Acta Scientiae Veterinariae, 2022. 50(Suppl 1): 835.

CASE REPORT
Pub. 835

ISSN 1679-9216

Bilateral Testicular Tumors in a Non-Cryptorchid Dog: Coexistence of Sertoli Cell and Interstitial Endocrine Cell Tumors

Eleonora Chiti[1], Esterina Fazio[2], Giorgio Cancedda[3], Pietro Medica[2] & Katiuska Satué[4]

ABSTRACT

***Background*:** Testicular neoplasms in dogs are more frequent than in other animal species, representing the most common tumors in elderly subjects after skin neoplasms. In cryptorchid subjects, the risk of neoplastic degeneration is high. The cytological examination is essential to differentiate the type of neoplasia and to determinate the best diagnostic approach. Aim of this report was to describe clinical and histopathological features of a dog with coexistence of Sertoli cell and interstitial endocrine cell tumors in a non-cryptorchid dog.

***Case*:** A 9-year-old non-neutered male dog, German Spitz breed, was presented to the veterinary clinic. On clinical examination, the dog had a body condition (BCS 6/9), pink mucous membranes, capillary refill time (CRT) < 2 sec, lymph nodes of normal size, afebrile, normal heart and respiratory rate. The abdomen was depressible to palpation, without pain, the skin appeared hyperpigmented, with generalized presence of comedones, pendular foreskin and absence of hair at the abdominal level, in the ventral portion of the trunk and neck, scant hair also at the level of the inner thighs and in perianal. At ultrasounds examinations, the right testicle presented a hypoechoic circular focal lesion, in the caudal pole, of 0.7 cm in diameter, well defined, echogenicity and a remnant of normal echostructure, smooth and regular margins; the left testicle showed an increase in size, irregular margins, with a heterogeneous echo structure, given by hypoechoic areas, referred to cysts, hemorrhagic or necrotic areas. The hemogram reported slightly microcytic and normochromic regenerative anemia. The leukogram showed monocytosis. The absence of the typical stress leukogram characterized by neutrophilia, lymphopenia and eosinopenia, and the reduction of ALP allows to rule out Cushing's disease. In order to rule out hypothyroidism due to the inhibitory effect of estrogens on the release of thyroid-stimulating hormone (TSH) the concentration of total thyroxine was analyzed, reporting normal values excluding hypothyroidism. Blood oestradiol 17-β (E2) concentration was increased, with a normal testosterone (T) concentration of 0.30 ng/mL. Given the suspicion of the presence of testicular tumors, castration was performed by the surgical excision of both gonads, after ruling out the presence of abdominal or pulmonary metastases by chest and abdominal radiography.

***Discussion*:** The clinical, histopathological findings supported the diagnosis of testicular tumors. According to the pathological report, both gonads presented parenchymal nodular neoplastic nodular areas referring to the Sertoli sustentacular cells in the right testicle, to the proliferation of Leydig interstitial cells in the left one. The nodule in the left testicle was unencapsulated and showed a solid-diffuse pattern. Neoplastic cells were irregular polygonal, medium to large in size with moderate nucleus/cytoplasm ratio and moderate anisocytosis. In the right testicle, the nodule was heterogeneous in consistency and a diffuse pattern was present. Neoplastic cells were polygonal morphology, had a moderate nucleus/cytoplasm ratio and were organized tubules lined and obliterated the extensive cell growth. Bilateral orchiectomy allowed to improve the clinical signs, and 3 months after surgery, the animal was in good health, with evident improvement skin lesions. The E2 analysis was repeated, detecting normal values, demonstrating that testicular neoplasm in this patient were involved in E2 production; also T concentration decreased considerably from 0.30 to < 0.07 values.

Keywords: Sertoli cell tumor, testicular neoplasm, neoplastic cells, Leydig cell tumor, castration, canine.

DOI: 10.22456/1679-9216.123553

Received: 3 July 2022 Accepted: 24 October 2022 Published: 23 November 2022

[1]Private Veterinary Practitioner, Veterinary Clinic Dr. Marinacci, Quattromiglia, CS, Italy. [2]Department of Veterinary Sciences, Veterinary Physiology Unit, Polo Universitario Annunziata, Messina, Italy. [3]Private Veterinary Practitioner, Ambulatorio Veterinario Associato Mascia Cancedda, Carbonia, SU, Italy. [4]Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, CEU Cardenal Herrera University, Valencia, Spain. CORRESPONDENCE: P. Medica [pmedica@unime.it]. Department of Veterinary Sciences, Veterinary Physiology Unit, Polo Universitario Annunziata. Via Palatucci n. 13. 98168 Messina, Italy.

INTRODUCTION

Testicular tumors are common in the canine species, and after integumentary tumors they rank second in order of prevalence. Primary testicular tumors are histologically classified into germ cell tumors, sex cord-stromal tumors, and mixed germ cell-sex cord stromal tumors [14]. Within these groups are the 3 most common canine testicular tumors, which have relatively similar incidence varying by study. Sertoli cell tumors (SCTs) and interstitial endocrine cell tumors, also named, Leydig cell tumors (LCTs) are sex cord-stromal tumors, while seminomas (SEM) are germ cell tumors [2].

In most cases, testicular tumors are incidentally discovered, are generally benign and orchiectomy is usually curative [10]. The present report describes an occasional case of a dog with a SCT in a testicle and a LCT in the contralateral one. Although most testicular tumors are diagnosed in geriatric animals aged 9 to 11 years (with a variability of 2 to 19 years) [3,9], there are breeds such as Boxer, Chihuahua, Miniature Poodle, Miniature Schnauzer, Cairn Terrier and Shetland Sheepdog that can be affected at an earlier age (7.2 years) [2,17].

Sertoli cell tumors most often originates in the cryptorchid testes (retained, in the abdominal cavity or at inguinal level) rather than in scrotal testicles and, in these cases, the age of presentation may be earlier [2,11,28]. In dogs, there are only few reports describing primary bilateral testicular tumors. On this basis, the aim of this report was to describe clinical and histopathological features of a dog with coexistence of Sertoli cell and interstitial endocrine cell tumors in a non-cryptorchid dog.

CASE

This was a study carried out at the Marinacci Veterinary Clinic and owner consents were obtained. A 9-year-old non-neutered male dog, German Spitz breed was attended with Sertoli cell tumors due to the presence of severe alopecia on the abdomen, trunk and neck.

In the medical history, the owners reported that the dog lived at home, without other pets, ate commercial food, was vaccinated regularly, and was regularly treated for ecto and endoparasites. The dog had not shown dysorexia or problems during defecation or urination. It had not received any therapy in the last 12 months and was itchy. On clinical examination, the dog had a body condition (BCS 6/9), pink mucous membranes, capillary refill time (CRT) < 2 s lymph nodes of normal size, afebrile, normal heart and respiratory rate. On abdominal palpation, there was no pain and the abdomen was depressible. Upon inspection, the skin appeared hyperpigmented, with generalized presence of comedones, pendular foreskin and absence of hair at the abdominal level, in the ventral portion of the trunk and neck, scant hair also at the level of the inner thighs and in perianal (Figure 1).

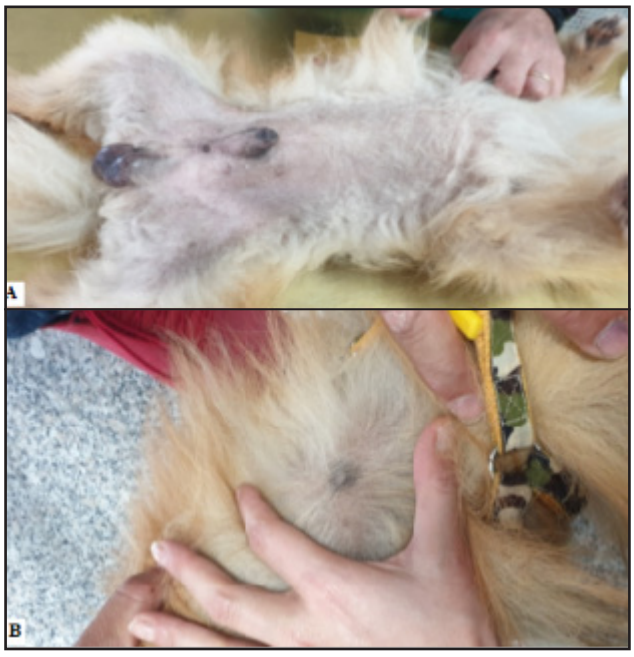

Figure 1. A- Alopecia and thinning of the hair on the ventral portion of the neck, abdomen, perianal area and inner thighs and slight asymmetry of the 2 testicles. B- Hyperpigmentation.

On palpation, the testes were both present in the scrotum, but showed a slight asymmetry between them. Asymmetry or enlargement of the testicles may be due to cancer, orchitis, testicular torsion, epidydimitis, inguino-scrotal hernia, spermatocele, hydrocele, sperm granuloma.

Based in that ultrasound evaluation was the most indicated imaging method for the evaluation of testicular tumors [6], ultrasounds of both testicles were performed (Ge Logiq5 Premium ultrasound machine)[1]. The right testicle presented a hypoechoic circular focal lesion, in the caudal pole, of 0.7 cm in diameter, well defined, echogenicity and a remnant of normal echostructure, smooth and regular margins (Figure 2A).

The left testicle showed an increase in size, irregular margins, with a heterogeneous echo structure, given by hypoechoic areas, referred to cysts, hemorrhagic or necrotic areas (Figure 2B).

The prostate was of normal size and shape, although with irregular margins. The echogenicity of the parenchyma was slightly increased in a heterogeneous manner, with small diffuse hypoechoic lesions. The adjacent peritoneum was normal (Figure 2C). The adjacent peritoneum, inguinal nodes and abdominal ultrasound did not show abnormalities. Other pathological conditions as epididymal granulomas, epididymitis were discarded [9].

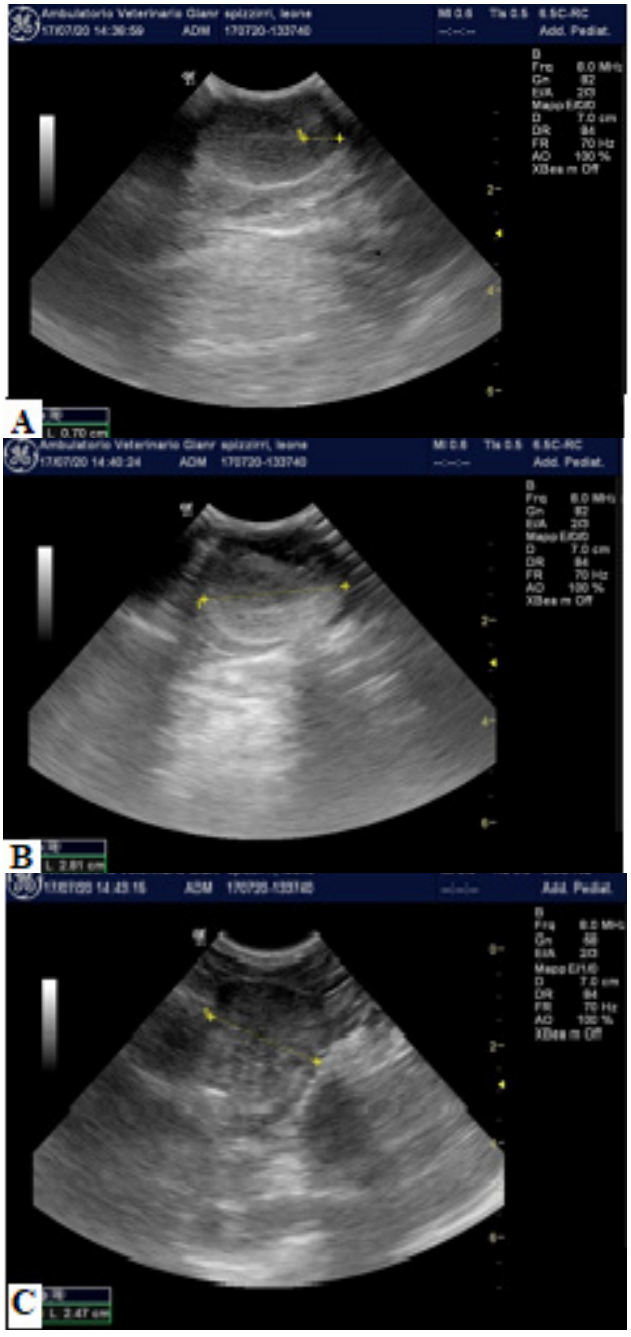

Figure 2. A- Left testis ultrasound: 0.70 cm hypoechoic focal lesion. B- Ultrasound of the right testicle: enlarged, heterogeneous echogenicity, with irregular margins and diffuse hypoechoic areas. C- Prostate ultrasound: heterogeneous parenchyma with diffuse anechoic and hypoechoic areas and irregular margins (Ge Logiq5 Premium).

The hemogram using a ProCyte Dx analyzer[2] reports slightly microcytic and normochromic anemia of a regenerative nature, without notable alterations in the blood smear. Although hyperestrogenism leads to bone marrow toxicity and therefore to non-regenerative anemia, leukopenia and / or thrombocytopenia [2], regeneration from anemia to normal leukocyte and platelet numbers in this patient could not confirm that estrogenic concentrations in this patient would have caused the spinal toxicity. The leukogram showed monocytosis without changes in other leukocyte cells [11].

Biochemical analysis shows ALP activity (14 UI/L) and CHOL concentration (95 mg/dL) below the specific reference ranges for the canine species (range ALP: 23-212 UI/L; range CHOL: 110-320 mg/ dL). Biochemical parameters, as glucose, BUN, creatinine, BUN / creatinine ratio, phosphorus, calcium, total protein, albumin, globulins, albumin / globulin ratio, ALT, GGT, total bilirubin, amylase and lipase were within the reference range for adult healthy dogs [13]. Thus, the absence of the typical stress leukogram characterized by neutrophilia, lymphopenia and eosinopenia, and the reduction of ALP allows to rule out Cushing's disease.

In order to rule out hypothyroidism due to the inhibitory effect of estrogens on the release of thyroid-stimulating hormone (TSH) from the anterior pituitary [1] the concentration of total thyroxine (TT4) was analyzed (Catalyst Dx chemical analyzer)[3], reporting normal values (1.4 µg/dL; range: 1.0 a 4.0 µg/dL), excluding hypothyroidism.

Noteworthy, blood oestradiol 17-β (E_2) concentration was increased (18.7 pg/mL: normal values: < 7 pg/mL [19]; while a normal testosterone (T) concentration of 0.30 ng/mL (range: 0.10 - 0.94 ng/mL) was detected. Neoplastic cells within testicular tumors may produce estrogen and/or T, resulting in excessive concentrations of these hormones. Higher concentrations of T have been reported in dogs with LCT [4,18] and large amounts of estrogen have been found in Sertoli cells.

However, the determination of a single concentration of T is not enough to verify testicular function due to the pulsatile pattern of this hormone. It would have been decisive to perform a GnRH stimulation test with any GnRH agonist and collect a blood sample 1 h later [12]. Probably, a T level of 0.3 ng / mL could have reached values > 1.0 ng / mL after stimulation. Although LCTs may produce T in a non-pulsatile

fashion, it cannot be concluded that T production in this dog was normal as it could have been a) low (in case of no peak after GnRH), or b) abnormally high after GnRH.

However, in healthy dogs the variability in E_2 concentrations exits. Hyperestrogenism leads to feminization of the male dog, which is observed in 25-50% of dogs with SCT and in 5% of dogs with LCT [18]. Hyperestrogenism (E_2: 10-150 pg/mL), is the responsible for some clinical signs present in this animal, such as bilateral symmetrical alopecia, hyperpigmentation and epidermal atrophy. Moreover, it affects skin pigmentation, decreases hair growth, delaying the initiation of the hair follicle growth phase, reduces the rate of hair growth and may induces other pathological conditions as gynecomastia, pendulous prepuce, linear preputial erythema, squamous metaplasia of the prostate gland and attraction of other male dogs. Bone marrow hypoplasia, resulting in pancytopenia, may also be seen as a consequence of hyperestrogenism [9,12]. Although hyperestrogenism can cause many clinical problems, concentrations of E_2 higher than the reference range may not be associated with clinical signs of hyperestrogenism [21]. Estrogens in the male dog may also cause reduction in the diameter of the hair [27].

A cytological study of the preputial epithelial cells was also carried out and revealed that the keratinized cells from the inner portion of the foreskin had a size and an appearance similar to the vaginal keratinized cells of bitches in heat (Diff Quick staining)[4] [Figure 3]. These keratinized or cornified epithelial cells are maybe be accounted to the hyperestrogenism present in the patient [22].

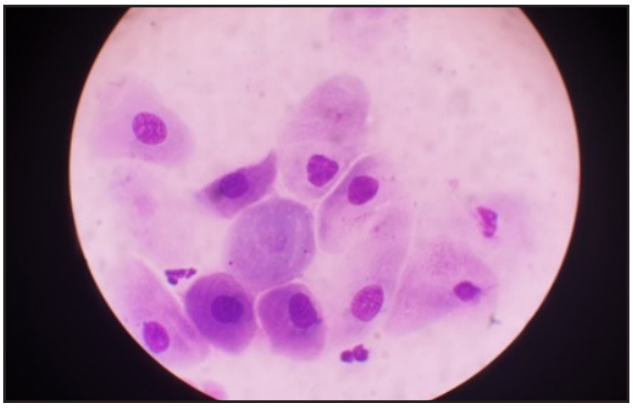

Figure 3. Cytology of the foreskin before orchiectomy. Keratinized epithelial cell population of the inner portion of the foreskin [Diff Quick; 30x-100x].

All endocrinopathies, hyperadrenocorticism, hypothyroidism, and also the idiopathic male feminization syndrome should be considered in the differential diagnosis of testicular tumors in dogs [10]. Inguinal hernia, orchitis, and testicular torsion may also be taken into account.

Given the suspicion of the presence of testicular tumors, castration was performed by the surgical excision of both gonads, after ruling out the presence of abdominal or pulmonary metastases by chest and abdominal radiography [10]. On the day of the surgery the patient was submitted to a careful physical examination. Food was withheld for 12 h and water for 6 h prior to surgery.

Before surgery, a 25 mm, 22-gauge catheter (Jelco)[5] was placed in the right cephalic vein for drug administration. The anesthetic protocol was the following: Tramadol® IM [3 mg/kg v.o., BID] and Midazolam® IM [0.25 mg/kg v.o., SID] were used for analgesia and sedation. Induction of anesthesia was performed with propofol IV to effect [3-5 mg/kg v.o., SID]. After endotracheal intubation, maintenance of anesthesia continued with propofol IV. A pre-scrotal orchiectomy (OR) with scrotal ablation was performed, using a 3-0 absorbable synthetic polyglactin 910 braided suture (Vicryl; Ethicon)[6]. Longitudinal cuts of the testicles, as well as fine needle aspirates, were done following orchiectomy. Longitudinal cuts were made from the cranial pole to the caudal pole of each testicle for macroscopic inspection (Figure 4). A slight testicular asymmetry was observed. Both testicles were less than 4 cm in length. The smallest right gonad presented a macroscopically homogeneous, glossy yellowish-white colour on section. Hemorrhagic and necrotic areas were evidenced in the left testicle in a large part of the parenchyma. Postoperative analgesia was maintained with NSAIDs. After the surgical procedures, patient received a single dose of Meloxicam® [0.2 mg/kg v.o., SID] subcutaneously and was carefully monitored. Once the presence of testicular lesions was identified, and in order to differentiate between normal, inflammatory, atrophic or tumor testicular tissue, a fine needle aspiration puncture (20-25 G gauge) was performed for cytological study [7,8,25]. Aspirates were spread on slides, air dried, stained with May-Grünwald Giemsa, and subsequently, observed under an optical microscope. In the right testicle, the presence of rounded and elongated neoplastic cells, occasionally palisading was identified. Neoplastic cells

had round nuclei and basophilic cytoplasm, containing large cytoplasmic vacuoles. Bashing of the cytological features the diagnosis was of SCT (Figure 5). On the left testis, pleomorphic cells with often peripheral nuclei and small vacuoles scattered in a moderately basophilic cytoplasm, compatible with LCT, were observed (Figure 6).

Postoperative treatment with NSAIDs and antibiotic was required for up to 5 days after surgery. One week after surgery, the dog returned to the clinic for a physical examination and evaluation of proper wound healing. In the routine check-up 3 months after surgery, the animal was in good health, with evident improvement skin lesions. The E2 analysis was repeated, detecting normal values for non-castrated adult canines (2.2 pg/mL). The reduction of E2 concentrations from 18.2 to 2.2 pg/mL demonstrated that testicular neoplasm in this patient were involved in estrogen production. In the same way, T concentration also decreased considerably from 0.30 to <0.07 values.

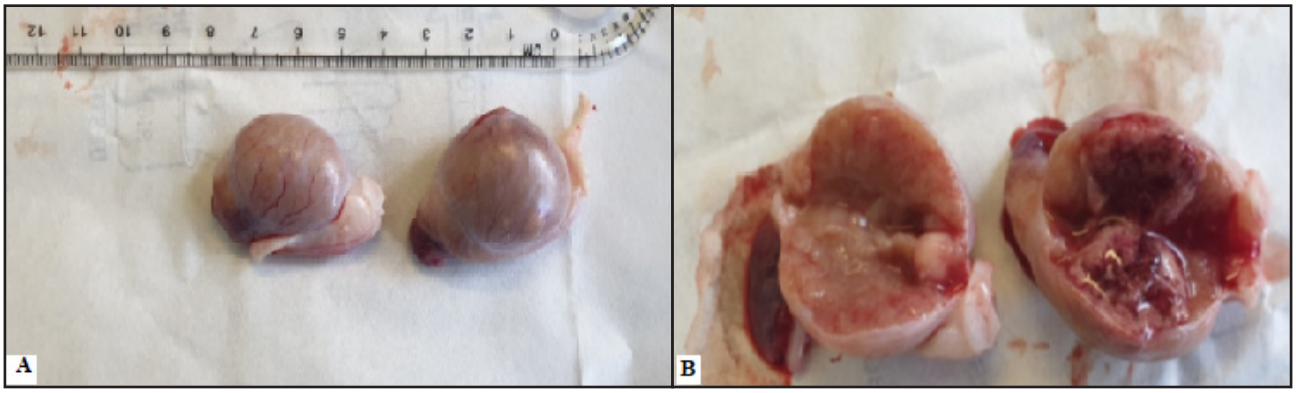

Figure 4. A- Macroscopic appearance of both testes after orchidectomy. The left testicle is slightly larger than the right (slight testicular asymmetry). B- Cross-section of canine testis. Homogeneous, glossy yellowish-white colour on section (LCT; left testicle). Hemorrhagic and necrotic areas were evidenced in the left testicle in a large part of the parenchyma (TCS; right testicle).

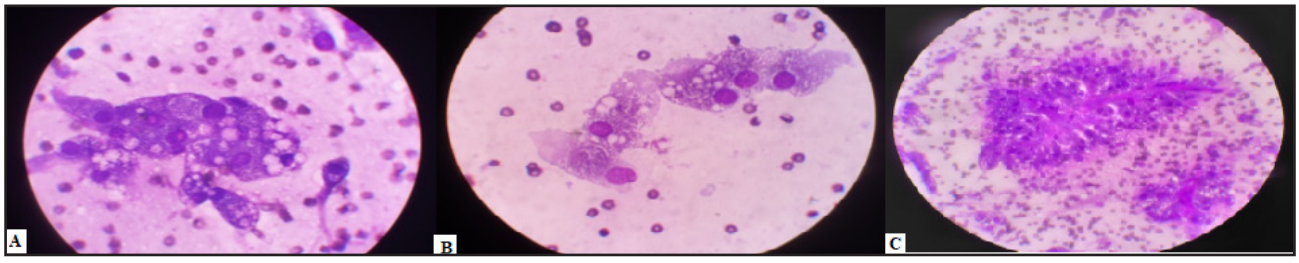

Figure 5. Cytological study of right testicle. Presence of: A- Elongated cells; B- Rounded cells; C- Cells sometimes arranged in a palisade; with rounded nuclei and voluminous cytoplasmic vacuolizations with clear margins [May-Grünwald Giemsa; 100x].

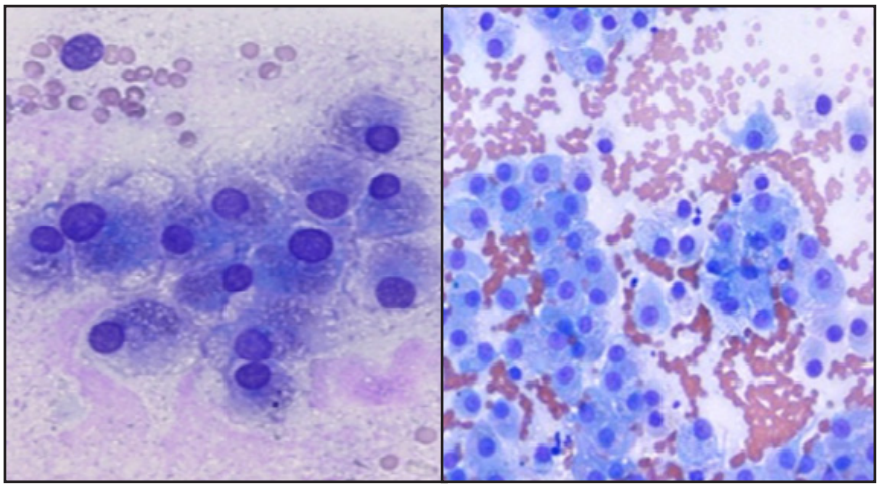

Figure 6. Cytological study of the left testicle. Pleomorphic cells of different sizes, fine chromatin, peripheral nuclei and vacuolized cytoplasm with small, very well defined vacuoles slightly scattered [May-Grünwald Giemsa; 100x].

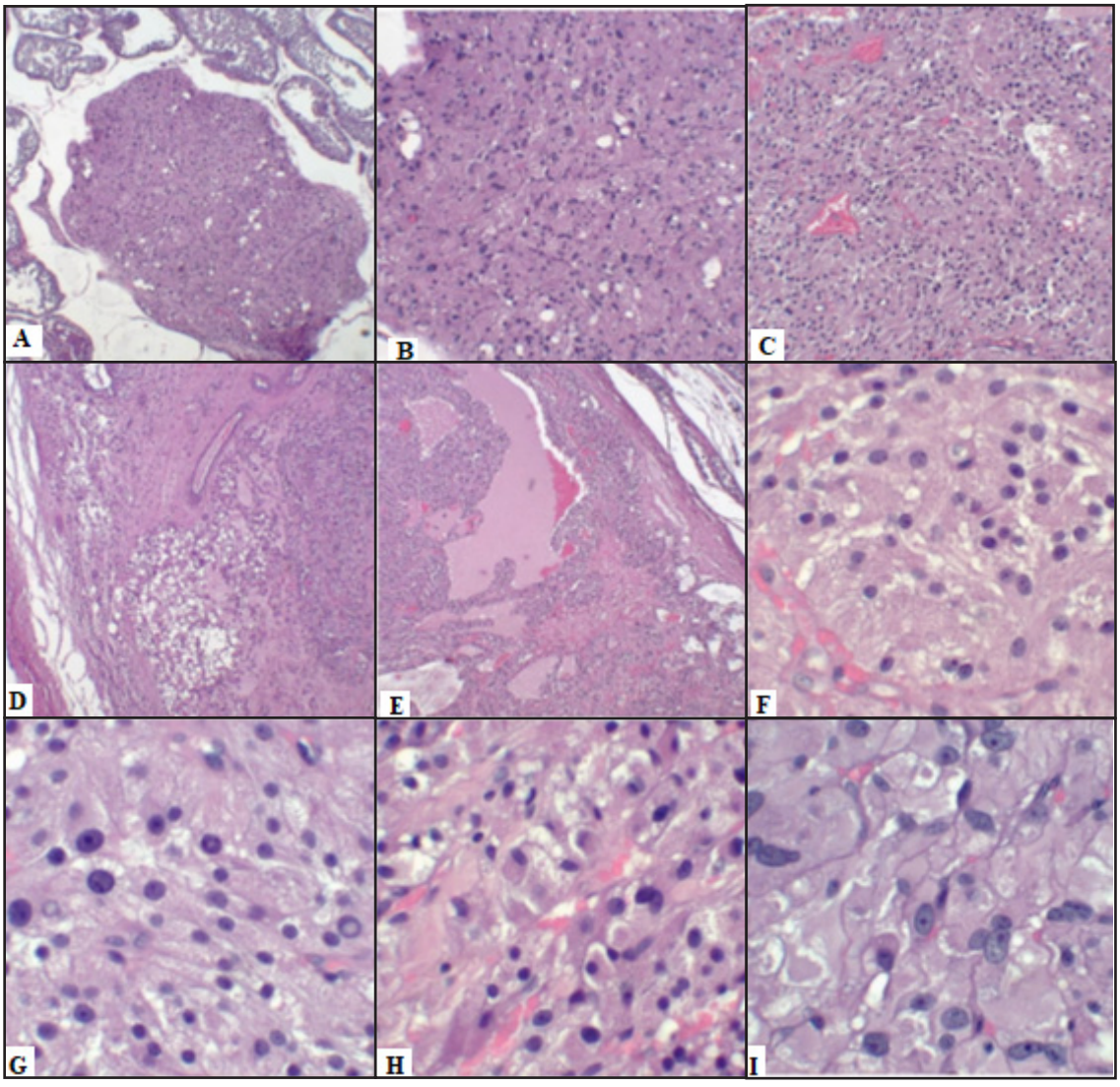

Figure 7. Histological pattern of SCT [A, B, C & D; H.E. 400x]: hyperplasia of spindle shaped cells with hyperchromatic nuclei. A population of irregular polygonal neoplastic cells with a cohesive columnar arrangement, elongated cytoplasm with mostly defined margins, moderately vacuolized, with round or oval internal nuclei with inhomogeneous thickened chromatin and small central nucleolus (diffuse SCT). Histological pattern of LCT [E, F, G, H & I; H.E. 400x]: polygonal neoplastic elements arranged in cohesive cords with vast vacuolized cytoplasm with numerous lipid vacuoles, and large, central round nuclei, and focal haemorrhages. [Images courtesy of Dr. G. Cancedda].

DISCUSSION

A retrospective study carried out on 301 testicular tumors in 206 dogs revealed that 86.4% and 13.6% had unilateral and bilateral tumors, respectively. In 5.8% of the dogs with bilateral tumors they presented bilateral seminomas (2.4%), followed by bilateral TCSs (1.5%) and bilateral LCT_s, detecting a single case of TCS and LCT and other mixed tumors such as seminoma and / or TCS and mixed germ cell-sex cord stromal tumours (MGCSCTs) in 1 dog each. The remaining 7.8% had multiple tumors in 1 or both testicles [25]. In case of unilateral tumors, the atrophy of the contralateral is common [23]. Of all testicular tumors, TCS represent 44% [4,5] and LCT the 25% [4].

TCS, being nodular and mostly multilobular frequently cause testicular deformation. They are mostly benign and malignant cases account to 10-20%. The 60% of these tumors causes paraneoplastic signs the "feminization syndrome" due to the increase in estrogens (hyperestrogenism). This causes non-pruritic bilateral symmetric alopecia, decreased libido, infertility, squamous metaplasia of the prostate, anemia due to medullary hypoplasia, hypothyroidism due to TSH inhibition, and, finally, atrophy of the contralateral testicle [17,29]. The 10-14% of TCS, behaves malignant,

with metastasis to regional lymph nodes and possible spread to other abdominal and thoracic organs [8]. The most common sites are the superficial inguinal lymph nodes, iliac and sublumbar; as well as the mesenteric ones. Metastases have been reported in abdominal organs, lungs, eyes, kidneys, spleen, adrenals, pancreas, liver, skin, and central nervous system [26].

Nonetheless, it is well known that metastases of canine testicular tumors are fairly rare, and their incidence reported around 10% [8]. Considering, canine malignant LCT_s, which are extremely rare, they may be associated to cutaneous metastases. The reason is unknown; however, this may be considered an important clinical aspect which should be taken into account [15,16]. The 10-15% of dogs with TCS have a bone marrow hypoplasia leading to anemia, thrombocytopenia, or leukopenia, or a combination of such abnormalities. Among the clinical manifestations that occur due to myelosuppression, are included lethargy, bleeding resulting from thrombocytopenia, petechiae, vomiting, anorexia, fever and pale mucous membranes [11].

LCT_s do not usually modify the testicular appearance, may cause a hormonal alteration characterized by hyperandrogenism, which is manifested by dermatological alterations (seborrhea, hyperpigmentation and alopecia); they can also cause hyperplasia of the perianal and tail glands, prostate hyperplasia, sexual hyperarousal and increased aggressiveness [2,3]. Moreover, other studies have proven that not only SCTs but also LCT_s can cause hyperestrogenism, with sign of feminization [14,24], due to an excess amount of estrogen in the body or to an imbalance between the estrogen and testosterone levels [20]. These data suggested that LCT_s can also be endocrinologically active. Similarly, also in men, estrogen-secreting LCT_s may cause hormonal alteration and frequently hyperestrogenism, with low plasma gonadotropin and testosterone concentrations, and increases E_2 levels that decreased to normal immediately after surgery. It is possible to argue that chronic hyperestrogenism produces hypothalamo-pituitary inhibition as well as direct steroidogenic blockade at the testicular level [19].

Since the diagnosis of testicular tumors must be confirmed by histopathology [27], in our clinical case the isolated fragments of altered testes were fixed for 24 h in 7% buffered formalin and were sent to the Pathological Anatomy Service for histopathological study. Samples were routinely processed and from the obtained paraffin blocks 4 μm thick sections were cut and stained with haematoxylin and eosin for microscopical examination WHO (World Health Organization) classification of canine testicular tumours was applied for the histological diagnosis [14].

According to the pathological report, both gonads presented parenchymal nodular neoplastic areas nodular areas referring to the Sertoli sustentacular cells in the right testicle, to the proliferation of Leydig interstitial cells in the left one. The nodule in the left testicle was unencapsulated and showed a solid-diffuse pattern. Neoplastic cells were irregular polygonal, medium to large in size with moderate nucleus / cytoplasm ratio and moderate anisocytosis. Neoplastic cells had distinct cell borders and a moderate amount of moderately eosinophilic cytoplasm frequently vacuolated. Nuclei were round to oval, with thickened chromatin, and a prominent and homogeneous central singular nucleolus. Moderate anisokariasis was present. In the right testicle, the nodule was heterogeneous in consistency and a diffuse pattern was present. Neoplastic cells were polygonal morphology, had a moderate nucleus/cytoplasm ratio and were organized tubules lined and obliterated the extensive cell growth. Neoplastic cells presented indistinguishable boundaries, eosinophilic cytoplasm and rounded to elongated nucleus with mild to moderate anisokaryosis, fine chromatin, and a single nucleolus. The expansive growth of the tumor caused the rupture of the tubular membrane, showing hemorrhagic and necrotic areas extending to a large part of the testicular parenchyma. In both tumors the presence of mitotic figures was less to 1 per 10 HPF. No inflammatory infiltrates were detected around the tumors. Based on the histological features, the presence of diffuse SCT in the right testicle and LCT in the left testicle were confirmed (Figure 7).

Hence, the evaluation of endocrine, biochemical profiles, and histopathological features of dog with primary testicular tumor can be used for comparative purposes in other dog breeds, as a monitor of health status for the diagnosis of certain oncological processes that could affect the specimens, impacting on their physiological state. For this purpose, bilateral orchiectomy resulted in the clinical improvement of patient.

MANUFACTURERS

[1]General Electric Company. Coventry, England.

[2]IDEXX Europe B.V. Hoofddorp, The Netherlands.

[3]Protectlife International Biomedical Inc. Taoyuang, Taiwan.

[4]Stapelia Diagnostica. Veroli, Italy.

[5]ICU Medical Inc. San Clemente, CA, USA.

[6]Johnson & Johnson. Hamburg, Germany.

***Acknowledgements*.** We express our gratitude to Dr. E. Chiti and G. Cancedda for your contribution.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 **Abdel-Dayem M.M. & Elgendy M.S. 2009.** Effects of chronic estradiol treatment on the thyroid gland structure and function of ovariectomized rats. *BMC Research Notes*. 2: 173.

2 **Agnew D.W. & MacLachlan N.J. 2017.** Tumors of the genital systems. In: Meuten D.J. (Ed). *Tumors in Domestic Animals*. 5th edn. Ames: John Wiley & Sons, pp.689-722.

3 **Alvarez-Manriquez L. & Padilla-Arellanes S. 2010.** Tumor de células de Leydig y seminoma en un perro. *Veterinaria Mexico*. 27(3): 174-179.

4 **Ciaputa R., Nowak M., Kielbowicz M., Antończyk A., Błasiak K. & Madej J.A. 2012.** Seminoma, sertolioma, and leydigoma in dogs: clinical and morphological correlations. *Bulletin of the Veterinary Institute in Pulawy*. 56(3): 361-367.

5 **D'Angelo A.R., Vita S., Marruchella G. & Di Francesco G. 2012.** Canine testicular tumours: a retrospective investigation in Abruzzo and Molise, Italy. *Veterinaria Italiana*. 48(3): 329-333.

6 **Domingos T.C.S. & Salomão M.C. 2011.** Meios de diagnóstico das principais afecções testiculares em cães: revisão de literatura. *Revista Brasileira de Reprodução Animal*. 35(4): 393-399.

7 **Dreimanis U., Vargmar K., Falk T., Cigut M. & Toresson L. 2012.** Evaluation of preputial cytology in diagnosing oestrogen producing testicular tumours in dogs. *Journal of Small Animal Practice*. 53(9): 536-541.

8 **Eslava M.P. & Torres V.G. 2008.** Neoplasias testiculares en caninos: un caso de tumor de células de sertoli. *Revista MVZ Córdoba*. 13(1): 1215-1225.

9 **Feldman E.C. & Nelson R.W. 2007.** Disorders of the Testes and Epididymis. In: Feldman E.C. & Nelson R.W. (Eds). *Canine and Feline Endocrinology and Reproduction*. 3rd edn. St. Louis: W.B. Saunders Co., pp.961-977.

10 **Grieco V., Riccardi E., Greppi G.F., Teruzzi F., Iermano V. & Finazzi M. 2008.** Canine testicular tumors: a study on 232 dogs. *Journal of Comparative Pathology*. 138(2/3): 86-89.

11 **Harvey J.W. 2012.** *Veterinary Hematology: A Diagnostic Guide and Color Atlas*. Amsterdam: Elsevier Health Sciences, pp.122-176. doi: 10.1016/B978-1-4377-0173-9.00006-3.

12 **Junaidi A., Williamson P.E., Martin G.B., Stanton P.G., Blackberry M.A., Cummins J.M. & Trigg T.E. 2007.** Pituitary and testicular endocrine responses to exogenous gonadotrophin-releasing hormone (GnRH) and luteinising hormone in male dogs treated with GnRH agonist implants. *Reproduction, Fertility and Development*. 19(8): 891-898.

13 **Kaneko J., Harney J.W. & Bress M.L. 2008.** Blood analyte reference values in small and same laboratory animals (Appendix IX). In: Kaneko J., Harney J.W. & Bress M.L. (Eds). *Clinical Biochemistry of Domestic Animals*. San Diego: Academic Press, pp.886-891.

14 **Kennedy P.C., Cullen J.M., Edwards J.F., Goldschmidt M.H., Larsen S., Munson L. & Nielsen S. 1998.** Histological classifications of tumors of the genital system of domestic animals. In: *World Health Organization International Histological Classification of Tumors of Domestic Animals.* Vol. IV. Washington DC: Armed Forces Institute of Pathology, pp.17-18.

15 **Kim O. & Kim K.S. 2004.** Seminoma with hyperestrogenism in a Yorkshire Terrier. *The Journal of Veterinary Medical Science*. 67(1): 121-123.

16 **Kudo T., Kamiie J., Aihara N., Doi M., Sumi A., Omachi T. & Shirota K. 2019.** Malignant Leydig cell tumor in dogs: two cases and a review of the literature. *Journal of Veterinary Diagnostic Investigation*. 31(4): 557-561.

17 **Liao A.T., Chu P.Y., Yeh L.S., Lin C.T. & Liu C.H. 2009.** A 12-year retrospective study of canine testicular tumors. *The Journal of Veterinary Medical Science*. 71(7): 919-923.

18 **Lopate C. 2010.** Clinical approach to conditions of the male. In: England G. & Von Heimendahl A. (Eds). *BSAVA Manual of Canine and Feline Reproduction and Neonatology*. 2nd edn. Gloucester: BSAVA, pp.191-196.

19 **Mattheeuws D. & Comhaire F.H. 1989.** Concentrations of oestradiol and testosterone in peripheral and spermatic venous blood of dogs with unilateral cryptorchidism. *Domestic Animal Endocrinology*. 6(3): 203-209.

20 **Mineur P., De Cooman S., Hustin J., Verhoeven G. & De Hertogh R. 1987.** Feminizing testicular Leydig cell tumor: hormonal profile before and after unilateral orchidectomy. *The Journal of Clinical Endocrinology & Metabolism*. 64(4):

686-691.

21 **Mischke R., Meurer D., Hoppen H.O., Ueberschär S. & Hewicker-Trautwein M. 2002.** Blood plasma concentrations of oestradiol-17beta, testosterone and testosterone/oestradiol ratio in dogs with neoplastic and degenerative testicular diseases. *Research in Veterinary Science*. 73(3): 267-272.

22 **Outerbridge C.A., White S.D. & Affolter V.K. 2016.** Alopecia universalis in a dog with testicular neoplasia. *Veterinary Dermatology*. 27(6): 513-e139.

23 **Patnaik A.K. & Mostofi F.K. 1993.** A clinicopathologic, histologic, and immunohistochemical study of mixed germ cell-stromal tumors of the testis in 16 dogs. *Veterinary Pathology*. 30(3): 287-295.

24 **Raskin R. & Meyer D. 2010.** *Canine and Feline Cytology: A Color Atlas and Interpretation Guid*e. 2nd edn. St. Louis: Saunders, 450p.

25 **Švara T., Gombač M., Pogorevc E., Plavec T., Zrimšek P. & Pogačnik M. 2014.** A retrospective study of canine testicular tumours in Slovenia. *Slovenian Veterinary Research*. 51(2): 81-88.

26 **Turek M.M. 2003.** Cutaneous paraneoplastic syndromes in dogs and cats: a review of the literature. *Veterinary Dermatology*. 14(6): 279-296.

27 **Valente P., Couto R., Gamba C., Vasconcelos A., Leme F., Ecco R. & Paes P. 2017.** Bone marrow bi-hypoplasia in a dog with a sertoli cell tumor. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 69(1): 95-100.

28 **Veronesi M.C., Riccardi E., Rota A. & Grieco V. 2009.** Characteristics of cryptic/ectopic and contralateral scrotal testes in dogs between 1 and 2 years of age. *Theriogenology*. 72(7): 969-977.

29 **Yu C.H., Hwang D.N., Yhee J.Y., Kim J.H., Im K.S., Nho W.G., Lyoo Y.S. & Sur J.H. 2009.** Comparative immunohistochemically characterization of canine seminomas and Sertoli cell tumors. *Journal of Veterinary Science*. 10(1): 1-7.