



WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 8

23 October 2019

Conference Moderator:

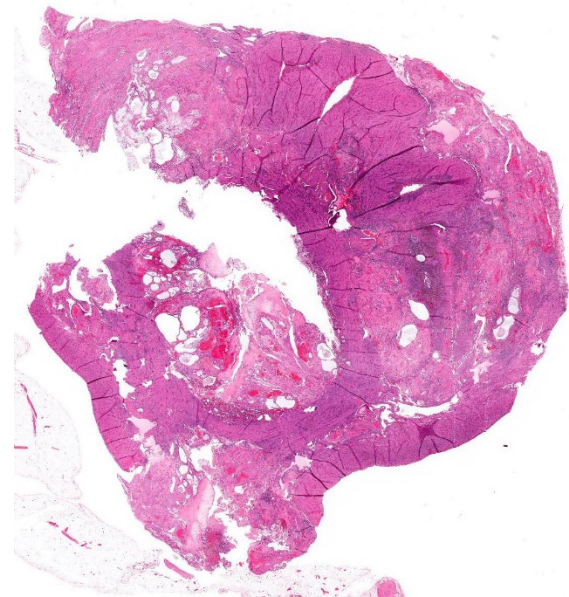
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East Lansing, Michigan

CASE I: D19-007212 (JPC 4135866).

Signalment: 21-month-old female Welsh Corgi mixed breed dog

History: Following spay procedure; a section of uterus was submitted for biopsy evaluation.

Gross Pathology: Arising from the splenic vein and connecting to the caudal vena cava in the region of the left kidney is a large shunt vessel up to 4 mm in diameter. Additionally, connecting the portal vein and caudal vena cava in the cranial abdomen are two small (1 mm in diameter), tortuous vessels. The liver is mildly reduced in size, weighing 67 g (2.9% of total body weight; normal is 3-4). The liver is diffusely markedly pale red to tan and moderately firm. Over the diaphragmatic surface of the right lateral liver lobe is a 2.0 x 0.8 cm region of hemorrhage and mild depression. The parenchyma subjacent to this focus is markedly firm. The right kidney is

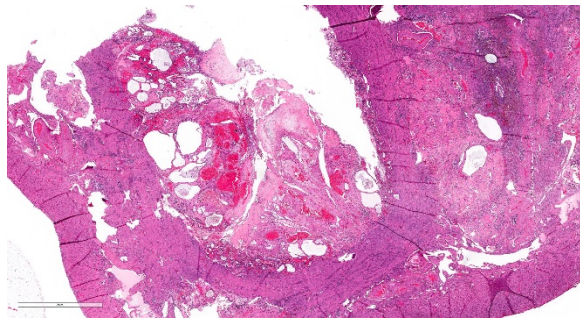


Uterus, dog. A cross section of uterus is submitted for examination. At low magnification, the endometrium is expanded by numerous dilated glands and areas of hemorrhage. The myometrium is focally expanded at left by plaques of collagen and bands of a dense cellular infiltrate. (HE, 5X)

mildly reduced in size compared to the left kidney and has multifocal chronic infarcts up to 2 cm in diameter. No abnormalities are identified externally in the brain.

Laboratory results: NA

Microscopic Description: Uterus: Diffusely and markedly expanding the endometrium, extending into the luminal space, and infiltrating into the myometrium are markedly ectatic endometrial glands which are admixed and/or surrounded by a coagulum of degenerate neutrophils, eosinophilic karyorrhectic and cellular debris, congested blood vessels, multifocal hemorrhage, hemosiderin, fibrin, and sloughed epithelial cells. These sloughed cells are similar to those lining remaining intact endometrium and are tall columnar epithelial cells with contain highly vacuolated cytoplasm. The myometrium is multifocally infiltrated by large numbers of lymphocytes, plasma cells and hemosiderin-laden macrophages. Multifocally, within the endometrial coagulum and the underlying myometrium are scattered aggregates of irregularly shaped syncytial trophoblast cells which contain up to 6-10 nuclei with abundant eosinophilic cytoplasm and multifocal mild mineralization. There is focal rupture of the serosa with transmural proliferation/invasion of endometrial glands,



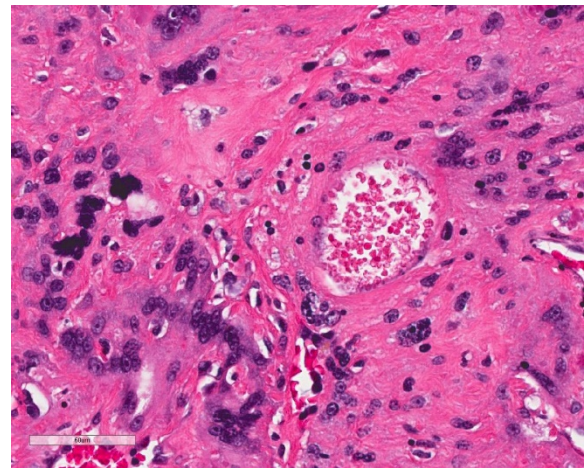
Uterus, dog. Higher magnification of the endometrium. Nodules of endometrium at left contain large ectatic glands; at right, the endometrium is largely replaced by a dense collagenous band. (HE, 20X)

hemorrhage, fibrin, and yellow pigment (hematoidin).

Contributor's Morphologic Diagnosis:

Uterus: Necrosis, hemorrhage, and mineralization with syncytial trophoblast retention, endometrial hyperplasia, and serosal rupture

Condition: Subinvolution of placental sites (SIPS)

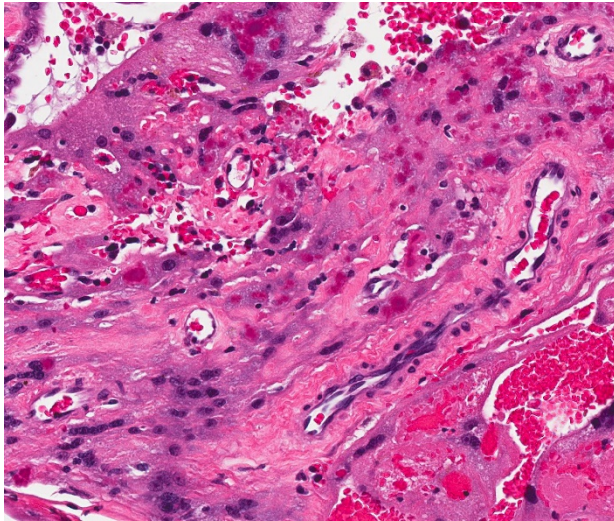


Uterus, dog. Bands of endometrial collagen are infiltrated by anastomosing cords of trophoblasts which demonstrate erythrophagocytosis and the presence of brightly eosinophilic hematin within their cytoplasm. (HE, 400X)

Contributor's Comment: Clinical presentation of subinvolution of placental sites (SIPS) typically consists of blood-tinged vaginal discharge that extends past the normally expected 7-10 days post-whelping¹, sometimes lasting for months. The condition occurs most commonly in young dogs and the cause is unknown. Prolonged bleeding and extensive loss of blood can lead to anemia and occasionally death. Affected dogs are also prone to ascending infections.

Normal uterine involution in dogs takes 12-15 weeks³. In dogs with SIPS gross lesions consist of some or all of the placental attachment areas (ellipsoidal enlargements)

being thickened, rough, grey to brown and hemorrhagic with the inter-placental sites appearing normal.



Uterus, dog. Numerous syncytiotrophoblasts remain within the collagenous bands as well. (HE, 400X)

Microscopically, SIPS are characterized by a luminal coagulum of abundant necrotic cellular debris, hemorrhage, and within the deeper layers of endometrium, syncytial trophoblasts or decidual cells that have a vesiculate nucleus and highly vacuolated cytoplasm due to progesterone stimulation. The retained trophoblastic cells fail to degenerate and subsequently invade into the deeper glandular layer and myometrium.² These trophoblastic cells also inhibit normal thrombus formation leading to prolonged bleeding.⁴ Mineralization and infiltration of lymphocytes, plasma cells, and macrophages often occur in these areas of necrosis. The syncytial trophoblasts or decidual cells may invade deeper layers including the myometrium and also may cause serosal rupture (as seen in this submitted case) leading to escape of the contents into the peritoneal cavity causing fatal peritonitis¹. Spontaneous recovery generally occurs in healthy dogs and in prolonged cases

ovariohysterectomy must be performed for resolution of the condition^{4,5}.

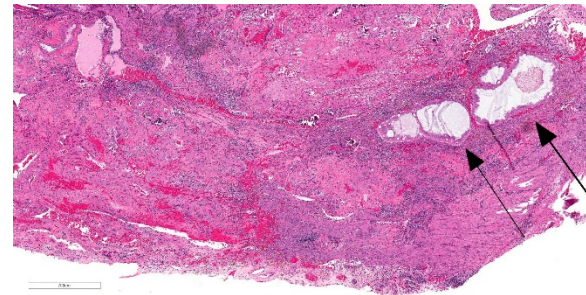
Other differentials for hemorrhagic vaginal discharge include endometritis, neoplasia, coagulopathies, brucellosis and other bacterial infections.

Contributing Institution:

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<https://cvm.ncsu.edu/research/departments/dphp/programs/pathology/>

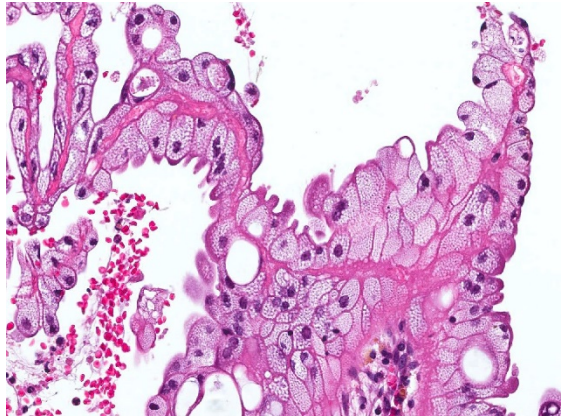
JPC Diagnosis: Uterus, Endometritis, chronic-active and necrohemorrhagic, diffuse, severe, with numerous myometrial trophoblasts and syncytiotrophoblasts, adenomyosis, endometrial progesterone effects, vascular thrombosis and mural rupture.



Uterus, dog. The endometrium (serosa at bottom) is markedly expanded by collagen, trophoblasts, inflammatory cells, and several endometrial glands (arrows). (HE, 33X)

JPC Comment: In domestic species, subinvolution of placental sites is a condition unique to the bitch. In humans, subinvolution of the placental site (also known as non-involution of the placental site or subinvolution of the uteroplacental arteries), is an uncommon cause of postpartum bleeding in older multiparous women. Unlike the dog, in humans it is most often diagnosed within several weeks postpartum, although cases have been diagnosed as long

as 6 years after the most recent parturition.⁶ In humans, this condition is different from the bitch, in that hemorrhage is usually the result of failure occlusion of the placental vessels.



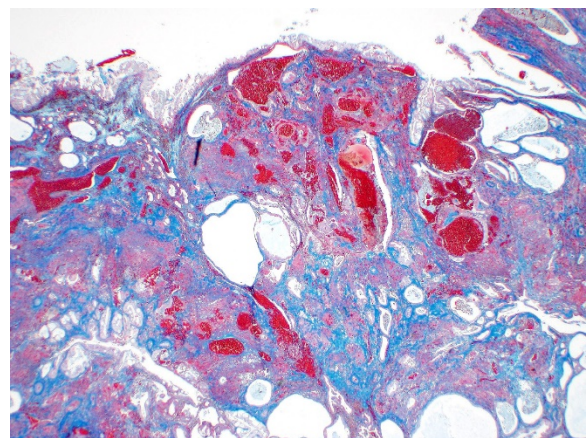
Uterus, dog. The epithelium lining the endometrial surface and endometrial glands is hypertrophic with numerous cytoplasmic vacuoles (progesterone effect). (HE, 324X)

During mid- to late gestation in humans, endovascular trophoblasts actually replace the endothelial lining of the uteroplacental arteries, expressing endothelial –type markers and angiogenic factors resulting in significant remodeling of the arterial bed to create low-resistance high-flow arteries to supply the needs of the developing fetus.⁶ Within days after birth, these trophoblasts disappear, resulting in endarteritis, thrombosis, and occlusive mural fibrosis of these vessels, which coincides with loss of the decidua and endomyometrium in the remainder of the uterus, and limits blood loss during this process. While the pathogenesis of this process has not yet been fully elucidated, it is likely that the lack of involution of placental vessels results from abnormal immunologic recognition, as the normal deposition of immunoglobulin (IgG, IgM, and IgG) and complement proteins (C1q, C3d, C4) seen in normally involuted arteries is not seen in those associated with subinvolution. Additionally, remnant

endovascular trophoblasts in non-involuting placental vessels express high levels of the anti-apoptotic protein Bcl-2.⁶

The moderator pointed out the focal area of rupture of the wall of this uterus, obviously complicating the prognosis in this case. The cause of the mural rupture is likely the result of ischemia due to multifocal thrombosis in this case. The moderator also commented on the presence of abundant hyaline collagen lacking fibroblasts within the section, which is a characteristic feature of involution in the dog. These collagen plaques form over the surface within 3 days of parturition to form a sort of hemostatic bandage over the profound vascularity of the deeper degenerating layers of the endometrium. The moderator also discussed the importance of discussing the complete disorganization of the endometrium in SIPS as compared to normal involution, which may be helpful in distinguishing between normal involution and SIPS in cases with incomplete history. The depth of the trophoblasts is also helpful in making this distinction.

The moderator also pointed out the presence of abundant reddish crystalline material in trophoblasts and syncytiotrophoblasts which



Uterus, dog. A trichrome stain demonstrates the amount of collagen within the remaining endometrium. (Masson's trichrome, 20X)

was interpreted as hematin, a blood breakdown pigment. Within these cells, as opposed to macrophages, the breakdown of hemoglobin is incomplete, more closely resembling hemoglobin crystals than hemosiderin granules.

References:

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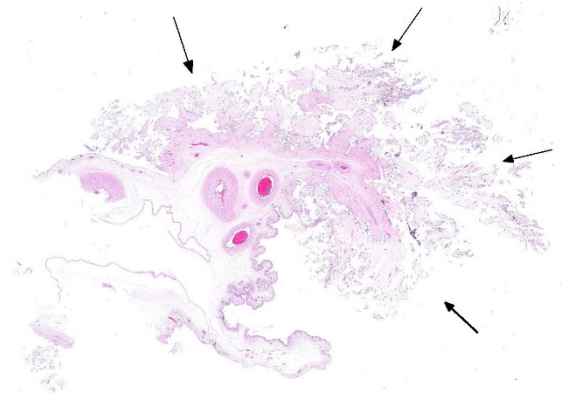
CASE II: 15-1137 (JPC 4066220).

Signalment: Three late term dairy goat fetuses (breed not specified)

History: Multiple late term abortions occurred in a large dairy goat herd. Submitted samples included fixed tissues, tissue pools, abomasal fluid and serum from one of the aborting dams.

Gross Pathology: The submitting veterinarian reported that the placentas had hemorrhages and pinpoint white foci on the cotyledons. Fetal spleens and livers were enlarged.

Laboratory results: Placentas were positive for *Chlamydophila* by antigen ELISA. PCR for *Toxoplasma gondii* was negative on placenta and brain. No *Campylobacter* was isolated and PCR for BVD was negative. FA for *Leptospira* was negative. A single dam serum was positive for antibodies to *Coxiella* but negative for *Brucella abortus*, BVDV and *Toxoplasma*. Immunohistochemistry for *Coxiella burnetii* on the placenta was positive.



Placenta, goat fetus. A cross section of chorioallantois with cotyledon (arrows) is submitted for examination. (HE, 5X)

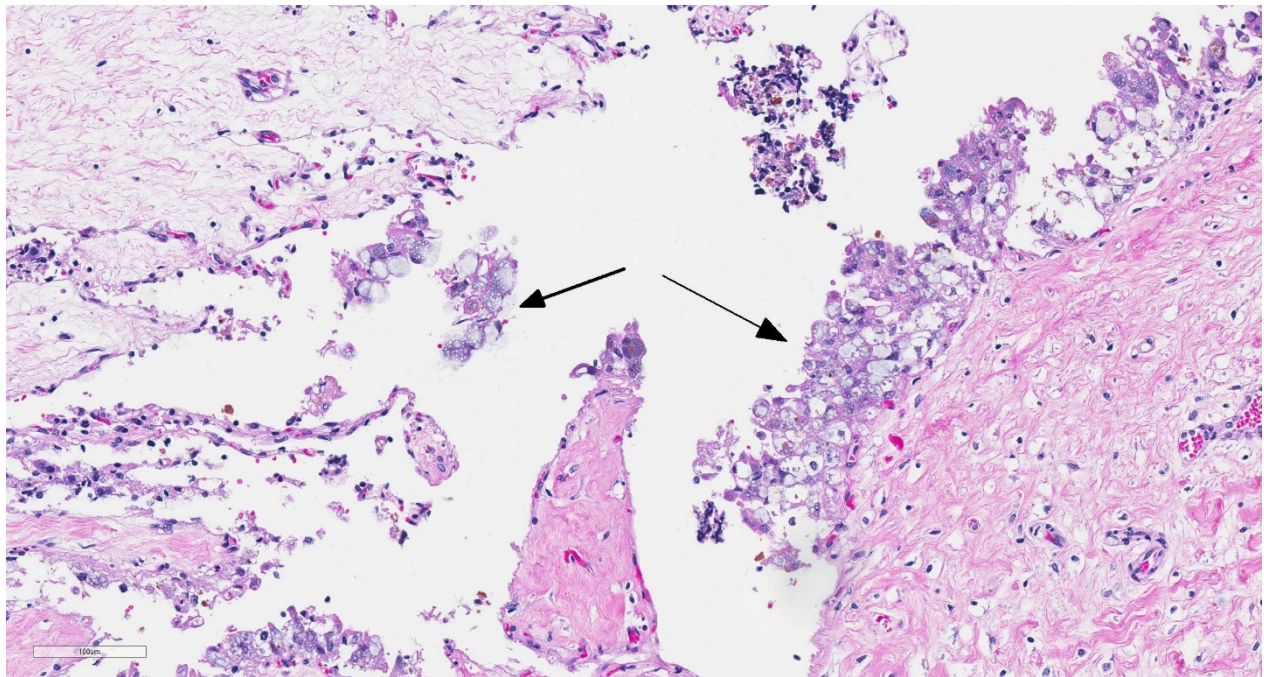
Microscopic Description: In sections of placenta from 2 fetuses, there were multifocal areas of necrosis and inflammation. Cotyledons and intercotyledonary placenta were infiltrated by neutrophils and macrophages. Fibrin admixed with degenerate inflammatory cells covered the cotyledonary surface. The intercotyledonary interstitium was infiltrated by inflammatory cells, and a few scattered blood vessels were necrotic and inflamed. Multiple chorionic epithelial cells were expanded by the

Contributor's Morphologic Diagnosis:

Multifocal necrotizing placentitis with vasculitis and intracellular bacterial organisms (*Coxiella burnetii*)

Contributor's Comment:

Coxiella burnetii is a well-recognized cause of abortion in sheep goats and, to a lesser extent, cattle.⁹ In one large study of goat abortions, *C burnetii* was second only to *Chlamydia psittaci* (*Chlamydophila abortus*) in numbers of cases of bacterial abortion in goats.⁶ Infected



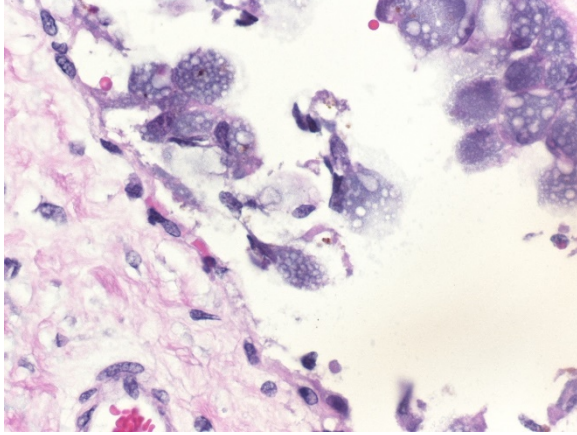
Placenta, goat fetus. There is marked loss of cotyledonary epithelium. Remaining trophoblasts ((right arrow) and sloughed trophoblasts are swollen by the presence of numerous intracytoplasmic bacilli. (HE, 142X)

presence of discreet vacuoles containing numerous bacterial organisms. Gimenez-stained sections highlighted the intracellular organisms. Gimenez-positive organisms were identified as *Coxiella burnetii* by immunohistochemistry.

Other lesions (not submitted) included multifocal gliosis within sections of cerebrum in two of the fetuses. No organisms were identified histologically. Sections of livers, lungs, spleens and kidneys had no histologic lesions.

animals remain as a reservoir, shedding large numbers of organisms during subsequent abortions or at normal parturitions. Organisms are also shed in feces and milk,³ Ruminant reservoirs are the major source of infection in people, in whom the organism causes the zoonotic disease Q fever.¹ Although cattle are major shedder of *C burnetii*, especially in milk⁶, the organism is not a major abortifacient in that species.⁵

Transmission is by inhalation or fecal-oral routes. Infected animals may abort in late



*Placenta, goat fetus. Higher magnification of trophoblasts with numerous *C. burnetii* within their cytoplasm. This pathogen gives infected cells a unique "bubbly" appearance. (HE, 400X) (Photo courtesy of: Department of Veterinary Microbiology and Pathology, and the Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA www.vetmed.wsu.edu)*

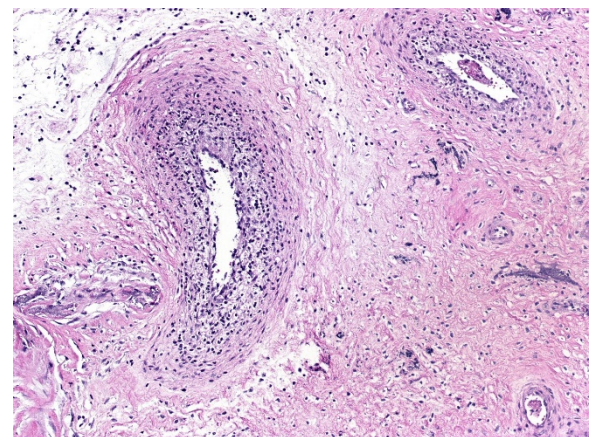
gestation or give birth to weak kids. Fetal lesions are minimal but gross evidence of placentitis is present.⁹ Characteristic histologic lesions are necrotizing intercotyledonary placentitis with intracellular organisms; vasculitis is less prominent than in chlamydial placentitis. Both *C burnetii* and *C abortus* are Gimenez-positive. In this case, since antigen ELISA for *Chlamydomphila* was positive, immunohistochemistry was needed to identify the organisms as *C burnetii*. *C abortus* should also cause multifocal necrosis in fetal liver and spleen, which was not seen in this case. This case was also interesting in that cerebral glial nodules suggested *Toxoplasma* infection, but PCR for *T gondii* on placenta and brain was negative.

The human disease Q fever, caused by infection with *C burnetii*, was first reported in Australia in 1935 but has a worldwide distribution.¹ Only 40% of infected people show any clinical signs. The more common acute disease manifests as flu-like symptoms, pneumonia or hepatitis; chronic infections

manifest primarily as endocarditis.² Most reported cases are of the acute disease. Cases in the US are most often reported from western and plains states where ranching and rearing of cattle are common. Cases are most commonly reported in spring in summer, coinciding with birthing season for ruminants. Incidence of disease increases with age and persons with immune compromise, or those in contact with livestock are at higher risk.

C burnetii is an obligate intracellular organism in the order Rickettsiales. It infects monocytes and macrophages and survives within the acidic environment of the phagosome. Intracellular survival depends upon inhibition of phagosome-lysosome fusion. Control of the infection requires intact cell mediated immunity; lymphocytes of people with chronic infections fail to proliferate in response to *C burnetii* antigen, whereas lymphocytes of people with acute infections do respond.²

Contributing Institution:

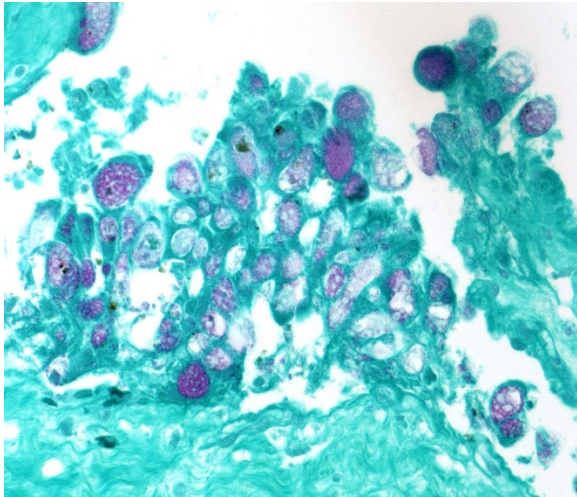


Placenta, goat fetus. There is loss of endothelium and there are numerous viable and degenerate neutrophils and cellular debris within the walls of chorionic vessels (vasculitis) (HE, 400X) (Photo courtesy of: Department of Veterinary Microbiology and Pathology, and the Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA www.vetmed.wsu.edu)

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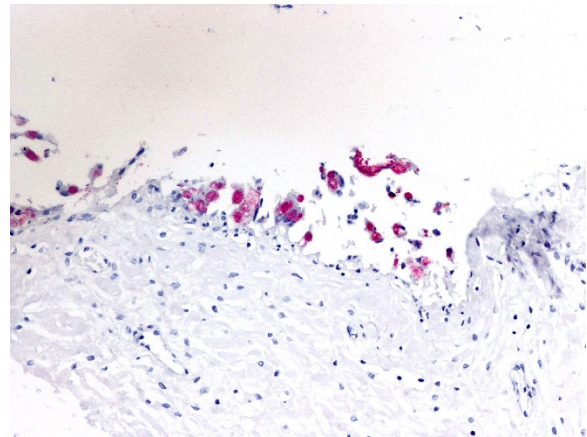
JPC Diagnosis: Chorioallantois: Placentitis, necrotizing, diffuse, severe, with multifocal moderate vasculitis, and numerous intratrophoblastic coccobacilli.

JPC Comment: *Coxiella burnetii*, the causative agent of Q fever, is a bacterium whose wall is similar to that of a gram-negative bacillus; however, its wall does not stain with Gram stains, requiring a Gimenez stain to disclose its location.⁴ As an obligate intracellular parasite, it produces a reproductive vacuole that resembles a phagolysosome with an acidic pH, acid hydrolases, and cationic peptides. To survive in this harsh environment, it has developed important buffering strategies, including the production of basic proteins, sodium-proton exchanges to combat oxidative stress, and osmoprotectants to



Placenta, goat fetus. Bacilli within trophoblasts stain positively with a Gimenez stain. (Gimenez, 400X) (Photo courtesy of: Department of Veterinary Microbiology and Pathology, and the Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA www.vetmed.wsu.edu)

prevent osmotic damage.⁴ In vitro experiments have determined that there are actually two variants of the coccobacillus, a reproductively active “large-cell variant” which is the replicative form, and an environmentally stable small cell variant, which has numerous cross-links in its peptidoglycan wall to give it additional resistance to environmental stresses such as desiccation, chemical, and heat stress.⁴



Placenta, goat fetus. Coccobacilli within trophoblasts stain immunopositively for C. burnetii. (anti-C. burnetii, 400X) (Photo courtesy of: Department of Veterinary Microbiology and Pathology, and the Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA www.vetmed.wsu.edu)

Q fever, short for *query* fever, was first identified by Dr. John Derrick in meat-packing workers in Queensland Australia in 1935 who developed an acute febrile illness but were negative on blood and serologic tests for known pathogens at the time.⁷ Transmission to guinea pigs and other laboratory animals ultimately resulted in the identification of rickettsia within the spleen. The clinical signs of Q fever – high fever, headaches and a slow pulse resembled other rickettsial diseases of the time including typhus and psittacosis, but lacked the rash common to the other two diseases.⁷

In humans, Q fever has been seen in all

countries except New Zealand. While ruminants have been the traditional reservoirs, in recent years, a number of additional species have been reported as shedding the bacillus, to include cats, marine mammals, psittacines, reptiles, and ticks.⁴ In the absence of a suitable host, *C. burnetii* also possesses the ability to utilize free-living amoebae as a reservoir for the small-cell, vegetative form.⁴ Q fever is most common seen in persons who come in contact with infected animals, including farmers, abattoir workers, lab workers, and veterinarians.⁷ Transmission has also been seen following ingestion of raw milk or fresh goat cheese.⁷ Q fever has many various acute and chronic manifestations, or may be asymptomatic. When it became a reportable disease in the US in 1999, case numbers increased by 250% in the next 5 years.⁴ While it most often presents as an influenza-like illness, more serious manifestations, such as pneumonia, hepatitis, or endocarditis are seen.¹⁰ Endocarditis is the most common chronic disease associated with *C. burnetii* infection in humans and has been seen following valve transplants.¹⁰ In cases of endocarditis, as vegetation may be absent or small, resulting in a long latent period.¹⁰

Between 2007 and 2010, a country-wide outbreak of Q fever occurred in the Netherlands with over 40,000 cases estimated, especially in the provinces of Noord-Brabant, Gelderland, and Limburg, centers of goat farming. The outbreak coincided with a marked increase in high-intensity dairy goat farming in proximity to urban areas. On some of these farms, abortion levels due to *Coxiella* exceeded 60%, leading to requirements for reporting of abortions and widespread immunization of goats. With cases still on the rise, in 2008, the Dutch government was forced to slaughter over 50,000 goats in order to minimize the public health risk.

The moderator discussed the presence of a brown granular pigment within the trophoblasts. While initially interpreted as hemosiderin by most participants, he offered the possibility of meconium and uptake by the trophoblasts. He noted the unusual lack of necrotic debris between the cotyledonary villi in this particular case. He also registered, during the description in this case, a personal enmity toward the words “admixed” and “coccobacilli”.

References:

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CASE III: VS17005 3 (JPC 4102155).

Signalment: 9-year-old, female rhesus macaque (*Macaca mulatta*)

History: This animal received 4Gy whole body irradiation three years prior to presentation. It had a history of menorrhagia and anemia (Hct 14.8%). A hysterectomy was performed to prevent further blood loss. During the procedure the uterus was found to be adhered to the right side of the abdominal wall.

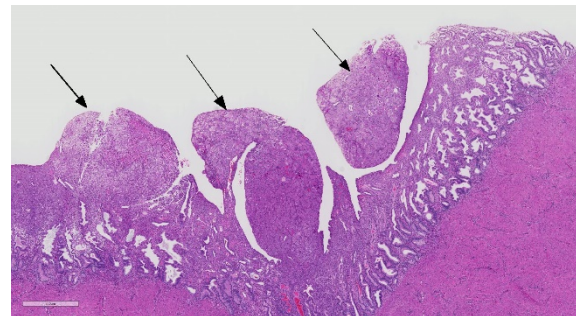
Gross Pathology: The uterus weighed 4.5g and the endometrium at the apex of the uterus was irregularly thickened up to 1.5mm.

Laboratory results: None.

Microscopic Description: The superficial endometrium is expanded and effaced in areas by an unencapsulated, poorly demarcated neoplasm composed of sheets of pleomorphic neoplastic cells supported by fine stroma, which projects into the lumen in some areas. Aggregates of macrophages, lymphocytes and few eosinophils and granular leukocytes are scattered throughout.

The neoplastic cells have variably distinct cell borders, are round to polygonal, have abundant eosinophilic vesicular cytoplasm, 1-12 oval nuclei with stippled chromatin, and 1-2 nucleoli. Mitoses are rare. A few cells, especially towards the lumen, have undergone degeneration and necrosis. The remaining endometrium has few scattered dilated glands lined by epithelial cells with basally arranged nuclei, without mitoses.

Contributor's Morphologic Diagnosis: Uterine trophoblastic tumor



Uterus, rhesus macaque. Multiple nodules of a neoplasm abut or occupy the superficial endometrium. Subjacent glands are mildly dilated. (HE, 28X)

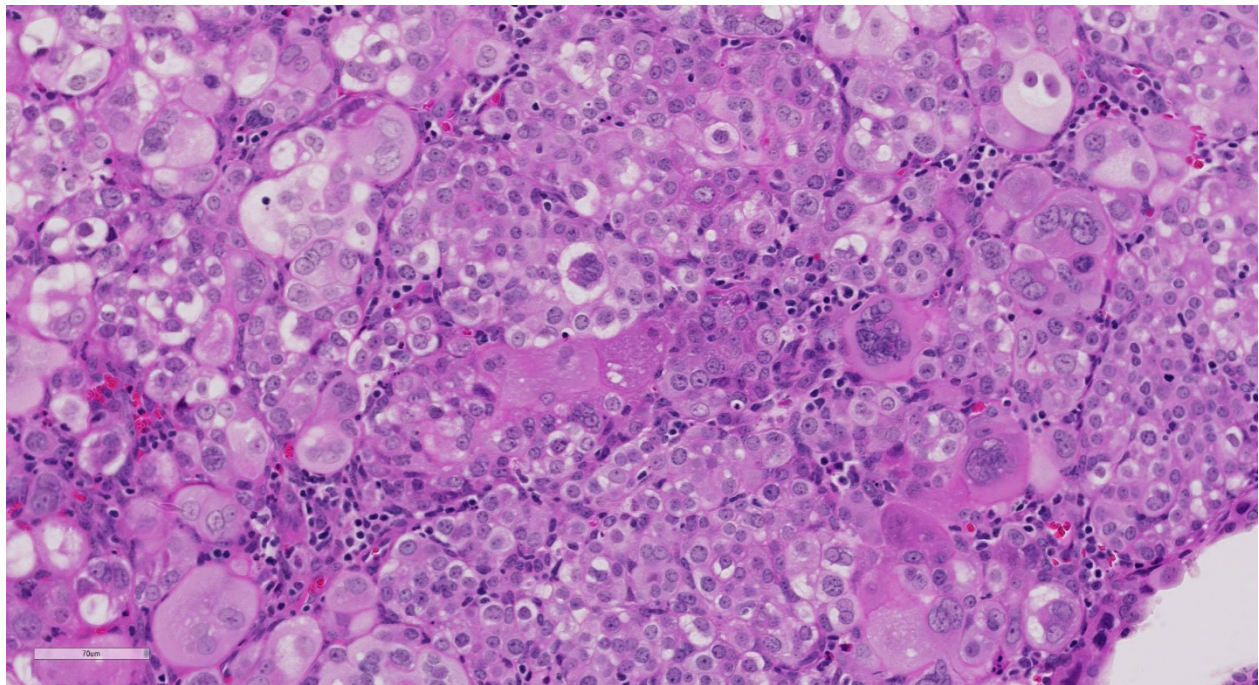
Contributor's Comment: Gestational trophoblast disease (GTD) is a rare disease of pregnancy, a result of abnormal differentiation of trophoblasts, but may also rarely develop from germ cells in the absence of pregnancy.¹³ Since trophoblasts lack the common regulatory pathways that prevent the development of neoplasms, either molar gestations/hydatiform moles or trophoblastic tumors can develop.¹⁶ Hydatiform moles are abnormal pregnancies characterized by aberrant chromosomal changes. Women greater than forty years of age and younger than 20 are more likely to develop molar gestations/hydatiform moles. Uterine epithelioid trophoblastic tumors have been reported in a red-tailed guenon (*Cercopithecus ascanius*)⁴ and an African green monkey (*Chlorocebus aethiops sabaues*).² Ovarian nongestational

trophoblastic tumors (2 choriocarcinomas and 1 epithelioid trophoblastic tumor) have been reported in captive macaques.^{6,7,10,15}

Trophoblast tumors can be classified into one of three subcategories: choriocarcinomas, placental site trophoblastic tumors, and epithelioid trophoblastic tumors. Choriocarcinomas are malignant tumors composed of bilaminar cytotrophoblasts and syncytiotrophoblasts without chorionic villi. In some cases, these represent a component of mixed germ cell tumors of the ovary. Pure nongestational choriocarcinomas are extremely rare in humans. Typically the neoplastic cells are strongly positive for human chorionic gonadotrophin (β -hCG) and weakly positive for human placental lactogen (hPL).¹⁴ Placental site trophoblastic tumors are composed of sheets of variably sized trophoblasts with single to multiple nuclei with atypia. These cells may permeate the myometrium and blood vessels.³ The cells are usually positive for hPL and variably

positive for β -hCG and placental-like alkaline phosphatase (PLAP).^{9,14} Epithelioid trophoblastic tumors are composed of sheets of monomorphic intermediate trophoblasts which resemble the chorionic membrane.³ The cells are variably immunoreactive for placental-like alkaline phosphatase (PLAP) and hPL but strongly immunoreactive for E-cadherin and epidermal growth factor receptor.^{9,14}

The clinical signs associated with GTD vary widely. Symptoms usually present within the first trimester and include bleeding and anemia, a larger than normal uterus for the gestational age, hyperemesis, pre-eclampsia, hyperthyroidism and respiratory distress. Abnormal fetal organs observed by ultrasonography and markedly elevated serum hCG can aid in the clinical diagnosis. Histopathology and immunohistochemistry provides a confirmatory diagnosis. The treatment options of trophoblast tumors depend on the stage of the tumor.^{13,16}



Uterus, macaque. The neoplasm is composed of two distinct populations of cells – uninucleated polygonal cells resembling cytotrophoblasts, and larger multinucleated cells resembling syncytiotrophoblasts. (HE, 205X)

Contributing Institution:

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JPC Diagnosis: Uterus: Choriocarcinoma

JPC Comment: The contributor has presented a concise yet informational description of a confusing set of neoplasms that are extremely rare in all species. These tumors are a subset of gestational trophoblastic disease, which as stated above, includes hydatiform moles.¹

Hydatiform moles (hydatiform meaning cystic, mole simply referring to a clump of growing tissue) arise during pregnancy from non-viable eggs that implant in the uterus, and are the most common of the gestational trophoblastic diseases. These eggs have lost the nucleus, and hence the maternal DNA. The mole, a parthenogenetic zygote, results from fertilization by a spermatozoa undergoing mitosis following combining with the egg (resulting in a 46 XX phenotype) or alternatively two haploid spermatozoa (resulting in a 46 X,Y karyotype.)¹ Even more rarely a normal egg may be fertilized by two sperm which reduplicate themselves (resulting in a 69, XXY karyotype) – known as a partial mole. Partial moles, due to the presence of maternal DNA may have discernable, but disorganized fetal elements within their structure.¹

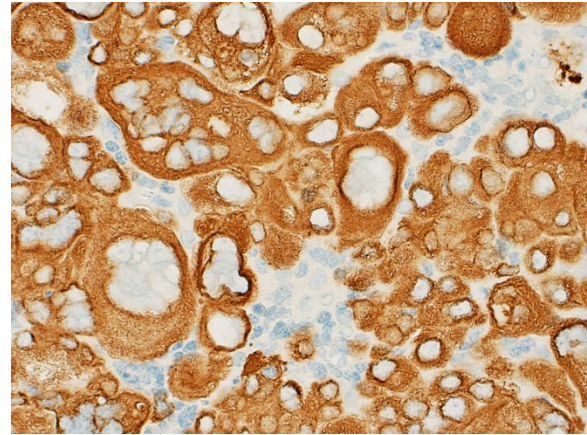
Moles may often be grossly differentiated from true trophoblastic tumors grossly and histologically by their grapelike appearance (owing to their development of chorionic villi, not seen in the true trophoblastic tumors. and histologically, but the presence

of chorionic villi, which are not present in the true trophoblastic tumors. They may be invasive, growing into the wall of the uterus, or actually turn into choriocarcinomas (this happens into 4% of tumors.)¹

The true trophoblastic tumors, as mentioned by the contributors, include choriocarcinoma, the placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT), and their differentiation histologically is based on morphology and immunohistochemistry (reviewed above in the contributor's comments.)¹² Choriocarcinomas are by far the most malignant and common of the three, often demonstrating significant invasion of the uterus and distant metastasis in approximately 40% of human cases. The other two tumors are far less common and both are derived from the intermediate trophoblast. PSTT is the rarest subtype with 1 per 100,000 pregnancies.⁹ These are slow growing malignancies that most affect women of reproductive age and are seen following a normal or ectopic pregnancy or miscarriage, with rare cases affecting post-menopausal women. Because of its similarities to a normal implantation site, it was initially called a "placental pseudotumor".⁹ The cellular population of the placental site trophoblastic tumor is monomorphic as composed to the dimorphic population of choriocarcinomas, and its immunohistochemical profile is the reverse, with strong staining for hPL and weak staining for hCG (as the choriocarcinoma stains strongly for hCG and weakly for hPL.¹¹ Interestingly, the differential levels of these hormones are often used as a clinical test for these particular neoplasms as well. The third neoplasm, epithelioid trophoblastic tumor (ETT) was first diagnosed in humans by Shih and Silverberg in 1998², and is also a neoplasm of intermediate trophoblasts (as is the PSTT) but resemble those of the chorionic villi, rather than the placental site

(as seen with the PSTT). The neoplastic cells stain weakly for hCG and HPL, but strongly for cytokeratin and inhibin α , epithelial growth factor, and e-cadherin.² A review of the immunohistochemical staining patterns for the various types of gestational trophoblastic diseases is presented in Table 1.

Since the submission of this case, this case has also been presented at the 2018 Satellite Symposium of the National Toxicology Program at the annual meeting of the Society of Toxicologic Pathologists, the minutes of which were subsequently published in *Toxicologic Pathology*. The presenter described the immunohistochemical findings for this neoplasm including a general immunopositivity for hCG and strong positivity in the multinucleated cells. The cells stained diffusely positive for pancytokeratin, but failed to stain for human placental lactogen, placental alkaline phosphatase, and p63. There were rare foci of CD10 immunopositivity, and these cells failed to stain with hCG and pancytokeratin. Based on these findings, the contributor and the participants at the conference prefer a diagnosis of choriocarcinoma. This is in agreement with the immunohistochemical stains run at the Joint Pathology Center,



Uterus macaque. Both cell populations are strongly positive for cytokeratin (AE1-AE3, 400X)

which demonstrated patchy but strong immunopositivity of the syncytiotrophoblastic population for hCG, and a weaker staining pattern for HPL and weak to absent staining of cytotrophoblasts for both. As expected, both populations stained strongly positive for cytokeratin, but this is not useful in differentiating any of the three trophoblastic tumors. Based on this findings and the histologic appearance of the tumor, we are in agreement with the contributor that this tumor is best classified as a choriocarcinoma.

Table 1. A Summary of the Immunohistochemical Staining Properties of Different Types of Gestational Trophoblastic Disease Compared to the Presented Lesion.

Immunohistochemistry Stain	ETT	PSTT	CC	Mole	PSN	Lesion
Human chorionic gonadotrophin	±	±	+	+	-	+
Human placental lactogen	±	+	±	+	-	-
Pancytokeratin	+	+	+	+	+	+
p63	+	-	±	+	+	-
CD10	-	±	±	+	-	Focal
Placental alkaline phosphatase	-	-	-	+	+	-
α -inhibin	+	+	+	+	+	-
MeI-CAM (CD146)	±	+	+	-	-	-
Ki67 index	Moderate	Moderate	High	Low	Low	-

Note: Select references were used to compile these data (Bentley 2003; Hui et al. 2014; Kalhour et al. 2009; Kommosi et al. 2001; Shih, Seidman, and Kurman 1999; Shih and Kurman 2004; Yokouchi et al. 2011). ETT = epithelioid trophoblastic tumor; PSTT = placental site trophoblastic tumor; CC = choriocarcinoma; PSN = placental site nodule.

Table 1. Immunohistochemical staining properties of trophoblastic diseases.⁵

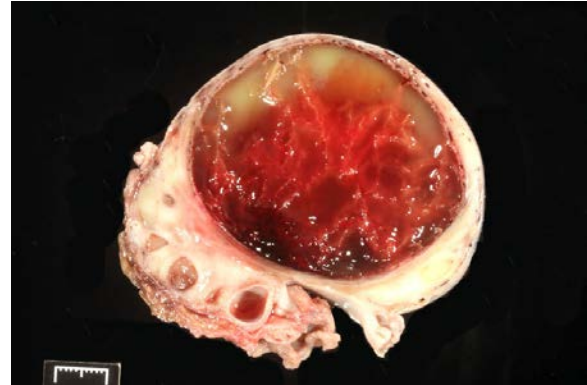
The moderator mentioned that based on the thickness of the uterus, the endometrium is likely hyperplastic (a change of little significance in this particular slide).

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with a flocculent, pleural effusion especially on the right where a hypoechoic fluid pocket at the level between ribs 9-15 in which gas was trapped was noted.



Ovary, horse. The left ovary weighed 268g and had a 4.6x4.6x5.2 cyst with a 1.5mm wall and hemorrhagic gelatinous content. (Photo courtesy of: Texas A&M University, College of Veterinary Medicine and Biomedical Sciences <http://vetmed.tamu.edu/vtpb>.)

CASE IV: TAMU 2017 WSC 2 (JPC 4102428).

Signalment: 7-year-old, American Quarter Horse mare, *Equus ferus caballus*

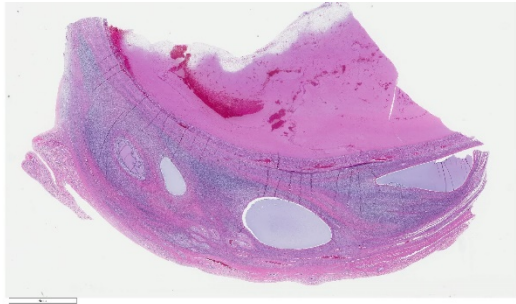
History: Four weeks prior to presentation at the end of April, this working Quarter Horse mare had had a tooth extraction under anesthesia. Approximately 2 weeks prior to presentation, the horse became dyspneic with a nasal discharge and coughing. She was treated with Uniprim (trimethoprim and sulfadiazine) without resolution, and at 5 days before presentation, her treatment was switched to doxycycline, banamine and Previcox (NSAID's Cox-2 inhibitor). Because the dyspnea worsened, she was taken to a veterinarian, and she was found to be febrile (103.6F) and severely dyspneic. She was referred to TAMU, and at presentation, the mare continued febrile, and dyspneic with a bilateral nasal discharge. Ultra sound showed bilateral consolidation

The chest drains were lavaged daily, and the mare receive LRS fluids, antibiotics (gentamicin then chloramphenicol) and antiinflammatories; however, the lesion and signs progressed, and she was euthanized after 3 days of hospitalization

Gross Pathology: A 422 kg (929 lb) seven-year-old, red roan, Quarter Horse mare with a white sock on the right hind and in adequate body condition is necropsied on April 27, 2017.

INTEGUMENTARY/SPECIAL

SENSES: Shaved areas include: 10x10cm on the right mid thorax and 10x10 cm on the right side of the mid-cervical neck. A 3 cm, linear, superficial abrasion is caudal to the right eye.



Ovary, horse. A section of ovary with a hemorrhagic cyst at top and normal ovary with visible primary and secondary follicles in the adjacent normal ovary. (HE 7X) (Photo courtesy of: Texas A&M University, College of Veterinary Medicine and Biomedical Sciences <http://vetmed.tamu.edu/vtpb>.)

RESPIRATORY: Approximately 5 L of yellow to grey, turbid fluid are in the pleural cavity, and a large amount of yellow, friable material is admixed with the fluid and firmly adhered to the lung (fibrinopurulent pleuritis). Diffusely, the costal and diaphragmatic pleura are dark red and markedly thickened (reactive mesothelium). The ventral lungs are diffusely firm and dark red (bronchopneumonia). In the middle of the right lung is a focal, 10x10x15 cm cavitation that connects to bronchi and contains a large amount of tan, casts of grey red, moist, friable material (Aspiration Pneumonia with sequestrum formation).

CARDIOVASCULAR (Heart weight: 4.19 kg; Right ventricular wall: 1 cm; Left ventricular wall: 3 cm): The pericardium is markedly and irregularly thickened and dark red (reactive mesothelium). The right side of the heart is mildly thickened (hypertrophy, pulmonary hypertension).

ENDOCRINE: A 1.5x0.5 cm, raised, pink to grey, mass is in the pituitary gland (pituitary adenoma, presumed).

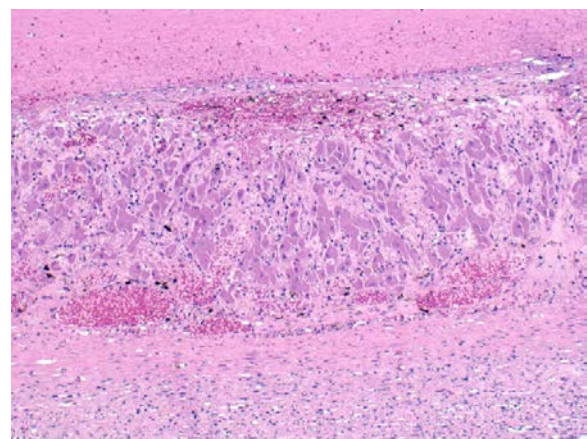
GENITAL: The left ovary (268 g) is oval with a 4.6X4.6X5.2 cm cyst having a 1-2 mm-thick, yellow wall and containing a

yellow and red, fibrillary and gelatinous content (submitted).

INTEGUMENTARY/SPECIAL SENSES, MUSCULOSKELETAL, HEMIC & LYMPHATIC URINARY (Right kidney weight: 1.47 kg; Left kidney weight: 1.56 kg),

DIGESTIVE, LIVER/PANCREAS (Liver weight: 9.1 kg), **NERVOUS** (Brain weight: 550 g): No significant findings.

Laboratory results: Hypergammaglobulinemia: globulins 5.4g/dL (92.2-3.8g/dL)
Mature neutrophils PMN 10,153/uL (2260-8580/uL)
Monocytosis 1287/uL (0-1000/uL)
Hyperfibrinogenemia 700mg/dL (100-400 mg/dL)
Thoracocentesis/trans-tracheal wash culture: Streptococcus zooepidemicus 4+, Bacteroides pyogenes 2+ and Fusobacterium spp. 4+



Ovary, horse. The wall of the cyst contains an inner layer of fibrovascular tissue and a thicker outer layer of well-vascularized luteinized thecal cells. (HE 100X) (Photo courtesy of: Texas A&M University, College of Veterinary Medicine and Biomedical Sciences <http://vetmed.tamu.edu/vtpb>.)

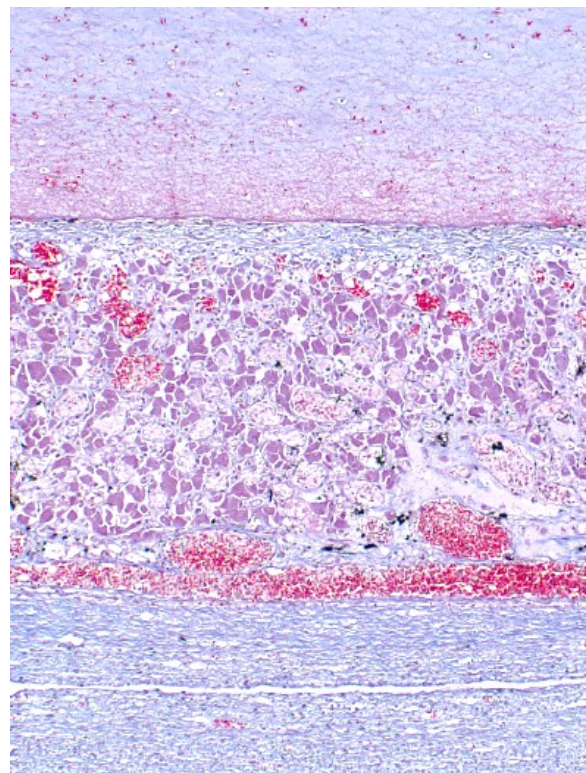
Microscopic Description: Ovary: The section consists of a border of ovarian capsule with a zone of ovarian cortex/stroma containing an occasional egg, various developing and atretic follicles and many hilar vessels. Below this cortical tissue is a band of normal fibromuscular ovarian stroma that ends as a discrete cyst. Moving centripetally into the cyst is a 5-15 luteal cell-thick lining in a fine, well vascularized stroma and with an inner thin layer of fibrous connective tissue. The central portion of the cyst has pools of free erythrocytes with fine, poorly organized fibrin and fibrillary material. The vessels in the luteal tissue are engorged and several have paucicellular, homogeneous walls (necrosis) with occasionally partial thrombosis. Luteal tissue has some siderophages, but most dark pigment seen is acid hematin. Occasional sections have a hilar artery with segmental, mild, mononuclear cell cuffing.

Contributor's Morphologic Diagnosis: Ovarian luteal cyst (Hemorrhagic anovulatory follicle-derived)

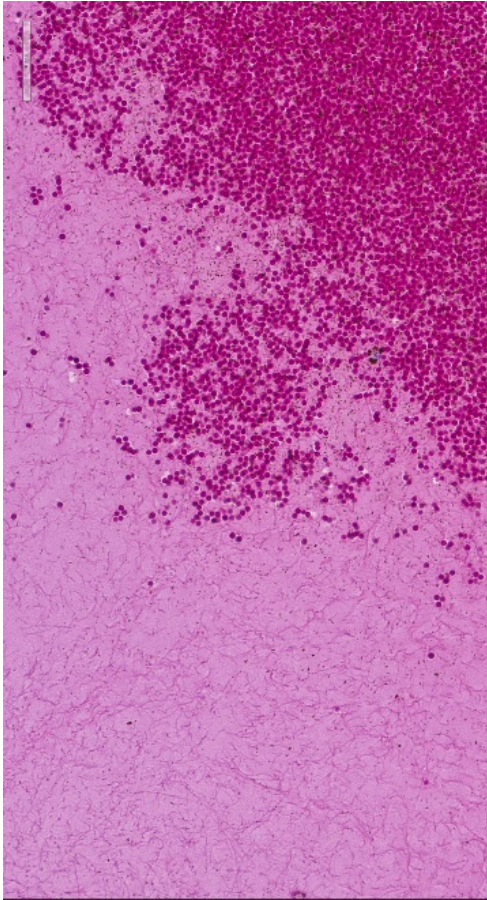
Contributor's Comment: This is an example of a hemorrhagic anovulatory follicle in the progression of becoming a luteal cyst. Hemorrhagic anovulatory follicles (HAFs) are a common but rarely described lesion seen in equine necropsies and equine biopsy submissions. It is odd that their description is not in veterinary pathology texts.

HAF's are an "event" described in the current veterinary literature by theriogenologists as a major cause of equine infertility^{1,2,3,4,5,6} and most recently in human medicine as a model for women's luteinized, unruptured-follicle syndrome.¹ This "event" must be discussed in view of the horse's reproductive physiology, because the HAF is similar if not identical to the transitional follicle, an ovarian structure

considered normal during the physiologic anovulatory period of the mare during winter's short photoperiod. Our case occurred at the end of April. The structure should be discussed with an understanding of the equine corpus hemorrhagicum (CH) and corpus luteum (CL). The CH and CL are triangular structures with the apex angled toward the ovulation fossa, and the CH fills in rapidly and usually completely to form a CL^{5,7} after ovulation and without significant fibrosis. Theriogenologists know HAFs are a common, if not the most common, lesion associated with anovulation and occur in 5-25% of mares during the breeding season.^{2,3,4,5,6} In ultrasound of HAFs, the follicular fluid has echogenic foci and swirling fibrin-like strands. Macroscopically on section, the hemorrhagic center is characteristically mottled yellow red,



Ovary, horse. A Masson's trichrome demonstrates the amount of collagen within the inner layer and exterior to the cyst wall. (HE 100X) (Photo courtesy of: Texas A&M University, College of Veterinary Medicine and Biomedical Sciences <http://vetmed.tamu.edu/vtpb>.)



Ovary, horse. The cyst contents are composed primarily of eosinophilic protein, some polymerized fibrin, and hemorrhage. (HE 100X) (Photo courtesy of: Texas A&M University, College of Veterinary Medicine and Biomedical Sciences <http://vetmed.tamu.edu/vtpb>.)

somewhat gelatinous and obviously different from a clot or thrombus. The blood does not flow out of an HAF when it is sectioned. Nor do you see characteristic layering of a large thrombus. Some mares will have several HAFs in a breeding season, and some mares are more predisposed to having them. They are more frequent in old mares. Although, like transitional follicles, HAFs are not treated routinely, some work has been done to discover how to induce them in order to understand the natural pathophysiology of the HAF in the breeding season. Variable success at inducing HAFs has been achieved with administration of prostaglandin, LH and NSAIDs using various regimen. Most

recently, dosing mares with the COX-2 inhibitor, flunixin meglumine, resulted in 100% HAF induction.¹

When do veterinary pathologists see them? They are common in necropsies of mares with chronic bacterial diseases (like this case) and in cases of endotoxemias and colics. Interestingly, they appear in biopsy services as specimen from ovariectomies where they clinically are diagnosed granulosa cell tumors by rDVM's. In such biopsy cases, history of an episode of colic in the previous 10 days is common/predictable. One can certainly think that products of sepsis and endotoxemias could both disrupt adenohipophyseal hormone release and generate interleukins and prostaglandins with the result of an anovulation. Most recently, our lab also sees them in ovaries coincident with abscesses induced by follicular aspirations, an increasingly common manipulation.

Are HAFs related to luteal cysts? The luteal tissue is well-vascularized and with DIC, hemorrhage accumulates in follicles that do not ovulate. Had the mare of this case survived, the hemorrhage would have increased due to the vascular lesions you see in the luteal wall. Follicle-sized cysts later form larger, spherical, blood-filled luteinized cysts. It is a rare ovarian hematoma that does not have a luteinized wall.^a

The old mantra was that "cystic ovarian disease as described in the cow does not occur in the mare",⁷ but there may be more similarities than previously believed. This is a case of an HAF progressing into a luteinized cyst and/or ovarian hematoma, but looked at from a pathologist's perspective and taken from a necropsy case.

Contributing Institution:

Texas A&M University, College of Veterinary Medicine and Biomedical Sciences (Departmental Web site address): <http://vetmed.tamu.edu/vtpb>

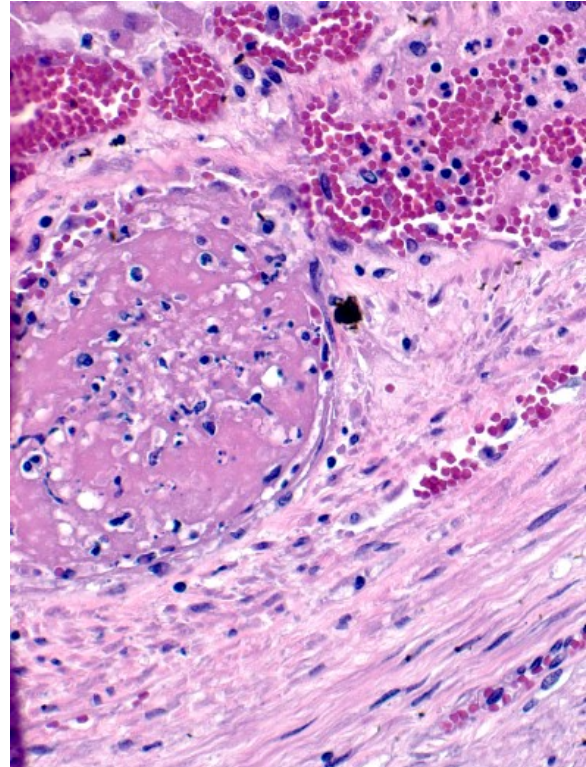
JPC Diagnosis: Ovary: Hemorrhagic luteal cyst (hemorrhagic anovulatory follicle OK)

JPC Comment: The contributor brings up excellent points on a fairly common cause of ovulation failure in older mares that rarely merits more than a line in most pathology texts. This lesion is a rarity in most surgical biopsy practices as especially as it is often successfully treated with prostaglandin therapy. It is most often seen as an incidental finding at autopsy, especially in animals dying suddenly of other causes.

Anovulatory hemorrhagic follicles (AHF), also known as persistent anovulatory follicles, will grow (especially during transition phases of spring and autumn) but fail to rupture, and has been estimated to occur between 4.4-13% of all ovulations in mares, with the higher percentages seen in older mares.⁴

While the true cause of this lesion is not known, quite a few theories abound as to its development. These include: a) insufficient gonadotrophic stimulation to induce ovulation; b) reduced follicular secretion of estrogen; c) physical blockage of passage of the developing follicle to the ovarian fossa; d) aberrant production of matrix metalloproteinases, required for tissue remodeling during ovulation and corpus luteal production, or e) overuse of ovulation-stimulating drugs, or f) abnormalities in angiogenesis during corpora lutea formation, or vascular regression during luteolysis.⁴

This slide is an excellent example of an AHF, with the characteristic wall consisting of a thin inner fibrovascular layer and a thicker outer layer of luteinized cells which is heavily vascularized and may be the source of significant ovarian hemorrhage which may be life-threatening. Immunohistochemistry



Ovary, horse. Multiple vessels within the luteinized thecal layer are thrombosed. (HE 400X). (Photo courtesy of: Texas A&M University, College of Veterinary Medicine and Biomedical Sciences <http://vetmed.tamu.edu/vtpb>.)

is not generally necessary for diagnosis, but these follicles often express an immunohistochemical profile similar to normal luteinized cells of corpora hemorrhagica and corpora lutea, with the exception of a decreased expression of Flk-1, a receptor for vascular endothelial growth factor (VEGF).⁴

The moderator commented briefly on the presence of thrombosed capillaries within this layer of luteinized cells surrounding the HAF, ascribing the thrombosis to the sepsis seen in this horse rather than the degeneration of the HAF itself.

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