WEDNESDAY SLIDE CONFERENCE 2011-2012

Conference 11

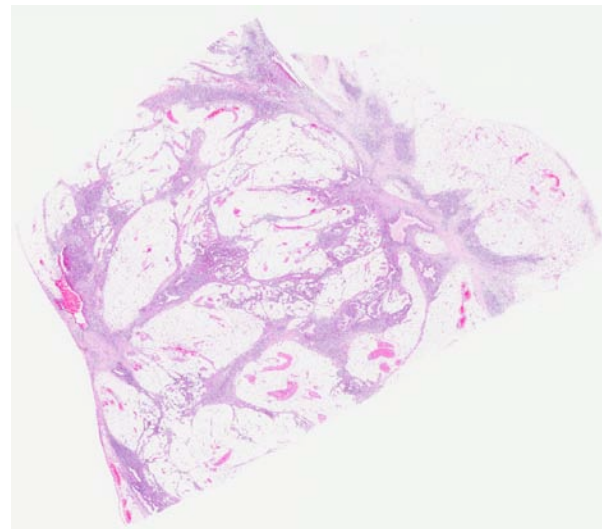**14 December 2011**

08108 (JPC 3134302).

Signalment: 15-year-old, female, rhesus macaque (*Macaca mulatta*).

History: This single-housed animal had a long-standing history of seizures induced by ketamine sedation. One day, care staff noted blood in the cage, but no external injuries were noted.   Clinical staff suspected epistaxis.   However, closer observation revealed she was not eating or drinking. A physical examination at that point revealed traumatic amputation of the rostral third of the tongue.   The veterinary staff and primary investigator elected for euthanasia.
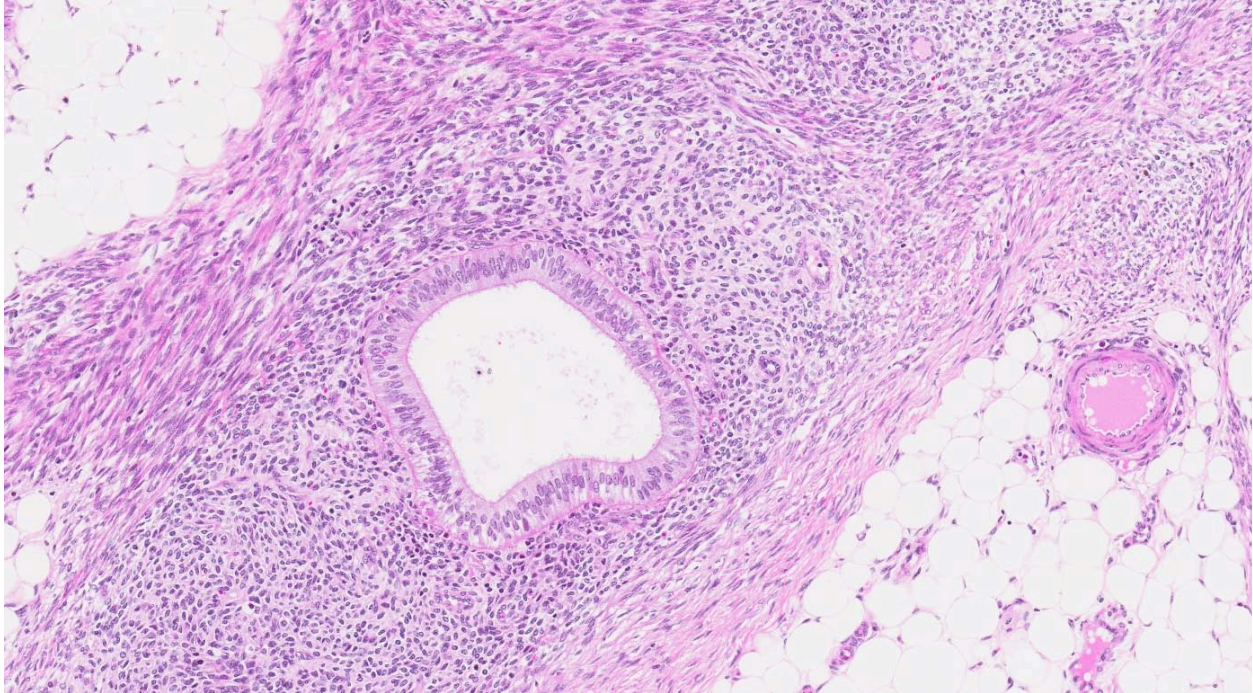
Gross Pathology: Examined was a 5.5 kg, intact, female rhesus macaque. The serosa covering 2 cm of the proximal colon was irregularly thickened and rough with prominent blood vessels and was covered by several bright red, multifocal to coalescing, raised lesions admixed with dark red fibrillar material (fibrin and hemorrhage). The corresponding mucosa was also rough and thickened in this area. An 8 mm x 6 mm x 5 mm, oval, grey-brown nodule was present adjacent to the left ovary.   The left oviduct is tortuous and thickened, and numerous small, coalescing, white to tan plaques cover the diaphragm and abdominal wall. The head of the pancreas was replaced by a 2.5 cm x 1.5 cm x 1.5 cm firm, yellow-brown mass with prominent, tortuous vessels and was attached to the stomach, duodenum and body wall by fibrous adhesions.   Multiple brown-red foci ranging in size from 1mm to 5 mm diameter were present in the peripancreatic and mesenteric adipose.   The urinary bladder was adhered to the uterus, also by a fibrous adhesion.

Laboratory Results: Blood work revealed BUN 39 mg/dl (21-31), creatinine 1.3 mg/dl (0.8-1.0), creatine kinase 3914 U/L (1-2692) and AST 120 U/L (28-58). The elevated BUN and creatinine levels are consistent

1-1. Mesentery, rhesus macaque. The mesentery is subdivided by dense bands of fibrous connective tissue which contain tortuous endometrial glands and stroma. (HE 4X)

1-2. Mesentery, rhesus macaque. Higher magnification image of an endometrial gland lined by tall columnar ciliated epithelium and surrounded by dense endometrial stroma. (HE 100X)

with mild dehydration resulting from the reduced oral intake of food and fluids. The traumatic tongue injury explains the rise in serum creatine kinase and AST as both of these enzymes have a muscle isozyme that is released when muscle is injured.

Contributor's Histopathologic Description: A section of mesenteric adipose is infiltrated by dissecting and intersecting streams of eosinophilic fibrillar material and elongated cells with scant deeply eosinophilic cytoplasm, indistinct cell borders and elongated, deeply basophilic nuclei with tapered ends (fibroblasts and fibrous connective tissue). These streams frequently contain groups of oval to polyhedral cells with pale eosinophilic cytoplasm and central, deeply basophilic nuclei often with perinuclear clearing (endometrial stromal cells). Abundant well-differentiated glands are present among the stromal cells which are lined by pseudo-stratified, ciliated, columnar epithelium punctuated by occasional clear cells. The glands contain dense amorphous to mildly granular eosinophilic or pale wispy eosinophilic material. Lymphocytes are scattered throughout the stroma along with fewer macrophages and neutrophils and rare eosinophils. Occasionally, lymphocytes and macrophages form aggregates within the stromal cells, and rarely small hemorrhages surrounded by macrophages containing greenish-brown pigment (hemosiderin) are noted in the stroma.

Contributor's Morphologic Diagnosis: Ovary, oviduct, diaphragm, intestinal serosa, and mesenteric adipose tissue: Endometriosis, multifocal, subacute to chronic, moderate to marked with marked fibroplasia.

Contributor's Comment: Developed in 1950 by Te Linde and Scott, the rhesus macaque was the first animal model for endometriosis. Presently the baboon model is utilized more frequently due to the ability to non-invasively monitor their menstrual cycle, continuous breeding in captivity, adequate volumes of spontaneous peritoneal fluid production and ease of vaginal transcervical uterine access.[4,6]

Endometriosis is an estrogen-dependent, chronic disease that occurs in menstruating species; which include human and non-human primates, the elephant shrew (*Elephantulus myuras jamesoni*) and one species of bat (*Glossophaga soricina*). Spontaneous endometriosis has only been reported in women and female non-human primates and the pathogenesis is not completely understood. It may be induced in other species through intra-peritoneal injection of viable endometrial tissue. Rodent models of the disease have been created in this manner. By definition, it is the presence of viable, ectopic, extra-uterine, functional endometrial glands with stroma in various sites throughout the pelvis and peritoneal cavity.[5,7]

The most widely accepted explanation of the pathogenesis is Sampson's three-fold transplantation: retrograde menstruation occurs, viable endometrial cells must be present, and adherence and implantation into structures in the peritoneum must successfully

2

occur. Retrograde menstruation alone is not enough for the condition to occur. In some baboon studies, retrograde menstruation has been reported in over 83% of animals. Endometrial cells have been reported in the peritoneal fluid of 59-79% of women during menses. However, endometriosis is not clinically present at these rates and is estimated to affect only 15-20% of women during their reproductive lives. Other theories include vascular and lymphatic dissemination, *in-situ* development from Wolffian or Müllerian duct remnants, and the development of metaplastic ovarian or peritoneal tissue. An alternate theory suggests induction and differentiation of mesenchymal cells that are affected by substances released by degenerating endometrial tissue following reflux into the peritoneal cavity. While the retrograde menstruation and transplantation theory is the most widely accepted, the cellular and molecular mechanisms that lead to the development of the disease are controversial.[2,4,7]

Angiogenesis, immune suppression (cytotoxic T-cells, NK cells), mesothelial lining injury and pro-inflammatory cytokines, matrix metalloproteinases, adhesion molecules, toxin (dioxin) exposure and genetic polymorphisms are all among the multitude of candidate factors in the creation of the proper peritoneal environment that must exist for successful survival, adhesion and implantation to occur. In women, an autoimmune component has also been proposed due to the demonstration of auto-antibodies and an association with other autoimmune diseases and immune mediated abortion. In humans, there is a 6-9 fold increase in prevalence among first-degree relatives.[2,4,7]

Briefly, an abnormal or "permissive" peritoneal environment in the face of endometrial reflux is thought to be central cause for this chronic inflammatory condition. Conditions that favor tubal reflux (e.g., cervical stenosis) in addition to increased sloughing and retrograde flow may increase tubal reflux and overwhelm peritoneal macrophage's ability to eliminate the sloughed endometrial material. However, it should be noted that conditions such as cervical stenosis are not present in all cases.[5,7]

Chronic inflammation and the release of certain cytokines in this inflammatory stage (TNF-alpha, IL-1, IL-6 and IL-8) may alter innate immunity and permit the viable cells to persist through decreased peritoneal macrophage, natural killer cell and cytotoxic T-cell activity. However, the decreased NK cell activity has also been described as constitutive rather than a result of cytokine or hormone induced immune suppression and secretory ICAM (sICAM) expression by endometrial tissue may bind LFA-1 and help prevent NK-cell recognition of endometrial implants.[2,5,7]

Endometrial cells from women with endometriosis have decreased rates of apoptosis, insensitivity to macrophage cytolysis, and enhanced gene expression of the anti-apoptotic gene Bcl-2, secretory ICAM (intercellular adhesion molecule), vascular endothelial growth factor (VEGF), and various matrix metalloproteinases which suggests increased intra-peritoneal viability, and ability to adhere and invade peritoneal membranes and tissues.[2,5,7]

JPC Diagnosis: Mesentery: Endometriosis.

Conference Comment: The contributor provided an excellent review on endometriosis, which is the extrauterine growth and proliferation of endometrial glandular and stromal cells in menstruating animals, most commonly in Old World primates. Three histologic features of endometriosis are the presence of endometrial glands, endometrial stroma, and hemosiderophages; at least two of these features are required for a diagnosis of endometriosis. Endometriosis presents grossly as blood-filled "chocolate cysts" which progress to fibrotic scar tissue in chronic cases. While implantation generally occurs in the pelvic and extrapelvic abdominal cavity, endometriosis has also been reported in the thoracic cavity, lungs, and brain. There are also rare reports of endometriosis in men undergoing estrogen therapy for prostate cancer and in premenstrual girls. As the first tenet of Sampson's threefold transplantation theory (retrograde menstruation) is clearly lacking in these cases, it is possible that another of the proposed pathogeneses for endometriosis (*in-situ* development from Wolffian or Müllerian duct remnants or development of metaplastic ovarian or peritoneal tissue) may still be reasonable theories in some individuals.[1]

Conference participants also discussed various risk factors in non-human primates which predispose them to endometriosis. These include low numbers of pregnancies, with increased numbers of menstruations throughout life, resulting in increased endometrial turnover compared to multiparous primates. Non-laproscopic abdominal surgical procedures, including hysterectomy and estradiol therapy are also implicated, as well as genetic predisposition and age. Aged non-human primates are more likely to develop endometriosis than older women because unlike human females, menstruation continues indefinitely due to lack of menopause.[3]

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CASE II: 43121 (JPC 3165086).

Signalment: 6-year-old, male, domestic rabbit (*Oryctolagus cuniculus*).

History: A male, sexually intact pet rabbit presented for enlargement of the left testicle in the scrotum. No other symptoms were found at clinical examination. Bilateral orchiectomy was performed.

Gross Pathology: The enlarged left testicle was firm and globular, in contrast to the elongated right one, and approximately 1.7 cm in diameter. The cut surface was flat, smooth and gray-white in color.
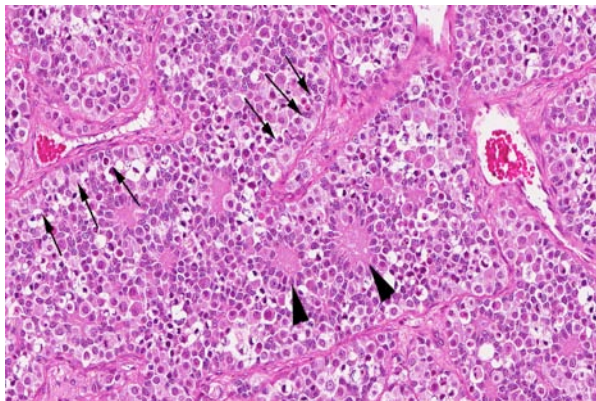
Contributor's Histopathologic Description: The left testicle was almost entirely replaced by tumoral tissue. The tumoral tissue consisted of discrete tubular structures separated by a fibrous stroma. Tubular structures were composed of an intermixture of large round cells similar to germ cells and spindle cells with elongated nuclei similar to Sertoli or granulosa cells. The spindle cells mainly located at the periphery of tubular structures formed a palisade surrounded by basement membrane (coronal pattern). Spindle cells sometimes surround an isolated or collected large round cell similar to a germ cell and took an appearance like follicular epithelium surrounding the ovum of the primary follicle (follicular pattern). Arrangement of a microfollicular pattern containing eosinophilic amorphous material (Call-Exner-like pattern) was also seen. The central eosinophilic amorphous material was PAS-positive. The large round cells (germ cell components) had abundant clear cytoplasm and round, vesicular nuclei with prominent nucleoli. The spindle cells (sex cord components) had scant clear cytoplasm and small oval or elongated nuclei with indiscernible nucleoli. Mitotic figures were occasionally observed in the germ cell components, but were not detected in the sex cord
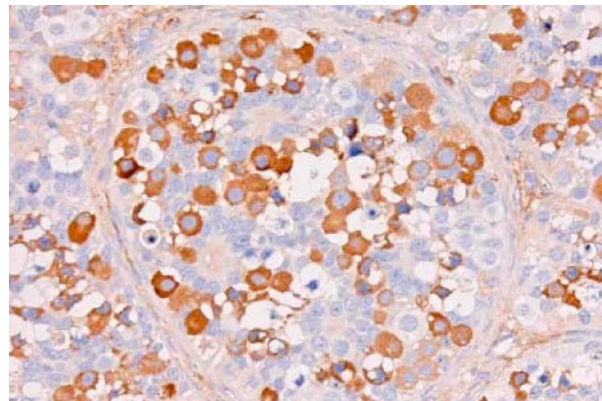

2-1. Testis, rabbit. The enlarged left testicle was firm and globular in contrast to elongated right one and approximately 1.7 cm in diameter. The cut surface was flat, smooth and gray-white in color. Photograph courtesy of Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka, 5730101, Japan

components. Aggregates of Leydig cells were present within the intertubular stroma. These cells were non-neoplastic and lacked crystals of Reinke. Immunohistochemically, the germ cell components were positive for c-kit and placental alkaline phosphatase (PLAP). The sex cord components stained for vimentin and Wilms tumor-1 (WT-1). Electron microscopic examination revealed that the germ cell components had abundant cytoplasm within a few organelles and large round nuclei with dispersed chromatin and prominent nucleoli in the neoplastic lesion. The sex cord components had many organelles composed predominantly of mitochondria in the primarily scant cytoplasm. The nuclei of cells of the sex cord components were irregular or oval shaped and contained few or modest nucleoli. Amorphous materials surrounded by sex cord components consisted of duplicated basal laminae.

Contributor's Morphologic Diagnosis: Testicle: Gonadoblastoma.

2-2. Testis, rabbit. Two distinct morphologies are present within neoplastic cells. The germ-cell phenotype are large round cells present in sheets, which resembles a seminoma. The second phenotype is a spindle cell which forms rosettes (arrowheads) and often palisades along the basement membrane (arrows). (HE 200X)
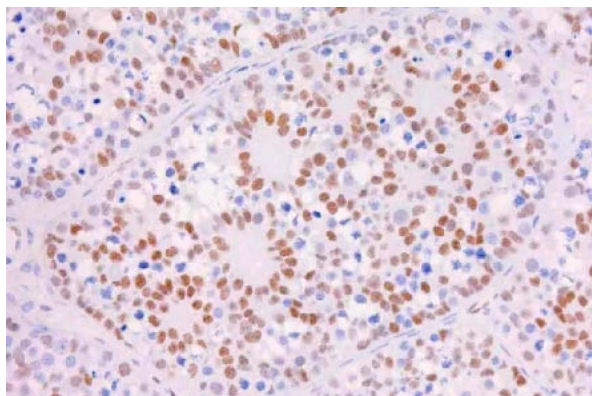

2-3. Germ cells show diffuse strong cytoplasmic immunoreactivity for Wilms tumor -1 antigen. Photograph courtesy of Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka, 5730101, Japan

Contributor's Comment: Tumors arising in testis of rabbits have rarely been reported. In domestic rabbits, one of the most common testicular tumors is the interstitial (Leydig) cell tumor.[3] Gonadoblastoma rarely develops, almost exclusively in dysgenetic gonads, or in those with an intersex syndrome in humans.[5] On the other hand, gonadoblastomas can be found in apparently normal ovaries and testes.[6] In animals, gonadoblastomas have only been reported in two dogs.[4,7]

Gonadoblastoma is histomorphologically defined as a tumor composed of two principal cell types: germ cell components similar to those of seminoma and sex cord components similar to Sertoli cells. In this case, immunohistochemical examination revealed both the germ cell derivative of the large round cells (c-kit and PLAP positivity) and the sex cord nature of the spindle cells (vimentin and WT-1 positivity) in addition to characteristic morphological features. Furthermore, the ultrastructural pattern of eosinophilic amorphous bodies that are comprised of whorled laminae is already verified in canines[6] and those of human gonadoblastomas.

The differential diagnosis includes mixed germ cell-sex cord-stromal tumor (MGSST) and sex cord tumor with annular tubules (STAT). MGSST generally lacks the discrete tubular structures seen in gonadoblastoma, and usually shows proliferative activity in the sex cord component, because unlike in gonadoblastoma, germ cells in MGSST are thought to be non-neoplastic and entrapped by neoplastic sex cord-stromal tumor. The histomorphological features showing characteristic three typical patterns (i.e. coronal, follicular, and Call-Exner-like) of sex cord elements within the discrete tubules is most characteristic and diagnostic for gonadoblastoma. In a gonadoblastoma, more than one pattern is usually found in an individual tubule.[6] The presence of these patterns supports the diagnosis of gonadoblastoma in this case. STAT has a growth pattern similar to gonadoblastoma and contains eosinophilic amorphous bodies and frequently calcified material, but lacks a germ cell component. Our case is conclusively distinguished from these diagnoses.

Gonadoblastoma typically occurs within dysgenic gonads in children or young adults. Approximately 80% of cases occur in phenotypic females, and most of the remaining 20% are in phenotypic male pseudohermaphrodites.[6] In more than 90% of patients with gonadoblastoma, a Y chromosome or fragment can be identified.[6] However, it has been found in the testes of normal men.[2] Our rabbit has no clinical symptoms such as alopecia, feminization, or cryptorchidism. Our case was apparently sexually intact judging from the intact right testis and normal development of genital organs, and has no dysgenic

2-4. Testis, rabbit. Sex cord-like cells show diffuse strong nuclear immunoreactivity for c-kit. Photograph courtesy of Department of Pathology, Faculty of Pharmaceutical Science, Setsunan University, 45-1 Nagaotohge-cho, Hirakata, Osaka, 5730101, Japan

gonads. However, it remains unclear whether this rabbit had Y chromosome fragments or not, because karyotypic analysis was not done.

JPC Diagnosis: Gonad: Gonadoblastoma.

Conference Comment: This neoplasm was considered benign because the tumor showed no evidence of invasive germ cells. It is postulated that gonadoblastomas result from abnormal proliferation of germ cells, which induce the sex cord mesenchymal cells around the germ cells to differentiate into granulosa-Sertoli-like cells.[8] A Call–Exner body surrounded by sex cord stromal cells indicates areas of the collection of degenerate and necrotic debris, similar to the phagocytosis of apoptotic spermatogenic cells by Sertoli cells in normal testicles, suggesting that sex cord components may have a tendency to assemble in the area of cell dissolution.[9]

Placental alkaline phosphatase (PLAP) only stains neoplastic germ cells, in addition to rare somatic epithelial malignancies, and not normal germ cells, which is useful in distinguishing this neoplasm from a mixed germ cell-sex cord-stromal tumor.[11] The transcription factor Wilms' tumor-1 (WT-1) protein, in addition to staining nephroblastomas, is a marker of Müllerian epithelial origin, and regulates the k-ras oncogene, which plays an important role in tumor suppression. Loss of WT-1 drives cells expressing oncogenic k-ras toward a senescence program, and inhibits the progression of certain types of neoplasia.[10] Sex cord stromal tumors are among several neoplasms in veterinary medicine that co-express vimentin and cytokeratin (AE1/AE3); others include canine prostatic carcinoma, feline bronchogenic adenocarcinoma, synovial cell sarcoma, ciliary body adenoma, renal carcinoma, amelanotic melanoma, meningioma, mesothelioma, and anaplastic carcinoma. However, due to the growing list of neoplasms that can express

both cytokeratin and vimentin, the diagnostic utility of vimentin is increasingly coming into question.[1]

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CASE III: Case 1 (JPC 4002497).

Signalment: Adult female dog, *Canis familiaris.*

History: This dog was euthanized due to clinical signs of depression, diarrhea, anorexia, and vomiting.

Gross Pathology: Gross findings included pelvic dilatation in the left kidney; multiple brown foci in the right kidney; bloody, opaque fluid in the uterus; multifocal dark red foci on the lungs; and petechia on the serosa of all sections of the intestine.

Contriubutor's Histopathologic Description: Uterus: The endometrium is diffusely expanded by an infiltrate of large numbers of lymphocytes, plasma cells, and fewer macrophages and neutrophils which surround and separate, and replace ectatic glands and numerous congested vessels. Ectatic glands are often filled with varying combinations and concentrations of degenerate and non-degenerate neutrophils, foamy macrophages, necrotic debris, and brightly eosinophilic proteinaceous secretory materials. Glands are often lined by tall columnar epithelium (attenuated to cuboidal in dilated glands) with abundant eosinophilic foamy cytoplasm and vesiculate nuclei (progestational epithelium). Multifocally, within all layers of the uterine wall, there are extensive areas of lytic necrosis containing large numbers of viable and degenerate neutrophils, necrotic debris, hemorrhage, fibrin, and edema. Additionally within all layers, vessels (both veins and lymphatics) are markedly dilated and often contain fibrin thrombi which are composed of abundant polymerized fibrin and numerous neutrophils, often with mural effacement and numerous viable and degenerate neutrophils, bright pink protein, and cellular debris within the vessel wall (vasculitis). Hemorrhage, edema, fibrin, and numerous viable and degenerate neutrophils expand the submucosa and muscularis, markedly separating submucosal collagen fibers and smooth muscle fibers. Smooth muscle

fibers are often swollen, brightly eosinophilic, and fragmented, with pyknotic or lytic nuclei, and low numbers of infiltrating neutrophils (degeneration and necrosis). The adjacent broad ligament is infiltrated with low to moderate numbers of neutrophils and there is abundant hemorrhage.
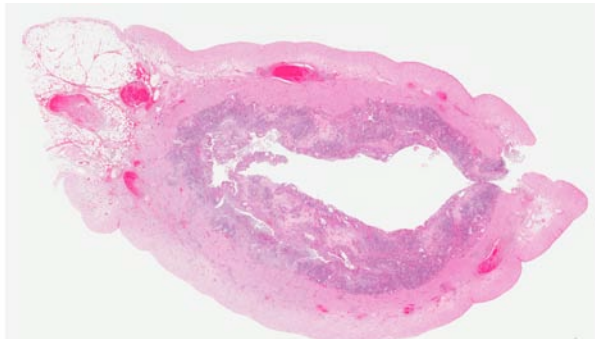
Contributor's Morphologic Diagnosis: Uterus: Cystic hyperplasia, endometrial, moderate.
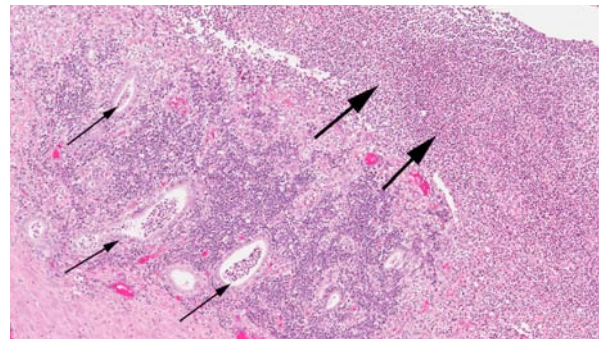Uterus: Necrosuppurative and lymphoplasmacytic endometritis, marked, with hemorrhage.
Uterus, myometrium: Vasculitis, necrosuppurative, with thrombus formation, marked.

Contributor's Comment: Although numerous findings were present in other organs, the primary cause of moribundity was considered to be septicemia arising from the uterus. In addition to bacteria being observed in the kidney at sites of necrosis, other tissues including peritoneal fat, heart, and lungs had necrosuppurative or hemorrhagic lesions arranged in an embolic pattern characteristic of septicemia. The source of the thromboemboli appeared to be the uterus, where there was marked necrosuppurative vasculitis in the myometrium. The cause of the uterine vasculitis was not determined but was likely a sequela to the cystic endometrial hyperplasia (CEH) and observed pyometra.
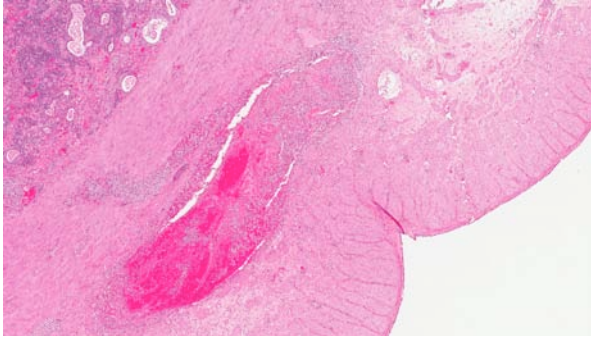
CEH most commonly occurs in intact bitches (most frequency in those animals that have not undergone pregnancies) and its pathogenesis is related to estrogen stimulation of the uterus followed by prolonged exposure to progesterone during diestrus. Progesterone acts to stimulate endometrial glandular secretion and to suppress contractions of the uterus, thus creating a uterine environment predisposed to bacterial growth.[11] Although CEH has long been thought to be a necessary predisposing factor for pyometra and certainly they are often observed together as in this case, each can occur independently
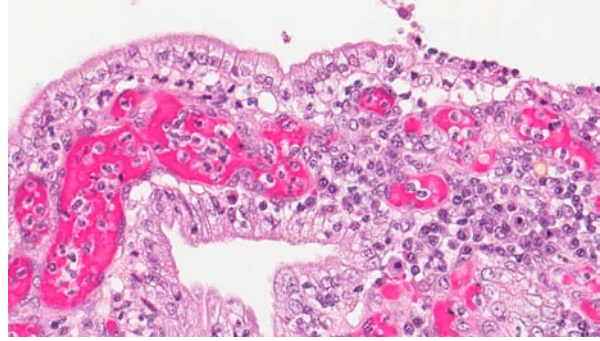

3-1. Uterus, dog. Cross section of the uterus of a dog showing a markedly thickened and inflamed endometrium.(HE 4X)

3.2. Uterus, dog. At higher magnification, the lumen contains numerous viable and degenerate neutrophils (large arrows), and the endometrium is expanded by large numbers of lymphocytes, macrophages, and neutrophils. Remaining endometrial glands are often ectatic (small arrows) and contain neutrophils and sloughed endometrial epithelial cells. (HE 200X)

3-3. Uterus, dog. Vessels throughout the muscular layers of the uterine body as well as the broad ligament exhibit vasculitis and partially occlusive fibrinocellular thrombi. (HE 120X)

3-4. Uterus dog. Endometrial endothelium is tall columnar and vacuolated under the influence of progesterone. (HE 400X)

of the other. New evidence suggests that pyometra should be considered a separate entity from CEH since CEH relies entirely on a hormone pathogenesis while pyometra is ultimately triggered by bacterial infection.[4,11] The most common bacterium isolated in canine pyometra is *Escherichia coli*, which is isolated in 59-96% of cases.[4] Endotoxin derived from the etiologic agent (which is most often a gram-negative bacteria) often results in sepsis, manifested by numerous cardiovascular, gastrointestinal, and overwhelming systemic inflammatory findings. In the absence of surgical intervention, death is common.[4]

The typical clinical presentation of pyometra is inappetance, depression, polydipsia, lethargy, abdominal distension due to an enlarged uterus, +/- vaginal discharge. Marked leukocytosis is the most common hematologic finding, although anemia, hyperglobulinemia, hypoalbuminemia, and elevation of markers of intrahepatic cholestasis are also often observed.[4,11] The incidence of pyometra in a colony of intact female beagles >4 years of age was 15%, and incidence increased with age.[5] Despite its bacterial component, antibiotic treatment is rarely effective. Ovariohysterectomy is the most reliable and successful treatment for canine pyometra.[5]

JPC Diagnosis: Uterus: Endometritis, chronic-active and suppurative, diffuse, severe, with mild chronic endometrial hyperplasia, vasculitis, and thrombosis.

Conference Comment: Progestational changes cause the endometrial epithelium to be enlarged, columnar, and vacuolated with pyknosis, as well as undergo pseudostratification or papillary proliferation, which are seen in this case. The underlying cause of endotoxin-derived polyuria/polydipsia, a consistent clinical finding with pyometra and associated septicemia, is a decreased response to antidiuretic hormone, glomerular dysfunction due to membranoproliferative glomerulonephropathy from immune-complex deposition, and renal tubular cell damage.[7]

The contributor mentioned the clinicopathologic finding of intrahepatic cholestasis, which is increased alkaline phosphatase, bilirubin, and cholesterol with an ALT within normal limits, which differentiates the findings from hepatocellular necrosis.[3]

Death from pyometra is often due to systemic inflammatory response syndrome (SIRS), resulting in the release of inflammatory mediators and the activation of neutrophils and platelets, resulting in multiple organs dysfunction syndrome and shock. Clinical criteria for diagnosing SIRS in dogs are at least two of the following: heart rate greater than 160 beats per minute, body temperature greater than 103.5°F or less than 100°F, respiratory rate of greater than 20 breaths per minute, pCO2 of less than 32, or a leukocyte count of greater than 12,000 or less than 4,000 or greater than 10% bands.[9]

SIRS caused by the lipopolysaccharide (LPS) endotoxin, as in this case, causes tachycardia and tachypnea in lower doses, and hemorrhagic diarrhea, vomiting, myocardial failure, and death in high doses. LPS binds lipopolysaccharide binding protein (LBP) in serum which in turn binds cell surface pattern-recognition receptor CD14. This activates clotting factor XII (Hageman factor), and induces toll-like receptor (TLR) 4. Also, the endothelium is activated, and the anticoagulant tissue factor production inhibitor (TFPI) and thrombomodulin are inhibited. Monocytes and macrophages are activated, releasing tumor necrosis factor alpha (TNF), IL-1, IL-6, IL-8, and other chemokines, which induces further inflammation and incites the release of acute phase proteins from the liver, such as LBP, mannose-binding protein, fibrinogen, C-reactive protein, serum amyloid A (SAA), hepatoglobin, hepcidin, C3 and C4, alpha-1 antitrypsin and alpha-1 acid glycoprotein. Many of the acute phase proteins bind bacterial components, such as the cell wall, and act as opsonins and fix complement. Some proteins that are reduced, also known as negative acute phase proteins, are transferrin,

albumin, pre-albumin, antithrombin, and alpha-2 macroglobulin (in cattle). Also, the complement cascade is directly activated, generating C3a and C5a.[6,7,8]

Animals that survive SIRS are predisposed to compensatory anti-inflammatory response syndrome (CARS), leading to decreased macrophage activity, T cell anergy, and apoptosis of lymphocytes.[2]

Conference participants also discussed some causes of endometrial hyperplasia, such as prolonged hyperestrogenism due to subterranean and red clover in ewes, which causes reduced fertility, dystocia, uterine prolapse due to hypotonicity, endometrial gland development in the cervix, and mammary gland engorgement. Sows develop endometrial cysts due to zearalenone mycotoxicosis, and cows develop cystic follicles and granulosa cell tumors from prolonged plant estrogen ingestion.[10]

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CASE IV: 112D (JPC 4003045).

Signalment: 18-month-old male mini-mule (donkey (*Equus asinus*) x minihorse (*Equus caballus*, subtype miniature) hybrid)

History: A 100kg mini-mule was presented for elective castration.
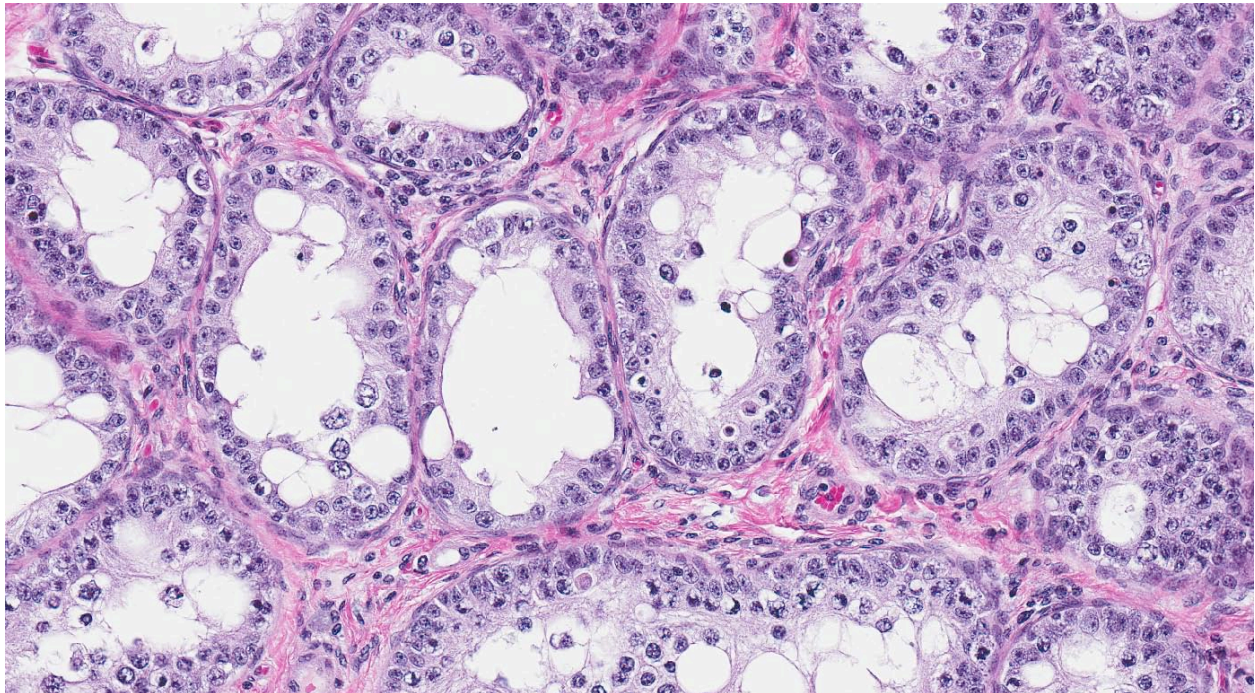
Gross Pathology: None.

Contributor's Histopathologic Description: Testis (cross-section): This ~2.5 x 2 cm (hypoplastic) testis is bounded by a thick tunica albuginea with two focally extensive (iatrogenic) disruptions of the capsule. Subjacent to one of the areas of capsular discontinuity is a large area of extravasated red blood cells (hemorrhage) within the interstitium; at the margin of this area and under the second area of capsular discontinuity, there is extensive interstitial pallor with separation of collagen fibers (edema). This acute hemorrhage and edema is interpreted as artifactual from the castration procedure.

The number of seminiferous tubules is decreased (testicular hypoplasia); a variable amount of fibrous stroma separates the tubules. There are prominent fibrous septae with many plump oval fibroblast nuclei and pigmented macrophages with pale gray-blue non-polarizing granular cytoplasm (lipochrome, lipofuscin, ceroid). Interstitial (Leydig) cells are difficult to discern, and are therefore less populous than expected.

Seminiferous tubules are diffusely hypocellular (hypoplasia) with diameters estimated at 120-130 m. All tubules contain Sertoli cells, often with marked apical micro- and macrovacuolation (degeneration), creating a wispy cytoplasmic appearance. Approximately 95% of seminiferous tubules contain at least one spermatogonium within the basal compartment; mitotic figures are occasionally noted. Meiotic figures are rare, and only a small fraction of the expected number of spermatocytes is identified; many have swollen clear cytoplasm with vacuolated nuclei (degeneration) or are individuated with hypereosinophilic cytoplasm and round condensed or fragmented nuclei (apoptosis). Neither elongating nor mature spermatids are identified. Rare multinucleated syncytial (degenerate) spermatocytes or spermatids are noted; some are apoptotic (may not be present in all sections). Approximately 5% of the seminiferous tubules contain only Sertoli cells with frothy, vacuolated apical cytoplasm. There are multiple small to moderately sized perivascular interstitial aggregates of mature lymphocytes. Clusters of seminiferous tubules without apparent lumina are noted (cause and significance not apparent).

Contributor's Morphologic Diagnosis: Testis: Severe spermatocyte degeneration with testicular hypoplasia and Leydig cell atrophy.

Contributor's Comment: A typical hypoplastic mule testis is examined. Testicular hypoplasia in mules is believed to result from the failure of autosomal

4-1. Testis, minimule – Diffusely, seminiferous tubules are largely devoid of spermatogonia and lined by a single layer of Sertoli cells. Remaining spermatogonia are shrunken (degenerate). Note: also the absence of interstitial cells. (HE, 192X)

chromosomal pairing in meiosis;[5] donkeys have a diploid chromosome number of 62, horses have 64, and mules have 63.[2] Donkeys also have more metacentric chromosomes and fewer acrocentric chromosomes than horses, making meiotic pairing physically difficult in mules.[2]

Because the size and development of the mule testis is entirely controlled by the donkey sire (which supplies the male mule's Y chromosome), it is more relevant to compare mule testes with donkey testes, but, as limited information is available for the jack, the stallion can also serve as a reference. The Y chromosome carries the Sry gene, which controls the development and differentiation of the testis, as well as the number of Sertoli cells and the length of the spermatogenic cycle.[5] The donkey Sry gene is significantly divergent from that of the stallion, and may be only partially successful in inducing and supporting testicular development in mules.[7] This is supported by the finding of a skewed sex ratio in mule foals (44 male:56 female) compared to horse foals (52.5 male:47.5 female)(reviewed in 7).

This testis has three features of hypoplasia: 1) it is small; 2) it has fewer seminiferous tubules than expected; and 3) the luminal diameter of the seminiferous tubules is decreased. The testis examined is about the size of that of a medium-sized dog, and is much smaller than expected for a 100kg animal. The average mule testis weighs 350g, while that of a jack weighs 750g, and that of a stallion weighs 900g.[4] Only a portion of the testis was submitted, thus the weight of this mini-mules testis was not measured. There is also a decrease in the number of tubules in this testis, possibly causing an apparent increase in the interstitial tissue. Mule testes are reported to have 73% fewer seminiferous tubules than jack testes, and the total length of mule seminiferous tubules is calculated to be only 57% of the total length of seminiferous tubules in jacks.[5] Finally, the average width of seminiferous tubules in mules is decreased; it was estimated at 120-130μm in this mini-mule. Previous quantitative reports suggest that the average width of seminiferous tubules in mules is 127μm and in jacks is 222μm.[5] It is 146μm in stallions.[3]

The number of Leydig cells is decreased in this testis, as is expected with fewer differentiating germ cells failing to provide sufficient crosstalk to support an appropriate number of Leydig cells. Mules have approximately 67% of the number of Leydig cells identified in jacks, and about 40% of the number seen in stallions,[4] but mule Leydig cells are ultrastructurally normal and identical to those of jacks and stallions.[4,5,6] The smaller number of Leydig cells in mule testes is believed to reflect the failure of spermatogenesis, rather than be the cause of it. This is supported by

anecdotal evidence that mules have very high libido among equids, presumably due to high levels of testosterone synthesized by the Leydig cells. Mules also typically demonstrate normal epididymal duct epithelium (a steroid-dependent phenotype).[1]

The number of Sertoli cells in this case is likely within normal limits, as is Sertoli cell function. Although the seminiferous tubular diameter is decreased compared to that seen in jacks, there is no waviness or buckling of the basement membrane, suggesting that the number of Sertoli cells is appropriate. Other references have shown that the number of Sertoli cells is equal in donkeys and mules,[5] and that they are ultrastructurally identical.[6] Additionally, lanthanum exclusion studies have shown that the blood-testis barrier is intact in mule testis.[6]

Failure of chromosomal pairing in the zygotene stage of meiotic prophase I leads to deletion of nascent germ cells by apoptosis. Apoptosis in the testis of mammals is a normal process, and is mediated by Fas on germ cells and FasL on Sertoli cells.[3] The number of germ cells undergoing apoptosis is increased after any insult, regardless of cause. It is also increased in testis with maturation arrest, as is present in mule testes.[3] Degeneration of seminiferous tubules in stallions frequently elicits a lymphocytic interstitial orchitis,[3] and that is the likely inciting cause of the inflammation seen in this case. Equine testes also commonly exhibit prominent fibrous tissue septa,[3] and the amount of interstitium in this testis may be within normal limits.

JPC Diagnosis: 1. Testis, seminiferous tubules: Hypoplasia, diffuse, severe.
2. Testis: Hemorrhage, focally extensive, severe.

Conference Comment: Conference participants felt the perception of decreased Leydig cells may be related to the relative decrease in tubules compared to the remaining stroma and interstitial cells. Participants also discussed a recent ultrastructural study of mule testicles which concluded that Sertoli and Leydig cell structure and steroidogenic capacity in mules is normal and not affected by chromosomal abnormality, as they are somatic in origin. The study also concludes that mule seminiferous tubules are able to sustain complete spermatogenesis and, the lack of complete spermatogenesis in mules is due mainly to the failure of homologous chromosomes to pair.[6]

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