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Intrahepatocytic small green cytoplasmic pigment granules can be seen in healthy hepatocytes and it is often interpreted as "bile" but it does not stain for bilirubin. This intrahepatocyte pigment is lipofuscin or hemosiderin. Lipofuscin is a "wear and tear" pigment and it is the most common intracellular pigment in hepatocytes. Bile pigment is extra cellular, it fills canaliculi between hepatocytes and forms light green to dark green to black bile casts called bile plugs or bile thrombi and is associated with cholestasis. Bile pigment is homogenous, "smooth" and extracellular, while lipofuscin and hemosiderin are heterogeneous, granular and intracellular.

Hemosiderin is a golden to brown-black pigment that accumulates in hepatocytes and macrophages when there is increased erythrocyte turnover (hemorrhage, hemolysis). Copper accumulation in inherited and acquired hepatopathies appears as pale green cytoplasmic granules on cytologic specimens. Bedlington Terriers, West Highland White Terriers and Doberman Pinschers are breeds at risk for copper storage disorders. More than one pigment can accumulate in the hepatocytes and to definitively identify the pigment special stains are necessary.

Vacular Hepatopathies: Vacular hepatopathy is usually due to lipidosis or glucocorticoids (endogenous = Cushing’s; exogenous administration). Fatty liver is a common disease in cats. Hypothyroidism and diabetes mellitus are the two most common causes of fatty liver in the dog. Cushingoid dogs do not have a fatty liver unless there is concurrent diabetes mellitus. Cushingoid dogs have enlarged livers with vacuolated hepatocytes due to the accumulation of intracellular glycogen. Vacular hepatic change occurs less commonly in a variety of other disorders including toxin exposure, inflammatory disease, nodular hyperplasia and idiopathic vacuolar hepatopathy. Lysosomal storage diseases are rare causes of vacuolar hepatopathy.

Inflammatory Reactions: All the classic types of inflammation (suppurative, granulomatous, lymphocytic, and eosinophilic) can occur in the liver. Inflammation must be distinguished from blood contamination, extramedullary hematopoiesis and chronic leukemias. Look for degenerative features of neutrophils, integration of inflammatory cells within clusters of hepatocytes and biliary epithelium, infectious agents (bacteria, mycotic agents, protozoa, viral inclusions) and peripheral WBC counts to confirm true inflammation. Infectious causes of hepatitis include bacterial septicemia, ascending cholangitis, clostridiosis, FIP, aspergillosis, histoplasmosis, coccidioidomycosis, cyttoxozoonosis, hepatozoonosis, leishmaniasis, mycobacteriosis, and infectious canine hepatitis (ICH). Lymphocytic-plasmacytic cholangiohepatitis is relatively common in cats and must be differentiated from well differentiated lymphoma and chronic lymphocytic leukemia.

Extramedullary Hematopoiesis (EMH)

The hallmark of EMH is the presence of normal appearing, late stage hemic precursors (erythrocytic, myelocytic, megakaryocytic) especially erythropoiesis. Differentials for EMH include blood contamination, inflammation, myeloid leukemias (erythroleukemia, erythremic myelosis, chronic myelogenous leukemia) and myelolipomas, rare masses composed of hemic cells and lipid laden stromal cells that only can be distinguished from EMH by histopathology. Of all the hematopoietic cell lines, megakaryocytes are the most obvious to help recognize or confirm EMH, although they will be in low numbers.

Hyperplasia

Cytologically, the hepatocytes may be indistinguishable from normal hepatocytes or hepatocytes from hepatocellular adenomas or well differentiated carcinomas. Ancillary tests such as ultrasound and histopathologic biopsy are necessary to definitively diagnose hepatocellular hyperplasia.

Neoplasia

My thumb rule is to diagnose obvious cases of lymphoma and malignant histiocytosis and send the other cases to a referral lab. The easiest neoplasms to recognize are those that have little associated inflammation, and the cell population is distinctly different from the large hepatocytes. In the situation where there are mononuclear cells with a small amount of discernible cytoplasm, diagnoses such as lymphoma, biliary carcinoma, or metastatic tumor are the most likely. The critical feature is that there are minimal or no hepatocytes, and instead, there is a population of small mononuclear cells that has replaced the liver. Although you may recognize a lymphoma because it essentially looks the same as it does in other tissues, trying to identify a hepatocellular or biliary carcinoma from a metastatic lesion from a carcinoit is something to reserve for a reference lab and histopathology. Diagnosing well-differentiated hepatocellular or biliary neoplasia is especially challenging because cytologically they appear indistinguishable from normal or hyperplastic cells. If there is a solitary large mass in the liver, and especially if it is associated with
SPLEEN

Normal Cytology: Cytologic evaluation of the spleen can be "unpleasant" (challenging). The spleen is a cellular organ and you must "read through" numerous normal cells and events. Small lymphocytes are the predominant cell type with lesser numbers of intermediate lymphocytes, and lymphoblasts; macrophages, plasma cells, occasional clumps of stromal cells, endothelial cells, mast cells and neutrophils are often seen; hemodilution is constant; EMH may be normal; and focal areas with increased numbers of intermediate lymphocytes or lymphoblasts may represent germinal or follicular centers and must be distinguished from lymphoma. Do not diagnose lymphoma from specimens with only focal areas of lymphoblasts.

Splenic Hyperplasia: Symmetrical enlargement suggests: congestion, hyperplasia or neoplastic infiltration. Congestion is associated with poor perfusion, torsion, or anesthesia and cannot be diagnosed by cytology. Infiltrative disorders such as infectious diseases (granulomatous - histoplasmosis, cytuxzeenosis, leishmaniasis, babesiosis, mycobacteriosis; pyogranulomatous - blastomycosis, sporothrixosis, canine infectious peritonitis) and hemic neoplasia may be diagnosed via cytology. Other causes of symmetrical splenomegaly are hemolytic anemia with marked erythrophagia, lymphoid hyperplasia secondary to bacterial or viral diseases (such as infectious canine hepatitis, canine ehrlichiosis, pyometra, hemobartonellosis), hypereosinophilic syndrome and extramedullary hematopoiesis.

Asymmetrical splenomegaly is frequently due to non-hemic neoplasia (hemangiosarcoma), nodular hyperplasia or hematoma. Splenic masses are more common in dogs than cats whereas diffuse symmetrical splenomegaly (mass cell tumor, myeloproliferative disease) is more common in cats than dogs. Hemangiosarcoma is the most common non-hemic malignant neoplasm in dogs. Other non-hemic neoplasms include fibrosarcomas, leymosarcomas, and metastatic neoplasia. The biologic behavior of splenic masses does not correlate well with either size or gross appearance of splenic lesions; e.g. hematomas are relatively common splenic masses in dogs and can be large (>6 cm), they are uncommon in cats.

Extramedullary Hematopoiesis (EMH): Megakaryocytes are the most obvious evidence of EMH whereas late stage hemic precursors (erythrocytic, myelocyctic, megakaryocytic) are the hallmark of EMH. Erythroid precursors usually predominate in marked EMH responses with lesser numbers of megakaryocytes and granulocyte precursors. In the cat EMH may be difficult to distinguish from myeloproliferative disorders such as erythroleukemia. Bone marrow biopsy is indicated in this situation. EMH is most frequently associated with leukemias, chronic hemolytic anemias, myelodysplastic syndromes, and hemangiosarcomas.

Primary Neoplasia: Hemic neoplasia (leukemia, lymphoma, mast cell tumor, multiple myeloma) usually causes diffuse splenomegaly and infiltration of other hemic organs such as bone marrow, liver and lymph nodes. The hallmark of hemic neoplasia is a very cellular specimen with sheets of homogeneous, uniform population of discrete round, neoplastic cells as compared to the normal mixed heterogeneous cellularity of the spleen.

Malignant Histiocytosis: Malignant histiocytosis (MH) has been reported in dogs, especially Bernese Mountain Dogs, Rottweilers, Golden Retrievers, Labrador and flat coated retrievers and cats. MH is a systemic disorder affecting spleen, lymph nodes, lung, bone marrow and rarely skin. Pulmonary forms are associated with hypercalcemia.

Cytologically, MH is "fun" to see as the abnormalities are obvious and numerous. A classical case is characterized by atypical histiocytes, giant cells, atypical mitosis and erythrophagia by tumor cells. The cells are large, discrete round cells with variable N:C ratio, moderate anisocytosis and anisokaryosis; cytoplasm may contain variably numbers of small vacuoles. Multinucleation can be dramatic. MH can be confused with granulomatous inflammation or even some round cell tumors.

Hemangiosarcoma: Clinopathologic changes associated with HSA are fragmentation anemia, acanthocytes, schistocytes, increased nucleated red cells and Howell-Jolly bodies, thrombocytopenia, neutrophilia and DIC. Often the majority of the mass is non-neoplastic tissue such as hematomata or necrotic parenchyma and therefore it is difficult to aspirate tumor cells from these lesions. Even in histologic preparations the majority of the mass is usually hematomata, necrosis and not the tumor. Ultrasound guided sampling is critical as is redirecting the needle and aspirating from several regions of the tumor. These tumors yield few neoplastic cells, do not expect to see numerous sarcomatous cells. When found the neoplastic cells are characterized by large plump spindle-shaped cells with large round to oval nuclei, and 1-2 prominent, irregularly shaped nucleoli. Anisocytosis, anisokaryosis, pleomorphism and bizarre cellular atypia can be marked. Cells can vary from unremarkable endothelial cells to anaplastic undifferentiated "round cells." Hemorrhage, erythrophagia, hemosiderin pigment, necrotic debris and neutrophils are frequently present in the background. Cellularity is usually low. Hemangiomatosa, a benign tumor of vascular endothelial cells, can not be diagnosed via cytology.

BONE CYTOLOGY

The most important reason to aspirate a bone lesion is to determine if the lesion is neoplastic or infectious. This
distinction is easy if the true lesion is aspirated, however, a common problem in bone tumors is that the majority of the lesion may be non-neoplastic. It consists of a large amount of reactive woven bone (seen on radiographs as a “sunburst” pattern, or Codman’s triangle), as well as areas of necrosis, inflammation, and fibrosis. Several tips to help get a good sample for either aspirational cytology or histology are: 1) take at least three samples from three different areas of the mass; 2) be sure to enter the medullary cavity with the needle; 3) most osteosarcomas arise in the medullary cavity of metaphyseal bone, therefore take samples from the metaphysis, possibly the epiphysis but not the diaphysis. If an osteosarcoma (OSA) is in the diaphysis they are often due to a prior fracture, metal fixation and/or poor healing.

**Osteosarcoma:** Correlating clinical information with cytology is essential to this diagnosis. If the clinical data indicates there is a lytic and proliferative lesion in the metaphyseal region of the appendicular skeleton in a large breed dog of middle-age or older, then there is a 95%+ chance the lesion is an osteosarcoma. Sometimes, the biggest problem is just finding the tumor cells because so much of the lesion is not neoplastic. Some osteosarcomas do not exfoliate well and, therefore, smears are of moderate to low cellularity. The neoplastic cells will be round to oval to angulated; some cells will have a “pointed” end (mesenchymal origin). Nuclei will be eccentric, there often is a clear zone adjacent to the nuclei (Golgi); and the cells usually have abundant, moderate to deeply basophilic cytoplasm (features of osteoblasts and plasma cells). There may be intracellular pink granules and extracellular red material (osteoid) as well as multinucleated giant cells. Osteoclasts can be distinguished from megakaryocytes because megakaryocytes have a single nucleus with multiple lobes that are connected, while osteoclasts have individual nuclei with cytoplasm visible between nuclei. Osteosarcomas have plasmacytoid features (the eccentric pattern of the nuclei with prominent Golgi and deeply basophilic cytoplasm) and therefore, multiple myeloma is a differential diagnosis. Plasma cells tend to exfoliate many more cells than do osteosarcoma and the cells are smaller, more uniform, round to oval, without the angular or spindeloid shape of osteosarcoma. Signalment, location and number of lesions, radiographs and clinical chemistry results will also help separate these two tumors.

**Plasma Cell Tumor; Multiple Myeloma:** If the animal has multiple punched-out lesions in bones, and has an increased concentration of total serum protein, especially with a monoclonal gammopathy, then you may not need aspirational cytology to establish the diagnosis. The critical job is to get a needle into one of the lytic lesions and get an adequate sample for cytologic examination. If the lytic bone lesion is not in a site that can be biopsied readily (e.g. vertebra), aspirate nearby bone marrow from femur, pelvis or humerus or if the spleen is enlarged, aspirate the spleen. The tumor cells should resemble plasma cells and will have some of the following features: round-to-oval shaped cells, eccentric nuclei, prominent perinuclear Golgi, deeply basophilic and moderately abundant cytoplasm, and clumped, dense chromatin that may be displaced along the nuclear margins. The three likely differentials are - plasma-cell neoplasia, lymphoma, or osteosarcoma (three bad tumors). Evaluate the cells carefully and correlate them with the rest of the clinical data to establish a definitive diagnosis. For instance, an osteosarcoma should be a solitary lesion in the metaphyseal region of a long bone, lymphoma should be located in multiple lymph nodes and soft tissues; multiple myeloma should have multiple bony lesions, with or without protein abnormalities in the serum or urine. Hypercalcemia can occur with myeloma (10% of cases) but it is most common with lymphoma (33% of cases) and is rare with OSA. Binucleation is a feature of plasma cell tumors and some can have considerable variability in nuclear and cell size and shape.

**Chondroma/Chondrosarcoma:** Criterion to differentiate benign vs. malignant cartilaginous tumors via cytology are not reliable. The tumor arises from cartilage; therefore it is more likely to be in axial skeleton (head, rib) or near a joint then appendicular skeleton. These tumors can be quite large before an owner presents the dog; they are rare in cats. Characteristically there is more matrix than cells. Smears are of low or very low cellularity. The diagnosis is suspected at 10-20X magnification by the visualization of abundant pink to light purple acellular background material that has encased mononuclear cells. At 400X the background material is nondescript, the nucleated cells do not lay flat, they are mononuclear, cell borders are indiscernible and cells or background may contain vacuoles. Cellularity in a well-differentiated chondroma/chondrosarcoma is much less than in osteosarcoma. Anaplastic chondrosarcomas in the nasal cavity can be confused with round cell tumors and carcinomas.

**Fibrosarcoma:** These tumors look cytologically like fibrosarcomas located in soft tissue. Tumor cells range from spindle to oval to polygonal. Nuclei will be oval to round. Multinucleation and nuclear variability help suggest malignancy, but location in bone basically confirms that. Golden Retrievers and other large breed dogs also have a fibrosarcoma that occurs in the maxillae and mandible that may appear cytologically and histologically as benign. However, these well-differentiated fibrosarcomas are aggressive, will invade soft tissue and bone and metastasize in about 20% of dogs. It can be difficult to distinguish granulation tissue from spindle cell tumors and histopathology may be necessary for this differentiation.

**Inflammatory Lesions:** Osteomyelitis is composed primarily of neutrophils with lesser numbers of macrophages, monocytes, lymphocytes, and some plasma cells. The diagnosis depends on seeing numerous neutrophils. Degenerative features in the neutrophils will depend on the presence of sepsis, which is likely. The best cells to examine for bacteria are neutrophils. If you suspect septic osteomyelitis, be sure to submit bacteriologic cultures for aerobic and anaerobic bacteria. A lesion that is interpreted to be osteomyelitis on cytology or histology, but that has a history of negative cultures, should be submitted for anaerobic bacteria and for mycotic growth. All of the yeast forms of fungi that are common to veterinary medicine can be associated with a mycotic osteomyelitis and all of them have the potential of producing hypercalcemia. Blastomycosis is the most common fungi that causes osteomyelitis and it can cause hypercalcemia. In the southwest, coccidioidomycosis is common. Both of these are primarily (“only”) found in their yeast phase and mycotic agents in hyphal forms are more
likely to be a different species, e.g. = *Mucor spp.*, *Aspergillus spp.*, etc.

**RESPIRATORY SYSTEM**

**Nose:** Normal cells include ciliated and nonciliated columnar epithelium. The nuclei in these cells are positioned basally, are round to oval, have one or more nucleoli, and cilia are on the pole opposite nuclei. Goblet cells are uncommon; they are columnar and have purple granules above the nucleus. A few mononuclear inflammatory cells are normal.

Nasal cytology is used to determine if an inflammatory (fungal) or a neoplastic disease is present. The clinical problems include: sneezing, nasal discharge, nasal bleeding, itching at nose, malodorous “head”, loss of turbinates and either a hole or a mass. Sampling the lesion is difficult. It is easy to make smears from some of the nasal discharge, but usually this yields just neutrophils, RBCs and is nondiagnostic. Both mycotic rhinitis and nasal neoplasms start deep in the nose, and samples need to be taken from this deep location. Submit several cytologic preparations and ask for them to be stained for fungi: PAS or GMS stains. Fungi are difficult to see in routinely stained preparations from mammals. Fungal hyphae are usually unstained or light blue with conventional stains. However, hyphae stain well when they are from birds, reptiles or snakes. The key to an accurate diagnosis of a nasal lesion is adequate sampling: get deep in the nasal cavity and take multiple pieces. Differentials include fungal, neoplasia, allergic and septic.

**Septic:** Bacterial rhinitis is usually secondary to another problem: trauma, allergy, fungal, cancer, viral, foreign body, etc. Therefore do not stop cytologic examination just because bacteria have been identified in neutrophils. Pathogenic bacteria are usually a monomorphic population. Neutrophils are degenerate and they are numerous. There will be excessive amounts of mucus, karyolitic strands and cellular debris. Contamination from the oropharynx has pleomorphic bacteria; there will be large squamous epithelial cells *Simonsiella spp.*

**Fungal:** There are two types of fungi that may cause rhinitis: hyphal forms such aspergillus and mucor, and yeast forms like blastomycosis and cryptococcus. The first types are the more difficult to diagnose, and the latter are relatively easy because they tend to be superficial in the mass and exfoliate easily. The hyphal forms are invasive, seated deeper, and they do not stain readily with the commonly used stains for cytology.

**Hyphal types:** Neutrophils and macrophages predominate; giant cells are characteristic but will be uncommon even if the cause is a mycotic rhinitis. Mucus is abundant it creates aggregates or “blobs” of thick blue material. Look in these globs of mucus for nonstained straight lines that separate the inflammatory cells and mucus. Focus in on these areas and try to discern a branch, or any indication that something in the straight line is staining. Reducing the light in the field of view by adjusting the iris diaphragm is helpful, this tends to highlight the straight lines and make them slightly refractile. You may need to get out pieces of the mass to sample it adequately enough to find the organisms. Multiple cytologic preparations are often needed.

**Yeast types:** All have the same type of inflammatory cells, but the degree of inflammation is different. Cryptococcosis, blastomycosis and rhinosporidiosis are all in a similar same size range (1-2 neutrophils) and 95% of the time they are extracellular, while histoplasmosis, toxoplasmosis and leishmania are all smaller than a neutrophil and most are intracellular.

**Cryptococcosis:** This is the most common cause of fungal rhinitis especially in cats. Organisms may be numerous, but the amount of inflammation is often minimal, supposedly due to their relatively nonantigenic capsule. These fungi also stain poorly. Look for unstained circles (yeast form) rather than straight lines (hyphae). The capsule does not stain at all. Cryptococcus has a large round halo, in the center of which is an oval to round yeast that is lightly stained. Budding is infrequent, if observed; it forms a thin neck, rather than a broad base like blastomycosis. Submit samples for culture.

**Blastomycosis:** Blastomyces dermatitidis usually does not produce rhinitis. Pneumonia, skin ulcers, and osteomyelitis are more common manifestations. Blastomycosis produces marked inflammation that is pyogranulomatous: i.e. neutrophils, macrophages, giant cells and other mononuclear cells. There is much more inflammation with blastomycosis than with cryptococcus. Blastomyces stains deep blue with Diff-Quick type stains and should be examined for with a 10X objective, not 100X objective. The organisms are easy to spot at low magnification as they appear as “blue-blobs.” Then you can proceed to high dry, and confirm their identification. Blastomycosis stain blue, the cell wall (they do not have a capsule) is usually close to the yeast and forms a second, pale blue line just beyond the yeast. The central body nearly fills the capsule and is roundish. Budding is uncommon and, if observed, should be broad based.

**Rhinosporidiosis:** Rhinosporidium sebeeri is an algae but it looks like a yeast in cytologic preparations. It is an uncommon infection, seen primarily in dogs and horses, especially in the southeast and southwest. The inflammation is purulent, and the organisms range in numbers and size. Rhinosporidiosis has a variety of stages, some are in the range of blastomycosis (20-40 µm; 1-2 X the size of neutrophils), and other stages are up to 100 µm in diameter to 500 µm (sporangium) or even 1 mm in diameter (visible to the naked eye). The most prevalent form seen in preparations is about the size of blastomycosis but Rhinosporidium do not have a capsule and they do not bud. Do not rely on labs to culture these "fungi" because they have only been cultured once and that was on a monolayer of tissue cells rather than routine culture media. Rhinosporidium sebeeri produces nasal polyps and it does not cause lysis of turbinates like mycotic rhinitis or nasal neoplasms.

**Neoplasia:** Nasal tumors are relatively common in dogs and cats. They produce a chronic mucopurulent nasal discharge. Sampling this discharge rarely yields neoplastic cells. The tumors tend to invade and destroy nasal turbinates. If inflammatory cells are present, they will be predominantly neutrophils with lesser numbers of mononuclear cells. The more ulcerative, invasive or necrotic...
the tumor, the more neutrophils will be present. Squamous cell carcinomas tend to have the most inflammation. If inflammation is marked and there are few suspect neoplastic cells then do not diagnose neoplasia. Consider doing some or all of the following: send slides to a referral lab, treat the inflammation with antibiotics and resample after the inflammation has decreased; resample now; culture for mycotic agents.

The key feature used to diagnose neoplasia is numerous mononuclear cells that are larger than adjacent inflammatory cells. Use the neutrophil as a "ruler" to assess sizes of other cells and their nuclei. Cellular preparations devoid of neutrophils are relatively easy to diagnose as neoplasia. Examine the mononuclear cells in question for variability in cell size (anisocytosis) and variability in nuclear size (anisokaryosis). The greater the cellular and nuclear variability the greater your level of confidence that neoplasia is present. Poorly differentiated sarcomas and carcinomas can be fairly monomorphic and resemble lymphomas. Many of these tumors are initially diagnosed as lymphomas but infrequently with special techniques (immunohistochemistry) confirm lymphoma. If it is lymphoma then the neoplastic lymphoid cells will also be in other locations. Don't worry about adenomas in the nose of domestic animals; if there is a nasal tumor, 100% are malignant. Polyps occur in the nose of all species, but adenomas are not reported.

The distinction of carcinoma vs. sarcoma is usually not needed and is often difficult to make on cytology, even with histology, especially if the tumor is poorly differentiated. Some nasal tumors are so pleomorphic that they appear to differentiate along epithelial and mesenchymal lines. Furthermore, nasal carcinomas and sarcomas are treated similarly. However, treatment for nasal lymphomas is different and therefore lymphoma needs to be differentiated from carcinomas and sarcomas. Poorly differentiated nasal tumors look lymphoid on cytology due to their size, round cell pattern, and lack of differentiation. If it is lymphoma, it will also be in another location. Nasal lymphoma does occur, especially in cats, but rarely as the only place lymphoma is present. Lymphomas arise in lymphoid tissue and, in 90% of the cases, are in several locations (multicentric lymphoma). Nevertheless, lymphoma can occur in the nose and there are nasal tumors that resemble lymphoma on cytology.

Summary Nasal Neoplasia: Much like mycotic infections the tumor is usually deeply seated in the nasal cavity and the diagnosis is dependent on aggressive sampling that goes “deep” and yields multiple pieces. Inflammation, if present is predominantly neutrophilic; tumor cells are mononuclear cells with anisocytosis and anisokaryosis that range from minimal to marked; could look lymphoid; may not exfoliate easily, therefore, make multiple preparations; correlate with clinical signs; adenomas do not exist.

Other entities that will be discussed are allergic rhinitis, and septic purulent rhinitis, tracheal washes and transthoracic pulmonary aspiration.

REPRODUCTIVE SYSTEMS

The most common tissues sampled are mammary and prostate glands, in an attempt to determine if neoplasia versus inflammation is present, and the vaginal mucosa, in an attempt to determine the stage of estrus. The most important component of prostatic cytology is getting an adequate sample. The main reason to use cytology in the evaluation of the mammary gland is to determine if a lump is inflammatory or neoplastic. Do not use aspirational cytology to determine the type of mammary tumor or if it is benign or malignant. Aspirational cytology is too imprecise a tool to answer this question. Even with histopathology it can be difficult to accurately predict biological behavior of certain mammary tumors. Some cases are easy to classify as benign or malignant but many cases fall in the gray zone between these two classifications.

Vaginal cytology: The primary purpose of vaginal cytology is in the staging of the estrous cycle of bitches. Correlate clinical signs with cytologic observations and “listen” to both: e.g. = The best time to breed the dog is when she will stand for breeding, regardless of what the cells look like under a microscope. Correlation of cytology and breeding with the concentration of serum progesterone is now commonly used to determine ovulation and therefore, when to breed the bitch. If limited breedings can be performed then the ideal time to maximize fertility is 2-4 days post ovulation which correlates with a serum progesterone that is >2 ng/ml (although some wait for it to be in the 4-10 ng/ml range). Ovulation of primary oocytes is about 2 days after the LH surge. Some guidelines are: serum progesterone should be less than 1.0 ng/ml during anestrus and the majority of proestrus; progesterone will then start to increase about 2-3 days before ovulation, increasing quickly to greater than 1.0 ng/ml and up to 4-10 ng/ml at the time of ovulation. If only one breeding is to be done then breed 2 days after the serum progesterone is in the 4-10 ng/ml range. This is approximately 2 days post ovulation. Bitches may vary in their individual concentrations and there is some variability from lab to lab. Consult reproductive books, chapters etc for additional information. Ideally the serum progesterone concentration would be correlated with vaginal cytology and if increasing concentrations of progesterone matches with greater than 90% of the nucleated cells being superficial cornified cells then chances for maximum fertility are optimal. Progesterone will continue to increase through estrus reaching concentrations of 15-90 ng/ml during diestrus. During anestrus progesterone should be <1 ng/ml. The concentration of progesterone then decreases over the next 6 weeks.

Normal: The wall of the vagina in anestrus is lined by a two-to-three cell-layer epithelium. The basal cells are rarely to never observed. The parabasal cells are the smallest nucleated epithelial cells in vaginal preparations. They have round nuclei and a high N:C ratio (1:2). The intermediate cells have characteristics in between those of parabasal and superficial cells. They are approximately twice the size of parabasal cells, have pale bluish cytoplasm, rounded edges and round, central nuclei. Superficial cells are the squamous epithelial cells that are large, have pyknotic nuclei or no nuclei, have straight cell edges and are cornified (keratinized). They may be folded, dense blue and resemble oral squamous epithelial cells. Estrogen causes an increase in thickness of the vaginal wall from a few cells to a thick cellular cornifying epithelium as proestrus proceeds to later stages. The stratified epithelium becomes cornified, red blood cells (uterine in origin) enter the vaginal vault and leukocytes are incapable of penetration. At the end of the estrus, the cornified layers slough, the epithelium reverts to the two-cell layer in late metestrus and there is an influx of leukocytes. Estrogen causes diapedesis of rbc's through
uterine capillaries. The majority of the red blood cells present are from a uterine source, but a small percentage may be from a vaginal source. Neutrophils may be seen in early proestrus, but are not seen in most bitches during late proestrus and estrus.

It is difficult to estimate the stage of the cycle from a single cytologic preparation. The following is primarily for estrus in dogs but the observations and principles are similar for cats with noted differences indicated.

Anestrus (from end of diestrus to next proestrus): This is the quiescent phase of the estrus cycle. Anestrus starts after diestrus ends and when progesterone drops to < 1 ng/ml, and ends with the onset of proestrus. Cytologically, it is characterized by parabasal and intermediate cells. There may be a few large intermediate noncornified (viable) epithelial cells, most of which will contain a centrally placed round (nonpyknotic) nucleus. Neutrophils and bacteria may be present in low numbers or are absent.

Proestrus: Estrogen increases and causes the vaginal epithelium to proliferate and become cornified (keratinized). During this stage there will be numerous red blood cells with low to moderate numbers of neutrophils, parabasal and intermediate cells (nucleated). In the later stages of proestrus, the red blood cells decrease and the squamous epithelium becomes more keratinized (fewer nuclei, more angular borders and increased eosinophilia to the cytoplasm). Epithelial cells begin to curl and the round cytoplasmic border is replaced by straight edges. The nuclei become pyknotic and lose detail. Leukocytes decrease markedly during proestrus and erythrocytes increase; however, many bitches will complete a cycle without exhibiting a bloody discharge or releasing erythrocytes in proestrus. The closer the animal comes to estrus, the greater the reduction of red blood cells and the greater the degree of cornification.

Estrus: Estrus is characterized by squamous epithelial cells with abundant cytoplasm (superficial cells) that have straight cell edges, and variable staining intensity to the cytoplasm (cornified, keratinized). Nuclei are pyknotic to absent; erythrocytes are decreased to absent; leukocytes are absent, debris is minimal and background is clear. Bacteria of various types may be present and are frequently present in high numbers. Early estrus is characterized by a near absence of red blood cells and a predominance (80-90%) of cornified superficial epithelial cells. In mid-estrus, the only cells present are superficial epithelial cells, most of which have pyknotic nuclei. In late estrus, superficial epithelial cells become more dense, intensely staining and may be found in clumps or sheets. These intensely staining epithelial cells are called squames, they are 90% of the cells and generally signify standing heat. Some bitches may retain intermediate cells throughout estrus. As the cycle proceeds from estrus to diestrus, leukocytes make their appearance. Diestrus is preceded by a one to two day period in which there is reappearance of small numbers of leukocytes. The cornified epithelial cells begin to disintegrate and debris accumulates. Somewhere during estrus is when the bitch should accept the male for breeding. Progesterone concentration can be monitored during estrus to determine the time of ovulation and optimize breeding and conception.

Diestrus is a period of time in which the bitch should no longer accept the male and is characterized cytologically by an influx of neutrophils and a change from non-nucleated superficial cells to nucleated epithelium with rounded edges. Key to the recognition of diestrus is a sharp decrease of superficial cornified epithelial cells and an increase of parabasal and intermediate (nucleated) cells. This pattern indicates ovulation has occurred. Parabasal and intermediate cells will increase and then comprise about 50% of the total nucleated cells. By the end of diestrus, there should be no non-nucleated superficial epithelial cells (the squamous epithelial cells should all have nuclei). Usually there are numerous neutrophils in the background. Neutrophils may be found within the epithelial cells and these cells are designated as “diestrus cells” (the old term was “metestrus cells”). Red blood cells may be present or absent. The bitch may attract males during diestrus, however, seldom will she allow the male to mount. Breedings during diestrus have reduced fertility.

Breeding: Progesterone concentrations that increase from 1ng/ml to 4-10ng/ml signify ovulation. If only one breeding will occur (e.g. frozen semen, A.I.) then breed two days after the serum progesterone is in the 4-10ng/ml range (approximately 2 days post ovulation). “Cytologically” they should be bred when > 90% of the vaginal epithelial cells are cornified (superficial cells). Diestrus signifies ovulation has occurred and breedings, once diestrus has started, have reduced fertility.

Whelping: Determine the first day of early diestrus and then proceed forward 57 days for the predicted day of whelping: 57 days from first diestrus smear = date of whelping. Whelping will occur 64-66 days from the first detected rise in progesterone above 1 ng/ml (semen needed!).

FELINE ESTROUS CYCLE

Vaginal cytology is used less frequently in queens but the principles and observations are essentially the same as used for dogs. One difference is that there will be fewer RBCs in proestrus and another is that neutrophils may not influx during diestrus. The background in the smear clears more during estrus in cats (this change is more consistent than the pattern of epithelial cornification), and there is an increase in anuclear superficial cells up to 40% of the total (compared to 90% in dogs). The estrous cycle is only about 8 days in length and ovulation requires coitus or simulation of coitus (A.I.; cotton swab). Proestrus in the queen is either unobserved, or of a very short duration (1-2 days).

PYOMETRA

The vaginal discharge is minimal in “closed pyometra” and may be massive in open pyometra. Preparations contain a sea of neutrophils, most of which are degenerate. Bacteria will be present intra- and extracellularly. Rarely are noncornified epithelial cells admixed because of the copious exudate. Bacteria are generally present in the exudate and are similar in type to those present in the uterus. Correlate cytologic features of degenerative neutrophils, bacteria etc. with physical exam findings and clinical pathology data. Polyuria is attributed to the endotoxins of E. coli that interferes with the action of antidiuretic hormones.
MISMATING

To determine a "mismating", use the swab technique to collect vaginal secretions and semen. Insert swab into vaginal vault and leave in place for 1-2 minutes. Place the swab in 1 ml of saline for approximately 2 minutes. Squeeze any fluid out of the swab and remove it from saline. Centrifuge saline at 3000 g for 5-10 minutes. Discard supernatant. Resuspend pellet and make a film (smear) on glass slide. Using this method, you should be able to determine a mating at 100% accuracy during the first 24 hours after "mating" and up to 75% accuracy during the first 48 hours. Any spermatozoa or sperm head that can be seen indicate a mating occurred. The closer to the purported mating the easier it is to see spermatozoa and the longer the post breeding interval the less likely to see these structures.

NEOPLASIA

Squamous cell carcinomas are possible in the vaginal mucosa of any species and in the prepulse of dogs and horses. Cytologic features are the same for squamous cell carcinomas described elsewhere. Leiomyomas are relatively common in the vaginal vault but the neoplastic cells rarely exfoliate. If a leiomyoma/sarcoma is aspirated it will appear cytologically as a spindle cell tumor. TVT occurs in the vagina of stray dogs. TVTs exfoliate numerous round cells with fairly abundant vacuolated cytoplasm and numerous mitotic figures. "Mucocutaneous lymphoma" can occur in the vaginal vault and appears similar to lymphomas elsewhere.

MAMMARY GLAND

Cytology is an excellent tool to differentiate mastitis from neoplasia. Cytologic examination of mammary lumps is not the best tool to identify a specific type of mammary tumor or to determine the biological behavior of a tumor and, hence, provide a prognosis. It is not necessary to use aspirational cytology on mammary lumps that you are already convinced are tumors. They should be excised and submitted for histopathology.

If the lesion is mastitis then the sample is predominately a purulent exudate, bacteria will or will not be observed intracellularly. The more degenerative the neutrophils, the more likely bacteria are present. In large animals, in addition to bacteria, the algae Prototheca is a cause of mastitis. Rarely blastomyoscopy is a cause in dogs.

A diagnosis of neoplasia is indicated for lesions that yield few or no inflammatory cells and numerous large epithelial cells that are "adhered" together. Mammary neoplasms in bitches may be a mixture of epithelial and mesenchymal cells (mixed mammary tumors). The mesenchymal cells are spindle shaped, have tapered ends and oval shaped nuclei. They may reside adjacent to clusters of clearly epithelial cells or only be present on separate slides (aspirates). Sometimes cartilaginous or osteoid material can be identified. Many mixed mammary tumors in the dog are benign.

Tumors that are clearly epithelial are in clumps, aggregates, acini, or morulae, something to indicate cell to cell adherence. Cell to cell adherence is the key to the diagnosis of an epithelial tumor. The more malignant the tumor the more variable will be the cytoplasmic and nuclear characteristics. More benign tumors tend to have uniform cells and nuclei. Carcinomas have marked variability of cell and nuclear morphology. Large cytoplasmic vacuoles are sometimes seen but seem to be more common in metastatic mammary carcinoma cells located in abdominal or pleural fluids. Once you have decided that a tumor is present, then thoracic radiographs, excisional biopsy, and/or aspiration/removal of regional lymph nodes are indicated. The ratio of benign to malignant mammary tumors in the dog is approximately 70:30 and is 20:80 in cats.

Mammary gland hyperplasia is seen in young intact female cats. Cytologically (and histologically) the samples consist of a mixture of "benign" appearing epithelial and mesenchymal/spindle cells. The lesion is a proliferation of both epithelial and stromal cells. The condition regresses following ovariohysterectomy. The lesion can also be induced by progesterone rich estrus inhibiting drugs.

PROSTATE

The normal epithelium is cuboidal to columnar epithelial cells with fairly abundant gray to lightly eosinophilic cytoplasm and uniform round to oval-shaped nuclei. Sometimes these cells will form sheets of 20 to 50 uniform cells. Degenerative changes are pronounced when the cells remain in urine specimens. When smears are made directly of prostatic discharges, the cell morphology is generally more definitive.

The abnormal conditions that are recognized are suppurative prostatitis, prostatic hyperplasia, and prostatic carcinoma. Prostatitis is characterized by numerous neutrophils degenerative or nondegenerative, with or without bacteria. Epithelial cells are not a major component of the cytologic preparation. The prostatic cells present may range from vacuolated degenerate appearing cells to hyperplastic cells. Prostatitis and prostate hyperplasia can occur concurrently in intact male dogs. In some prostatic abscesses, there may be no epithelial cells present. The entire specimen is composed of degenerated neutrophils with bacteria.

Cystic prostatic hyperplasia is a relatively common problem in intact older dogs; it is characterized by minimal or no inflammation and relatively normal appearing prostatic epithelium but cells and clumps of cells are in greater numbers. The cells may be arranged in columns or in large rafts. There is minimal variation in cell size and shape, as well as nuclear size and shape. Uniformity of cells and nuclei is the key to recognizing hyperplasia.

Prostatic cysts may exist as a separate lesion or as part of prostatic hyperplasia. The fluid is of low cellularity, and cells are macrophages or benign prostatic epithelium. Cholesterol clefts and hemosiderin may be identified. Cysts with hemorrhage tend to be red or chocolate brown in color. Some cysts will have an ossified wall that is clearly visible on radiographs.

Adenomas of the canine prostate are not recognized. In prostatic carcinomas, the features to look for are the same as in any other malignant epithelial tumor: moderate to marked variability in the size and shape of cells, as well as the size and shape of nuclei and nucleoli. Prostatic carcinoma cells have less cytoplasm and larger nuclei than do hyperplastic cells. Nuclear chromatin is fine and one or more nucleoli are seen. Anisocytosis and anisokaryosis can be marked and are helpful in recognizing this tumor (as in any malignancy). The rafts of normal appearing epithelial cells are gone or are the minority of the preparation. Cells form balls or morulae rather than flat sheets. The morulae are of variable
Small Animal - Cytology

composition and the cells within them are anaplastic (variable).
Transitional cell carcinoma (TCC) can occur in the prostatic urethra. These carcinomas are markedly anaplastic and highly malignant. The cytologic appearance of prostatic TCC is the same as TCC located in the urinary bladder.

TESTICLE

If the clinical question is to determine if there is orchitis or epididymitis, then aspirational cytology can be useful. If neoplasia is suspected clinically, then castration and submission of the entire testicle for histopathology is the best means to determine a morphologic diagnosis and prognosis. The cytologic criterion is straightforward, as is the gross appearance of most testicular tumors.

**Seminoma** - Grossly, it is white and soft. Cytologically it is composed of numerous blast cells that resemble lymphoblasts; little to no cytoplasm is characteristic but some cells have considerable cytoplasm; binucleation and multinucleation are features. Prominent large nuclei and nucleoli are characteristic. Mitotic figures are often seen. This tumor has the greatest degree of cellular and nuclear pleomorphism. If you think the tumor looks like a lymphoma then it is a seminoma.

**Interstial cell tumor** – Grossly these tumors are yellow, soft with areas of hemorrhage and some cysts. Cytologically the tumor cells are round to polyhedral to columnar-shaped cells with round nuclei and vacuolated cytoplasm. Benign looking cells with abundant cytoplasm and cytoplasmic vacuoles is the key to the recognition of this tumor. This is the most common testicular tumor of dogs. Occasionally tumor cells appear to reside on capillaries aspirated during the procedure.

**Sertoli cell** - Grossly, white and firm. Cytologically these tumors may not exfoliate easily because of all the fibrous tissue. The cells are columnar to angular with lightly basophilic to gray cytoplasm and variably placed nuclei. Cytoplasm tends to stain lightly and contain one or more vacuoles. The vacuoles are not as numerous or prominent as in interstitial cell tumors. Tumor cells may resemble a sarcoma due to their spindeloid shape. Sertoli cell tumors secrete estrogen and rarely have been associated with aplastic anemia but are commonly associated with other signs of hyperestrogenism (gynecomastia, alopecia, hyperpigmentation). They are more common in retained testes. They are reported in Schnauzers with male pseudohermaphroditism and uterine remnants with abdominal testicular tissue.

**PENIS PREPUCE**

**Balanoposthitis** is common in dogs and is characterized by neutrophils (80-90% of the nucleated cells), usually nondegenerate and low to moderate numbers of squamous epithelial cells. The squamous epithelial cells are individualized (no rafts), mature, have abundant cytoplasm and central nuclei. They have no features of malignancy and do not exfoliate in rafts or sheets of different sizes.

**SCC** – Cytologic preparations from these tumors may have numerous neutrophils, but they are not as plentiful as in balanoposthitis. The squamous epithelial cells will be more numerous, some will be individualized and many will be in varying sized rafts, groups or clumps. Within these clumps the cells will vary in size, color and shape. Nuclei and nucleoli will be large prominent and they will be varied in numbers, shapes and sizes.

**TVT** – Typically this tumor exfoliates numerous round cells in a pattern that resembles lymphoma. They have more cytoplasm than do lymphomas and the cytoplasm often has one or more vacuoles. Nuclei are round and chromatin is often fine with prominent nucleoli. Mitotic figures can be numerous. If you think the diagnosis is lymphoma but the sample is from the penis or vagina, then TVT is more likely.