S, et al: Prostatic sarcomatoid carcinoma in a dog: cytologic and immunohistochemical findings, *Vet Clin Pathol* 36:368–372, 2007.
Piseddu E, Masserdotti C, Milesi C, et al: Cytologic features of normal canine ovaries in different stages of estrus with histologic comparison, *Vet Clin Pathol* 41:396–404, 2012.
Powe JR, Canfield PJ, Martin PA: Evaluation of the cytologic diagnosis of canine prostatic disorders, *Vet Clin Pathol* 33:150–154, 2004.
Reed LT, Balog KA, Boes KM, et al: Pathology in practice. Granulomatous pneumonia, prostatitis and uveitis with intralesional yeasts consistent with Blastomyces, *J Am Vet Med Assoc* 236:411–413, 2010.
Rehm S, Stanislaus DJ, Williams AM: Estrous cycle-dependent histology and review of sex steroid receptor expression in dog reproductive tissues and mammary gland and associated hormone levels, *Birth Defects Res B Dev Reprod Toxicol* 80:233–245, 2007.
Restucci B, Maiolino P, Martano M, et al: Expression of beta-catenin, E-cadherin and APC in canine mammary tumors, *Anticancer Res* 27:3083–3089, 2007.
Restucci B, Maiolino P, Paciello O: Evaluation of angiogenesis in canine seminomas by quantitative immunohistochemistry, *J Comp Pathol* 128:252–259, 2003.
Ribeiro GM, Bertagnolli AC, Rocha RM, et al: Morphological aspects and immunophenotypic profiles of mammary carcinomas in benign-mixed tumors of female dogs, *Vet Med Int* 2012:432763, 2012.
Riccardi E, Greco V, Verganti S, et al: Immunohistochemical diagnosis of canine ovarian epithelial and granulosa cell tumors, *J Vet Diagn Invest* 19:431–435, 2007.
Rijsselaere T, Van Soom A, Tanghe S, et al: New techniques for the assessment of canine semen quality: a review, *Theriogenology* 64:706–719, 2005.
Romanucci M, Marinelli A, Sarli G, et al: Heat shock protein expression in canine malignant mammary tumours, *BMC Cancer* 6:171, 2006.
Root Kustritz MV: Collection of tissue and culture samples from the canine reproductive tract, *Theriogenology* 66:567–574, 2006.
Root Kustritz MV: Managing the reproductive cycle in the bitch, *Vet Clin North Am Small Anim Pract* 42:423–437, 2012.
Root Kustritz MV: The value of canine semen evaluation for practitioners, *Theriogenology* 68:329–337, 2007.
Saba CF, Rogers KS, Newman SJ, et al: Mammary gland tumors in male dogs, *J Vet Intern Med* 21:1056–1059, 2007.
Salgado BS, Monteiro LN, Grandi F, et al: What is your diagnosis? Ascites fluid from a dog with abdominal distension, *Vet Clin Pathol* 41:605–606, 2012.
Sato T, Maeda H, Suzuki A, et al: Endometrial stromal sarcoma with smooth muscle and glandular differentiation of the feline uterus, *Vet Pathol* 44:379–382, 2007.
Schafer-Somi S, Spergser J, Breitenfellner J, et al: Bacteriological status of canine milk and septicaemia in neonatal puppies—a retrospective study, *J Vet Med B Infect Dis Vet Public Health* 50:343–346, 2003.
Schubach TM, de Oliveira Schubach A, dos Reis RS, et al: *Sporothrix schenckii* isolated from domestic cats with and without sporotrichosis in Rio de Janeiro, Brazil, *Mycopathologia* 153:83–86, 2002.
Sfacteria A, Mazzullo G, Bertani C, et al: Erythropoietin receptor expression in canine mammary tumor: an immunohistochemical study, *Vet Pathol* 42:837–840, 2005.
Shille VM, Lundstrom KE, Stabenfeldt GH: Follicular function in the domestic cat as determined by estradiol-17β concentrations in plasma: relation to estrous behavior and cornification of exfoliated vaginal epithelium, *Biol Reprod* 21:953–963, 1979.
Sigurdardottir OG, Kolbjornsen O, Lutz H: Orchitis in a cat associated with coronavirus infection, *J Comp Pathol* 124:219–222, 2001.
Simeonov R, Simeonova G: Computerized morphometry of mean nuclear diameter and nuclear roundness in canine mammary gland tumors on cytologic smears, *Vet Clin Path* 35:88–90, 2006a.
Simeonov R, Simeonova G: Fractal dimension of canine mammary gland epithelial tumors on cytologic smears, *Vet Clin Path* 35:446–448, 2006b.
Simon D, Schoenrock D, Baumgartner W, et al: Postoperative adjuvant treatment of invasive malignant mammary gland tumors in dogs with doxorubicin and docetaxel, *J Vet Intern Med* 20:1184–1190, 2006.
Simon D, Schoenrock D, Nolte I, et al: Cytologic examination of fine-needle aspirates from mammary gland tumors in the dog: diagnostic accuracy with comparison to histopathology and association with postoperative outcome, *Vet Clin Pathol* 38:521–528, 2009.
Singer J, Weichselbaumer M, Stockner T, et al: Comparative oncology: ErbB-1 and ErbB-2 homologues in canine cancer are susceptible to cetuximab and trastuzumab targeting, *Mol Immunol* 50:200–209, 2012.
Sirinarumitr K, Johnston SD, Kustritz MV, et al: Effects of finasteride on size of the prostate gland and semen quality in dogs with benign prostatic hypertrophy, *J Am Vet Med Assoc* 218:1275–1280, 2001.
Smith FO: Canine pyometra, *Theriogenology* 66:610–612, 2006.
Smith J: Canine prostatic disease: a review of anatomy, pathology, diagnosis, and treatment, *Theriogenol* 70:375–383, 2008.
Snead EC, Pharr JW, Ringwood BP, et al: Long-retained vaginal foreign body causing chronic vaginitis in a bulldog, *J Am Anim Hosp Assoc* 46:56–60, 2010.
Solano-Gallego L, Raskin RE, Meyer D: The authors respond. Letter to the editor, *Vet Clin Path* 40:411–413, 2011.
Sontas BH, Turna O, Ucmak M, et al: What is your diagnosis? Feline mammary fibroepithelial hyperplasia, *J Small Anim Pract* 49:545–547, 2008.
Sontas BH, Yüzbaşıoğlu Öztürk G, Toydemir TF, et al: Fine-needle aspiration biopsy of canine mammary gland tumours: a comparison between cytology and histopathology, *Reprod Domest Anim* 47:125–130, 2012.
Sorenmo K: Canine mammary gland tumors, *Vet Clin Small Anim* 33:573–596, 2003.
Sorenmo KU, Goldschmidt MH, Shofer SF: Evaluation of cyclooxgenase-1 and cyclooxygenase-2 expression and the effect of cyclooxgenase inhibitors in canine prostatic carcinoma, *Vet Comp Oncol* 2:13–23, 2004.
Sorenmo KU, Shofer FS, Goldschmidt MH: Effect of spaying and timing of spaying on survival of dogs with mammary carcinoma, *J Vet Intern Med* 14:266–270, 2000.
Sycamore KF, Julian AF: Lipoleiomyoma of the reproductive tract in a Hunt-away bitch, *N Z Vet J* 59:244–247, 2011.
Taniyama H, Hirayama K, Nakada K: Immunohistochemical detection of inhibin-a, -βB, and -βA chains and 3β-hydroxysteroid dehydrogenase in canine testicular tumors and normal testes, *Vet Pathol* 38:661–666, 2001.
Teske E, Naan EC, van Dijk EM, et al: Canine prostate carcinoma: epidemiological evidence of an increased risk in castrated dogs, *Mol Cell Endocrinol* 197:251–255, 2002.
Thrall MA, Olson PN, Freemyer EG: Cytologic diagnosis of canine prostatic disease, *J Am Anim Hosp Assoc* 21:95–102, 1985.

Torres LN, Matera JM, Vasconcellos CH, et al: Expression of connexins 26 and 43 in canine hyperplastic and neoplastic mammary glands, *Vet Pathol* 42:633–641, 2005.

van Garderen E, Schalken JA: Morphogenic and tumorigenic potentials of the mammary growth hormone/growth hormone receptor system, *Mol Cell Endocrinol* 197:153–165, 2002.

Van Israel N, Kirby BM, Munro EA: Septic peritonitis secondary to unilateral pyometra and ovarian bursal abscessation in a dog, *J Small Anim Pract* 43:452–455, 2002.

Verstegen J, Dhaliwal G, Verstegen-Onclin K: Mucometra, cystic endometrial hyperplasia, and pyometra in the bitch: advances in treatment and assessment of future reproductive success, *Theriogenol* 70:364–374, 2008.

Ververidis HN, Mavrogianni VS, Fragkou IA, et al: Experimental staphylococcal mastitis in bitches: Clinical, bacteriological, cytological, haematological and pathological features, *Vet Microbiol* 124:95–106, 2007.

Walker JT, Frazho JK, Randell SC: A novel case of canine disseminated aspergillosis following mating, *Can Vet J* 53:190–192, 2012.

Wanke MM: Canine brucellosis, *Anim Reprod Sci* 82-83:195–207, 2004.

Watts JR, Wright PJ, Lee CS: Endometrial cytology of the normal bitch throughout the reproductive cycle, *J Small Anim Pract* 39:2–9, 1998.

Watts JR, Wright PJ, Lee CS, et al: New techniques using transcervical uterine cannulation for the diagnosis of uterine disorders in bitches, *J Reprod Fertil* 51:283–293, 1997. Suppl.

Wenzlow N, Tivers MS, Selmic LE, et al: Haemangiosarcoma in the uterine remnant of a spayed female dog, *J Small Anim Pract* 50:488–491, 2009.

Winter MD, Locke JE, Penninck DG: Imaging diagnosis-urinary obstruction secondary to prostatic lymphoma in a young dog, *Vet Radiol Ultrasound* 47:597–601, 2006.

Wright PJ: Application of vaginal cytology and plasma progesterone determinations to the management of reproduction in the bitch, *J Small Anim Pract* 31:335–340, 1990.

Yager JA, Scott DW, Wilcock BP: The skin and appendages: neoplastic disease of skin and mammary gland. In Jubb KVF, Kennedy PC, Palmer N, editors: *Pathology of domestic animals*, ed 4, San Diego, 1993, Academic Press, pp 706–737.

Zambelli D, Cunto M: Semen collection in cats: techniques and analysis, *Theriogenology* 66:159–165, 2006.

Zanghì A, Nicòtina PA, Catone G: Cholesterol granuloma (Xanthomatous metritis) in the uterus of a cat, *J Comp Pathol* 121:307–310, 1999.

Zappulli V, De Cecco S, Trez D, et al: Immunohistochemical expression of E-cadherin and β-catenin in feline mammary tumours, *J Comp Pathol* 147:161–170, 2012.

Zinkl JG: The male reproductive tract: prostate, testes, and semen. In Cowell RL, Tyler RD, Meinkoth JM, DeNicola DB (eds): *Diagnostic cytology and hematology of the dog and cat*, ed 3, St. Louis, 2008, Mosby, pp 369–377.

Zuccari DA, Santana AE, Cury PM, et al: Immunocytochemical study of Ki-67 as a prognostic marker in canine mammary neoplasia, *Vet Clin Pathol* 33:23–28, 2004.