Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2025-2026

Conference #12 — 19 November 2025

CASE I:

Signalment:
Stillborn male Rocky Mountain bighorn sheep (*Ovis canadensis*) lamb, estimated gestational age of greater than 140 days

History:
This bighorn sheep lamb is a stillborn from a ewe that is part of a free-ranging population from Washington state. This ewe birthed a full-term healthy lamb during the previous lambing season; however, two different ewes in the same herd had aborted, and toxoplasmosis was diagnosed as the cause.

Gross Pathology:
Crown-rump length was 48.5 cm. No obvious gross abnormalities were seen within the examined organs.

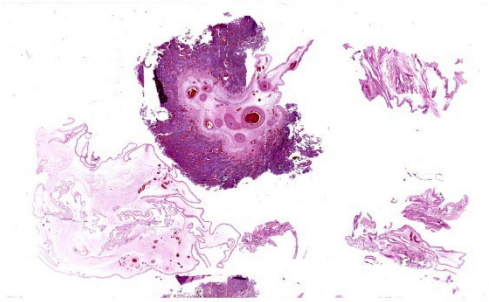

Figure 1-1: Placenta, lamb. One section of cotyledon and several sections of chorioallantoic membranes are submitted for examination. (HE, 6X)

Laboratory Results:
Neospora and *Toxoplasma* duplex PCR on paraffin-embedded brain tissue: *Toxoplasma* detected, *Neospora* not detected.

Microscopic Description:
Placenta: Trophoblasts are rarely expanded by single intracytoplasmic protozoal cysts up to 15 µm in diameter with thin, refractile, bright eosinophilic walls which surround dozens of elongate 2 x 1 µm bradyzoites. Multiple regions within the cotyledonary placenta comprising approximately 15% of the total examined area are effaced by hypereosinophilic cellular and pyknotic to karyorrhectic nuclear debris (lytic necrosis) and fibrillar to amorphous eosinophilic material (fibrin). Small caliber blood vessels within the cotyledonary placenta are occasionally occluded by dense fibrin coagula with loss of the subjacent endothelium (fibrin thrombi).

Contributor's Morphologic Diagnoses:
Placentitis, necrotizing, acute, multifocal, moderate with thrombosis and intralesional protozoal cysts morphologically consistent with *Toxoplasma gondii.*

Contributor's Comment:
Toxoplasma gondii is an obligate intracellular parasitic protozoan in the phylum Apicomplexa, and the cause of toxoplasmosis. Members of the *Felidae* family are definitive hosts of *T. gondii*. Following infection through ingestion of tissues containing *T. gondii* cysts,

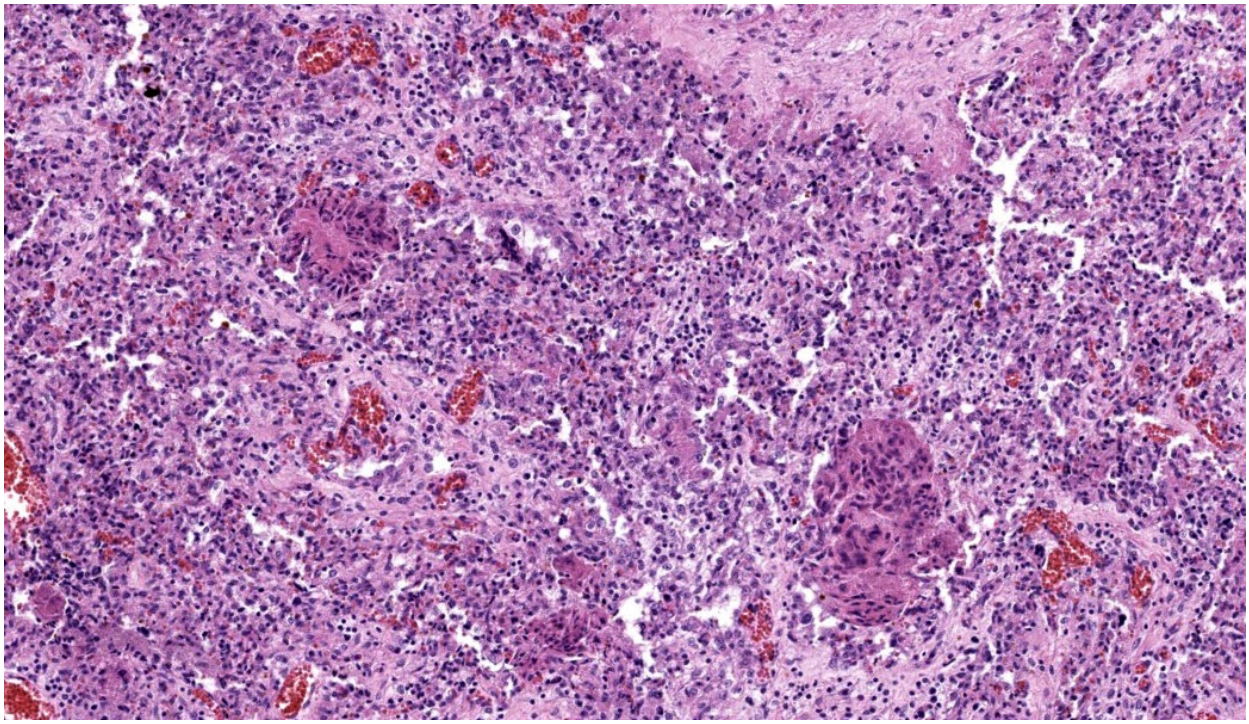

Figure 1-2: Placenta, lamb. There are multifocal areas of lytic necrosis that are scattered through the chorionic vili. (HE, 229X)

felids typically shed oocysts in their feces for one to two weeks. All other homeothermic vertebrate species are susceptible to infection and become intermediate hosts through ingestion of food, water, or soil contaminated with oocysts. Following ingestion, sporozoites are released from the oocyst and multiply asexually within the intestinal lamina propria as tachyzoites. Within hours, tachyzoites disseminate hematogenously or via lymphatics to other organs. *T. gondii* forms tissue cysts within the organs of intermediate hosts, with muscular and neural tissues preferentially affected. Tissue cysts persist for the life of the host and will perpetuate the life cycle if ingested by a felid.[3]

The outcome of infection is dependent upon individual host and parasite factors, including host susceptibility, immune status, parasite virulence, and parasitic life stage to which the host is exposed. Infected animals may remain subclinical or progress to systemic infection, abortion, and/or death.[5] Spread of tachyzoites to the placenta and fetus of a pregnant animal typically results in necrotizing lesions within the placenta and fetal brain, though other organs may also be involved.[2] In this case, protozoal cysts associated with mononuclear inflammation were also identified within the brain, lungs, and adipose tissue. Toxoplasmosis and neosporosis can cause similar lesions, and the tissue cysts of each species cannot be reliably distinguished using histology alone. Therefore, definitive diagnosis relies on ancillary testing such as PCR or immunohistochemistry.[6]

The reproductive consequences of *T. gondii* infection are well-studied in domestic sheep, with one meta-analysis reporting detection of *T. gondii* using molecular methods in 42% of aborted sheep fetuses from 11 countries.[7] However, its impact on wild sheep populations is poorly understood. Only one case of confirmed toxoplasmosis in a bighorn sheep has been published to date.[1]

Contributing Institution:
Washington Animal Disease Diagnostic Laboratory, Washington State University (https://waddl.vetmed.wsu.edu/)

JPC Diagnoses:
Placenta: Placentitis, necrotizing, subacute, multifocal to coalescing, moderate, with intratrophoblastic and intrahistiocytic apicomplexan zoites.

JPC Comment:
This year's 12th conference was moderated by the JPC's own MAJ Anna-Maria Travis, an enthusiast of reproductive pathology, who led participants through a "Call the Midwife"-themed conference, complete with a fancy English tea party. As participants donned bowties and/or fasteners, sipped hot tea out of fine bone china, and enjoyed scones with homemade clotted cream, this first case demonstrated a classic entity that is an absolute "must-know" for any diagnostic pathologist.

The contributor's comment gives a great overview of the life cycle of *Toxoplasma gondii*, which participants were asked to recount during conference. In many species, *Toxoplasma gondii* is known to cause disseminated disease, infections of the CNS (leukoencephalomalacia), and abortions (except in cattle). It is considered an economically important cause of abortions in sheep and goats, especially in late pregnancy, and generally results in classic gross lesions in the placental cotyledons. Affected cotyledons are bright red (in contrast to their normal deep purple color) and contain numerous 1-3mm white flecks/foci of necrosis and mineralization. The intercotyledonary chorioallantois is usually spared but may be edematous.

Fetal leukoencephalomalacia has been reported in fetal lambs infected with

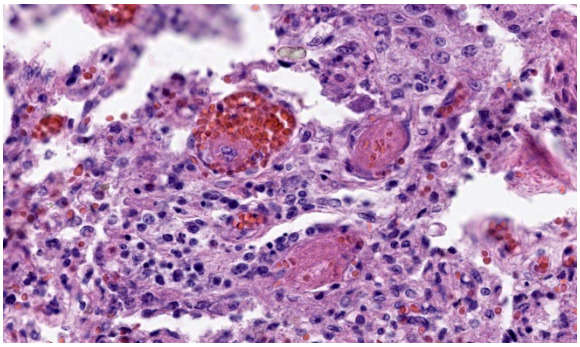

Figure 1-3: Placenta, lamb. Placental vessels occasionally are occluded by fibrin thrombi. (HE, 229X)

Toxoplasma gondii.[4] In fetuses with leukomalacia, histologic loss of oligodendrocytes and increased numbers of both astrocytes and microglia in areas of necrosis and the immediately-surrounding neuropil are common findings.[4] These lesions are similar to those seen in sheep used experimentally for inflammation syndrome and hypoxic models of periventricular leukomalacia in humans. It has been hypothesized that a fetal inflammatory syndrome resulting in hypoxia of the CNS may be involved in the pathogenesis of early abortion in ovine toxoplasmosis.[4]

Ewes do not typically show any signs of infection, and the effects on the fetus depend on the stage of gestation. In early gestation, fetal death with resorption or mummification is common. In mid-gestation, there can either be fetal death with resorption/mummification or there may be stillborn lambs. Occasionally, a fetus infected during this time frame may survive to term, but they are usually weak and do not survive long. In late gestation, the fetus will develop an immune response and may survive.

Important differentials to consider in ovine placentitis were included in conference discussion and covered: *Neospora caninum*, which causes similar lesions in the aborted placenta to *T. gondii* and requires PCR to differentiate; *Chlamydia abortus*, which causes a necrotizing placentitis with vasculitis that, in

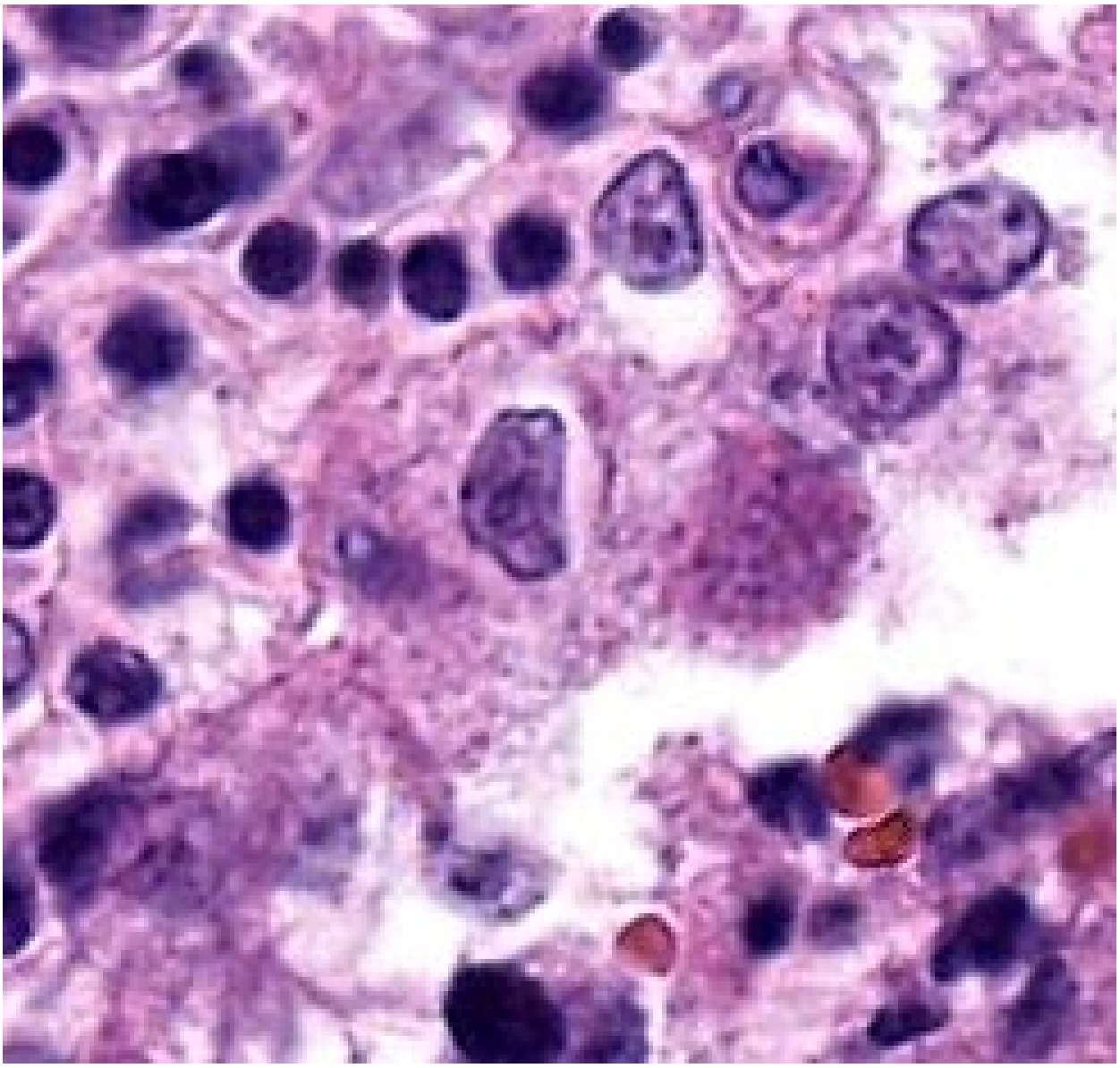

Figure 1-4: Placenta, lamb. Trophoblasts occasionally contain a 12-15um apicomplexan schizont consistent with Toxoplasma gondii (HE, 520X)

contrast to *T. gondii*, will affect the intercotyledonary areas and produce a leathery thickening of the placenta; *Coxiella burnetti*, which will cause similar lesions to *Chlamydia abortus*, but without vasculitis; *Brucella ovis*, which produces a thick, brown exudate that covers the chorionic surface of the placenta, causes leathery thickening of the intercotyledonary spaces, and is associated with vasculitis; *Campylobacter fetus*, which results in relatively non-specific edematous changes to the fetus, an exudative placentitis, and characteristic targetoid hepatic necrosis of the fetal liver; and, lastly, *Listeria monocytogenes*, which is associated with intratrophoblastic gram-positive bacilli and a necrotizing and suppurative placentitis of both the cotyledons and the intercotyledonary spaces.

Wrapping up this case's discussion, MAJ Travis touched on a few helpful tips regarding infectious ruminant abortions that pathologists should keep in mind when evaluating these

cases. These pointers can be summarized as follows: Bacterial and mycotic infections generally compromise the placenta and deprive the fetus of oxygen/nutrients, resulting in fetal death from placental insufficiency rather than direct infection of the fetus. Viral infections, however, tend to move right on through the placenta without causing it too much damage, but will go on to infect and kill the fetus via virus-induced organ damage. In general, mares get bacterial and mycotic placentitis via cervical infection, whereas cows get bacterial or mycotic placentitis via hematogenous spread. Finally, the most important organs to collect when working up an abortion case are the placenta, abomasum or stomach fluid, liver, lung, kidney, brain, eyelid (surface and palpebral conjunctiva) from bovine fetuses especially, and serum from the dam at the time of the abortion followed by another serum sample two weeks later to check for any rise in titers.

References:

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CASE II:

Signalment:
9-year-old FS Chihuahua (dog, *Canis lupus familiaris)*

History:
This dog was previously diagnosed with an intermediate grade mammary carcinoma.

Gross Pathology:
This was a 2 x 1.5 x 1.5 cm, tan, firm, subcutaneous mass from the left axilla. It was not overtly associated with a mammary gland.

Laboratory Results:
N/A

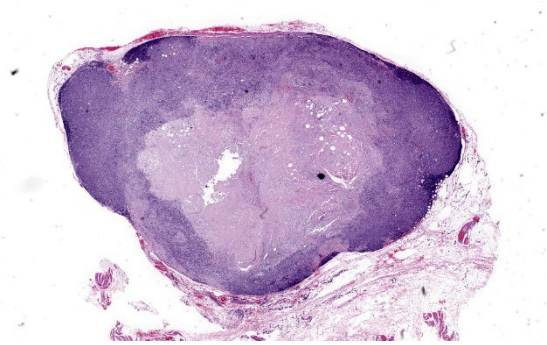

Figure 2-1: Lymph node, dog. A lymph node is effaced by a neoplasm – remnant lymph node is present beneath the capsular surface. Approximately 33% of the neoplasm is necrotic. (HE, 17X)

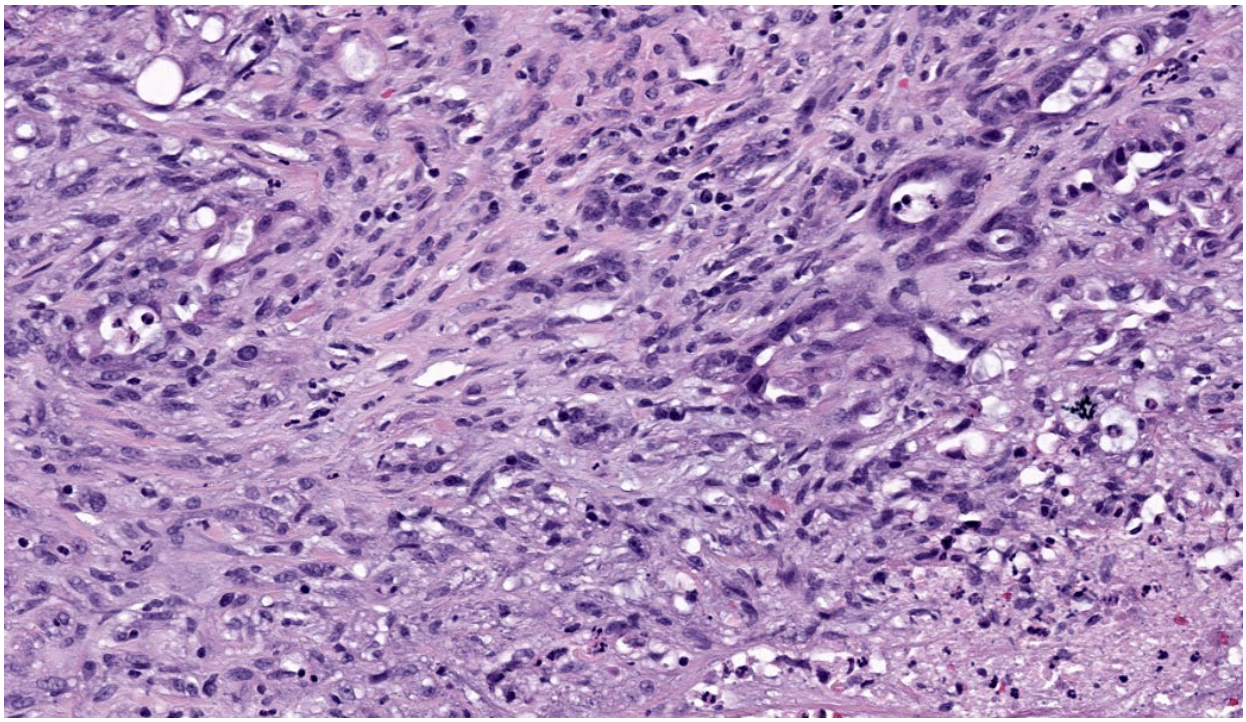

Figure 2-2: Lymph node, dog. There are two cell types within the neoplasm. The predominant cell type is myoepithelium arranged in long streams and bundles, and scattered throughout are cuboidal epithelium forming glands. (HE, 458X)

Microscopic Description:

Axillary mass: Examined are two sections of haired skin and mammary tissue containing an encapsulated structure rimmed by lymphocytes (lymph node, presumptive) that is 95% effaced by a large neoplasm composed of two cell populations. The first, and most numerous, population consists of spindle-shaped cells arranged in haphazard bundles and whorls immersed in a pale basophilic matrix and supported by a fine fibrovascular stroma. These cells have indistinct borders and a moderate amount of wispy to vacuolated pale basophilic cytoplasm. Nuclei are ovoid to elongate with evenly dispersed chromatin and 1-2 indistinct nucleoli. Anisokaryosis is moderate. The second population consists of polygonal cells arranged in tubules. This population has more abundant eosinophilic cytoplasm, but the nuclear morphology is nearly identical to the first population. Mitoses are 34 in 2.37 mm^2, including bizarre mitoses. There is extensive central necrosis of the mass, which abuts the surgical margin of the submitted tissues.

Immunohistochemistry results: The polygonal cell population exhibits strong, diffuse membranous and cytoplasmic positivity for MNF116 (pancytokeratin). This population is negative for smooth muscle actin (SMA), consistent with epithelial cells. The spindle cell population exhibits moderate to strong, predominantly membranous positivity for MNF116, as well as moderate to strong membranous and cytoplasmic positivity for SMA, consistent with myoepithelial cells. There is a smaller group of streaming spindle cells that are negative for MNF116 and positive for SMA, consistent with myofibroblasts (desmoplastic response). Controls stain appropriately.

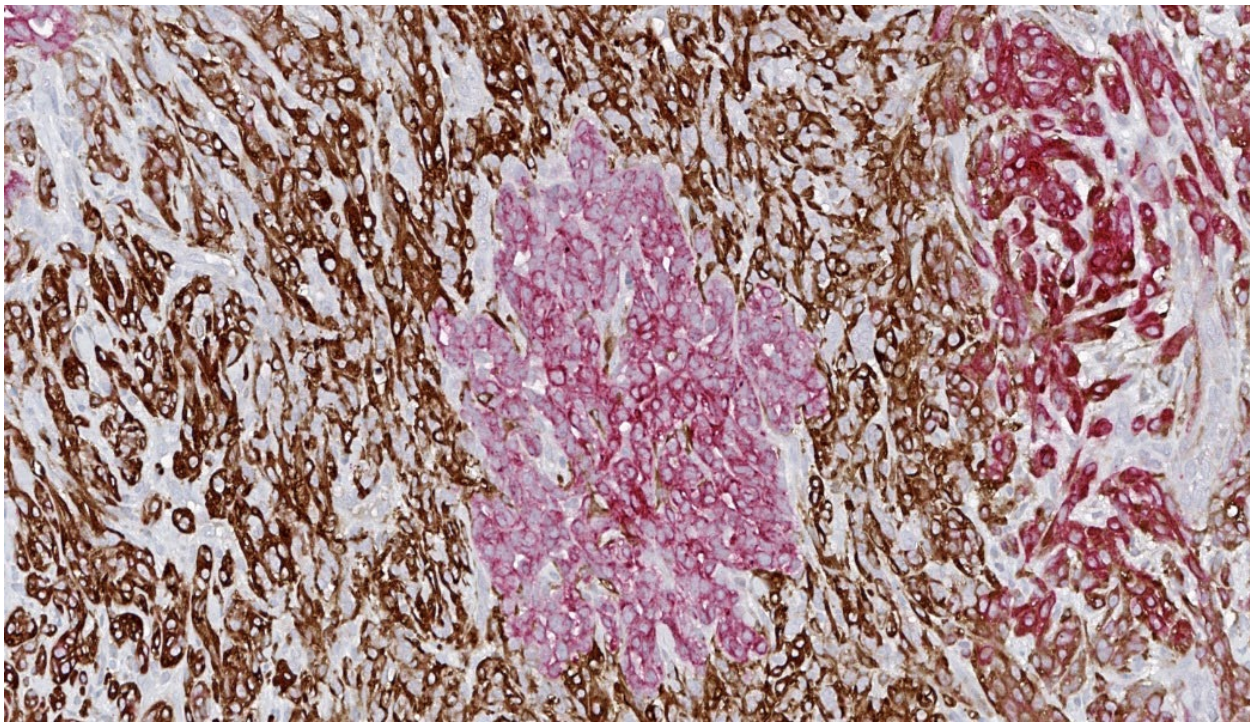

Figure 2-3: Lymph node dog. Neoplastic epithelial cells exhibit strong cytoplasmic positivity for cytokeratin (brown chromogen), and neoplastic myoepithelial cells exhibit moderate cytoplasmic positivity for smooth muscle myosin (red chromogen). (anti AE1/AE3 and SMMH, 400X).

Contributor's Morphologic Diagnoses:
Axillary mass: Mammary carcinoma and malignant myoepithelioma, grade II

Contributor's Comment:
The diagnosis of canine carcinoma and malignant myoepithelioma (CAMM) was first introduced in the review article *Classification and Grading of Canine Mammary Tumors* (2011) by M. Goldschmidt, et. al.[2] In previous classification systems for canine mammary tumors, any tumor containing both luminal epithelial and myoepithelial cells was considered either a complex tumor (without a mesenchymal component) or a mixed tumor (with a mesenchymal component), without differentiating between tumors whose myoepithelial population was benign and those with a malignant myoepithelial component.[3,4] Recognizing CAMM as a separate entity in the 2011 classification system required two major changes in our approach to mammary tumors. First, the use of immunohistochemistry (IHC) to accurately characterize and classify mammary tumors became widespread enough that consistently accurate classification of mammary tumors was possible, allowing the behavior of individual tumor types to be studied more closely.[2] Secondly, our definition of complex mammary carcinomas evolved to only include those tumors with a *benign* myoepithelial component, in recognition of the fact that mammary tumors in which both the epithelial and myoepithelial populations are malignant might behave differently than those in which the myoepithelial population is benign.[2-4] In fact, a follow-up 2-year prospective study of 229 dogs showed that dogs with CAMM were more than 10 times more likely to experience tumor-related death than dogs with complex carcinoma, validating the decision to separate these two entities.[5]

The myoepithelial cell population in canine complex mammary tumors is positive for

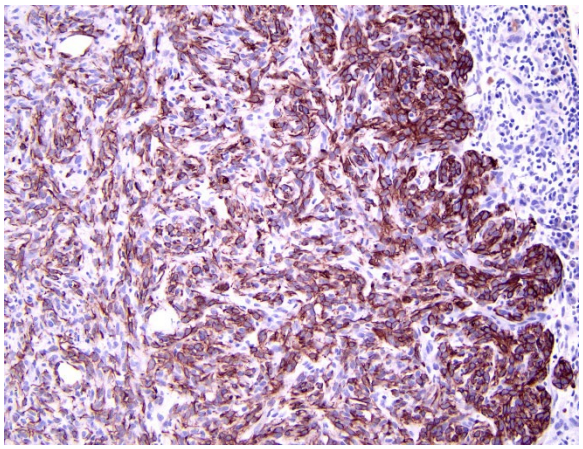

Figure 2-4: Lymph node, dog. Myoepithelial cells exhibit strong membranous positivity for MNF116. (anti- MNF116, 200X) (Photo courtesy of: Veterinary Medical Diagnostic Laboratory, University of Missouri, https://vmdl.missouri.edu/)

smooth muscle actin (SMA), calponin, vimentin, p63, and high molecular weight basal cytokeratins CK5, CK6, and CK14, while the epithelial population expresses luminal cytokeratins CK8, CK18, and CK19.[8] Interestingly, the myoepithelial population in CAMM has higher expression of CK5, CK14, SMA, calponin, and p63 (characterized as an "intermediary" or less well-differentiated phenotype), compared to the myoepithelial population in complex carcinoma (which is more similar to that of terminally differentiated myoepithelial cells).[6] CAMM myoepithelial cells also have significantly higher Ki67 expression than those in complex carcinoma, in accordance with their more aggressive biological behavior.[6] Conversely, the benign myoepithelial population in canine complex mammary carcinomas has been theorized to play a tumor suppressor role, accounting for the better prognosis of complex carcinoma when compared to simple carcinomas that have no myoepithelial component.[1,7] However, if this is true, the tumor suppressor activity appears to be a feature specific to terminally differentiated myoepithelial cells, rather than the malignant, intermediary-like myoepithelial population present in CAMM.[6]

The present neoplasm most likely represents metastasis of a primary mammary carcinoma to the axillary lymph node, which is supported by the patient history, anatomic location of the mass, and the presence of a compressed population of mature lymphocytes enclosed within a thin fibrous capsule, consistent with remnant nodal architecture. However, given the presence of mammary tissue in the adjacent subcutis, the possibility remains that this is a primary tumor arising from mammary tissue in an unusual location (i.e., the axilla). Either way, the morphologic features of the myoepithelial population (pleomorphism, anisokaryosis, and a high mitotic rate, including bizarre mitoses) are consistent with malignancy, supporting a diagnosis of CAMM over complex mammary carcinoma, in which the myoepithelial component is uniform with minimal anisocytosis and anisokaryosis.[2]

Definitive diagnosis of CAMM requires IHC to confirm the presence of both a myoepithelial and an epithelial component.[2] The present mass has three subpopulations of cells with differing immunophenotypes. The first is the luminal epithelial cell population, which is positive for MNF116 and negative for SMA.

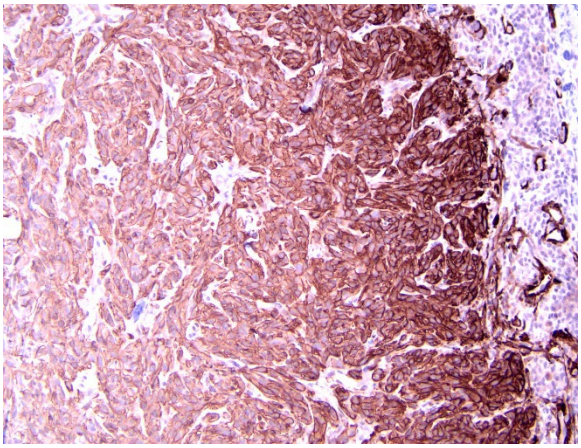

Figure 2-5: Lymph node, dog. Myoepithelial cells exhibit strong cytoplasmic positivity for smooth muscle actin. (anti- SMA, 200X) (Photo courtesy of: Veterinary Medical Diagnostic Laboratory, University of Missouri, https://vmdl.missouri.edu/)

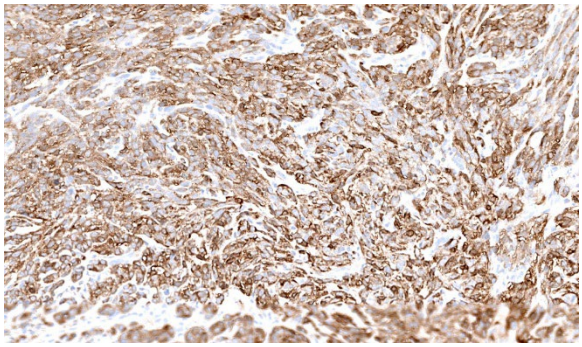

Figure 2-6: Lymph node, dog. Myoepithelial cells exhibit strong cytoplasmic positivity for calponin. (anti- calponin, 350X).

MNF116 is an antibody that reacts with cytokeratins 5, 6, 8, 17, and possibly 19; thus, it is expected to stain both luminal epithelial cells and myoepithelial cells. The second group of cells, the myoepithelial population, is positive for both MNF116 and SMA, as expected. However, there is a third population, which is composed of streaming spindle cells that are positive for SMA but not MNF116. These cells are consistent with a desmoplastic response characterized by myofibroblast proliferation, which is a feature shared with highly aggressive mammary carcinomas such as anaplastic carcinoma.[2]

Contributing Institution:
Veterinary Medical Diagnostic Laboratory
University of Missouri
https://vmdl.missouri.edu/

JPC Diagnoses:
Lymph node: Malignant epithelial and spindle cell neoplasm, metastatic.

JPC Comment:
This second case stimulated lively discussion amongst participants regarding both anatomic location and diagnosis. Some participants were successfully able to get to lymph node, while others hedged a bit more and felt comfortable with calling it subcutaneous tissue with a particularly immunogenic neoplasm. Either way, it was a tough call, but the presence of a capsule and subcapsular sinuses are what helped push participants off the fence and onto Team Lymph Node.

MAJ Travis led participants through a "Mammary Neoplasms 101" conversation, covering the major classifications of mammary tumors. These include ductular, simple (involve just neoplastic epithelium), complex (epithelium and myoepithelium), mixed (Epithelium, myoepithelium, and either bone, cartilage, and/or adipose tissue), and myoepithelial. Within each of these are subcategories, but those five main classifications are a "must-know" starting place when working up mammary neoplasms as a pathologist. Current criteria for histologic grading of canine mammary neoplasms includes evaluation of percentage of tubule formation, nuclear pleomorphism, and mitotic count.[2]

Dogs have the highest incidence of mammary neoplasms of any domestic species.[2] Luckily for dogs, the majority of these tumors are clinically benign, which contrasts with most other domestic species that have mostly malignant mammary neoplasms. In dogs, these are typically progressive, and development of neoplasia can proceed from ductular or lobular hyperplasia to dysplasia and onwards to neoplasia. Even within benign adenomas, there can be progression to non-invasive adenocarcinoma and further progression to metastatic mammary adenocarcinoma if these neoplasms are left to grow on the affected dog. Mammary sarcomas in dogs are much less common than epithelial neoplasms but are significantly more aggressive and metastatic when they do occur. Additionally, if a dog develops one mammary neoplasm, it is common for multiple others to develop over time. The prognosis for each mass may vary greatly, and different types of mammary

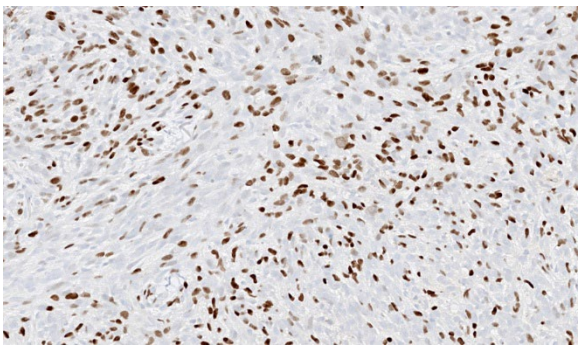

Figure 2-7: Lymph node, dog. 66% of myoepithelial cells exhibit strong nuclear positivity for P-63. (anti-P63, 350X).

tumors can be found in the mammary glands of an individual animal.[1,2]

On to the difficulty in providing an appropriate morphologic diagnosis. When deciding how to morphologically diagnose WSC cases, the JPC tries to provide a diagnosis that would be achievable from the H&E slide alone (as this is what is available to participants around the world before each conference). As such, and after much deliberation, participants ultimately decided on a diagnosis of metastatic malignant epithelial and spindle cell neoplasm, as there were no exclusively defining features of a mammary-specific neoplasm on the H&E slide. All agreed, however, that the IHC profile of this neoplasm is supportive of the contributor's diagnosis of canine mammary carcinoma and malignant myoepithelioma (CAMM), especially given the immunoreactivity of the neoplastic cells for calponin and the spindle cells to both p63 and smooth muscle myosin-heavy chain, which is more specific for myoepithelium than just smooth muscle actin.

Following these spirited discussions, Dr. Bruce Williams deployed some well-timed humor by asking attendees, "Having never been to a tea party before, is this what people *normally* talk about?" Probably not, Dr. Williams, probably not.

References:

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CASE III:

Signalment:
2 year intact female cynomolgus macaque (*Macaca fascicularis*)

History:
A 2-year-old female cynomolgus macaque in a vehicle control group of a 3-month safety assessment study was necropsied per standard GLP procedures at the end of the study timeline.

Gross Pathology:
No gross lesions were noted at the time of necropsy.

Laboratory Results:
N/A

Microscopic Description:
Uterus: Regionally effacing the endometrium, displacing and disrupting the normal glandular and stromal architecture, is a poorly circumscribed, nonencapsulated, infiltrative proliferation of highly pleomorphic round to polygonal neoplastic cells arranged in sheets. The neoplastic proliferation is composed of a biphasic population of uninucleate or multinucleate cells with distinct cell borders, moderate to marked amounts of eosinophilic cytoplasm, and large round vesiculate to finely stippled nuclei and 1-4 variably prominent nucleoli, consistent with a trophoblastic (cytotrophoblasts and syncytiotrophoblasts, respectively) lineage. There are 2 mitotic figures in 10 high power fields (400x). Moderate numbers of lymphocytes and plasma cells are scattered throughout the adjacent endometrial stroma and outer myometrium. Dilated endometrial glands occasionally contain a variable amount of eosinophilic amorphous to basophilic flocculant debris and few macrophages or lymphocytes. Regional erosion of endometrial epithelium is present. The ovary lacks corpora lutea, consistent with sexual immaturity.

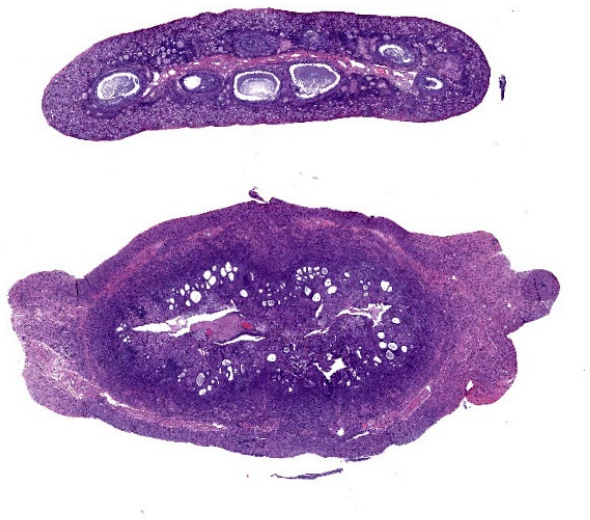

Figure 3-1: Uterus, cynomolgus monkey. A section of normal ovary (top) and uterus (bottom) are submitted for examination. At subgross magnification, there is multifocal dilation of endometrial glands. (HE, 10X)

Slides not provided:

Immunohistochemistry on the uterine neoplasm is performed using anti-human chorionic gonadotropin (hCG), anti-pancytokeratin AE1/AE3, anti-human placental lactogen (huPL), anti-p63, anti-CD10, anti-placental alkaline phosphatase (PLAPH), anti-progesterone receptor (PR), anti-estrogen receptor α (ERα), and anti-Ki67 antibodies and is visualized using 3,3′-diaminobenzidine or Fast Red as a chromogen. Occasional neoplastic cells with high degrees of atypia express cytoplasmic labeling for hCG. Neoplastic cells have strong cytoplasmic and membranous labeling for pancytokeratin AE1/AE3. Labeling for huPL and CD10 demonstrates rare neoplastic cells expressing moderate to strong cytoplasmic immunolabeling; however, the majority of neoplastic cells are negative. Few neoplastic cells have weak nuclear p63 labeling. Labeling for Ki67 demonstrates moderate to

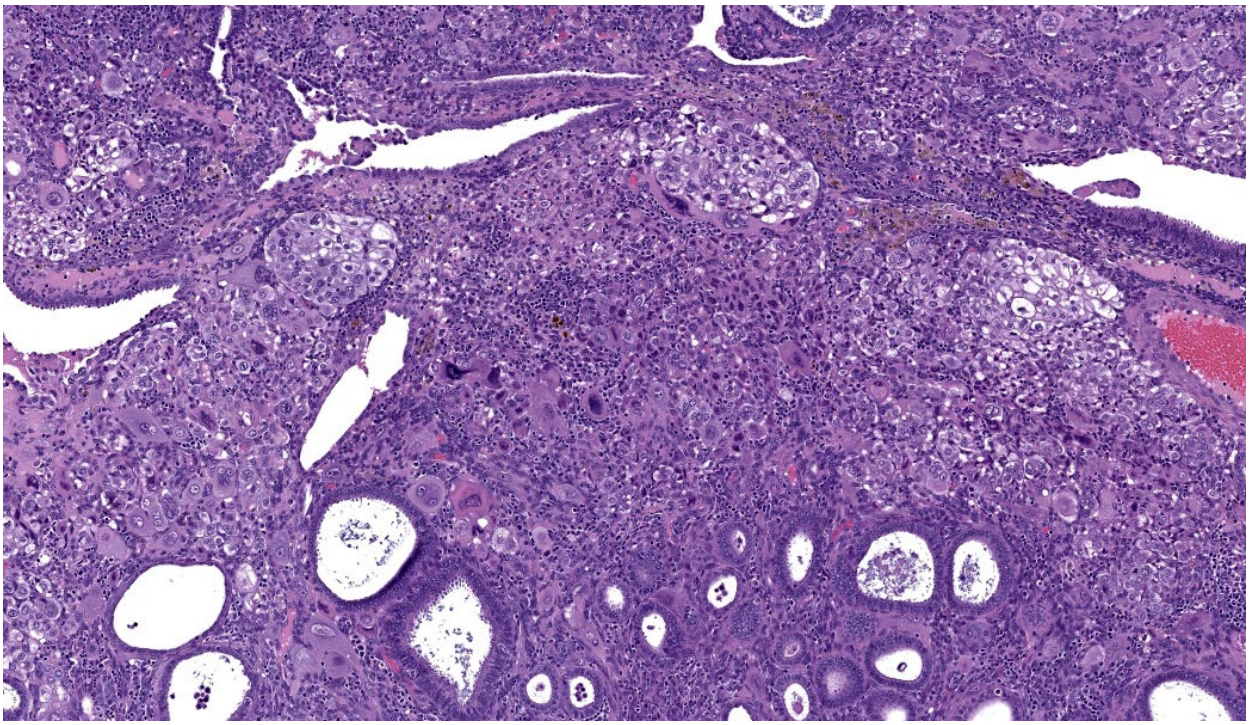

Figure 3-2: Uterus, cynomolgus monkey. Expanding the endometrium, there is a multilobular neoplasm which is composed of nests of uninucleate cells resembling trophoblasts and multinucleated cells resembling syncytiotrophoblasts. There are also numerous lymphocytes infiltrating this tissue. (HE, 150X)

high numbers of proliferative neoplastic cells regionally. Overall, PR and ERα expression is limited due to the immature age of the animal, but is present in the expected locations in the endometrial stroma and glandular epithelium, and absent in the neoplastic population. Neoplastic cells do not label for PLAPH. These immunohistochemical findings further support a diagnosis of choriocarcinoma.

Contributor's Morphologic Diagnoses:
Uterus: Choriocarcinoma

Contributor's Comment:
Choriocarcinoma is a neoplasm of trophoblastic lineage that most often arises in women following normal or abnormal pregnancies.[1] Diagnostic features include highly pleomorphic, polygonal to round cells with abundant cytoplasm that are both uninucleate (cytotrophoblast or intermediate trophoblast-like) and multinucleate (syncytiotrophoblast) cells, and often a high mitotic rate with metastasis. Morphologic features on histologic evaluation of hematoxylin and eosin-stained uterus from this sexually immature cynomolgus macaque were consistent with choriocarcinoma.

In human medicine, choriocarcinoma falls under the umbrella entity of gestational trophoblastic disease. Other neoplasms in this entity include placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT). PSTTs are composed of a monomorphic population of large, pleomorphic cells derived from implantation-type intermediate trophoblasts. Most cells are uninucleate, but it is not uncommon to see occasional scattered multinucleate cells. ETTs arise from chorionic-type intermediate trophoblasts and present as nests, cords, or solid masses with a monomorphic population of small, round cells.[1] Choriocarcinomas tend to have a distinct morphology compared to PSTT and ETT with the presence of uninucleate and multinu-

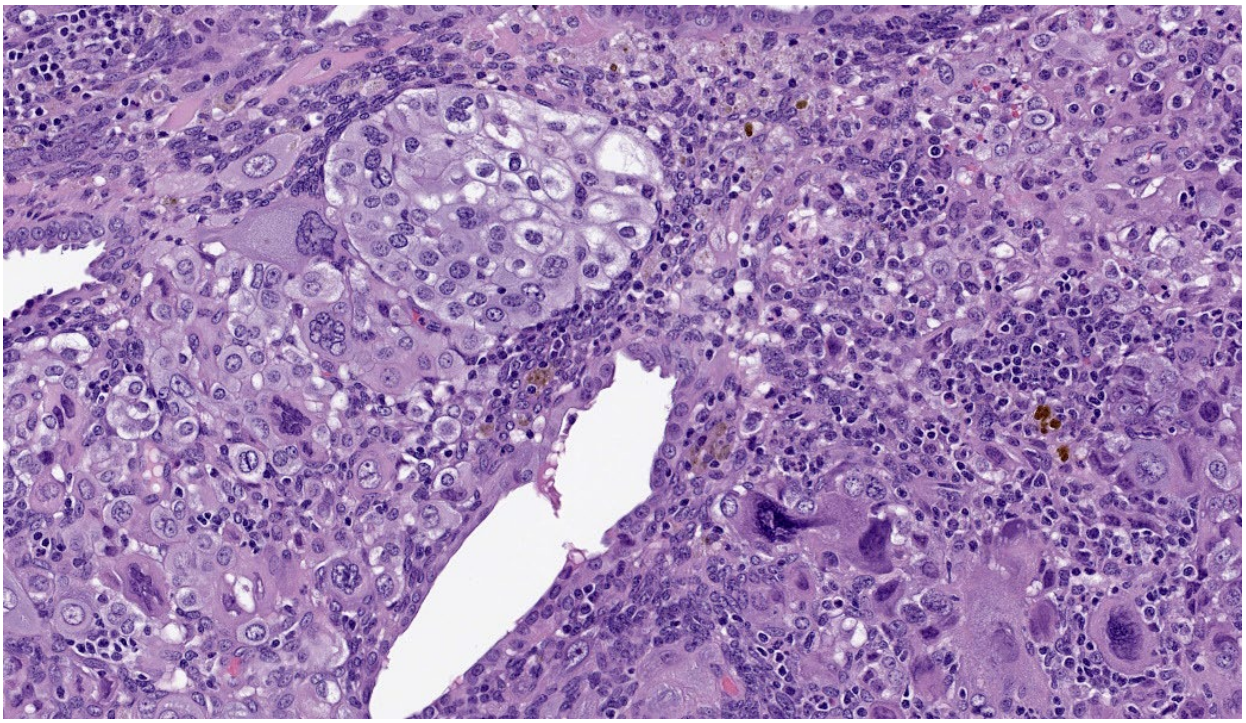

Figure 3-3: Uterus, cynomolgus monkey. Higher magnification of nests of uninucleate neoplastic cells resembling trophoblasts, and fewer multinucleated neoplastic cells resembling syncytiotrophoblasts. There are rare hemosiderin-laden macrophages scattered throughout. (HE, 381X)

cleate cells; however, a panel of immunohistochemical markers is commonly used to confirm diagnosis. Characterization with antibodies against hCG, pancytokeratin AE1/AE3, huPL, p63, CD10, PLAPH, and Ki67 provides further support for a diagnosis of choriocarcinoma. These immunohistochemical markers are helpful in distinguishing between PSTT, ETT, and choriocarcinoma (see table).[5]

Marker	PSTT	ETT	Chori-ocarci-noma	Lesion
hCG	+/-	+/-	+	+
huPL	+	+/-	+/-	Rare
p63	-	+	+/-	Rare weak
Pancyt-okeratin AE1/AE3	+	+	+	+
CD10	+/-	-	+/-	Rare
PLAPH	-	-	-	-
Inhibin-α	+	+	+	Not per-formed
Ki67 In-dex	Mod-erate	Mod-erate	High	High

This neoplasm has been reported in laboratory and domestic species and is noted in the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) for non-proliferative and proliferative lesions in non-human primates (NHP), mice, and rabbits.[2-4] In women, non-gestational choriocarcinoma can occasionally arise in the ovary and have only rarely been reported as a primary tumor in the uterus.[7] While non-gestational choriocarcinoma of the ovary has been reported in NHP, there are no published examples of a primary non-gestational choriocarcinoma of the uterus.

Spontaneous neoplasms in nonrodent species are rare in routine toxicology work due to the young age of animals used in nonclinical toxicity testing. Knowledge of spontaneous background lesions is necessary to distinguish from potential test article-related effects in any species.

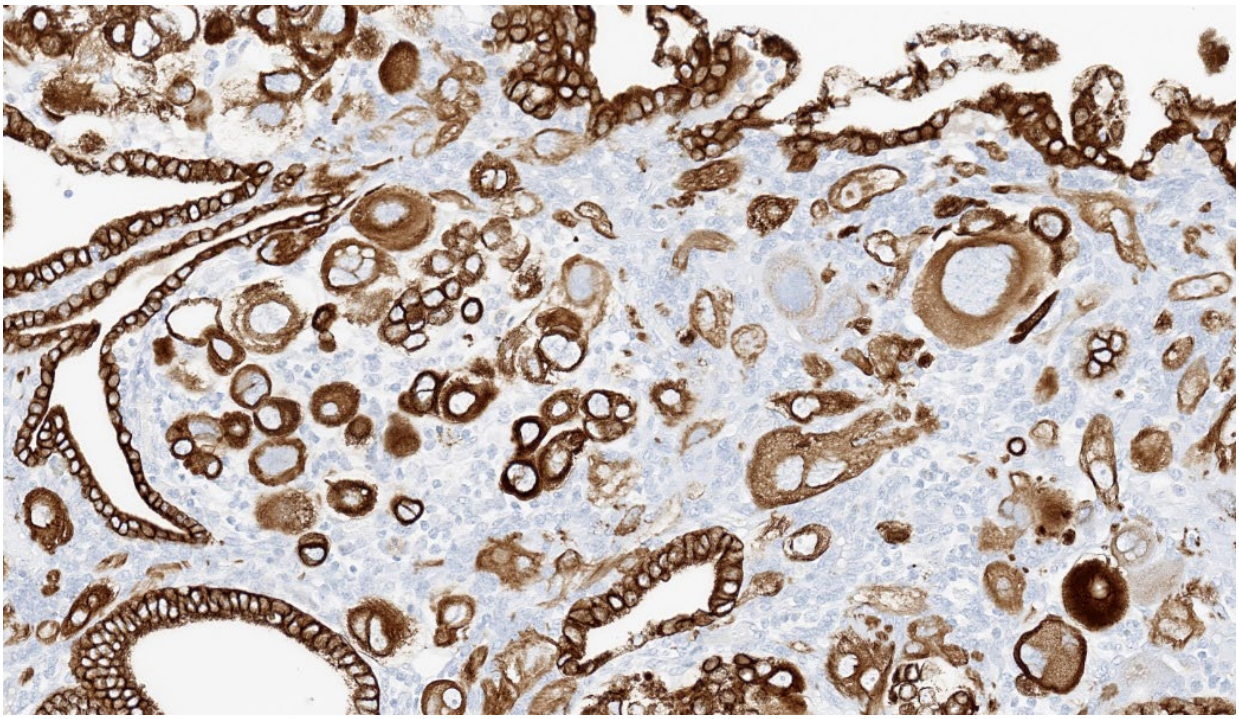

Figure 3-4: Uterus, cynomolgus monkey. The neoplastic cells (both uni- and multinucleated) demonstrate strong cytoplasmic immunopositivity. (anti-AE1/AE3, 381X)

Contributing Institution:
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JPC Diagnoses:
Uterus: Choriocarcinoma.

JPC Comment:
The contributor of this case provides a great overview of choriocarcinomas, their main differentials, and the common immunohistochemical profiles expected with these neoplasms in their comment. These topics covered much of what was discussed in conference for this case.

The topic of the IHCs prompted a review of what each of the pertinent markers is for and what some of the IHC targets do physiologically. For example, choriocarcinomas are readily immunoreactive for human chorionic gonadotropin (hCG). In the body, hCG (or any of the species-specific CGs, for that matter), is produced by the placental trophoblasts (the cell of origin in choriocarcinoma) and stimulates the release of progesterone from the corpus luteum (CL) to maintain the pregnancy. Human placental lactogen (HPL) also functions as a marker for trophoblasts and is released by the placenta to, among other functions, stimulate growth of the mammary glands for lactation. CD10 marks endometrial stroma. Inhibin, released from granulosa cells (GC) to regulate production of follicle-stimulating hormone (FSH), will be immunoreactive in granulosa cells (including in some neoplastic GC populations). Although choriocarcinoma is classified as a germ cell tumor, it is weakly to not at all immunoreactive for placental-like alkaline phosphatase (PLAP), which is a marker of germ cells. This is thought to be due to the neoplastic choriocarcinoma cells being less well-differentiated or potentially even de-differentiated compared to regular syncytiotrophoblasts, which are usually immunoreactive for PLAP.[6] Lastly, p63, a member of the p53 "Guardian of the Genome" family of genes, will be immunoreactive in

myoepithelial cells, squamous epithelial cells, and a few others. The table provided by the contributor provides an excellent resource for cross-comparison of IHC profiles between these in choriocarcinomas and their differentials.[5]

Choriocarcinomas are categorized as gestational (associated with pregnancy) or non-gestational (formed in the absence of pregnancy). Some participants were astutely able to deduce from the types of follicles found in the accompanying ovary on the slide that this was very likely a juvenile animal. This, coupled with the lack of gestational change to the uterus, led some to make the jump to a non-gestational choriocarcinoma. Kudos are due to the participants that managed to reason through those subtleties! As mentioned in the contributor's comment, there are no published examples of primary non-gestational uterine choriocarcinoma in non-human primates.

While most participants were readily able to reach the diagnosis of "choriocarcinoma" from the H&E section in the absence of any immunomarkers,there was quite a bit of discussion on if this neoplasm would truly classify as a choriocarcinoma or if it would be considered "choriocarcinomatous differentiation" due to the young age of the animal. This is a term occasionally utilized in research settings, usually in the context of early choriocarcinoma-like changes seen with decidual reactions in NHPs. Although there was no evidence of decidual reaction in this case, we consulted with our JPC MD reproductive subspecialists, who agreed with the diagnosis of choriocarcinoma. The term "choriocarcinomatous change/differentiation" is not utilized in human medicine and is not listed in the NHP INHAND guide for terminology related to this neoplasm.[3] As such, the JPC refrains from the usage of this term.

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CASE IV:

Signalment:

2-years-old, female, rabbit, *Oryctolagus cuniculus domesticus*

History:

This rabbit was taken to the veterinary hospital with the main complaint of bleeding from the genitals. Because uterine bleeding was sus

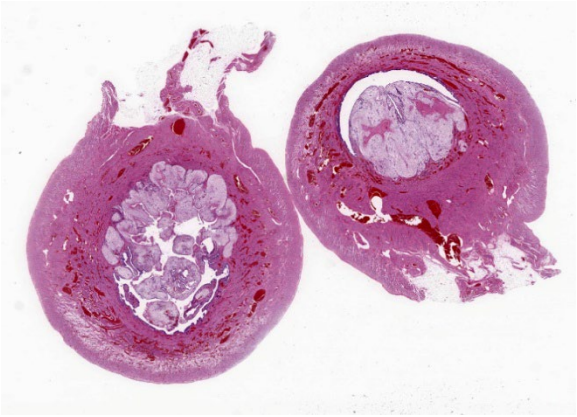

Figure 4-1: Uterus, rabbit. Two sections of the uterus demonstrate a polypoid multinodular thickening of the uterine mucosa predominantly on the mesometrial side. The endometrium in this area exhibits profound pallor. (HE, 12X)

pected, a total hysterectomy and oophorectomy were performed. A mass-like lesion was identified in the right uterine horn.

Gross Pathology:
The midbody of the right uterine horn had a circular lump (0.6 cm in diameter). The cut section of this lump appeared white and solid.

Laboratory Results:
N/A

Microscopic Description:
The endometrium on the mesometrial side of the uterus proliferated with the formation of large nodules and numerous polyps. The surface of the nodules and polyps was covered by endometrial epithelium, however, approximately 50% of the epithelium were eroded. The polyps and nodule contained numerous blood vessels with normal endothelium, along with cells that resembled large-vacuolated decidual cells, arranged in sheets. The decidual cells had distinct cell boundaries, a round shape, abundant transparent cytoplasm, and nuclei ranging from oval to irregular shapes. Mitotic figures were also occasionally observed. Just beneath the endometrial epithelium, the decidual cells exhibited a spindle-shaped morphology. (Fig.1 and 2)On the antimesometrial side of the uterine, endometrial hyperplasia was mild, decidual cells in the endometrial stroma were sparse, and proliferation of huge giant cells resembling trophoblast was prominent. The huge giant cells displayed a variety of morphologies, including round, spindle, to pleomorphic, with notably large, highly atypical nuclei and abundant cytoplasm. Multinucleated giant cells were also observed.

The immunohistochemical analysis demonstrated that normal endometrial epithelium is positive for progesterone receptor (PgR), keratin AE1/AE3, and CAM5.2, and negative for CD10, SMA and desmin. Normal endometrial stromal cell is positive for PgR and CD10, and negative for keratin AE1/AE3, CAM5.2, SMA and desmin. Decidual cell is positive for PgR and CD10, and negative for keratin AE1/AE3, CAM5.2, SMA and desmin. The huge giant cells exhibited positive for keratin AE1/AE3, CAM5.2 and CD10, and negative staining for PgR, SMA and desmin.

Contributor's Morphologic Diagnoses:
Uterus: Decidual reaction

Contributor's Comment:
Rabbits have a hemodichorial and bidiscoid type of placenta. Histologically, the placenta of rabbits is composed of the labyrinth zone, the junctional zone, the decidua zone, and the mesometrium.[3,6,7] In the labyrinth zone, there are two layers of trophoblasts, an outer and inner layer separating the maternal blood spaces from the fetal blood vessels. The outer trophectoderm is comprised of the syncytiotrophoblasts. The inner trophectoderm is one layer of cytotrophoblasts overlying fetal blood vessels. The junctional zone is composed of glycogen cells with eosinophilic cytoplasm

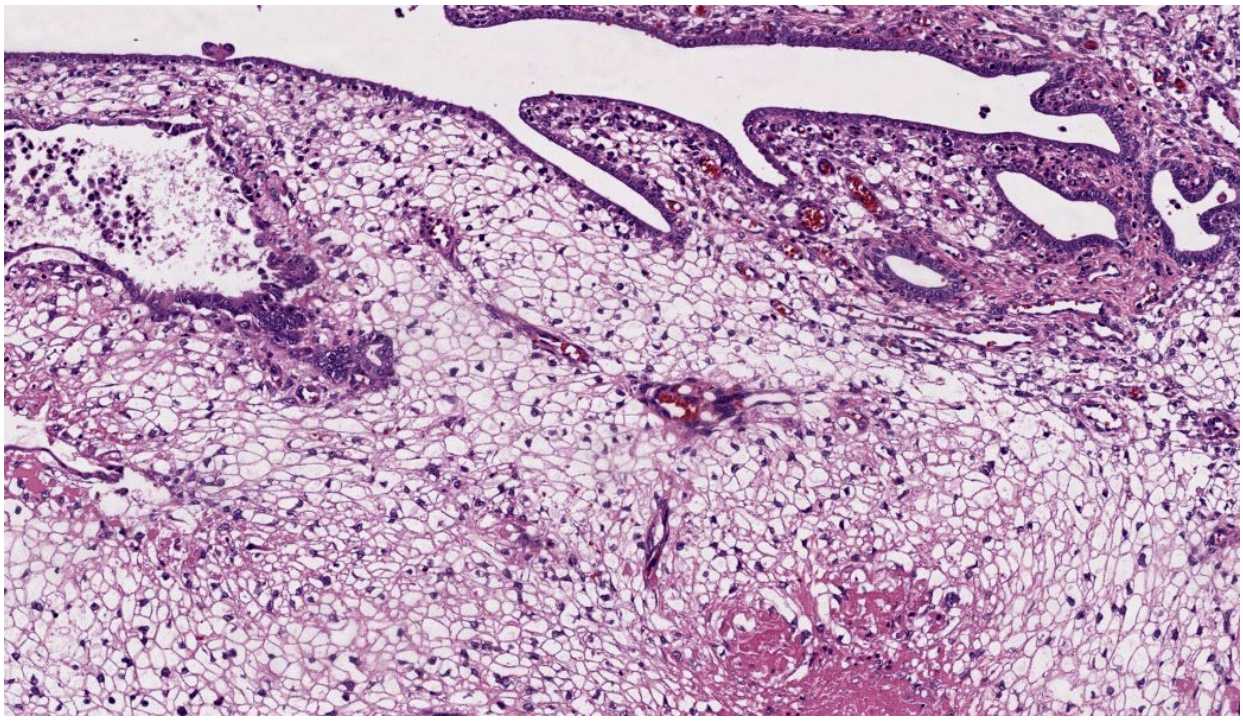

Figure 4-2: Uterus, rabbit. Endometrial stromal cells are swollen with clear cell change (decidual reaction) markedly separating tortuous endometrial glands. (HE, 230X)

containing PAS-positive substances and giant cells having often 2-3 number of nuclei. The decidua originates from stromal cells of the mesometrial endometrium. Before 11 days of pregnancy, it consists of proliferating spindle cells, subsequently, vacuolated cells known as decidual cells begin to appear. The proliferation of decidual cells continues, and from 13 days of pregnancy, necrosis of decidual cells begins to be observed. In mid-gestation, the decidual zone is divided into the zone of necrosis and the zone of separation.[3,6,7] The zone of necrosis develops with dilated blood vessels as pregnancy advances. This zone is detected under the junctional zone and is composed of necrotic tissue. The zone of separation, which is composed of the cells having large foamy cytoplasm, becomes thinner without necrosis as pregnancy advances.[3,6,7]
During pregnancy in rabbits, a characteristic feature is the presence of giant cells on the obplacental (antimesometrial) region. These giant cells are referred to as obplacental giant cells to distinguish them from certain cell populations of the definitive (chorioallantoic) placenta and decidua.[1] Immunocytochemistry shows that the giant cells are positive for cytokeratin and vimentin, but are negative for desmin and Factor VIII-related antigen. The cells are positive for cytokeratin from their inception, but only become vimentin-positive between Days 12 and 15 of pregnancy, a change seemingly related to their detachment from epithelial tissue to take on an independent existence.

The case was characterized by the proliferation of vacuolated cells in the endometrial stroma covered by normal endometrial epithelium. In the presence of an embryo and normal placental formation, trophoblasts attached to the outer surface of the endometrial epithelial cells.[3,6,7] However, in this case, the absence of trophoblast cell proliferation clearly indicates that normal placental formation has not occurred. It can thus be concluded that only vacuolated cells of maternal origin are

proliferating. The vacuolated cells exhibited a morphology similar to that of decidual cells during normal placental formation and frequently contained PAS-positive granules, further supporting this interpretation. Immunohistochemical staining revealed positivity for CD10, negativity for keratin and positivity for progesterone receptors. These findings are consistent with the staining pattern of endometrial stromal cells and provide further confirmation of the origin of these cells from the endometrial stroma. Sensitization by progesterone is necessary for the initiation of decidualization of the endometrial stroma, and stable progesterone activity is necessary for the stable existence of decidual cells.[2] In this case, the expression of progesterone in both the endometrial stroma and epithelium indicates that a decidual reaction has occurred overall.

In contrast, numerous giant cells were observed on the obplacental (antimesometrial) region. These giant cells are similar to obplacental giant cells formed in the pregnant uterus of rabbits with regard to both cell morphology and location. [1] The origin of these cells remains unclear. The absence of obvious trophoblasts and the formation of giant cells in the endometrial stroma beneath the normal endometrial epithelium suggest a uterine origin. However, immunostaining revealed positivity for CK and CAM5.2 (epithelial marker) and CD10 (positive for endometrial stromal cell), and negative for PgR (positive for both endometrial epithelial and stromal cell), which did not correspond with the staining patterns of endometrial epithelial or stromal cells. Consequently, it was not possible to ascertain their origin with any degree of certainty.

The decidual reaction is a well-documented phenomenon in rats, which can be categorized into two distinct types. The first type is characterized by the formation of nodules in the uterine cavity, exhibiting a high degree of structural organization and regional variation.

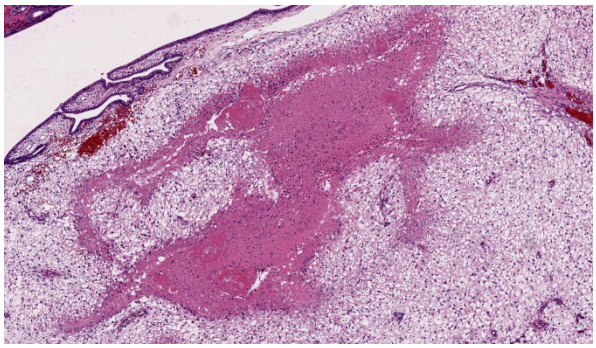

Figure 4-3: Uterus, rabbit. There are geographic areas of necrosis within the decidualized endometrial stroma. (HE, 103X)

The second type is referred to as focal decidualization, which is marked by the proliferation of decidual cells within a specific area of the endometrial stroma. Decidual cells in rats are large cells with oval nuclei and eosinophilic cytoplasm, characterized by PAS-positive small vacuolated cytoplasm. In the present case, although the lesions in rabbits corresponded to the focal decidualization observed in rats due to the absence of clear organization, the morphology of decidual cells differed, with rabbits exhibiting distinct vacuolated cells. The substantial disparities in placental formation among different animal species imply that the morphology of decidual reactions also exhibits considerable variation.

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JPC Diagnoses:

Uterus, endometrial stroma: Decidual reaction, subacute, focally extensive, severe, with trophoblastic giant cells.

JPC Comment:

This final case provided a not-oft seen entity in diagnostic pathology, but one that is frequently encountered in research animals. Many thanks to the contributor for providing

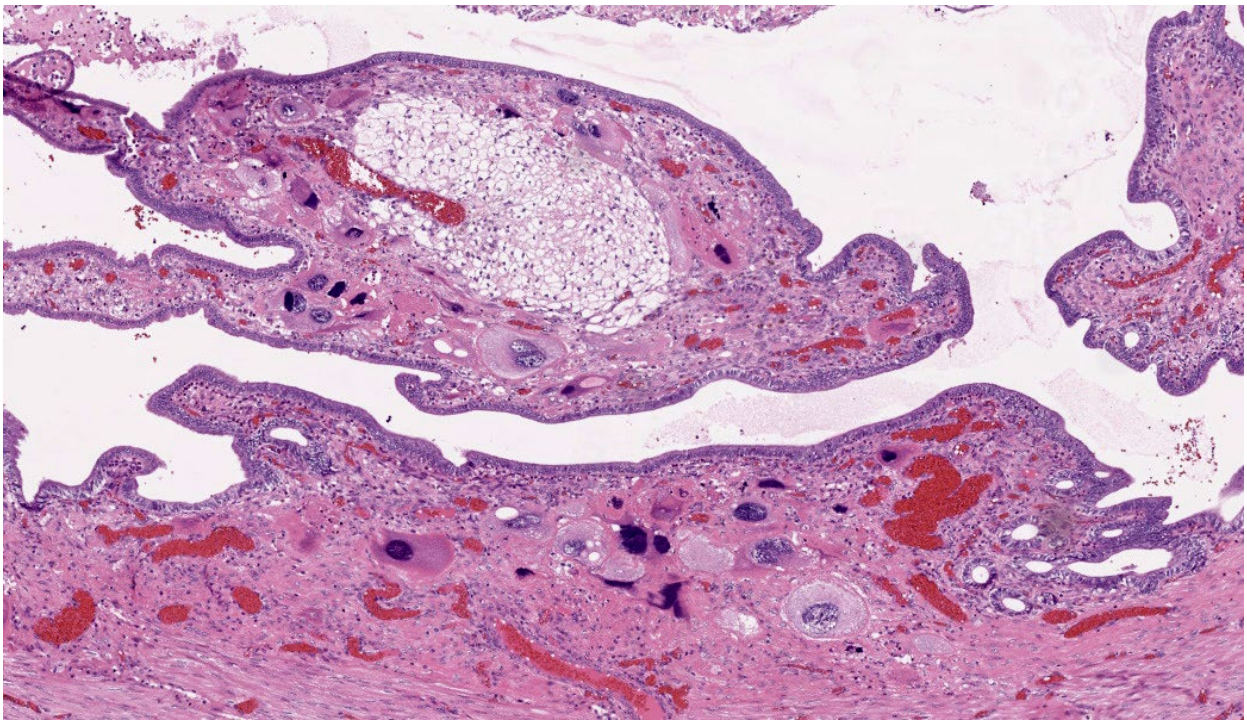

Figure 4-4: Uterus, rabbit. In one area of the section, adjacent to a nest of decidualized endometrial stroma, giant cells resembling trophoblasts and syncytiotrophoblasts infiltrate the endometrium. (HE, 121X)

this highly educational case, along with an excellent write-up on rabbit placentation and decidual reactions. Being able to identify a decidual reaction as such and not mistaking it for another lesion is an important part of understanding the normal reproductive physiology of species with a deciduate uterus. The degree of proliferation in this case caused some participants to pause a suggest possible diagnoses such as deciduoma vs focal decidualization, but the ultimate consensus following consultation with reproductive specialists was that this is consistent with the spectrum of decidual reaction in a rabbit. MAJ Travis used this case as an opportunity to review pertinent reproductive physiology, which encompassed much of this case's discussion and will be summarized here.

Ovarian follicle development starts with primordial follicles, which are surrounded by a single layer of squamous epithelial follicular cells. Primordial follicles then develop into primary follicles that are encircled by a single layer of plump cuboidal follicle cells.[10] In primary follicles, the zona pellucida, a thick, glycoprotein-rich layer, forms between the primary oocyte and the adjacent follicle cells. In the later developmental stages of the primary follicle, the follicular cells surrounding it undergo stratification into the stratum granulosum/membrana granulosa, which is avascular.[10] At this point, the follicle cells are now known as granulosa cells. Simultaneously, the stromal cells surrounding the late primary follicle form a sheath of connective tissue, known as the theca folliculi, that differentiates into the theca interna, which is an inner, highly vascularized layer containing cuboidal secretory cells, and the theca externa, composed of an outer layer of smooth muscle and collagen. The cells of the theca interna have many luteinizing hormone (LH) receptors and synthesize an androgen precursor to estrogen. However, without the help of granulosa cells, thecal calls cannot convert this androgen precursor into estrogen. The granulosa cells, under

Figure 4-5: Uterus, rabbit. High magnification of the pleomorphic giant cells. (HE, 121X)

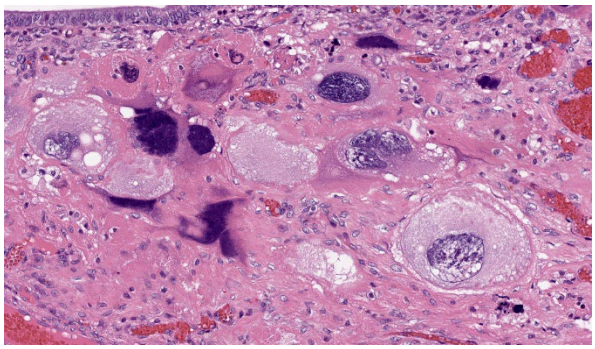

Figure 4-5: Uterus, rabbit. High magnification of the pleomorphic giant cells. (HE, 121X)

the influence of follicle stimulating hormone (FSH) from the anterior pituitary gland, catalyze the conversion of the precursor to estrogen, which enables further proliferation of granulosa cells.

Primary follicles develop into secondary (also called antral) follicles, which are characterized by a proliferation of granulosa cells that increase the follicle's size in the presence of sufficient FSH, growth factors, and calcium. Fluid-filled cavities appear among the granulosa cells and coalesce to form a single, crescent-shaped cavity (the antrum).[10] As this antrum enlarges, its lining of granulosa cells forms a thickened layer around the primary oocyte. This layer is called the "cumulus oophorous." These cells immediately surrounding the oocyte remain after ovulation and become known as the corona radiata.[8]

Finally, the mature/graafian follicle, housing a mature secondary oocyte, causes a bulge on the ovarian surface. Increased pressure in the antrum leads to attenuation of the granulosa cells and the development of the single layer of corona radiata cells around the secondary oocyte.[10] Approximately 24hrs prior to ovulation, the anterior pituitary releases a surge of FSH and LH in response to the rise in estrogen production from the follicle. Following this surge, LH receptors on the granulosa cells are downregulated and no longer produce estrogens, resulting in a decrease in estrogen that enables a subsequent rise in progesterone. During ovulation, the secondary oocyte is released from the mature follicle. Increased antral pressure effectively causes the oocyte, with its corona radiata, to explode out of the follicle. The peristaltic action of the theca externa's smooth muscle layer enables the freed oocyte to be propelled towards the fimbria of the oviduct. From there, the oocyte adheres to the fimbriae and is then actively transported into the uterus by ciliated cells that line the uterine tube.

Following the rather traumatic exit of the oocyte from the follicle during ovulation, the ruptured follicle experiences bleeding from the capillaries of the theca interna into the follicular lumen, earning it the name "corpus hemorrhagicum" (CH). The remaining follicular wall, composed of granulosa and thecal cells, is thrown into deep folds as the follicle collapses. The granulosa cells and the theca interna then differentiate into granulosa luteal and theca luteal cells through a process known as "luteinization" to form the "corpus luteum" (CL). Granulosa luteal cells are large and compose roughly 80% of the CL. They synthesize estrogen, progesterone, and inhibin (inhibin regulates production of FSH). By contrast, theca luteal cells are small and make up the remaining ~20% of the CL. They secrete androgens and progesterone. As the CL begins to form, blood vessels rapidly grow in to form a rich and complex vascular network. Progesterone and estrogen produced by the CL stimulate the growth and secretory activity of the endometrium to prepare for implantation of a fertilized oocyte. In cases where fertilization occurs, chorionic gonadotropin (CG) is produced by the early placenta, which stimulates consistent progesterone secretion from the CL to maintain the pregnancy. If no fertilization occurs, there is no CG production and the CL degenerates, leaving a scar known as the "corpus albicans" (CA). Over time, the CA shrinks and fades away.[10]

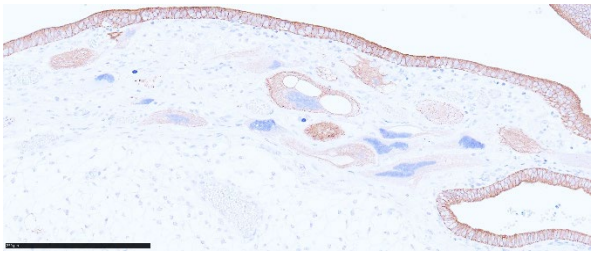

Figure 4-6: Uterus, rabbit. Giant cells demonstrate variably strong cytoplasmic immunoreactivity for cytokeratin. (anti-AE1/AE3, 121X)

Once ovulation has occurred, an oocyte has roughly 24hrs to be fertilized.[8] Sperm cells, upon contact with an oocyte, penetrate the oocyte by binding to zona pellucida receptors and releasing enzymes to degrade the corona radiata. Once a spermatozoon gains entry to the oocyte, the nucleus of the sperm's head is incorporated into the oocyte, where it will release its DNA and start the formation of the zygote. The fertilized oocyte then undergoes a series of changes while passing through the uterus, dividing many times to form a ball of cells called a morula (Latin for "mulberry"), which is still surrounded by a zona pellucida. Through subsequent cell divisions, the embryo loses its zona pellucida and forms a hollow sphere (blastocyst) with a centrally located cluster of cells called the "embryoblast", which will develop the amniotic sac, fetal yolk sac, and fetus. The embryoblast is surrounded by a layer of cells that will form trophoblasts, which ultimately become the placenta.

Trophoblasts, when they contact the uterine wall, begin to proliferate and invade the endometrium. Trophoblasts are biphasic and include cytotrophoblasts, which form the mitotically active inner cell layer, and syncytiotrophoblasts (which are a further differentiation of cytotrophoblasts) that form the outer layer, are not mitotically active, are frequently multinucleated, and actively invade the epithelium and underlying uterine stroma to facilitate implantation of the embryo.[8] Syncytiotrophoblasts secrete chorionic gonadotropin to support the CL and maintain the pregnancy in a sort of, "I'm still here and active, don't let me die," feedback loop.

Animals with a deciduate (meaning "falling off/shedding") uterus have a portion of the endometrium, primarily the antimesometrial portion, that undergoes morphologic changes that can be seen histologically. The maternal portion of the endometrium that undergoes these changes and ultimately tears away with the placenta is called the "decidua".[8] The decidua provides physical support, nutrition, immunological protection, and hormonal support to the developing embryo.[8] Species with a deciduate uterus include rabbits, humans, rodents, and non-human primates, and the histologic appearance of the decidual reaction varies between species.[3,7] Animals with a deciduate uterus shed their placenta at parturition and it includes all but the deepest layer of the endometrium. The process of decidualization involves stromal cells, also called "decidual cells", that become large and rounded in response to increased progesterone. There are three regions of the decidua named based on their relationship to the site of embryo implantation and include the decidua basalis (endometrium that underlies implantation site), the decidua capsularis (thin portion of the endometrium between the implantation site and the uterine lumen), and the decidua parietalis (the remaining endometrium of the uterus).

In the absence of pregnancy, a decidual reaction is thought to be a proliferative response of stromal endometrial cells that is histologically similar to decidual implantation sites. It is associated with pseudopregnancy and nonspecific physical stimuli. It requires both estrogen and progesterone to be present, and there must be some form of physical stimulus to induce the reaction.[2] In research, this can be done by scratching the endometrium and then supporting with estrogen and progesterone administration. In toxicological studies, the presence or absence of a decidual reaction is considered

a sensitive indicator of estrogenic or anti-estrogenic activity.[3,4,10]

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