The Armed Forces Institute of Pathology
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CASE I – 04-2871 (AFIP 2936449)

Signalment: Three near term fetuses, mixed sex, unknown breed, ovine (*Ovis aries*).

History: Six out of forty yearling ewes in the flock became mildly lethargic and would often stand alone in a corner away from the rest of the flock. Affected ewes had slight vaginal discharge followed by abortion. The older ewes had no clinical signs or abortions. All ewes were vaccinated, and the yearling ewes were vaccinated twice, including vaccinations for *Campylobacter* species.

Gross Pathology: The cotyledons in the placenta submitted with one of the fetuses were edematous and mottled with multiple 1-4 mm diameter, white to tan foci. The intercotyledonary placenta occasionally contained similar foci.

Laboratory Results: A pure culture of *Campylobacter jejuni* was isolated from the abomasal fluid of all three lamb fetuses.

There was no bacterial growth on aerobic or *Brucella* cultures. Fluorescent antibody testing of the lung, liver, kidney, placenta, and thyroid gland was negative for bovine viral diarrhea (BVD) virus (used to test for border disease virus). Fluorescent antibody testing of the placenta, lung, and liver was negative for *Chlamydophila abortus*. Fetal titers were negative for BVD virus at less than 1:2, negative for *Toxoplasma gondii* at less than 1:8, and negative for bluetongue virus.

Contributor's Morphologic Diagnoses: 1. Placenta: Multifocal, necrotizing and suppurative placentitis with mineralization, vasculitis, and thrombosis
2. Liver: Multifocal, suppurative and necrotizing hepatitis
3. Liver: Extramedullary hematopoiesis

Contributor's Comment: There is moderate postmortem decomposition in the submitted placenta, with loss of most of the trophoblastic epithelial cells. The chorionic villi contain multiple foci of intact and degenerate neutrophils with necrosis and mineralization. The chorioallantois is edematous and contains a few perivascular and multifocal infiltrates of macrophages, neutrophils and lymphocytes. The tunica media of a few small arterioles in the chorionic villi is infiltrated with intact and degenerate neutrophils and the vascular wall is necrotic. A few of these affected arterioles contain fibrin thrombi. In the submitted liver, there are multifocal areas of necrosis filled with variable numbers of neutrophils. There is extramedullary hematopoiesis in the liver. In the placenta of another lamb (not submitted), there is mild suppurative inflammation with many of the trophoblasts containing numerous gram-negative coccobacilli. In the lung of all three lambs (not submitted), there is suppurative bronchopneumonia.

Campylobacter species are small, curved, highly motile, noncapsulated, microaerophilic, gram-negative bacilli. In sheep, the most common manifestation of *Campylobacter* infections are late term abortions, stillbirths, premature births, the birth of weak lambs, and occasional ewe fatalities due to metritis.[1,2] The most common ovine *Campylobacter* is *Campylobacter fetus* subspecies *fetus*, but *Campylobacter jejuni* can also infect sheep.[1-4] One study showed an increase in ovine abortions caused by *Campylobacter jejuni* versus *Campylobacter fetus* subspecies *fetus* in the later years (1983-1989) of the study.[5] In this same study, *Toxoplasma gondii*, *Campylobacter* species, and *Chlamydophila abortus* were the most common identifiable causes of ovine abortions. Placentitis was the most prominent lesion.[5]

The macroscopic and microscopic lesions in aborted fetuses and their placentas are similar in infections with *Campylobacter fetus* subspecies *fetus* and *Campylobacter jejuni.* The lesions include necrotizing edematous placentitis, fetal suppurative bronchopneumonia, and multifocal hepatic necrosis.[1-3] The most common lesion seen with *Campylobacter* abortions in sheep is placentitis.[4] In some cases, the placentitis is macroscopically apparent. The liver lesions can be large enough to be seen grossly and have a "target" appearance.[1,3]

The transmission of *C. jejuni* and *C. fetus* subsp. *fetus* is believed to be orally due to fecal contamination of water and feedstuffs.[1] The *Campylobacter* organisms then become transiently bacteremic with localization of the bacteria in the gut and bile.[1] In nonimmune ewes, *Campylobacter* can localize in the uterus during the

bacteremic phase.[1] In nonimmune ewes that are pregnant, *Campylobacter* first localizes in the hilar zone of the placentomes causing vascular necrosis and thrombosis.[4] This results in separation of the chorion with invasion of the chorion and chorionic capillaries, with subsequent necrosis.[4] If the fetus survives the hypoxia secondary to the necrosis of the placenta, then the fetus can be invaded by the *Campylobacter*, and in some cases, undergoes fetal death.[4]

AFIP Diagnoses: 1. Chorioallantois (cotyledon): Placentitis, necrotizing, suppurative, diffuse, severe, with multifocal vasculitis, thrombi, and mineralization, breed not specified, ovine.
2. Liver: Hepatitis, necrotizing, neutrophilic, random, mild.

Conference Comment: The contributor provides a thorough overview of campylobacteriosis in sheep. The placentitis is characterized by an edematous intercotyledonary chorioallantois and friable, yellow cotyledons. Grossly, about 25% of the fetuses have multiple, yellow, "targetoid", areas of hepatic necrosis that are characteristic of the disease. *Flexispira rappini* causes similar lesions in the placenta and fetus, but infections are sporadic.[6]

Infectious causes of abortion in sheep include the following (C = cotyledonary; IC = intercotyledonary):[6,7,8]

Organism	Placental Lesions		Fetal Lesions
	Gross	Histological	
Campylobacter fetus fetus	C = friable, yellow IC = edema, exudate	Often vasculitis, inflammation severe in chorionic villi with gram-negative bacteria	Liver: large targetoid areas of necrosis
Toxoplasma gondii	C = pinpoint white foci of necrosis IC = edema	Chorionic epithelial hypertrophy and hyperplasia with rare intracellular zoites	Focal necrotic lesions in the brain, liver, kidney, lung
Neospora caninum	C = necrosis IC = normal	Zoites rarely seen within trophoblasts	Multifocal encephalitis with gliosis and necrosis
Chlamydophila abortus	C = necrosis IC = brown exudate	Necrotizing placentitis with neutrophilic vasculitis and organisms within trophoblasts	Inflammatory/necrotic foci in the liver, lungs, muscle, etc.
Coxiella burnetii	C = less affected IC = thick, yellow, with exudate	IC necrotizing placentitis with gram-negative rickettsial organisms within chorionic epithelium	Inconsistent; lymphocytic infiltrates in the lungs, kidneys, liver
Brucella ovis	C = necrosis IC = brown exudate	Vasculitis; gram-negative bacilli intra- and extracellularly	Nonspecific
Listeria monocytogenes	C = necrosuppurative IC = necrosuppurative	Severe diffuse necrosuppurative placentitis with gram-positive bacteria within chorionic epithelial cells	Hepatomegaly with numerous 1mm yellow necrotic foci

Other less common causes of ovine abortion include *Salmonella dublin, S. typhimurium*, *S. abortusovis*, Ovine orbivirus (Bluetongue virus), Ovine pestivirus (Border disease), and bunyaviruses (Akabane virus, Cache Valley virus, Rift Valley fever virus). [6,7,8]

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CASE II – UCONN 2004#2 (AFIP 2942012)

Signalment: Four-year-old, female, mixed-breed dog (*Canis familiaris*).

History: The dog is a free-ranging outdoor dog from the U.S. Virgin Islands. A protruding vulvar mass of two weeks duration was surgically removed.

Gross Pathology: A section of the vulvar mass, measuring 3 cm x 1.5 cm x 1.5 cm is submitted for histopathologic examination. The tissue has a uniform pale tan color and is homogeneous on cut section.

Contributor's Morphologic Diagnosis: Vulva: Transmissible venereal tumor, canine.

Contributor's Comment: Vulva. The section is of non-haired, non-cornified, stratified squamous epithelium and associated submucosal connective tissue, within which there is a raised, sessile, superficially eroded mass. The mass is well-demarcated, nonencapsulated, highly cellular and expansile, extending into the submucosa. The mass is composed of loosely packed sheets and cords of round cells, separated by fine strands of fibrovascular connective tissue. Cells are uniform, with scant eosinophilic cytoplasm and distinct cytoplasmic margins. Nuclei are large, round with marginated chromatin and a single prominent nucleolus or occasionally two nucleoli. There are moderate numbers of mitotic figures (2 – 4/HPF). At the deep and lateral margins, the stroma is infiltrated by small numbers of lymphocytes, fewer plasma cells, and macrophages. Toluidine blue stain shows few mast cells in subepithelial tissues; there are no metachromatic granules detected in the cytoplasm of tumor cells.

Canine transmissible venereal tumor (CTVT) is a naturally occurring contagious round cell neoplasm with a primarily histiocytic immunophenotype, with immunopositivity to lysozyme and vimentin.[1] Tumors are primarily found in the mucus membranes of the external genitalia of dogs of both sexes. Commonly, single or multiple masses are found on the caudal part of the penis, from crura to bulbis glandis and glans penis, and, in females, in the posterior part of the vagina and at the junction of the vestibule and the vagina. The tumor occurs also extragenitally in the nasal and/or oral cavities. This tumor is most often seen in young, roaming, sexually active dogs. CTVT is transmitted only by the transplantation of viable tumor cells to mucus membranes at coitus or during other contact. CTVT have been reported in the lymph nodes and skin, and occasionally in the tonsils, liver, pancreas, spleen, lungs, and kidneys. These neoplasms typically regress without treatment via an IgG-mediated immune response; however, metastasis does occasionally occur.[2,3] Distinctively, karyotyping of the cells of this tumor reveals 58-59 chromosomes, with 13-17 metacentric, compared to the normal canine component of 78 with 2 metacentric.[2]

The differential diagnosis includes mast cell tumor, histiocytoma, plasmacytoma, cutaneous lymphoma, and, grossly, other neoplasms of the vagina and vulva including papilloma, squamous cell carcinoma, epidermoid carcinoma, fibroma, and leiomyoma.

There is patchy worldwide distribution of this tumor, with endemic areas in the Caribbean, where this dog lived.[2] Transmission to the fox, coyote and jackal is possible.[4]

AFIP Diagnosis: Vulva (per contributor): Transmissible venereal tumor, mixed-breed, canine.

Conference Comment: Canine transmissible venereal tumor (CTVT) is the only known naturally occurring tumor that can be transplanted as an allograft across major histocompatibility (MHC) barriers within the same species, and to other canids, such as foxes, coyotes, and wolves.[5]

The histogenesis of CTVTs is not yet certain, but immunohistochemical studies suggest the cells are of histiocytic origin. The cells are immunohistochemically positive for vimentin, lysozyme, ACM1 (an epitope on canine mononuclear phagocyte stem cells), and alpha-1-antitrypsin (a good marker for benign and malignant histiocytes). These antigens are not expressed by other mesenchymal round cells, except those of histiocytic origin. Nonetheless, CTVT cells are unique in that they contain only 59 chromosomes. The normal diploid number of chromosomes in the somatic cell of the dog is 78.[5]

Like histiocytomas, the growth pattern of CTVTs includes a progressive growth phase, a static phase, and a regression phase. The progressive growth phase occurs after sexual transmission and is characterized by rapid proliferation of neoplastic cells. The static phase follows and is characterized by indolent local tumor growth or progression with metastasis. Some tumors regress spontaneously. CTVTs evoke both humoral and cell mediated immune responses, and as seen in this case, infiltrating lymphocytes may be present within and around the neoplasm during the regression phase.[5]

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References:

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CASE III – 2004B (AFIP 2937641)

Signalment: 2-year-old, CD-1, male mouse (*Mus musculus*).

History: This mouse was from a control group on a 2 year carcinogenicity study. There were no adverse antemortem findings.

Gross Pathology: At necropsy, the left lobe of the seminal vesicle was irregular, with red/brown discoloration.

Contributor's Morphologic Diagnosis: Seminal vesicle: Granular cell tumor, benign.

Contributor's Comment: Granular cell tumors are generally a single mass in the male and female genital tract of the mouse, but may occur in other organs. These benign tumors typically grow by expansion but can include a more infiltrative pattern. Individual cells have abundant pale cytoplasm filled with numerous eosinophilic, course granules, and small, round to oval nuclei. The cytoplasmic granules are consistent with secondary lysosomes (residual bodies) and are PAS-positive and diastase resistant. The histiogenesis of the granular cells is unknown, although Schwann cells or primitive mesenchymal cells have been proposed as the cell of origin. Synonyms include myoblastoma and benign Abrikossoff's tumor. [1]

AFIP Diagnosis: Seminal vesicle: Granular cell tumor, mouse, murine.

Conference Comment: Granular cell tumor (GCT), once called granular cell myoblastoma, is an uncommon neoplasm of uncertain origin. They have been most frequently reported in the dog and horse, but also occur in laboratory rodents, cats, and birds. In the dog, GCT occurs most commonly in the tongue, but they have been reported in the ear, lip, palate, cerebral cortex and meninges, heart, lymph node, orbit, and the skin. In the horse, GCT appears to be exclusively a tumor of

the lung, and is frequently found in association with the bronchi, but may be disseminated throughout the lung.[2] Granular cell tumors have been described in the genital system, brain and meninges in mice and rats.[1,3] In cats, GCTs have been reported in the tongue, palate, vulva, and digits.[2]

Grossly, the neoplasm is often nodular, whitish, and firm. Microscopically, the tumor very characteristically consists of nests of large, round to polygonal cells with prominent, coarsely granular, eosinophilic cytoplasm. The cytoplasmic granules are PAS (periodic acid-Schiff) positive and diastase resistant. The circumscribed nature of many of the tumors and a lack of mitotic activity suggests a benign course. However, some reported GCTs were invasive and/or mitotically active. Granular cell tumors are immunohistochemically variably positive for vimentin, S-100 protein, and neuron specific enolase (NSE), emphasizing the heterogeneous nature of these tumors. Ultrastructurally, the cells contain packed lysosomes and phagosomes (myelin bodies).[4]

Contributor: Merck Research Laboratories, Department of Safety Assessment, WP45-227, Sumneytown Pike, West Point, PA

References:

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CASE IV – CASE #2 (AFIP 2940162)

Signalment: Seven-week-old, male Sprague-Dawley rat.

History: Administered 200 mg/kg/day ethylene glycol monomethyl ether (EGME) for 4 days.

Gross Pathology: None

Contributor's Morphologic Diagnosis: Testis: Stage-specific spermatocyte necrosis, and spermatocyte and round spermatid loss.

Contributor's Comment: EGME is a well-characterized experimental testicular germ cell toxicant in rats.[1] One day following a single 200 mg/kg dose, degeneration of spermatocytes (both those undergoing meiotic division and early pachytene stage) in Stage XIV tubules is evident. Occasionally, spermatocyte degeneration is also evident among Stage I and XIII tubules.

In rats examined following dosing with EGME for 4 consecutive days (such as the submitted case), similar spermatocyte degeneration predominantly within Stage XIV tubules is evident, as is depletion or absence of round spermatids and spermatocytes among Stage I-V tubules. There are occasional Stage IV or V tubules having absence of pachytene spermatocytes but retention of round spermatids, consistent with loss of Stage XIV early pachytene spermatocytes (but not spermatocytes undergoing meiotic division) following exposure to EGME on day 1 of dosing. Most conspicuous are tubules having Sertoli cells, spermatogonia, and elongate spermatids present, but lacking spermatocytes and round spermatids (presumably Stage I to VI tubules). These represent tubules having lost all spermatocytes when at Stage XIII or XIV, some time in the previous 4 days. Some of the affected tubules contain multinucleate germ cells (syncytia of round spermatids). However, Stage VII through XII tubules are generally unaffected.

Staging of the seminiferous tubular epithelium is useful for identifying temporal changes in germ cell associations in the course of spermatogenesis.[1,2,3] Each stage identifies a morphologically distinct array of spermatogonia (proliferating diploid germ cells), spermatocytes (meiotic [tetraploid] germ cells), and round and elongate spermatids (differentiating haploid germ cells) at a particular phase of development, supported in layers by basilar Sertoli cells. Staging schemes are based on light microscopic morphologic characteristics (usually related to details of spermatid development) and vary among species and among investigators describing them. Stages are designated by a Roman numeral and are of variable temporal duration (ranging from 7 [e.g. Stage IX] to 58 hours [Stage VII] in the rat). The most widely accepted staging scheme for the rat has one 12.9-day cycle divided into Stages I through XIV. Four and a half cycles (56 days) are required for spermatogenesis - development of a mature rat (step 19) spermatid from a type A1 spermatogonium. Maturation of sperm (spermiogenesis) is described by morphologic changes of spermatids designated by Arabic numeral as steps 1 through 19 over the course of one and a half cycles. Familiarity with spermatogenic staging aids recognition and description of testicular injury in acute toxicologic studies.

In the rat, Stage I through VII tubules are characterized by a single layer of pachytene spermatocytes and two populations of spermatids (both round and elongate). At Stage VIII, step 19 spermatids are released into the lumen and the round (step 8) spermatids begin to elongate. Stage IX through XIII tubules have two layers of spermatocytes (the luminal layer being large pachytene spermatocytes, and the basilar layer smaller preleptotene, leptotene, or zygotene spermatocytes) and a single layer of elongating spermatids. Stage XIV tubules have the luminal spermatocytes undergoing meiotic division to secondary (diploid) spermatocytes and then (haploid) round spermatids, and basilar spermatocytes progressing to the pachytene stage.

AFIP Diagnosis: Testis, seminiferous epithelium: Degeneration, necrosis and loss, segmental, with multinucleated germ cells, Sprague-Dawley rat, rodent.

Conference Comment: The contributor provides a thorough overview of the staging of seminiferous tubules and the importance of stage specific changes that may occur with testicular germ cell toxicants. For the toxicologic pathologist, the ability to identify the tubular stages of the spermatogenic cycle and a sound understanding of the spermatogenic process are essential in order to detect and characterize toxic effects to the male reproductive system. There are several excellent references listed below which cover this topic.

Contributor: Abbott Laboratories, Department of Pathology, AP13A/R 469, 100 Abbott Park Road, Abbott Park, IL

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