Sertoli Leydig cell tumour in a bitch, 10 years after spaying

A case report

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Introduction

In order to sterilize and to sensibly stop oestrus or to prevent recurrence of vaginal hyperplasia and hormonal changes, the ovarectomy or the ovariohysterectomy is the best recommended method. The operation, performed in young dogs reduces the incidence of mammary gland tumours. The relative risk for developing mammary gland tumours drops to 0.5%, when spaying is performed before the first oestrus. Between the first and the second oestrus, the operation reduces the risk to 8%, between the second oestrus and 2.5 years of age the risk is further reduced to 26%. Furthermore, ovariohysterectomy is recommended to treat pyometra and pseudopregnancy (15, 22, 26).

The present investigation describes a rare case of a spayed bitch, which showed oestrus 10 years following neutrering. We report the clinical, sonographical, histological and immunohistological findings of an abdominal neoplasm. The causal factors of the neoplasma e. g. embryologic and histogenetic are discussed.

Case report

Clinical findings

A German shepherd bitch was spayed after her first oestrus. She was regularly vaccinated and treated against ectoparasites and endoparasites. Ten years later, the dog came into heat again (vaginal bleeding, swollen vulva, male attraction). The results of the haematological and biochemical investigations revealed no abnormalities.

Sonographic examination revealed a 2.1 × 2 cm large mass below the left kidney. The tissue and a uterus specimen were surgically removed and histopathologically examined.

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Histological findings

Both specimens were fixed in 10% buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E). Histologically, the uterus specimen showed up to 200 µm broad uterine

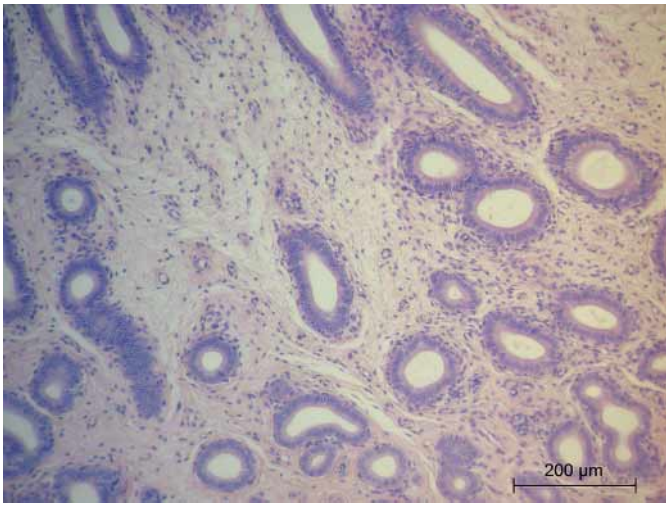

Fig. 1 Uterus, multifocal secretoric hyperglandular hyperplasia, periglandular fibrosis of the uterine glands (H&E stain)

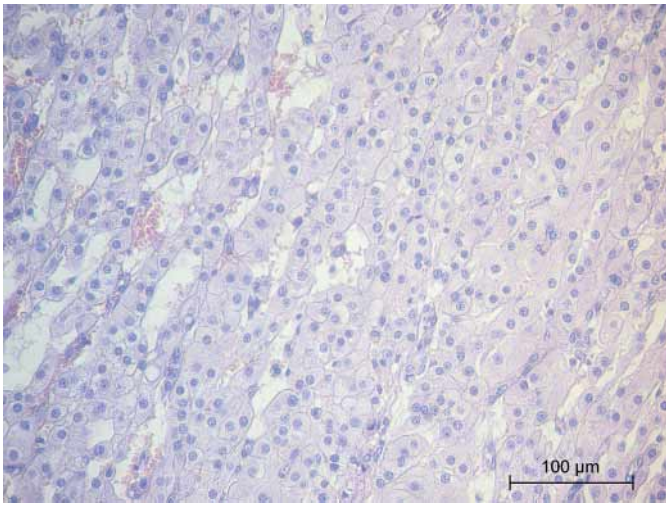

Fig. 2 Leydig cell tumour: cuboid to oval, polyhedral cells packed in sheets or as cords separated by thin strands of connective tissue (H&E stain)

Antibodies	Clone	Type of antibody	Dilution	Results SLCT	Results uterus
Vimentin	Vim3B4	monoclonal	1:50	+++	++
EMA	E29	monoclonal	1:50	–	nd
S-100-Protein		polyclonal	1:2000	–	nd
Cytokeratin	clone AE	monoclonal	1:50	–	++
Progesterone receptor	PR 10A	monoclonal	1:50	–	–
α-Inhibin	R1	monoclonal	1:100	+++	nd
Oestrogen receptor α	ER-α	monoclonal	1:50	–	+++

– non-staining; + = weakly positive, ++ = positive: +++ strongly positive
EMA = epithelial membrane antigen; SLCT = Sertoli Leydig cell tumour, nd = not determined

Table 1
Immunoreactivity of the tumour and the uterus specimen

mucosa with multifocally secretoric hyperglandudar hyperplasia and periglandular fibrosis of the uterine glands (▶Fig. 1). Furthermore, multiple endometrial cysts with eosinophilic material and scanty granulocytes in their lumen were noted.

At gross morphology, the mass below the left kidney was surrounded by fat tissue. Although it was completely laminated and in total embedded for microscopy, it did not show any presence of ovarian tissue in all the localizations examined histologically. Instead, the main histological structures consisted of two kinds of cells: cuboid to oval, polyhedral cells, and tall and slender tumour cells. The cuboid to oval tumour cells packed in sheets or as cords separated by thin strands of connective tissue, were arranged radially around blood vessels, with the small, round dark nuclei at the cell periphery in rosette formation. The cytoplasm was eosinophilic, often vacuolated. Here and there, prominent cell polymorphism and small cysts were noted. Mitotic figures were seen occasionally. There was neither angioinvasion, nor necrosis (▶Fig. 2). The above described features fit to a Leydig cell tumour (9, 11). The tall and slender to columnar tumour cells were arranged in well-formed tubules and arranged perpendicular to the basement membrane with scanty eosinophilic secrete in the lumen. The cells and their nuclei were round to oval and uniform. Mitotic figures were not present. These patterns correspond to a Sertoli cell tumour (9, 11) (▶Fig. 3). At an edge, a fallopian tube-like papillary proliferation with ciliated epithelial cells was noted (▶Fig. 4).

Immunohistological findings

In order to specify the immunophenotype of the tumour and the uterus specimen, the following antibodies were used according to Gómez-Laguna et al. (7) and Dabbs (3) (▶Table 1). Sections were deparaffined in xylene, rehydrated in a graded ethanol series, treated with 3% hydrogen peroxide solution at room temperature for 20 minutes, and washed three times with phosphate-buffered saline (PBS; pH 7,4, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPOH_4, 2 mM KH_2POH_4). The labelled streptavidin biotin

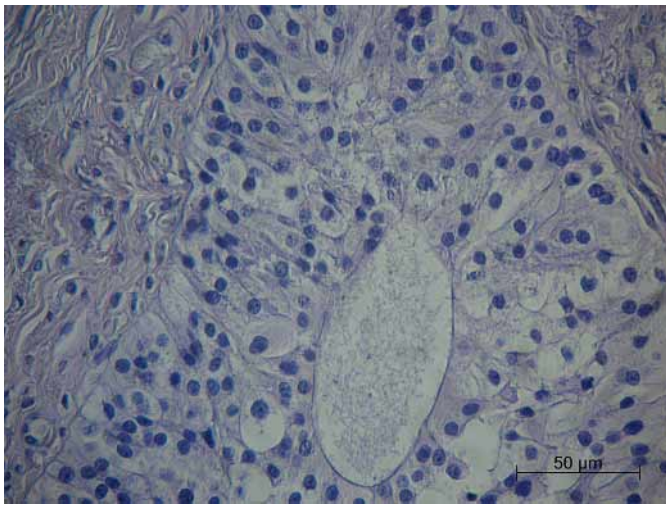

Fig. 3 Sertoli cell tumour: slender to columnar tumour cells, arranged in well-formed tubules and perpendicular to the basement membrane with scanty eosinophilc secrete in the lumen (LSAB)

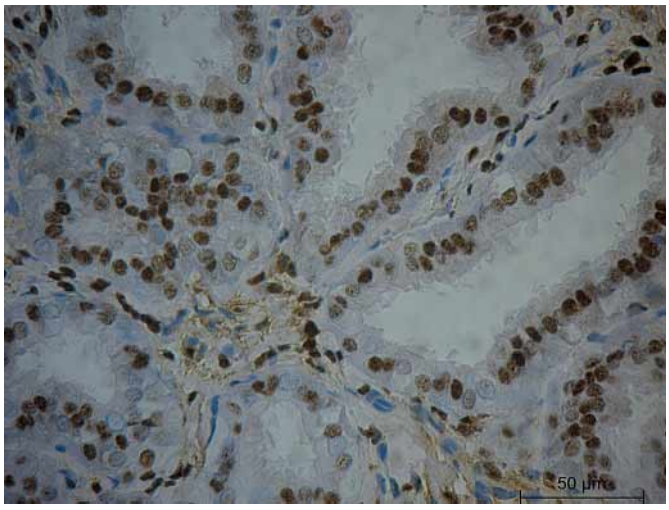

Fig. 4 Strong intranuclear ER-α expression in the tube-like epithelial cells (LSAB)

complex (LSAB) method was used by Flex/HRP (HRP) (Fa. Dako GmbH, Hamburg).

In the uterus specimen, the epithelial cells of the glands were strongly and specifically positive for estrogen receptor-α (ER-α) in their nuclei (▶Table 1, Fig. 5) and for cytokeratin in their cytoplasm (▶Table 1) and did not express progesterone receptor (PR). The stroma cells of the uterus mucosa weakly expressed vimentin in their cytoplasm (▶Table 1).

Both the cuboid to oval as the slender to columnar tumour cells strongly expressed vimentin and α-inhibin (▶Fig. 6, Table 1) in the cytoplasm, while the tumour cells did not express ER-α, cytokeratin, PR, S-100-protein and epithelial membrane antigen (EMA, ▶Table 1). The nuclei of the tube-like epithelial cells were strongly positive for ER-α, while their cytoplasm weakly expressed cytokeratin (▶Fig. 4, Table 1).

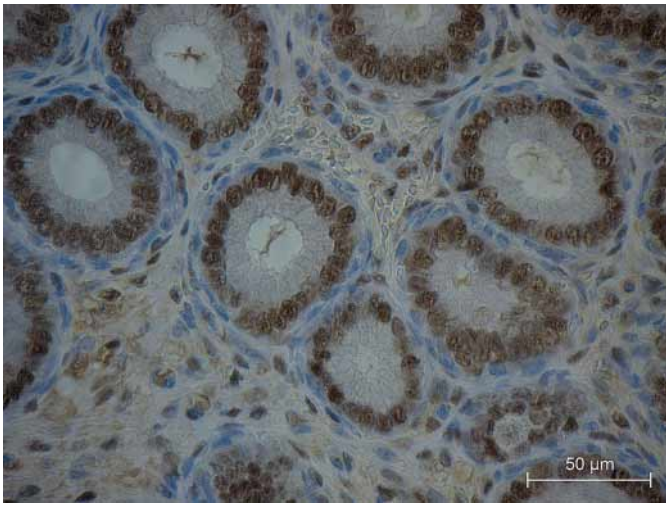

Fig. 5 Intranuclear ER-α expression in the epithelial cells of the uterine glands (LSAB)

Discussion

Canine ovarian neoplasms are uncommon (1, 11, 19, 24, 27). Their incidence ranges between 0.5 and 1.4% of all canine tumours (24). They occur more frequently in older, multiparous bitches or in bitches with ovarian remnant syndrome (7, 10, 12–14).

Sertoli Leydig cell tumours in male dogs are not uncommon (15, 21, 23). The neoplasia is occasionally reported in intact bitches (20) but is very rare in spayed female dogs. Ichimura et al. (8) recently reported a luteal and Sertoli cell tumour in a spayed bitch. Histogenetically, the neoplasma in the present case may have one of the four following origins: a) hermaphroditism, b) ectopic ovary, c) ovarian remnant syndrome and d) embryologic genetic defect.

a) Hermaphroditism is a very rare phenomenon. True hermaphrodites have both ovarian and testicular tissue (ovotestis). Affected animals are most phenotypically female with masculinization (6, 9).

b) Ectopic ovary, the result of the duplication of an ovary, is a rare anomaly with the additional ovary originating separately or splitting from an already developing ovary as an accessory ovary (6, 12).

c) The ovarian remnant syndrome develops in previously spayed bitches, which come into heat again. The length of time from the original surgery and the re-occurrence of oestrus signs in reported cases varied from 2 weeks to 3 years. The period mimics generally normal oestrous cycles in length (6, 18). The ectopic ovary as well as the ovarian remnant continue the cycle even without the presence of the uterus. The bitch in the present case has not shown any signs of oestrus during the last 10 years. Recently, Ball et al. (2) reported neoplasms in dogs and cats with ovarian remnant syndrome 120 months after spaying. A specification of the reported neoplasms was not performed (2). The authors suggest that errors in surgical technique are the most common reason for ovarian remnant syndrome in dogs and cats (2).

d) Both female and male gonads form from the urogenital system, which develops from the mesoderm (17). Primordial germ cells originate early from the fetal yolk. In humans, they develop about the 5th week of gestation from the endoderm of the embryo's yolk sac. They then migrate to bilateral swellings of the pelvic mesoderm (gonadal ridges) above the mesonephroi (primitive kidneys) to form paired bipotential gonads (6, 9, 12). After the gonadal migration, the gonadal ridge becomes sex cords. Further developments depend on the presence or absence of the Y chromosome, genetic factors and endocrine products. Theoretically, differentiation and thus transformation into cancer cell can occur at any point of the migration trace; this reflects random naturally occurring events. Sex cord stromal neoplasms are positive for α-inhibin and negative for EMA (3) as in the present investigation. Sex cord stromal tumours occur intragonadally as well as extragonadally. Doxsee et al. (5) reported cases of Sertoli Leydig cell tumours in previously neutered male dogs and cats. The tumours appeared 5–8 years following castration and were located in the spermatic cord, within the scrotal skin or at the site of the pre-

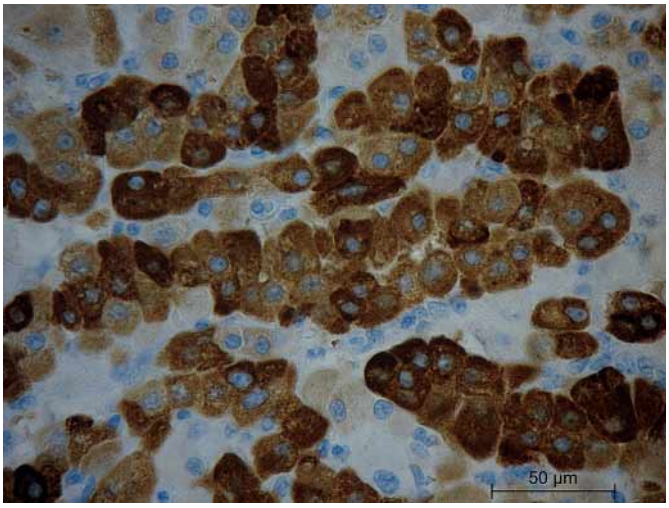

Fig. 6 α-Inhibin expression in the tumour cells (LSAB)

Conclusion for practice

Ovariohysterectomy is a prudent method for preventing many diseases in dogs and cats, such as pyometra, mammary and ovarial tumours. Even after castration, bitches occasionally come into heat again. This disorder can be observed up to 10 years after spaying. Sertoli Leydig cell tumour is a very rare neoplasma in the bitch. It might, however, occur more often than suspected since, in contrast to human medicine, most of the material removed during surgery is not subjected to pathological examination. Veterinary practitioners should enlighten pet owners regarding the possibility of this neoplasma.

scrotal castration incision site (5). However, the authors did not examine the neoplasms for α-inhibin expression, which represents a key antibody for separating the rare sex cord stromal tumours from gonadal neoplasms (2, 7).

Sex cord stromal tumours are not uncommon in humans. They represent 8% of all primary ovarian neoplasms (3, 4). Sertoli Leydig cell tumours are rare in women, accounting for less than 1% of ovarial neoplasmas. They typically occur in reproductive-age women (average 25°years). Younger girls are rarely affected (4).

Sex cord stromal tumours have the potential to be hormonally active and vary considerably in regards to the pattern of steroidogenesis. Depending of the predominating cells, the expressed hormones can be progestin, estrogen or androgen and cause prolonged anoestrus to nymphomania and masculinization, respectively (26). In the present case, the tumour produced estrogen. The intranuclear estrogen receptors were detected immunohistologically in the epithelial cells of the tube and the uterine glands with ER-α antibody. Concerning the biological behaviour of the tumour, signs of malignancy and metastasis were both absent. Since tumour removal (16 months) our patient has been healthy and failed to show any signs of oestrus. These findings correspond to those of Doxee et al. (5), who noted neither neoplasm-related deaths nor metastasis (5). We propose that veterinary practitioners should enlighten pet owners regarding the eventuality of this rare disease.

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Conflict of interest

The authors confirm that they do not have any conflict of interest.

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