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Case report

Ovarian sex-cord stromal tumor in Yorkshire Terrier dog

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Abstract

A 12-year-old, spayed Yorkshire Terrier dog with a history of progressive abdominal distension was diagnosed with an ovarian sex-cord stromal tumor. Microscopically, the residual ovarian tissue sample was composed of 2 different tumor cell populations: a luteal-like cell and Sertoli cell components. These cells were notably immunopositive for vimentin, inhibin-α and neuron-specific enolase (NSE). On the basis of all findings, the tumor was diagnosed as luteoma and Sertoli cell tumor of the ovary developing from the ovarian remnant tissue.

Keywords: Dog, Luteoma, Ovarian sex-cord stromal tumor, Sertoli cell tumor

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INTRODUCTION

Primary ovarian tumors are relatively uncommon tumors in dogs; however, they have been frequently reported in old, multiparous dogs or dogs with ovarian remnant tissues (Vissiennon et al., 2010). Overall, primary ovarian tumors account for 0.5%–1.2% of all canine tumors (Namazi et al., 2015). Generally, ovarian tumors are classified into 3 different types depending upon the origin of the cells. These types include epithelial cells, germ cells and sex-cord stromal cells (Flores et al., 2019; Patnaik and Greenlee, 1987). Of these, the epithelial ovarian tumor is known to be the most common tumor in dogs (Patnaik and Greenlee, 1987; Sforna et al., 2003).

Sex-cord stromal tumors (SCSTs), tumors arising from primitive sex cord or stromal cells or both, are usually categorized into various types depending upon the dominant cell population. Those are granulosa cells, theca cells, luteal cells and interstitial endocrine cells (Arlt and Haimerl, 2016). Among these subtypes, the granulosa cell tumor is the most common. In dogs, SCSTs have been demonstrated in Cocker Spaniel, Rottweiler, Boxer, German Shepherds, Fox Terriers, Mongrels and Yorkshire Terriers (Deluchi et al., 2018; Diez-Bru et al., 1998; Namazi et al., 2015; Sung et al., 2007; Yamini et al., 1997).

Ovarian remnant syndrome (ORS) is a complicated problem resulting from a failure to completely remove the ovarian tissue during ovariohysterectomy (OVH) or ovariectomy (OVE). However, Sontas explained that the causes of ORS may include the presence of ovarian residue resulting from a surgical technical error or the presence of accessory ovarian tissues at the adjacent tissue (Sontas et al., 2007). These remnant tissues will continue to secrete sex hormones and the bitches will then present clinical signs of proestrus, estrus, ovarian or mammary tumors, or stump pyometra (Ball et al., 2010; van Nimwegen et al., 2018). The symptoms mentioned above may occur within a week to several years following the neutering of the animal (van Nimwegen et al., 2018; Vissiennon et al., 2010). Furthermore, these remnant tissues will continue to function as normal ovary and may be risk of developing ovarian tumor (Oliveira et al., 2012).

In our study, the clinical signs, histopathological and immunohistochemical features, and diagnostic imaging findings of luteoma and Sertoli cell tumor of the ovary in a 12-year-old, female Yorkshire Terrier dog with ORS are described

HISTORY, CLINICAL DIAGNOSIS and FINDINGS

A 3.0 kg, 12-year-old, female Yorkshire Terrier dog was presented at the Oncology Clinic, Small Animal Hospital, Chiang Mai University Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University with a history of progressive abdominal distension for 1.5 months. The owner mentioned the observance of certain clinical signs including serous vaginal discharge and the continued attraction of male dogs after an ovariohysterectomy (OVH) had been performed 9 years prior. Upon physical examination, all vital signs were observed to be normal. The dog, however, had revealed serous vaginal discharge, abdominal distension, immature cataracts, dental tartar and halitosis at the time of the initial presentation. Hematological and

blood chemical profiles (Table 1) revealed mild thrombocytosis (696 x 10^3/µl; reference range 211–621 x 10^3/µl), elevated blood urea nitrogen (BUN, 39.8 mg/dl; normal range 8–28 mg/dl), mildly elevated alanine aminotransferase (ALT, 118 U/L; normal range 10–109 U/L), hyponatremia (131.1 mmol/L; normal range 142–152 mmol/L), hypokalemia (3.48 mmol/L; normal range 3.9–5.1 mmol/L) and hyperchloremia (125.4 mmol/L; normal range 110–124 mmol/L). Abdominal radiograph illustrated a soft tissue density mass at the middle part of the abdomen resulting in caudal displacement of the small intestine shadow (Figure 1). Abdominal ultrasonographic examination revealed a multilocular cystic mass (3.74 x 3.09 cm) located between the spleen and the cranial pole of the left kidney. Free abdominal fluid was also detected. However, no evidence of tumor invasion into the adjacent organ was diagnosed (Figure 2).

Table 1 Hematological and blood chemical profiles of the present case

Parameters	Results	Normal range*	Unit
Hematology			
Hct	53	35 – 57	%
Hgb	16.7	11.9 – 18.9	g/dl
RBC count	7.62	4.95 – 7.87	x10^6 /µl
MCV	70.0	66 – 77	fl
MCH	21.9	21 – 26.2	pg
MCHC	31.3	32 – 36.3	g/dl
WBC count	10.06	5 – 17.00	x10^3 /µl
Band neutrophil	0.00	0.00 – 0.45	x10^3 /µl
Segment neutrophil	7.72	2.90 – 12.00	x10^3 /µl
Lymphocyte	1.50	0.40 – 2.90	x10^3 /µl
Monocyte	0.54	0.10 – 1.40	x10^3 /µl
Eosinophil	0.30	0.00 – 1.30	x10^3 /µl
Basophil	0.00	0.00 – 0.14	x10^3 /µl
Platelet count	696	211 - 621	x10^3 /µl
Platelet smear	Adequate	Adequate	-
Blood chemistry			
BUN	39.80	8.00 – 28.00	mg/dl
Creatinine	0.99	0.50 – 1.70	mg/dl
ALP	44.00	1.00 – 114.00	U/L
ALT	118.00	10.00 – 109.00	U/L
Total protein	6.50	5.40 – 7.50	g/dl
Albumin	3.10	2.30 – 3.10	g/dl
Globulin	3.40	2.70 – 4.40	g/dl

* (Kenneth et al., 2003)

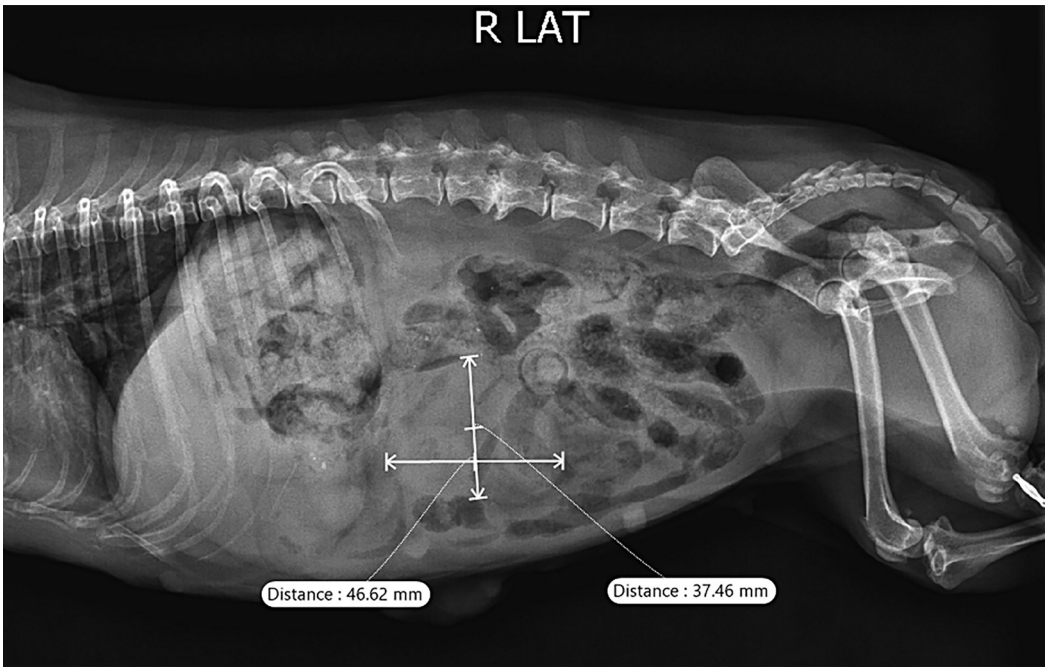

Figure 1 Radiographic image in right lateral view illustrates a soft tissue density mass (4.7 x 3.7 cm) at the middle part of abdomen (arrows).

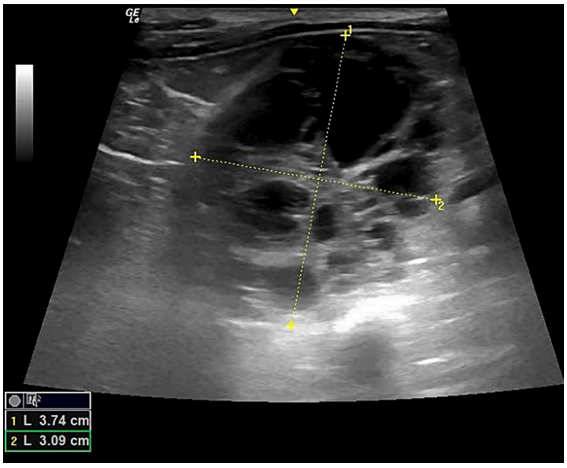

Figure 2 Ultrasonographic image illustrates a multilocular cystic mass in the abdominal cavity. B-mode (10 MHz probe).

CASE MANAGEMENTS

Ultrasound-guided abdominocentesis was performed. A fluid sample was subsequently analyzed. The specific gravity of fluid sample was 1.024. Total nucleated cell count and protein values were 930 cells/µl and 3 g/dl, respectively. On the basis of these findings, a diagnosis of transudate was made. The owner, then, requested exploratory laparotomy of the dog for tumor removal. In accordance with the gross examination, an abdominal tumor (4.8 x 3.3 cm) was well-circumscribed and encapsulated by a thin fibrous capsule. Multiple cystic

lesions were observed (Figure 3). On the cut surface, these lesions had thin walls and contained clear fluid, whereas the tumor tissue was grayish-white in color and firm consistency. The tumor sample was fixed in 10% neutral buffered formalin and then submitted for pathological examination. Tissue sections were cut 4 µm-thick and then stained with hematoxylin and eosin (HE). In addition, immunohistochemistry was performed using an avidin-biotin complex (ABC) method as previously described (Boonsri et al., 2021). The following primary antibodies were described in Table 2. In addition, normal skin, testis and pancreas, were also used as positive controls.

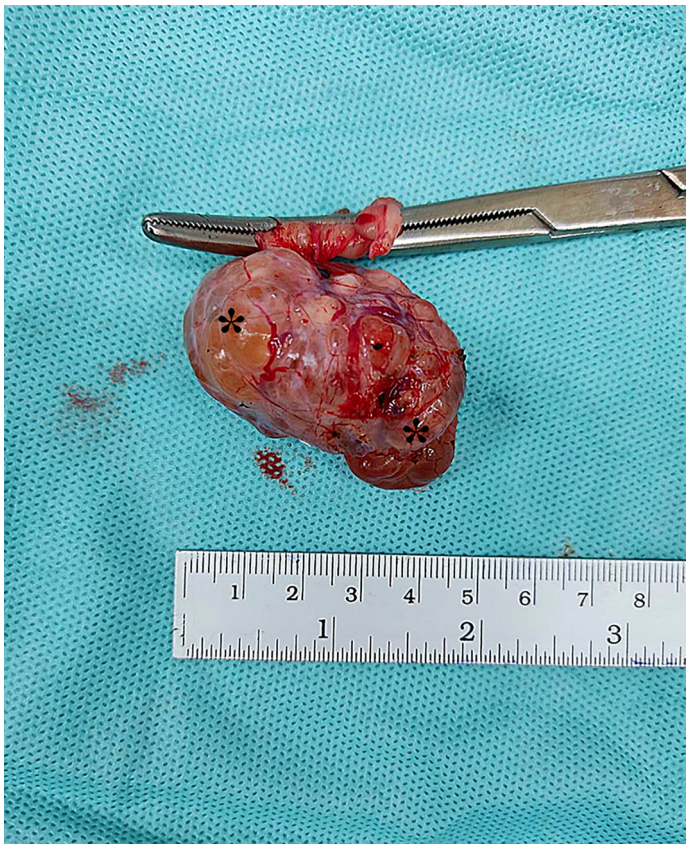

Figure 3 Left ovarian remnant tissue (4.8 x 3.3 cm). On gross examination, the lesion is well-circumscribed and encapsulated by a thin fibrous capsule. Multiple cystic lesions are observed (asterisk).

Table 2 Primary antibodies used for immunohistochemistry*

Antibody	Type	Dilution	Cellular expression	Source
Cytokeratin	mAb (AE1/AE3)	1:300	Cytoplasm	Abcam, Cambridge, MA, USA
Vimentin	mAb (V9)	1:300	Cytoplasm	Abcam, Cambridge, MA, USA
Inhibin-α	mAb	1:100	Cytoplasm	Cell Maque™
NSE	mAb (5E2)	1:600	Cytoplasm	Zeta corporation, CA, USA

* NSE = neuron specific enolase; mAb = monoclonal antibody

This specimen was microscopically confirmed to be ovarian remnant tissue that was comprised of 2 different tumor cell populations. The large population of tumor cells arranged in multiple lobules were separated by connective tissue stroma. These cells were round to polygonal-shaped with an abundant clear vacuolated cytoplasm. Their nuclei were eccentric, round to ovoid-shaped with prominent nucleoli (Figure 4A). In some areas, the tumor cells were polygonal-shaped with granular acidophilic cytoplasm. The nuclei were round and hyperchromatic with single prominent nucleoli (Fig. 4B). The second population was comprised of vacuolated tumor cells that resembled the Sertoli cell component. These cells were tall columnar cells with indistinct cell borders. Their nuclei were round to ovoid-shaped with a single prominent nucleolus. The tumor cells were in palisade arrangement and were surrounded by fibrous stroma (Figure 4C). No evidence of vascular infiltration was detected. Luteal cysts were noted to be scattered throughout the specimen. They had a fluid-filled, central cavity that was lined with several layers of luteinized cells (Figure 4D). In addition, adenomatous hyperplasia of the subsurface epithelial structure was observed (Figure 5). Based on the histopathological findings, a diagnosis of luteoma together with Sertoli cell tumor was made. Furthermore, other SCSTs should be included in the differential diagnosis. Immunohistochemical examination, however, illustrated that the tumor cells from 2 different cell populations reacted positively for vimentin, inhibin-α and neuron-specific enolase (NSE). However, no immunoreaction for cytokeratin was identified (Figure 6). On the basis of all findings, a diagnosis of luteoma and Sertoli cell tumor of the ovary developing from the ovarian remnant tissue was made.

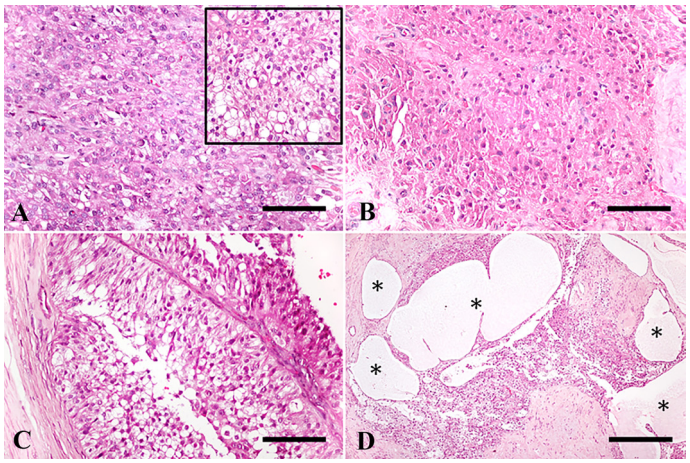

Figure 4 Luteal-like cell component (A – B). The tumor cells are round- to polygonal-shaped with abundant cytoplasm. Clear vacuoles within the cytoplasm are also noted (A; inset). Some of them are polygonal-shaped with granular acidophilic cytoplasm (B). The Sertoli cell component (C). The tumor cells are in a palisade arrangement and are surround by fibrous stroma. Luteal cysts are observed scattered throughout the specimen (D; asterisks). HE. 400x. Scale bar = 50 µm.

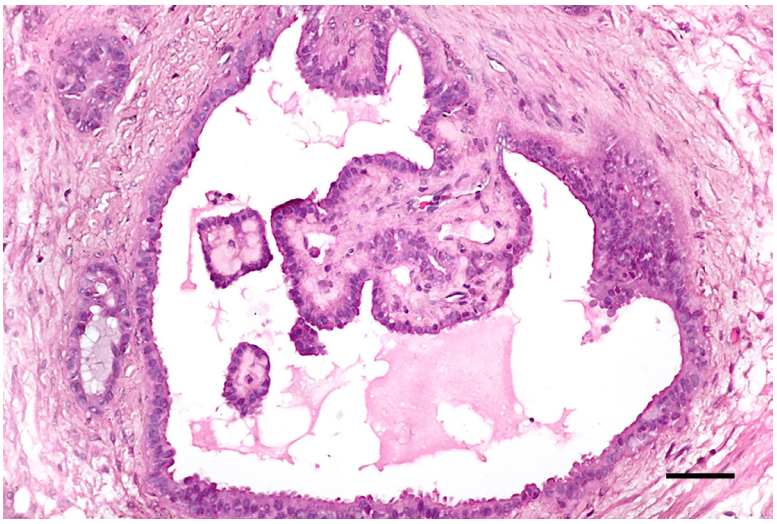

Figure 5 Adenomatous hyperplasia. A tubulopapillary proliferation of tall columnar cells is observed. These cells have eosinophilic cytoplasm. HE. 400x. Scale bar = 50 µm.

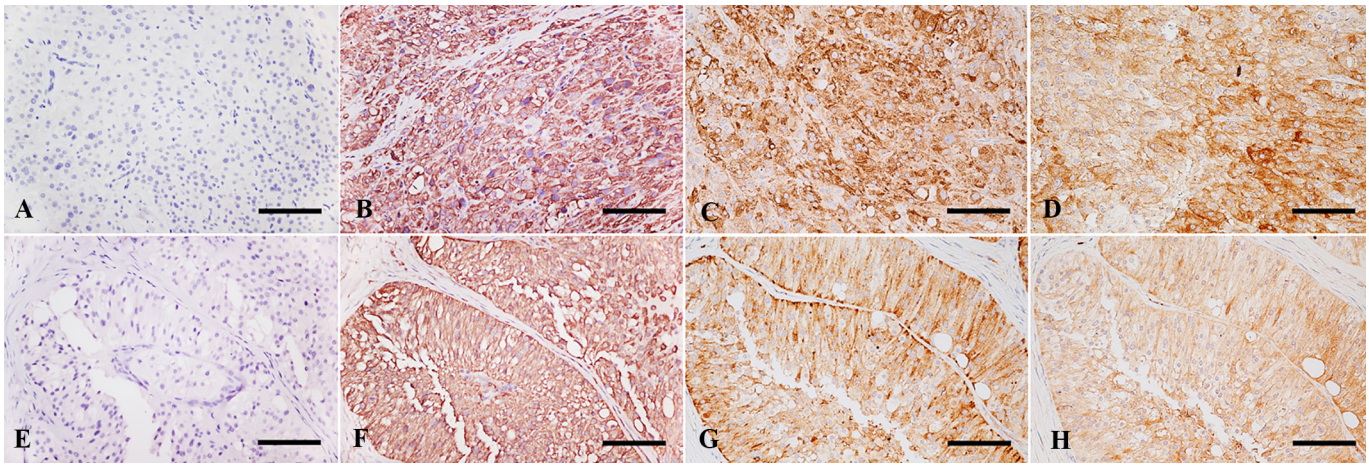

Figure 6 The tumor cells of luteal-like cell (A – D) and Sertoli cell (E – H) components are immunonegative for cytokeratin (A & E). These cells, however, are immunopositive for vimentin (B & F), inhibin-α (C & G) and NSE (D & H). DAB chromogen, hematoxylin counterstain. 400x. Scale bar = 50 µm.

During 7 days of follow-up observations after the operative procedure, the dog was found to be bright, alert and responsive with normal vital signs. At that time, a sex hormone examination was conducted. Consequently, follicle stimulating hormone (FSH), progesterone and estrogen levels were measured, and the results revealed normal FHS (<0.05 mIU/ml; chemiluminescent microparticle immunoassay), normal progesterone (<1.00 ng/ml; reference range 1 – 12 ng/ml (estrus) and > 12 ng/ml (diestrus); vCheck cProgesterone test kit, Gyeonggi, Korea) and normal estrogen (<10 ng/ml; reference range 30 – 150 ng/ml; chemiluminescent microparticle immunoassay) levels.

After twenty-one days of follow-up observations, the dog was bright, alert and responsive with normal vital signs. All hematological parameters were within the normal range. A blood chemical profile, however, revealed mildly elevated ALT. For sonographic examination, no evidence of peritoneal effusion and tumor recurrence was detected.

DISCUSSION

In the present study, the animal showed signs of entering heat as well as the continued attraction of male dogs during a 9-year period after the OVH. This suggested the possibility that the residual functioning ovarian tissues were still producing reproductive hormones. Unfortunately, a limitation of this study was the lack of sex hormone measurement prior to the operation. We believe these parameters are likely to be a tool that could be used to confirm the reproduction condition of the present case.

Canine ovarian tumors are commonly demonstrated in senior, intact female dogs or dogs with ovarian remnant syndrome. These tumors can be categorized into 3 types depending upon the cell of origin. Luteoma and Sertoli cell tumors of the ovary belong to SCSTs and are mostly considered to be benign tumors. In our study, interestingly, most luteal-like cells contained clear vacuoles within the cytoplasm. These cells were observed to be interspersed among other cell components that had a granular acidophilic cytoplasm resembling the Leydig cells. Nielsen explained that the luteoma is generally divided into two subtypes including lipid cell tumor and Leydig-like tumor (Nielsen et al., 1976). This suggests that the luteoma portion of the present study was composed of these two subtypes. The evidence of tumor invasion, moreover, was not detected in the present case indicating that the luteoma and Sertoli cell tumor of the ovary display benign behavior.

NSE is a marker for neuroendocrine origin. Normal Sertoli cells and Sertoli cell tumors are strongly positive for this marker (Owston and Ramos-Vara, 2007). Other than the Sertoli cell component, the luteal-like cell component of the present case also reacted positively for NSE as has been mentioned previously (Ichimura et al., 2010). Although there have been several previous studies that demonstrated NSE expression in SCSTs, other sarcomas and/or carcinomas also express NSE, suggesting that this marker is not a specific marker for SCST confirmation (Durkes et al., 2012). Inhibin-α is a specific marker for human and canine SCSTs. Ovarian epithelial tumors and germ cell tumors, however, are usually negative for inhibin-α (Akihara et al., 2007; Ichimura et al., 2010; Kudo et al., 2019; Namazi et al., 2015). In the present study, the tumor cells from 2 different components were strongly positive for inhibin-α together with vimentin, whereas those cells were negative for cytokeratin indicating that these tumor cells arose from sex-cord stromal origin.

To date, several studies have been conducted with regard to the exact pathogenesis of ovarian tumors that are associated with ascites development. These tumors may contribute to the impairment of vascular permeability and lymphatic drainage resulting in fluid accumulation within the peritoneal cavity (Sangisetty and Miner, 2012). According to the present case, the dog

we studied had abdominal distension at the time of its initial presentation. However, this condition was not detected during the three weeks of follow-up observation indicating that the exact cause of ascites may have been resolved. SCSTs of the ovary are uncommon tumors in dogs. It is noteworthy to recognize that inhibin-α, as well as vimentin and NSE, can be used as the antibody panel for immunohistochemistry in order to confirm canine ovarian SCSTs. However, morphological characteristics of tumor cells and histological patterns are very important to distinguish among subtypes of SCSTs.

To our knowledge, this is the first case report of canine luteoma and Sertoli cell tumor of the ovary developing from the ovarian remnant tissue after 9 years of OVH to have been conducted in Thailand.

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