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BILATERAL PRIMARY OVARIAN LEIOMYOMA IN A LION (*Panthera leo*): A CASE REPORT

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Abstract- Ovarian leiomyomas are very uncommon in carnivores, as smooth muscle tumors in these animals most frequently develop from the tubular genitalia. A rare case of bilateral, ovarian leiomyoma in a 13-year-old female lion (*Panthera leo*) is reported here. Although she was kept in the Zoo with another female and with a male, she was never diagnosed pregnant, in contrast to her cohort that conceived twice in the same period. The female was in good physical condition, and due to the need of anaesthesia to moving her to a different park, a decision on performing routine ultrasonographic evaluation of the genital organs was made. On transabdominal ultrasonography a bilateral altered echogenicity of the ovaries was observed. A compact mass with homogeneous isoechogenicity was detected on both ovaries; also ultrasonographic signs of mild uterine hyperplasia were observed. Consequently, the decision for ovariohysterectomy was formed. Resected material was sent for analysis, which confirmed the cystic endometrial disease, co-existing with a bilateral primary ovarian leiomyoma. Morphological evaluation of enlarged ovaries showed the existence of bilateral smooth muscle tumor that has been further characterized by specific histochemical and immunohistochemical analysis. In this paper the morphological, ultrasonographic and pathological features of this rare condition in a zoo lioness are described.

Keywords- ovarian leiomyoma; primary ovarian tumor; smooth muscle tumor; histochemistry; *Panthera leo*

Introduction

Leiomyomas are benign smooth-muscle tumors, which occurs, in all the species, more frequently in the tubular genitalia (oviducts, uterus, cervix or vagina) than in the ovary. Primary ovarian leiomyomas are uncommon ovarian disorders [1-3]. They are usually described as unilateral, solitary tumors that most frequently remain asymptomatic, thus being observed as incidental findings during ultrasonographic evaluation of the genital tract or at necropsy [1,3]. In the women, ovarian leiomyomas are frequently found in association with uterine smooth muscle tumors, and in such case they are considered as secondary leiomyomas [4,5], which seem to develop more frequently than primary leiomyomas and are usually associated with other ovarian lesions [6-8].

Genital leiomyomas developing mainly from the uterus but also from the cervix and the vagina, are common findings in domestic dogs, cats, cows and sows [3,9]. They have also been occasionally reported in wild felids [10,11]. Cooper and Valentine [1] refer to the existence, in Cornell files, of only five ovarian leiomyomas (one in a sow, three in bitches and one in a leopard), all of them being unilateral situations, while McEntee [3] found eight cases in bitches and only one case in a queen. Besides these references, only sporadic ancient reports on ovarian leiomyomas exist, either in domestic or wild felids [12,13]. The origin of primary ovarian leiomyomas remains unclear. It has been proposed to originate from the smooth muscle cells existing in the walls of blood vessel in the ovarian hilus [7,14] or from the smooth muscle fibres of the mesovarium [9,15].

For some authors, genital leiomyomas observed in Zoo animals could be associated with progestagen treatments, although the survey accomplished by Chassy, et al. [10] did not confirm this hypothesis. On their work the authors also refer the lion as having the higher frequency of occurrence of genital leiomyomas from the 23 different represented species. On that survey, even if the animal species was not referred, the presence of ovarian leiomyoma arising from the ovarian pedicule was reported, frequently accompanying the presence of smooth muscle tumors on other locations of the genitalia.

Sporadically, some cases of ovarian leiomyoma have been described in carnivores and ferrets [1,3,12] most of them secondary to other genital leiomyomas. However, to the authors' knowledge, it has never been described a bilateral primary ovarian leiomyoma, which has been diagnosed in a 13-year-old female lion (*Panthera leo*) during a routine ultrasonographic evaluation of the genital organs.

Case description

A 13-year-old female lion (*Panthera leo*), kept for almost 10 years at the Zoo da Maia (Porto, Portugal), in a heterosexual group, was submitted to routine exams as it was planned to moving her to another park. This female came from the Lisbon Zoo, where despite living in a heterosexual group, never conceived. At the Zoo da Maia she entered a group with a couple of lions, which produced two litters during this period. No accurate reproductive records were kept, and the existence of regular ovarian cycles could not be ascertained. Besides regular deworming (Doramectin 1%, 2ml, PO; Dectomax®, Pfizer) and occasional otitis, this female was considered to be otherwise healthy. The estimated weighted of the lioness was 160kg.

Under anaesthesia, blood samples were collected to hematological

and biochemistry analysis [Table-1], along with the ultrasonographic evaluation of the genital tract, by using a GE ultrasound scan (LOGIQ_e, General Electrics Healthcare Europe GmbH) and a multiple frequency probe (a 4-10 mHz veterinary probe 8C-RS; General Electrics Healthcare Europe GmbH).

Table 1- Hematological and serum biochemical values in the zoo lioness on the day of the ultrasound evaluation. (*Reference values for *Panthera leo* females older than 3 years [22]).

Parameter	Value	Reference values* [Min; Max]
Complete Blood Count		
RBC (x10 E12/L)	7.2	7.89 [5.51; 10.50]
Packed-cell volume (L/L)	0.365	0.390 [0.280; 0.512]
WBC (x10 E9/L)	15.98	13.79 [6.160; 26.70]
Neutrophils (x10 E9/L)	11.79	10.21 [0.038; 22.70]
Eosinophils (x10 E9/L)	1.57	0.507 [0.000; 2.880]
Basophils (x10 E9/L)	0.02	0.058 [0.000; 0.386]
Monocytes (x10 E9/L)	0.62	0.465 [0.000; 2.873]
Lymphocytes (x10 E9/L)	1.95	2.068 [0.033; 6.400]
Platelets (x10 E12/L)	0.38	0.320 [0.009; 0.734]
Serum biochemistry		
Albumin (g/L)	27.1	34 [25; 56]
Alkalin phosphatase (U/L)	10.2	19 [0; 89]
Alanine aminotransferase (U/L)	38.9	52 [0; 195]
Creatinine (mmol/L)	190.94	239 [62; 424]
Blood Urea Nitrogen (mmol/L)	22.81	10.71 [4.998; 23.21]
Total protein (g/L)	90	74 [62; 90]
Cholesterol (mmol/L)	5.01	4.628 [1.43; 7.722]
Triglicerids (mmol/L)	0.37	0.506 [0.044, 1.793]

The ultrasonography revealed a solid, isoechoic, compact mass located caudally to the kidneys, which occupied two-thirds of the ovary. There were also visible anechoic structures compatible with ovarian follicles, but no corpus luteum was detected on the ultrasound scans [Fig-1a]. In addition, altered ultrasonographic appearance of the endometrium compatible with cystic endometrial hyperplasia was recorded: increased thickness of the uterine walls and the accumulation of hypoechoic fluid within the uterine lumen [Fig-1b].

The results of blood analysis were found to be normal for the age and species despite the fluid accumulated into the uterus, thus discarding the existence of a pyometra on the moment of evaluation.

On the basis of the ultrasonographic findings and the previous history of infertility, and for the increased risk for developing pyometra due to uterine disease, the ovariohysterectomy was decided.

For the anesthesia, an association of Tiletamine-Zolazepan (Zoletil® 100, Virbac; diluted in 1.5 ml of solvent) was used in a dosage of 5mg/kg. A dart with 3.5 ml was used for sedation and a second intravenous administration (additional 200mg of Zoletil® 100; Virbac) was performed 20 minutes later, to maintain the anesthesia throughout the surgery. The lioness fully recovered from the general anesthesia within 24 hours.

Following the surgical procedures, the resected ovaries and the uterus were sent for the surgical pathology lab for analysis, fixed in 10% formalin. A marked increased of the ovarian dimensions and consistency was detected upon examination in both the ovaries. One ovary measured 5.5 x 1.5 x 3 cm and the other 6.5 x 4 x 2 cm at their greatest dimensions. Both ovaries exhibited a similar gross appearance. On the ovarian surface were visible several smooth and shiny, nodular yellowish masses that compressed the functional ovarian parenchyma into one ovarian margin [Fig-2a] [Fig-2b]. Some follicular structures were observed in both the ovaries, the largest with 2.5 cm in diameter. In addition, nearby one of the ovaries, a para-ovarian cyst, emerging from the oviduct, with 1.5 cm in diameter was found.

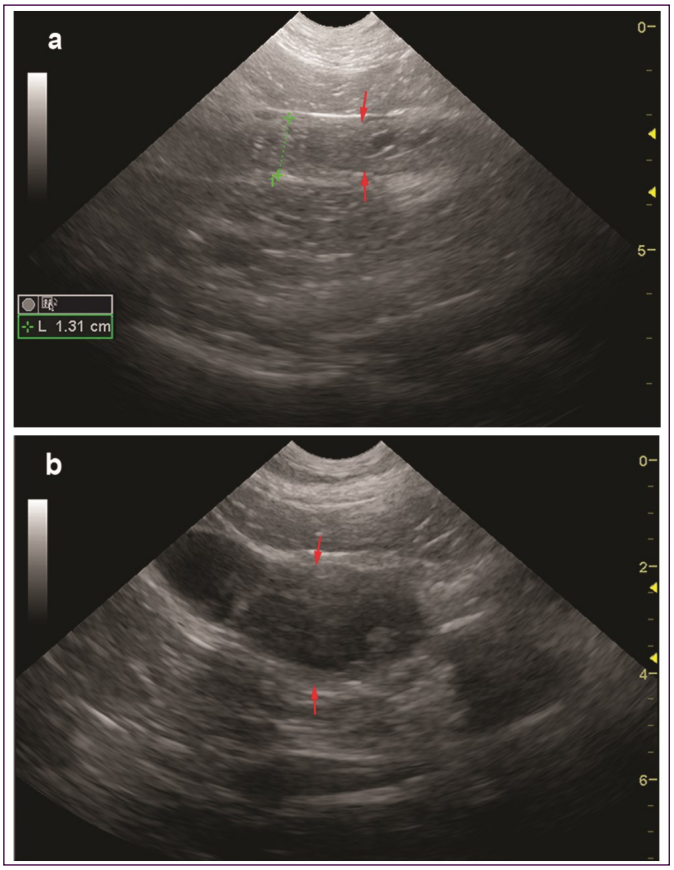

Fig. 1- Ultrasound scans of the ovaries and uterus in a *Panthera leo*: **(a)** In the ovaries an increase in size was recorded along with the existence of a solid mass (delimited between arrows) displaying a regular isoechoic pattern; **(b)** An increase in the size of uterine horns associated to a slight increase in the uterine wall thickness and to the accumulation of a limited amount of fluid within the uterus was found.

The longitudinal cut surface of the ovaries showed the presence of solid and firm masses, whitish in color, and evidencing a whorled pattern [Fig-2c], [Fig-2d]. upon gross examination the existence of antral follicular structures was confirmed [Fig-2d]. The uterine horns were enlarged, with 3 cm in diameter. On longitudinal section a reduced amount of fluid and small cystic structures of about 0.1cm at the endometrial surface were observed [Fig-2e], [Fig-2f].

The tissue was processed for light microscopy using standard methods, sectioned at 3µm thickness and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

On microscopic examination, the ovarian masses were found to be composed of a cellular proliferation of fusiform

cells with a typical smooth muscle appearance and cigar-shaped nuclei, proliferating according to a fascicular, irregular pattern [Fig-3 a], [Fig-3b], clearly resembling a leiomyoma. In some marginal areas of the tumor, smooth muscle proliferation emerging from the blood vessels walls was observed [Fig-3a]. Although moderate nuclear atypia and large vesicular nuclei were observed, mitoses were rare. No signs of necrosis were detected. In one ovary, a larger follicle was visualized, with a thin granulosa layer but without

hemorrhage. On the cortex of both ovaries several smaller antral and atretic follicles were observed; an old corpus luteum was detected in one ovary. Adjacent to the ovaries, oviduct sections were analyzed, which presented some cystic epithelial structures. On the uterus, moderate cystic distension of the endometrial glands was observed, with a diminute accumulation of mucinoid material in the uterine lumen [Fig-3c]. No evidences of inflammation were detected nor the uterus exhibit signs of leiomyoma. The uterine disturbance was classified as mucometra associated with cystic endometrial hyperplasia grade I.

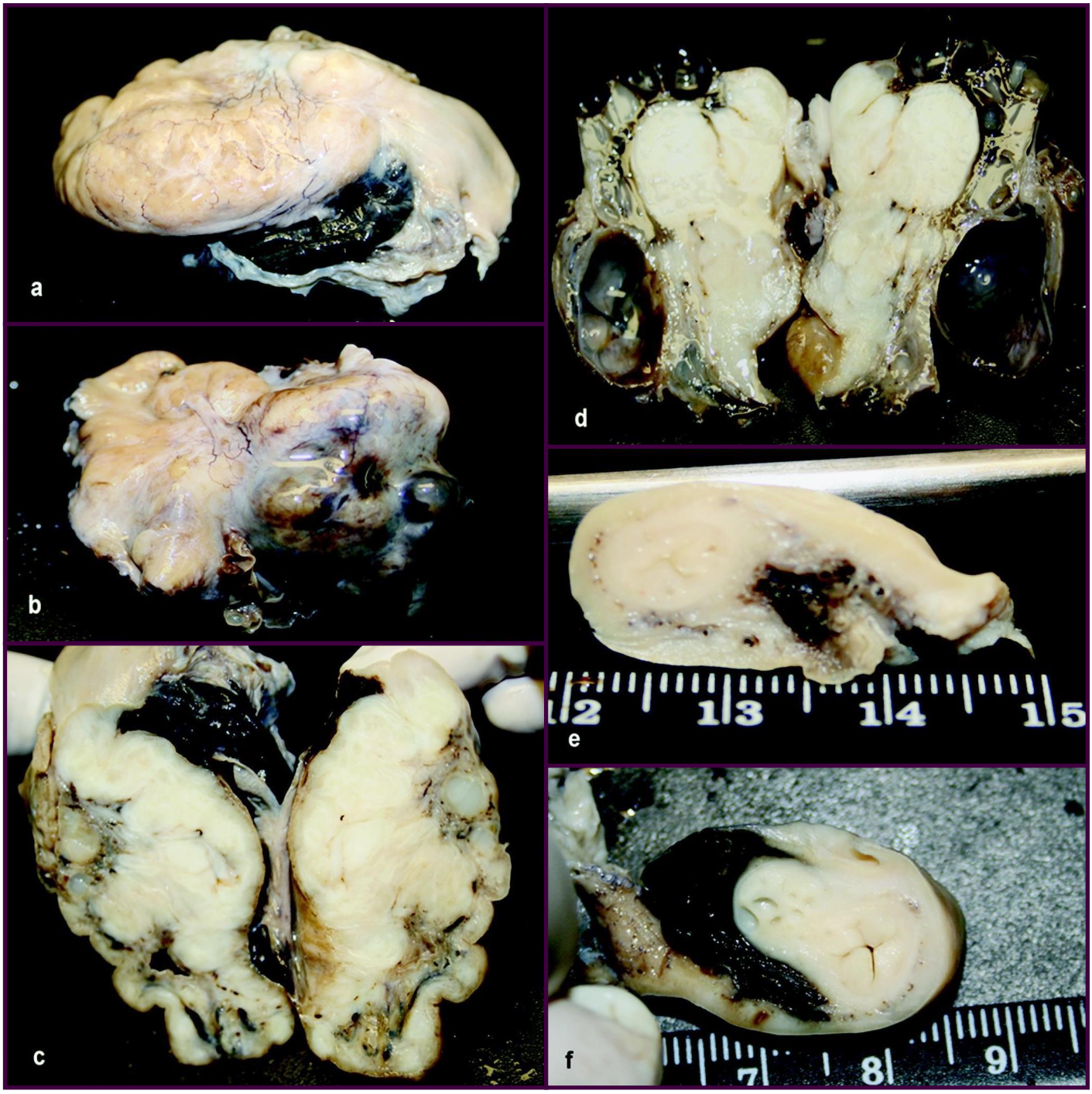

Fig. 2- Macroscopic evaluation of the ovaries and uterus of a female Panthera leo. **(a)** In one of the ovaries, a large yellowish mass occupying the majority of the ovary was observed; the mass was nodular and firm. **(b)** On the other ovary, a similar mass was found in one of the ovarian margins. **(c)** On the cut surface of the ovary presented in **(a)**, it was visible the extension of the tumor, which occupied almost the totality of the ovarian parenchyma. **(d)** The longitudinal cut surface of the ovary depicted in **(b)** showed the same features displayed by the contralateral tumor: a solid whitish mass displaying a whorled, fascicular pattern. On this ovary, it was also observed the existence of a large antral follicle. On both images **(c)** and **(d)**, the reduction of the ovarian parenchyma is apparent, due to the compression exerted by the tumor. **(e)** Transversal cut of one uterine horn showing an increased size; it is also possible to notice the existence of small cystic structures inside the endometrial folds **(f)** On another transversal cut, medial extension of the cystic structures into the myometrial layer (adenomyosis) was also observed.

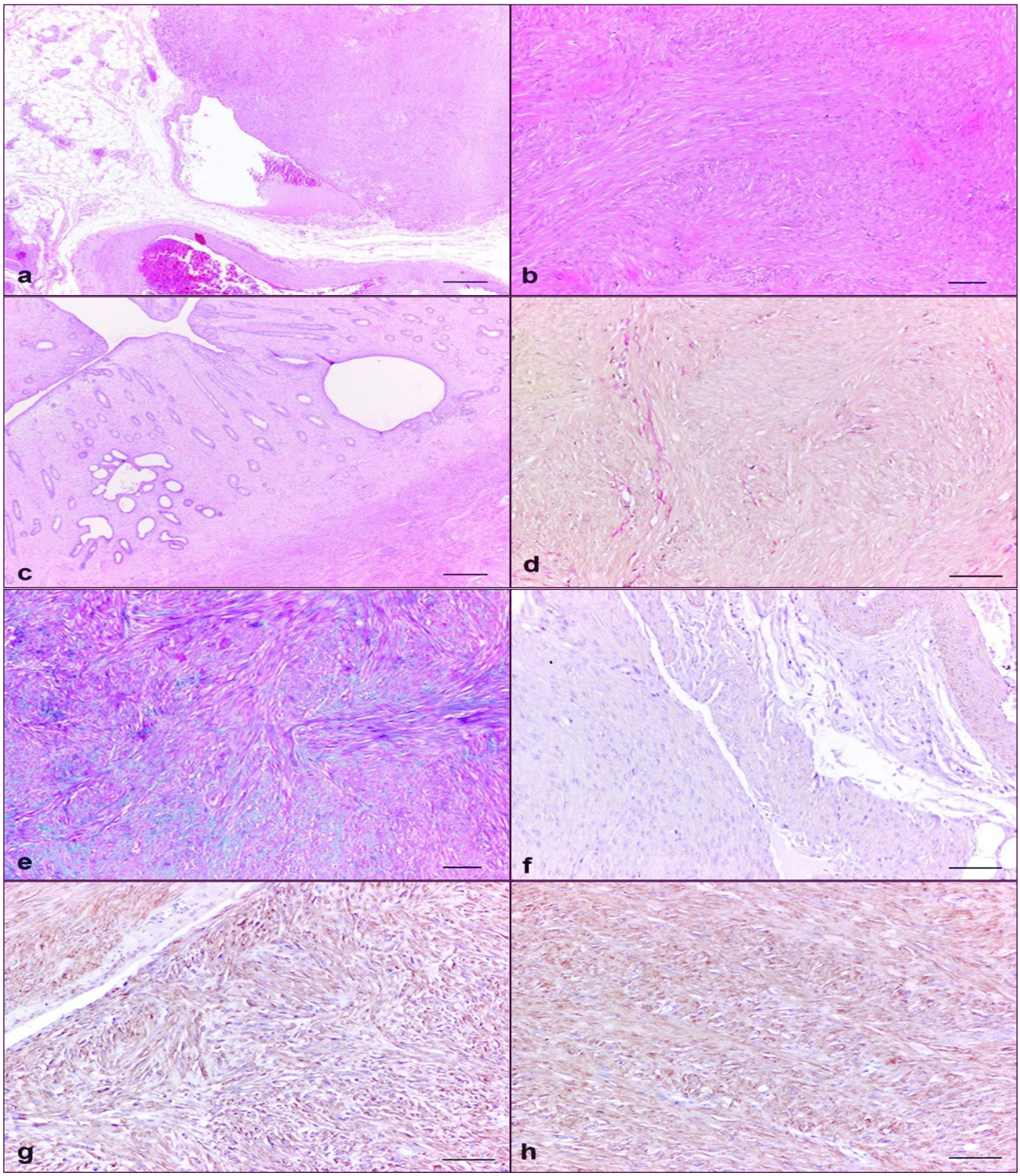

Fig. 3- Microscopic evaluation of ovarian and uterine sections and characterization of the ovarian leiomyoma. **(a)** Proliferation of fusiform cells arising from the media layer of an hilar vein (scale bar= 300µm; H&E). **(b)** The tumor was composed by well differentiated, typical smooth muscle cells displaying a whorled interlacing fascicular pattern (scale bar= 100µm; H&E). **(c)** The endometrial evidenced signs of cystic endometrial hyperplasia: loss of the typical endometrial gland architecture with pronounced dilatation of the glandular elements of the medium and deeper glandular layer (scale bar= 300µm; H&E). **(d)** With Van Gieson staining, the muscle fibers stain in orange, and collagen in red. With this staining, the reduced collagen content of the tumor stroma was evidenced (scale bar= 70µm). **(e)** The small amount of collagen was also confirmed with the Masson trichrome staining, in which collagen stains in blue and the muscle fibers in red (scale bar= 100µm). **(f)** The positive SMA immunoexpression by the neoplasic cells suggesting a smooth muscle cell tumor (scale bar= 70µm; counterstained with Gill´hematoxylin). (g, h) A positive and homogeneous immunoexpression for desmin in the tumor confirms the smooth muscle origin for this tumor (scale bar = 70 µm; counterstained with Gill´hematoxylin).

Due to histological features, the differential diagnoses for the ovarian masses included fibroma, thecoma and leiomyoma. To further characterize the lesions and to differentiate between these two tumors, a histochemical approach was essayed, by using Van Gieson and Masson Trichrome stainings. These staining are specific for evidencing the connective tissue, in particular the collagen fibers. Both the staining showed a reduced collagen content within the tumor [Fig-3d], [Fig-3e]. In addition, immunohistochemistry studies were performed on the formalin-fixed paraffin-embedded tissue utilizing the streptavidin-biotin-peroxidase method and the primary antibodies against large spectrum cytokeratin (CK AE1/AE3, at 1:50; Dako®, Labometer Lda), Vimentin (at 1:100; Novocastra®, Newcastle upon Tyne, UK), α-smooth muscle actin (SMA, at 1:200; Novocastra®, Newcastle upon Tyne, UK) and desmin (at 1:100; Novocastra®, Newcastle upon Tyne, UK). The tumor cells displayed a strong and diffuse positive staining for SMA and desmin [Fig-3f], [Fig-3h], but were negative to vimentin and the CK AE1/AE3.

The moderate expression of SMA and desmin by the tumor cells was strongly suggestive of a smooth muscle cell origin, which was further supported by the reduced collagen content of the extracellular matrix. Based on the histological observations and on the results of additional histo- and immunohistochemical studies, a final diagnosis of primary bilateral ovarian leiomyoma co-existing with grade I cystic endometrial hyperplasia and cystic oviductal hyperplasia was established.

Discussion

Primary ovarian leiomyomas are very rare benign tumors in either domestic animals or humans, and are usually unilateral in occurrence [1,3,16]. Even in the cases reported on the Chassy´ survey in wild carnivores, all the situations were unilateral [10]. Generally, they growth asymptomatically and are only detected at routine ovariohysterectomy or at necropsy [3,10]. The case presented in this report is somewhat unusual in that the tumor had developed in both the ovaries and into an extent that it occupied almost two-thirds of the ovarian parenchyma.

Ovarian leiomyomas are considered to be small sized tumors with low growth rates, which allow them to remain silent for long periods. That seemed to be the case in the situation reported herein. The situation reported here was discovered at the surgery, although on ultrasound scans findings suggestive of a solid mass on both ovaries were detected, along to signs of uterine cystic endometrial hyperplasia, the later being the major motive for surgery. No other clinical signs have been detected previous to this evaluation, despite the fact that the lioness has never been pregnant. The imaging features of an ovarian leiomyoma have not been described in the veterinarian literature, but a homology could be established to the classical uterine leiomyoma ultrasound features, which are typically evidenced as well delimited lesions of homogeneous isoechoic to hypoechoic pattern [17,18].

Macroscopic evaluation of the ovaries evidenced an important increase in size, if the ovarian dimensions are compared to those reported by Rowlands and Sadleir [19] when describing the ovarian features of three superovulated lioness (between 3 x 1.7 cm and 4 x 2 cm). The diameter of the uterine horns was also increased when compared to data reported on that study (3 cm vs. 1 cm, respectively), which was related to the existence of cystic endometrial hyperplasia, which promotes the thickness of the endometrial walls. Despite the uterine disease, this female was otherwise healthy, as it was confirmed by the blood analysis. This finding is compatible with the initial stages of the disease and to the absence of inflammation or pyometra. It is also in accordance with the fact that these tumors are reported to persist in silent for long periods before being detected [1,3]. Cystic endometrial hyperplasia is not uncommon in zoo felids, and has been associated with contraceptive treatments [20]. In the case reported here, never a progestagen treatment has been used in this female.

It is important that ovarian leiomyomas be differentiated from other spindle cell tumors of the ovary, such as fibromas and thecomas. Histochemical and immunohistochemical studies can be used to differentiate them. Fibromas are usually rich in collagen content, which can be visualized using Van Gieson and Masson´s trichome stainings. Using these histochemical techniques, collagen fibers will stain in red and blue, respectively. In the case presented here, a limited amount of collagen was present within the tumor, consistent with very limited production of a collagen extracellular matrix of the tumor cells, in contrast to what happens in fibromas, which tend to produce high amounts of collagen [5,7,8]. The low collagen content of the neoplastic stroma suggested the presence of a leiomyoma.

By using immunohistochemical analysis it is possible to demonstrate the smooth muscle differentiation of the tumor cells. Leiomyomas show diffuse positivity for desmin and smooth muscle actin (SMA), and are negative for vimentin [5,7,8]. By contrast, thecoma does not express SMA and so it can be differentiated from leiomyomas that stain for SMA [5,7,8]. Fibromas can stain for smooth muscle actin but are typically negative for desmin [5,8].

In the case described here, tumor cells express SMA and desmin, but not vimentin or large spectrum citokeratins. This information, along to the low collagen content of the extracellular matrix, confirmed the initial diagnosis of ovarian leiomyoma.

The origin of primary ovarian smooth muscle tumors remains controversial. Fallahzadeh, et al. [21] and Güney, et al. [7] suggested that they originate from the walls of the hilar blood vessels. However, based on veterinary described situations, it has been proposed that ovarian leiomyomas arise from the smooth muscle fibers of the mesovarium [9,15]. On the situations included in the survey conducted by Chassy, et al. [10], the ovarian leiomyomas were found in the ovarian medulla and pedicle. In the case reported here, some microscopic images are suggestive of that the tumor may originate from the blood vessels walls localized in the hilus of the ovary, as shown in the [Fig-3a]. This finding seems to be in accordance with previous reports from human medical literature.

An overall increase on the incidence of genital leiomyomas in the genus *Panthera* was observed and could indicate a predisposition for the development of those tumors in animals from that genus [10]. In addition, an age predisposition has been reported for those animals [10], as it was already stated for domestic animals [16]. In the situation described here, the primary ovarian leiomyoma developed in an old *Panthera leo* female, although it could not be ascertain if it was a long lasting pathological process or not. Nevertheless it is worthy to register that this female never conceived despite the existence of a proven fertile male in the cohort group. A potential influence exerted by the bilateral ovarian leiomyoma condition over the female regular cyclicity and her fertility could not be excluded.

In conclusion, a rare case of bilateral primary ovarian leiomyoma was described here. As in typical leiomyoma it had developed in the absence of clinical signs, and despite of its size and partial com-

pression of the ovarian parenchyma, suppression of the ovarian activity was not detected. Its unique sign was the presence of a solid, isoechogenic mass located in both the ovaries on ultrasound scans. Despite its rarity, ovarian leiomyoma should be considered in the differential diagnosis of ovarian conditions. Nevertheless, appropriate diagnosis requires tumor sampling and histochemical or immunohistochemical analysis.

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Conflict of Interest

The authors disclose no relevant financial interests or affiliations with any commercial interests.

Contributors

N.A. performed the clinical evaluation of the female and the surgery. J.G. performed the ultrasound examination. R.P.-C. and M.A.P. received the excised organs for analysis. M.A.P. was responsible for the pathology analysis. R.P.-C. was also involved in the data analysis and interpretation, as well as the manuscript writing and the reviewing of the literature. All the authors have approved the final article, agree with the present disclosure and approved the manuscript for final publication.

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