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A subvulvar leiomyosarcoma in a Simmental cow - a case report

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ABSTRACT

In this case, a mass located subcutaneously in the perineal subvulvar region of a six-year-old Simmental cow was examined clinically, pathomorphologically and immunohistochemically. Macroscopically, the solitary, whitish-yellow- tumor mass was 19x15x6 cm in size, weighed 1610 grams, and had a lobular structure with a few small cystic formations in the section. Histopathological examination revealed that the tumor parenchyma had smooth muscle-like cells with abundant cytoplasm, pleomorphic cells with blunt-ended or cigar-shaped nuclei, anisocytosis, anisokaryosis and karyomegaly. Immunohistochemically, strong positive expression for α-SMA, vimentin, Ki67 and slight positive for desmin were found, while immulolabeling for pancytokeratin (AE1/AE3), S-100, CD31 and CD34 were negative. In conclusion, on the basis of these findings, the tumor was diagnosed as leiomyosarcoma.

Key words: surgery; histopathology; α-SMA; vimentin; desmin; Ki67; perineal subvulvar leiomyosarcoma; Simmental cow

Introduction

Smooth muscle tumors are very rare in domestic animals. They frequently occur in the gastrointestinal and urogenital tracts. This type of tumor may be classified as benign (leiomyomas) or malignant (leiomyosarcomas-LMS) (COOPER and VALENTINE, 2002). It has been established that 10-50% of such tumors originate from smooth muscles and, among them, approximately 10% are considered malignant (KLIJANIENKO and LAGACE, 2011). In retrospective studies dealing with bovine genital neoplasms, smooth muscle accounted for 0.03% of the tumors and only one (0.0006%) had features of a LMS (PIRES et al., 2017). LMS develops in four different clinical settings: in the retroperitoneum, cutaneously and subcutaneously, deep-seated, and of vascular origin (KLIJANIENKO and LAGACE, 2011). LMS tumors of smooth muscles can originate from the wall of the uterus or gastrointestinal tract, soft tissues that are rich in a smooth muscle, or the wall

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of great vessels (ITOGA et al., 2013). LMS exhibit malignancy with slow invasion and rare metastasis in cows (ENGINLER et al., 2011).

Grossly, LMSs may be as well circumscribed as leiomyomas, but are larger and softer and have a tendency for fresh tumor necrosis, hemorrhage, and cystic degeneration (ACKERMAN and ROSAI, 2004). Microscopically, LMSs are defined as smooth muscle tumors with nuclear atypia in combination with any level of mitotic activity (MIETTINEN, 2016). Prognosis is difficult to estimate. Superficial or cutaneous LMSs are generally small and have a good prognosis, whereas retroperitoneal tumors are large and difficult to excise. They cause death by both local extension and metastases (ROSENBERG, 2017).

LMSs often grossly resemble leiomyomas, but they are invasive rather than circumscribed, and may ulcerate mucosa (HULLAND, 1990). The cells resemble those in leiomyomas in general cytology, but there is generally some degree of anisokaryosis, multiple nucleoli, scattered bizarre nuclei, and obvious mitotic activity (YENER et al., 2001). There may be necrosis in the mass, and mild lymphocytic or eosinophilic inflammatory cells (ENZINGER et al., 1988).

This paper describes the clinical, pathomorphological, histological and immunohistochemical features associated with a leiomyosarcoma seen in the subvulvar region of a six-year-old Simmental cow.

Materials and methods

A six-year-old Simmental cow, weighing approximately 750 kg, was presented to a private veterinary clinic in the Gevas district of Van province with a subvulvular mass. On clinical examination the animal was in average condition, without any symptoms of systemic disease. A round mass was protruding from the subvulvar region. On palpation, the tumor mass was firm and not painful. It was covered by perineal skin. There was no ulcerative lesion or abrasion on the surface of the tumor mass. The cow was restrained and the tail was bandaged. Caudal epidural anesthesia was performed with the administration of 8mL of 2% lidocaine (Jetokain; Adeka, Turkey). Additional local infiltration anesthesia, within the area surrounding the pedicle of the tumoral mass, was performed with the same anesthetic agent using a volume of approximately 15-20mL. Following anesthesia, the vulva was retracted from either side with uterine forceps and the mass was revealed. An oval incision was made on the skin surface at a distance of approximately 2cm from the margin of the mass. Following incision, blunt dissection with scissors was used to increase the depth of the incision without interfering with the edge of the mass. The subcutaneous tissue was closed. The major blood vessels were ligated where necessary. The skin was apposed with +3 nylon (Dermalon, Davis & Geck) in a simple interrupted pattern. There was slight postoperative swelling and edema in the perineal area, but the cow defecated normally. Postoperative parenteral antibiotics (Clemipen-Strep; Topkim, Turkey) for five days and local wound healing pomads (Bepanthene plus; Roche, Turkey) were administered daily for 14 days. The mass was submitted for histopathological examination to the Department of Pathology (Veterinary Faculty, University of Yuzuncu Yil, Van, Turkey). Eight months after the operation the owner reported that the cow had no signs of recurrence of the mass.

Macroscopically, the mass was found to be 19x15x6 cm in diameter and weighed 1610 gram. The tumor was solitary, whitish-yellow in color, and the sectioned surface had a lobulated appearance. The tumor contained cystic formations, without regions of superficial ulceration (Fig. 1 A-C).

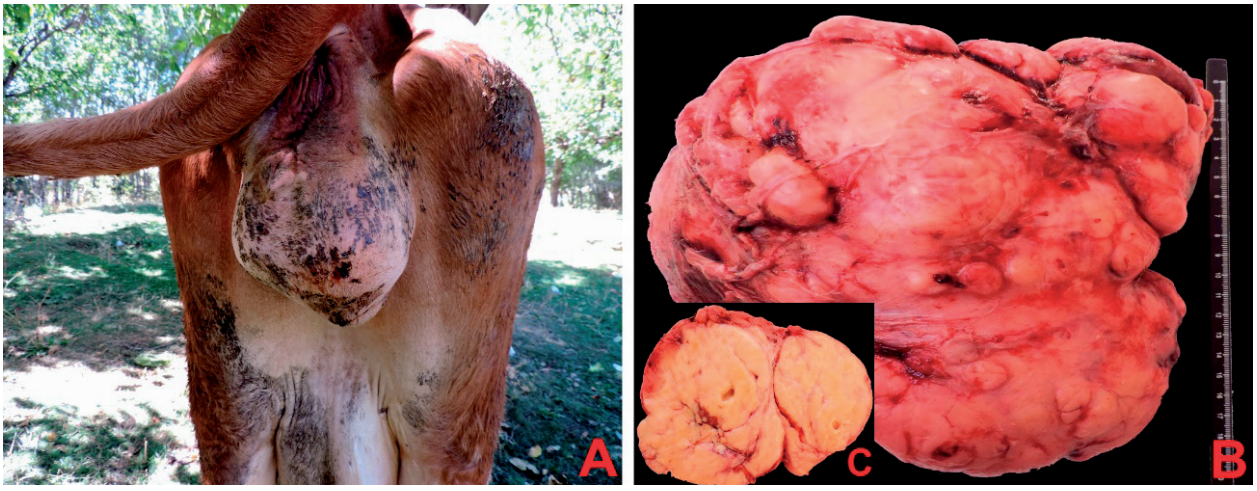

Fig. 1. Macroscopic view of the tumor mass on the perineal subvulvar area (A); macroscopic view of the excised tumor (B); macroscopic view of cut surface of the mass (C)

The tissue samples were fixed in 10% neutral buffered formalin. After processing routinely, serial tissue sections of the tumor mass were stained by the Hematoxylin and Eosin (H-E) technique, and submitted for immunohistochemistry (IHC). IHC was performed according to the avidin-biotin-peroxidase complex (ABC) method (NADJI and MORALES, 1986) using antibodies for some tumor markers, such as α-SMA, vimentin, desmin, pancytokeratin (AE1/AE3), S-100, Ki67, CD31 and CD34 (Table 1). After deparaffinizing and rehydrating, sections were rinsed in distilled water. Then after quenching the endogenous peroxidase activity with 3% H_2O_2 (v/v) for 20 min, the slides were washed twice in 0.01 M phosphate-buffered saline (PBS) for 5 min. After the sections were put into an antigen retrieval solution (citrate buffer) in a container, the lid of the container was closed and exposed to heat in an oven twice, each session lasting for 20 min. Before adding the primary antibody, the slides were incubated with blocking serum (Histostain-Kit, Invitrogen, CA, USA) for 15 min to block nonspecific binding. Sections were incubated with the primary antibodies and kept at 4 ºC overnight in a humidified chamber. Slides were washed 4 times in 0.01 M PBS for 5 min, incubated with biotinylated secondary antibody (Histostain-Kit, Invitrogen, CA, USA) for 20 min at room temperature, and then washed 4 times in 0.01 M PBS for 5 min.

Table 1. Primary antibodies used on differential diagnosis of the liomyosarcoma

Antibody	Host	Dilution	Source	Catalog no.
α-SMA	Mouse/monoclonal	1 : 400	Dako	M0851
Vimentin	Mouse/monoclonal	1 : 200	Dako	M0725
Desmin	Mouse/monoclonal	1 : 200	Dako	M0760
Pancytokeratin	Mouse/monoclonal	1 : 100	Novocastra	NCL-AE1/AE3
S-100	Mouse/monoclonal	1 : 100	BioGenex	MU058-5UC
Ki67	Mouse/monoclonal	1 : 100	Novocastra	NCL-Ki67p
CD31	Mouse/monoclonal	1 : 50	Cell Marque	131M-95
CD34	Mouse/monoclonal	1 : 50	Cell Marque	134M-15

After incubation with secondary antibody, sections were incubated with streptavidin–peroxidase conjugate (Histostain-Kit, Invitrogen, CA, USA) for 20 min, and then washed 4 times in 0.01 M PBS for 5 min, following enzymatic incubation. An ABC staining kit was used for labeling and the reaction product was visualized with 3.3.9-diaminobenzidine chromagen (DAB) (Histostain-Kit, Invitrogen, CA, USA) for 1-2 min. After development of DAB reactions, the sections were counterstained with Gill hematoxylin. The sections were then passed through alcohol and xylene, and mounted directly with Entellan mounting medium (Merck, Millipore-Sigma, St. Louis, MO). Negative controls were used in order to confirm the staining. For this purpose, the negative control slides underwent reactions with PBS instead of reacting with primary antibodies. The slides were examined and photographed using a light microscope (E-400, Nikon, Tokyo, Japan) equipped with a video camera (DS-U3, Nikon, Tokyo, Japan).

Results

Histopathological investigation revealed that the tumor parenchyma was composed of densely smooth muscle-like cells, with abundant cytoplasm, pleomorphic cells with blunt-ended or cigar-shaped nuclei, and a central nucleus, and rare tumoral giant cells, admixed with variably dense collagenous stroma. Some sections had pleomorphic cells with marked nuclear atypia including anisocytosis, anisokaryosis, karyomegaly, irregular nuclear contours and prominent nucleoli. There were some mitotic figures. The bundles within lobules of neoplastic smooth muscle were arranged in a very irregular pattern, traversing and intersecting at obtuse angles from one another (Fig. 2 A-B).

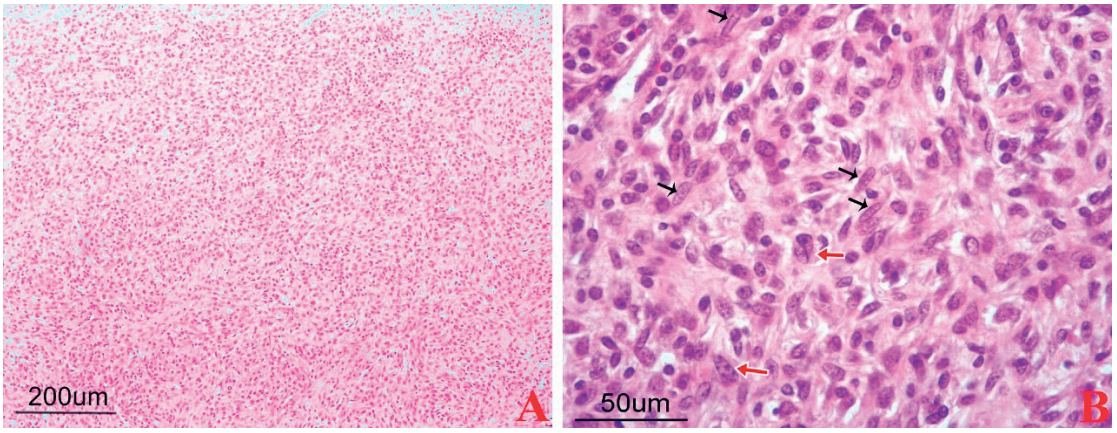

Fig. 2. Microscopical view of the tumor (A); many of the spindle shaped smooth muscle-like neoplastic cells (B) containing pleomorphism and nuclear atypia (black and red arrows), H&E

Immunohistochemical staining of the mass tissue sections showed strong α-SMA, vimentin and Ki67 immunoreactivity, slight desmin immunoreactivity revealing uniform intensive staining of neoplastic cells, whereas immunolabeling with; pancytokeratin (AE1/AE3), S-100, CD31 and CD34 was negative (Fig. 3 A-D).

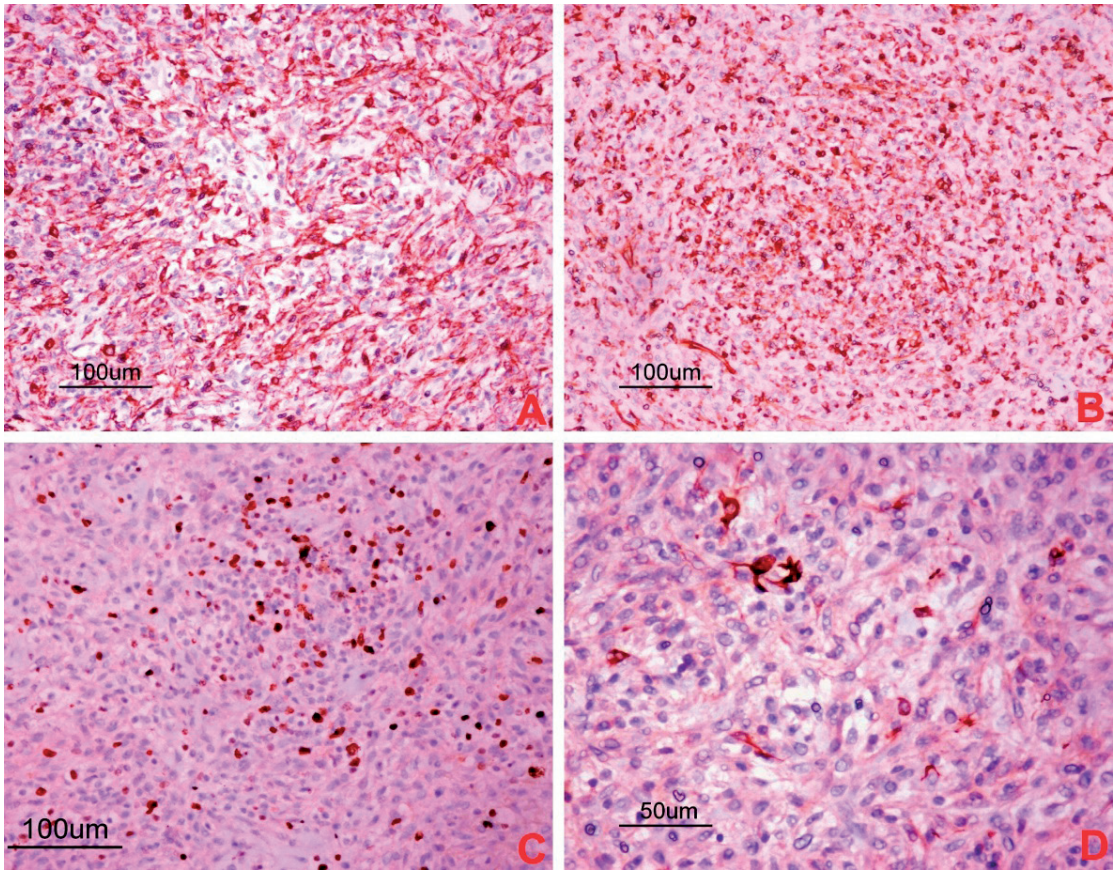

Fig. 3. Immunohistochemical study results: most neoplastic cells show strong positive immunoreactivity to α-SMA (A), Vimentin (B), Ki 67 (C); few neoplastic cells show positive cytoplasmic immunoreactivity to desmin (D); avidin biotin peroxidase complex method

Discussion

LMSs were previously determined in the vaginas of pregnant women, the uterus and cervix of goats, the uterus of sheep, the vagina and vulva of bitches, and the vulva of cats (WHITNEY et al., 2000; BEHZATOĞLU et al., 2003; HOSSEIN et al., 2009; NAKAMURA et al., 2010; ENGINLER et al., 2011) and cervical leiomyosarcoma in women and cows (MEHRA et al., 2015; RAVAL et al., 2016). Perineal LMS has rarely been reported in humans (RICE et al., 1990) and also in a heifer (MUELLER, 2009). However, no case of perineal subvulvar LMS has been reported in cows.

Leiomyosarcoma is a slow-growing neoplasm and it has also been reported in cows of different ages, such as 4 years old (AVCI et al., 2010; ENGINLER et al., 2011), 7 years old (RAVAL et al., 2016), and 12 years old (SAUT et al., 2013). In this present case, LMS was seen a six year-old cow.

LMSs of different diameters and weights, localizations, colors and shapes have been reported according to animal species. SUH and PARK (2008) reported that the tumor was grossly black-brown in color and approximately 6.0×5.5×5.0 cm in diameter. They also defined that the vaginal mass was well demarcated, nonencapsulated, and may protrude from the vulva. It has been stated that it was solid, smooth, and firm in cross-section, with a homogeneous greyish white color (AVCI et al., 2010). Additionally, SAUT et al. (2013) detected that a mass taken from the vaginal wall was a large 5x20x26cm, 1.4kg, firm, poorly encapsulated, and

unevenly surfaced growth. On gross examination of the tumor presented in this case, the tumor mass was measured as 19x15x6 cm in diameter and it weighed 1610 gram. It was solitary, encapsulated and, whitish-yellow in color. Moreover, it had a lobular structure and a few small cystic formations in the section surface.

Histopathologically, a LMS is characterized by the presence of spindle tumor cells with a fibrillated, acidophilic cytoplasm, cellular polymorphism, mitoses, multiple nucleoli, rare tumor giant cells, anisokaryosis, and regular elongated, blunt-ended or cigar-shaped configuration nuclei (HASHIMOTO et al., 1986; COOPER and VALENTINE, 2002; SAUT et al., 2013). The mitotic index as well as the atypical features and pleomorphism vary considerably from tumor to tumor. An average 1-2 mitoses have been reported in leiomyosarcomas. Areas of tumor necrosis, hemorrhage and inflammation are common, which result in marked edema and distortion of histological features (COOPER and VALENTINE, 2002). In this study, histopathological examination revealed that the unencapsulated tumor was composed of densely spindle shaped cells arranged in short interlacing bundles admixed with collagenous stroma. The nucleus of some of the spindle cells was characteristically large, with blunt ends or cigar-shaped. Additionally, this tumor exhibited marked pleomorphic cells and atypia, rare tumoral giant cells and moderate mitoses, but no necrosis was defined. According to these findings, the tumor was diagnosed as malignant.

Although the microscopic features of LMS are characteristic and can be easily recognized by pathologists, there are areas where this neoplasm may be confused morphologically with other neoplasia including fibromas, fibrosarcomas, hemangiopericytoma or malignant schwannomas (HASHIMOTO et al., 1986; HULLAND, 1990; YENER et al., 2001). In a large number of tumor cases, differentiation by immunohistochemistry is the standard for an accurate histopathological diagnosis (ENZINGER et al., 1988; YENER et al., 2001; AVCI et al., 2010). Smooth muscle actin is only expressed in smooth muscle neoplasms and in non-smooth muscle lesions with myoid differentiation, such as nodular fasciitis and myofibroblastic lesions (FRANQUEMONT, 1993). Vimentin is one of the other important intermediate filament proteins of the mesenchymal cells and is the essential key protein of the cytoskeleton (KOKKINOS et al., 2007). This is in contrast to keratin, which is the intermediate filament found in epithelial cells. Mesenchymal and endothelial cells commonly stain vimentin positive and thus act as a measure for internal quality control in immunoreactivity (FUYUHIRO et al., 2010). Desmin is an intermediate filament found in skeletal, cardiac and smooth muscles (ENZINGER et al., 1988). Typical leiomyosarcomas generally demonstrate prominent actin reactivity similar to that seen in benign leiomyomas, but desmin reactivity is variable and may be absent (RANGDAENG and TRUONG, 1991). ANDREASEN and MAHAFFEY (1987) were able to define desmin in only 14 of 22 LMSs investigated by the immunohistochemical method. Fewer desmin-positive cases might be detected in their studies, because prolonged formalin fixation has been shown to have a decreasing effect on tissue antigenicity in many studies. Ki67 is a nuclear protein that evaluates the growth fraction of neoplastic cell populations (INWALD et al., 2013). In this case, immunohistochemical staining for α-SMA, vimentin, desmin, CD31, CD34, pancytokeratin (AE1/AE3), Ki67 and S-100 protein was used to characterize the immunophenotype of the tumor. Strong α-SMA, vimentin, Ki67 and slight desmin immunoreactivity were found as positive but the others were negative in the present case. Positive immunolabeling for α-SMA, vimentin, desmin and Ki67 was able to support the histological features of LMS.

Histopathology, based on increasingly advanced immunohistochemistry methods, is routinely used in the diagnosis of neoplastic diseases. Comparisons may be helpful for a differential diagnosis between neoplasms (RUBISZ et al., 2019). Malign schwannomas exhibit variable morphology known as Antoni A and/or Antoni B patterns, and it also gives positive immunoreactivity for S-100 protein (ENZINGER et al., 1988). In this case, the tumor cells did not react with S-100 protein positivity and no findings of malignant schwannomas were

observed. Fibrosarcoma is determined by its parallel monomorphic spindle-shaped fibroblasts. In fibrosarcomas, vimentin is mostly the only positively stained marker (AUGSBURGER et al., 2017). These tumors are also typically mildly or focally immunopositive with smooth muscle actin as a sign of myofibroblastic differentiation (FISHER et al., 2002). Hemangiopericytomas are exhibited microscopically by spindle cells containing cytoplasmic processes arranged in whorls around the blood vessels in a fingerprint pattern formed by tumor cells. In hemangiopericytomas, CD31 and CD34 are taken as markers to define the vascular lineage (MITARAI et al., 1998; AVCI et al., 2010). The tumor cells gave a negative reaction to CD31 and CD34 antibody in this case. In this leiomyosarcoma, the detection of blunt-ended or cigar-shaped nuclei and a positive immunoreaction for α-SMA, vimentin, desmin is reported to be useful in differential diagnosis.

Conclusion

In conclusion, according to pathomorphological, histopathological and immunohistochemical findings the first case description of a bovine perineal subvulvar leiomyosarcoma was reported in a Simmental Cow originating from Turkey. However, the results indicate that for the differential diagnosis of this type of tumor, histopathological findings should be supported by immunohistochemical staining.

Conflict of interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SAŽETAK

U šest godina stare krave simentalske pasmine klinički, patomorfološki i imunohistokemijski obrađena je tvorba smještena supkutano u perinealnoj subvulvarnoj regiji. Solitarna, žutobjelkasta tumorska tvorba bila veličine 19 x 15 x 6 cm i težila je 1610 g. U režnjevitoj građi uočeno je nekoliko manjih cističnih formacija. Histopatološka pretraga pokazala je da tumor parenhima ima stanice nalik na glatke mišićne stanice s obilnom citoplazmom i pleomorfne stanice sa zaobljenom jezgrom ili jezgrom u obliku cigare, te s anizocitozom, anizokariozom i kariomegalijom. Imunohistokemijski je pronađena jaka pozitivna ekspresija za α-SMA, vimentin, Ki67 i slabo pozitivna za dezmin, dok je imunoobilježavanje za pancitokeratin (AE1/AE3), S-100, CD31 i CD34 bilo negativno. Na temelju ovih rezultata zaključujemo da se radi o tumoru leiomiosarkomu.

Ključne riječi: kirurgija; histopatologija; α-SMA; vimentin; dezmin; Ki67; perinealni subvulvarni leiomiosarkom; krava simentalske pasmine