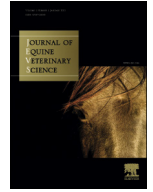




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Case Report

Multiple Uterine Subserosal and Submucosal Intramural Leiomyomas in a Mare: An Immunohistochemical Study



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ABSTRACT

A 32-year-old, pluriparous, oriental horse breed mare was presented to the Veterinary Teaching Hospital of the Veterinary Medical School of Messina, with clinical symptoms characterized by vulvar blood drainage for suspicious abortion. Ultrasonographic examination showed the presence of blood in uterine body and a complex-mass structure, echo dense with cystic areas with anechoic content at the basis of the right horn. Uterine leiomyoma was suspected, a partial ovary hysterectomy was proposed to the owner; despite this, the owner refused to submit the mare to surgery. The mare spontaneously died after 18 months, and necropsy was performed. At macroscopic postmortem examination, the right uterine horn showed a well-demarcated pedunculated nodular bulge, variegated in color with large hemorrhages (22 × 18 × 15 cm), and a capsulated sessile nodule (7 × 4 × 3 cm) involving the smooth uterine musculature. Histologic analysis suggested a diagnosis of subserosal leiomyoma (large mass) and submucosal intramural leiomyoma (small nodule).

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1. Introduction

Uterine tumors are rarely detected in domestic animals. Only the 0.4%–1.6% of the neoplasms diagnosed in equine species have a uterine origin [1]. Moreover, several kinds of tumors have been reported as incidental uterine findings in horse, such as fibroma, fibrosarcoma, adenocarcinoma, lymphosarcoma, leiomyoma, leiomyosarcoma, fibroleiomyoma, and rhabdomyosarcoma [1]. Leiomyoma is a benign neoplasm derived from the external smooth muscle of the uterus. This neoplasm seems to be the uterine tumor more frequently reported in mares [2]. In mares, uterine leiomyomas are generally benign and peduncu-

lated, even if they could also appear as intramural masses [3]. Small uterine tumors (<5 cm) can be hard to find and/or could be an incidental finding during clinical evaluation; very often, they are clinically asymptomatic. Uterine neoplasms of bigger size (>5 cm) are commonly associated to clinical signs, such as persistent infertility, abdominal pain, protrusion of the neoplastic tissue through vulva, recurrent uterine hemorrhage, and secondary endometritis. Furthermore, they can be palpable via transectal inspection or detected at ultrasound examination during a clinical evaluation of the genital system of mares with hypofertility or via hysteroscopy [3–7]. These benign neoplasms may interfere with fertility and consequently should be surgically removed as an elective treatment choice, determined by size and localization of the tumor; by contrast, malignant tumors are generally not eligible to treatment because of their usually poor prognosis. However, in cases of very large and extremely invasive tumors,

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complete or partial ovary hysterectomy has been generally suggested [3]. Considering that only few sporadic data about multiple uterine leiomyomas are present in literature on mares, we would describe herein a case report concerning the occurrence of this unusual pathology in an old mare.

2. Materials and Methods

A 32-year-old, pluriparous, mare was presented to the Veterinary Teaching Hospital of the Veterinary Medical School of Messina in 2012, with clinical symptoms characterized by vulvar bloody discharge for suspicious abortion. Anamnestic data referred by the owner revealed that the mare had delivered eight times, the last one in 2010, all eutocic; moreover, the mare was mated incidentally about 3 months ago. Periodically blood discharge was registered during estrus. At the time of the first clinical evaluation, the mare was healthy and in good nutritional status; the external genital system was normal, with a slight ventral–cranial inclination of the vulvar rhyme subsequent to depression of the anus. At vaginoscopic examination, vestibulus and vagina were normal; cervix was flaccid, semi-open with evident blood clot. Rectal exploration showed dilatation of the uterine body and the right uterine horn. The body had a fluctuant consistence for the presence of fluids, whereas uterine horn was filled of something referable to a fetal structure. Ultrasound examination, carried out using a portable echograph Aquila Esaote with a linear multifrequency (5–8 MHz) probe, showed an echoic corpuscular fluid within the body, referable to blood, and at the basis of the right horn an echoic, complex-mass structure with cystic spaces with an anechoic content was seen (Fig. 1). Left ovary showed small follicular structures, whereas right ovary showed a preovulatory follicle, 3.9 cm in size.

After verifying the absence of fetal structures, hysterectomy was carried out by a flexible 10-mm fibroscope, provided with a light source and insufflating and/or aspirating system Olympus CLK-532, connected to an external monitor and an image acquiring system. The presence of blood did not permit a clear visualization of the uterine mass, although biopsies were obtained from both endometrium and mass; tissue samples were fixed in 10% buffered formalin at room temperature (RT) for 24 hours and later processed for histologic evaluation. After these biopsy procedures, a blood sample was obtained and sent to the laboratory for hemochromocytometric and biochemical examinations, as well as to check 17- β estradiol (E_2) and progesterone (P_4) levels. On the basis of histologic evaluation, partial ovary hysterectomy was proposed to the owner that refused surgery.

Thus, the mare was periodically monitored, and when it showed uterine hemorrhage, it was treated with an antihemorrhagic (menadione sodium bisulfite/polyvinilpirrolidone, 1 mL/10 kg/d intramuscular) as palliative care. Mare spontaneously died 18 months later, and the owner required necropsy.

At necropsy, all organs were evaluated. Samples taken from both uterine masses were fixed in 10% neutral formalin for 48 hours at RT and then embedded in paraffin at

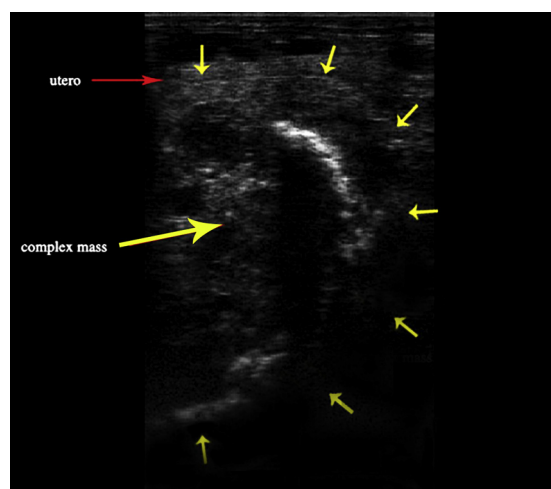


Fig. 1. Basis of the right uterine horn (red arrow): complex-mass, echoic, with cysts filled by anechoic content (yellow arrows).

56°C. From each tissue block, 4- μ m-thick sections were cut and routinely stained with hematoxylin and eosin, mounted on silane-coated glasses, and then dewaxed in xylene and rehydrated in graded ethanols. Parallel sections were stained by Masson trichrome; moreover, additional sections mounted on silane-coated slides were subjected to immunohistochemical investigations. For this purpose, sections were treated in a moist chamber: (1) with 0.1% H_2O_2 in methanol to block the intrinsic peroxidase activity (30 minutes at RT); (2) with normal sheep serum to prevent unspecific adherence of serum proteins; (3) with monoclonal primary antibodies against (anti-human) smooth muscle actin (SMA; DakoCytomation, Copenhagen, Denmark; working dilution [w.d.], 1:250), desmin (DakoCytomation; w.d., 1:200), and vimentin (DakoCytomation; w.d., 1:125); (4) with sheep anti-mouse immunoglobulin antiserum (Behring Institute; w.d., 1:25; 30 minutes at RT); and (5) with mouse anti-horseradish peroxidase-anti-peroxidase complexes (Dako Cytomation; working dilution [w.d.], 1:25; 30 minutes at RT). For the demonstration of peroxidase activity, the sections were incubated in darkness for 10 minutes with 3-3' diaminobenzidine tetrahydrochloride (Sigma Chemical Co, St Louis, MO), in the amount of 100 mg in 200 mL of 0.03% hydrogen peroxide in phosphate-buffered saline (PBS). The nuclear counterstaining was performed by Mayer hemalum. Moreover, human tissues obtained from uterine leiomyoma, cardiac rhabdomyoma, and retroperitoneal sarcoma were used as a positive control. To test the specificity of each immunostaining to deny the possibility of nonspecific reaction, serial sections of each specimen were tested by replacing the specific antisera by either PBS or normal rabbit serum. The results obtained were negative.

3. Results

Necropsy performed on the mare showed good skeletal development and body constitution, good nutrition state, and evident hyperextension of the abdominal cavity.

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