Case Report

Management of a granulosa-theca cell tumor in a female Rottweiler

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Abstract

A 4-year, multiparous Rottweiler female dog, was presented for breeding management. Dog had clinical signs of proestrus and estrus for > 6 weeks, including cornification of vaginal epithelium and low circulating progesterone concentrations (< 2 ng/ml). Transabdominal ultrasonography of the reproductive tract and measurement of circulating hormones (antimüllerian hormone, Inhibin-B, and progesterone) suggested granulosa-theca cell tumor. Affected ovary was removed via laparoscopy. Histopathology confirmed granulosa-theca cell tumor; dog resumed cyclicity 4 months after surgery, had normal estrus and was successfully mated with a young stud dog. Dog was diagnosed pregnant (30 days after LH surge) via transabdominal ultrasonography; 4 amniotic sacs were detected, and 3 grew to term but were not viable at delivery (via cesarian surgery).

Keywords: Persistent estrus, granulosa-theca cell tumor, laparoscopic ovariectomy

Background

Ovarian tumors are rare in mammals, but among domestic animals, they appear to be most common in female dogs and cows.[1–5] Primary ovarian tumors can be categorized into 3 groups; epithelial, germ cell, and sex cord-stromal. Granulosa-theca cell tumors (GCT) are sex cord-stromal cell-based ovarian tumors that account for 0.5 to 1.2% of all canine tumors. GCTs arise from granulosa cell cords of atretic follicles[5] and are not locally invasive; however, in 10 to 20% of cases they can metastasize.[2,5] A mass effect of the GCT causing clinical signs is very uncommon. However, GCTs can be challenging to diagnose as they do not always have the classical honeycomb-like appearance that is recognizable via ultrasonography.[6] Most GCTs are unilateral in all species, although bilateral tumors were described in a female dog.[5]

The most common presentation associated with canine GCTs are signs of estrus that persist for > 6 weeks.[7] Clinical signs of persistent estrus are associated with constant secretion of estrogen and include behavioral signs such as standing/sexual reflexes (flagging and postural changes), receptiveness to intact males, and anatomical changes, including vulvar swelling and serosanguineous vulvar discharges.[3–5,8–12] However, clinical signs associated with estrogen can also be associated with normal physiologic cyclicity, ovarian remnant syndrome, ovarian cyst(s), ovulation failure, and exposure to exogenous sources of estrogen. Diagnosis of GCT has also been previously reported in ovariohysterectomized dogs with concurrent ovarian remnant syndrome.[13-15] Therefore, determination of when clinical signs of estrus begin, and ongoing vaginal cytology and serum progesterone measurements are required to facilitate differentiation of a GCT from other causes of estrogen-associated conditions.

Long-term exposure to estrogen arising from continuous secretion of steroid hormones from neoplastic granulosa cells of a GCT can have detrimental effects on bone marrow and uterine tissue, resulting in anemia and infertility, respectively.[3-5,8-12] Exposure to sustained elevated concentrations of estrogen affects the hematopoietic system in 3 stages: a brief increase in platelets followed by severe thrombocytopenia, then development of bone marrow granulocyte hyperplasia with neutrophilia and, finally, bone marrow recovery or aplasia, dependent on the magnitude of hormonal insult.[16,17] GCTs can also produce progesterone that in combination with estrogen, causes stimulation of endometrial growth, proliferation, and glandular secretions.[8] Direct exposure to these steroid hormones results in the development of cystic endometrial hyperplasia (CEH), which not only increases the risk of developing a life-threatening pyometra but can lead to sub/infertility through early embryonic loss and impaired placentation.[8,18,19]

Bioassay panels of reproductive hormones in mares and cows are routinely used to help differentiate ovarian

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pathology and diseases. Increased serum concentrations of antimüllerian hormone (AMH), inhibin B and/or testosterone, all produced by granulosa cells, have been demonstrated to be useful biomarkers for identifying functional GCTs in cows and mares.[20–25] In mares, AMH secretion can be identified in normal growing and small antral follicles.[26] However, studies comparing normal ovarian function with GCTs determined that bioactive AMH was more strongly expressed in mares with GCT.[20,26] Elevated serum concentrations of inhibin and testosterone are present in 87 and 67% of mares with a GCT, respectively.[25–28] Inhibin B isoform is the only isoform of inhibin that reliably indicates ovarian neoplasia in mares.[22] When AMH, Inhibin B and testosterone are used together as a screening panel in mares, 100% sensitivity is achieved.[23] Unfortunately, a screening panel for canine GCT has not yet been established. Previous studies reported serum concentrations of AMH in dogs with GCTs to range from 1.12 to ≥ 23 ng/ml.[21,29,30] At the time of this report, no studies were identified regarding the use of inhibin-B serum concentrations for canine GCTs diagnosis. As a result, no reference range has been established. Testosterone has also not been reported as a bioassay in the dog for GCT diagnosis and was not measured in this case. Currently, in dogs, measurement of AMH and inhibin needs to be interpreted in conjunction with other clinical and diagnostic findings (e.g. ovarian anatomical changes on ultrasonography) for a presumptive diagnosis of GCT.[20,21]

Ultrasonographic examination of the ovaries is a valuable screening and diagnostic tool for determining the presence of pathological changes associated with ovarian parenchyma in many species.[20] Previous publications in dogs have demonstrated that ovarian tumors, particularly GCTs, have a wide range of alterations to echogenicity and shape.[6]

In this case report, we discuss the steps taken to diagnose GCT at a very early stage of development in a young, genetically valuable breeding female dog and a novel treatment approach to remove the ovarian tumor whilst maintaining fertility.

Case presentation

A 4-year, multiparous Rottweiler female dog, was presented 7 days after the onset of clinical signs associated with proestrus (i.e. bloody vulvar discharge and vulvar edema). A routine breeding examination was performed, which included a complete physical examination, screening for *Brucella canis* (Brucella Multiplex, Cornell University, Ithaca, NY). Additionally, complete blood count (CBC) and chemistry analyses were performed. No abnormal findings were reported. Starting on day 8 (from the first signs of proestrus observed by the owner), blood samples were obtained every other day to measure serum progesterone concentrations (IMMULITE® 1000 Immunoassay System, Colorado State University, Fort Collins, CO) and vaginal smears were taken for cytological evaluation of vaginal epithelial cells every 2nd or 3rd day to determine stage of estrus and detect LH surge (LH0). This was performed, as is routinely done, to facilitate optimal timing of insemination (> 90% superficial keratinized vaginal epithelial cells) with frozen-thawed canine semen.[31–35] Progesterone concentrations ranged from 0.90-1.85 ng/ml but never exceeded 2 ng/ml, and vaginal cytology never changed from > 90% superficial cell (Table).

Table. Serum progesterone profile and vaginal cytology

Day of cycle from onset of clinical signs	Progesterone concentrations (ng/ml)	Vaginal cytology (percent superficial cornification)
8	0.923	> 90
10	0.976	> 90
13	0.971	> 90
16	0.924	> 90
21	1.030	> 90
23	0.895	> 90
27	1.16	> 90
30	1.03	> 90
35	1.85	> 90

Differential diagnoses

Differential diagnoses for persistent estrus included an anovulatory follicle/ovulation failure, an ovarian follicular cyst, exogenous exposure to estrogens through diet, oral treatment, or inadvertent exposure to human hormonal creams and lastly, hormone-secreting ovarian neoplasia (e.g. GCT).[6,7,32,36,37] Intersex conditions were not considered as a differential diagnosis due to previous history of successful litters.[38,39]

Diagnosis

Initial B-mode ultrasonographic examination (Canon® TUS-AI700 ultrasound machine with a multi-frequency micro-convex (8M1C) transducer) of the reproductive tract (ovaries, uterus) was performed on day 40 of estrus. Enlarged (19-21 mm in diameter) left ovary had a heterogeneous hypoechoic region within the caudal pole (Figure 1A). Right ovary was normal in appearance and size (Figure 1B). Differentiation between an anovulatory follicle undergoing luteinization, an ovarian follicular cyst, or ovarian neoplasia was not possible.

On day 57, progesterone concentrations remained lower (< 2 ng/ml) and > 90% vaginal epithelial cells were cornified. Another transabdominal ultrasonography was performed; right ovary was still normal in appearance, size with ultrasonographic heterogenicity and had no follicular activity, left ovary had increased in size (19-21 to 20-22 mm) and the heterogeneous hypoechoic region within caudal pole was still present (suspected to be neoplastic). Left ovary had begun to take on a honeycomb-like appearance (Figure 2), a common finding with GCTs in mares.[40] Using color Doppler, moderate peripheral vascularity and a few small internal vessels associated with the heterogenous hypoechoic structure on left ovary were visualized. Increased size, level, and location of angiogenesis and the honeycomb-like appearance of left ovary were all suggestive of GCT.

At each transabdominal ultrasonographic examination, uterus was notably enlarged (~ 10.4 mm diameter/width/thickness) for a young, multiparous dog, and echogenic changes (cystic structures) observed throughout the endometrium (Figure 3), indicative of cystic endometrial hyperplasia (CEH).[41]

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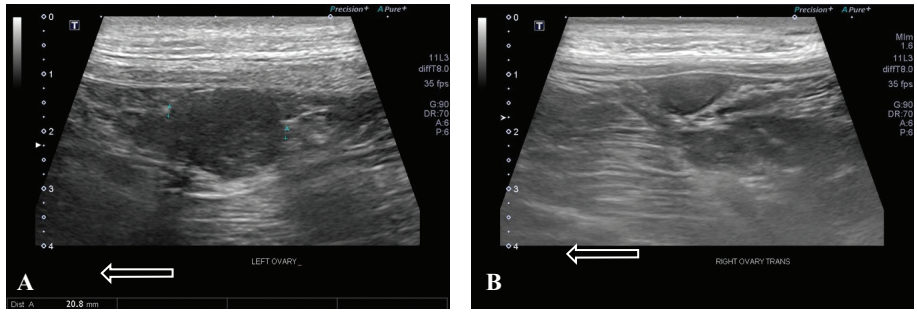

Figure 1. A. Ultrasonographic image of 'abnormal' left ovary (note enlarged ovary with a heterogeneously hypoechoic region within the caudal pole) on day 40 of estrus; distance between calipers: 20.8 mm and arrow pointing cranial side. B. Ultrasonographic image of 'normal' right ovary on day 40 of estrus and arrow pointing cranial side.

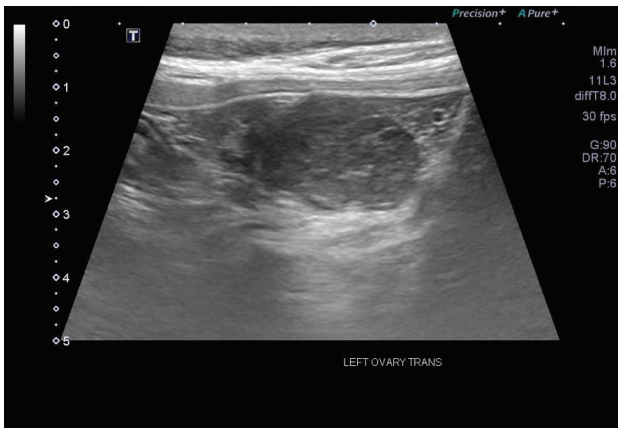

Figure 2. Ultrasonographic image of 'abnormal' left ovary on day 57 of estrus (note: honeycomb-like appearance suggestive of early stages of differentiation of a GCT)

On day 57, serum was submitted (University of California Davis, Davis, CA) for inhibin-B (AL163, Ansh Labs, Webster, TX) and AMH (Canine AMH AL116, Ansh Labs, Webster TX). Inhibin concentrations were 182.4 pg/ml, and AMH concentrations were 0.89 ng/ml. The laboratory reported that AMH concentrations were within the normal reference range for an intact dog in estrus (0.12 to > 12 ng/ml). However, there is currently no normal reference range for canine inhibin, but concentrations detected were considered highly elevated compared to equine normal reference range of 2 to 100 pg/ml. Progesterone concentrations (Enzyme immunoassay, University of California Davis) were 9.3 ng/ml. CBC and biochemistry revealed nonregenerative anemia, severe thrombocytopenia, and leukopenia. Prothrombin time and partial thromboplastin time were unremarkable, but fibrinogen was elevated. Biochemistry markers, including fibrinogen, were consistent with chronic inflammation associated with myelosuppression. Serum estrogen and estradiol concentrations were not measured as the cytological assessment of vaginal smears remained at > 90% superficial/cornified vaginal epithelial cells.

Based on these clinical, ultrasonographic, and endocrine results, a preliminary diagnosis of GCT was made. Due to the development of nonregenerative anemia, it was decided that immediate surgical removal of left ovary was necessary to eliminate the source of estrogen and to confirm our diagnosis. No additional measurements nor follicular activity of the contralateral ovary were determined due to the critical state of the patient (bone marrow suppression).

Treatment

Before surgery, blood crossmatching (centrifuged EDTA blood samples of donor and recipient) was carried out, and a unit of DEA 1.1 (+) blood was given intraoperatively to improve low oxygen-carrying capacity caused by bone marrow suppression. Unilateral ovariectomy (laparoscopic laparotomy) was performed. Briefly, a 3 cm incision was made in caudal abdomen on the ventral midline to facilitate laparoscope introduction. Abdomen was insufflated to 11 mm Hg, and left ovary was visualized (Figure 4) and transected using a Ligasure™ (Medtronic). Incision site was widened to exteriorize left ovary from abdomen (Figure 5). Right ovary appeared small with no grossly evident follicular activity during the procedure. Hemorrhage was minimal and controlled using monopolar electrocautery. Abdominal wall was closed, and a Tegaderm™ (Nexcare™, 3M) bandage was placed over the incision site. Dog recovered uneventfully and was discharged the following day. Removed left ovary was submitted for histopathology.

Outcome

Left ovary (~ 8.5 x 4 cm) had prominent multinodular white to tan ovarian tissue with well-demarcated white, variably friable, and partially caveated mass (3 cm); expanding around the mass were regions of frank blood (hemorrhage). Circumscribing the mass was white to yellow thick fibrous capsule. Remainder of the ovarian tissue was tan with variably sized cystic structures (developing follicles; Figure 6). Histopathology confirmed that the tumor effaced over 60% of left ovary and was highly cellular, encapsulated and well-demarcated sex cord neoplasm. Neoplastic cell population was arranged in a rare glandular/pseudo-rosette structure, with the remaining population located within interlacing streams supported by collagenous stroma. Anisocytosis and anisokaryosis was moderate with 3 mitotic figures per mitotic area. Histopathology findings were consistent with a poorly differentiated GCT. Remaining ovarian tissue within left ovary was histologically normal, with ovarian follicles observed in varying stages of maturation (Figure 7). A portion of left uterine horn was also submitted for histologic evaluation, and endometrium was described as hyperplastic, measuring ~ 2 mm in thickness with variably ectatic endometrial glands containing scant

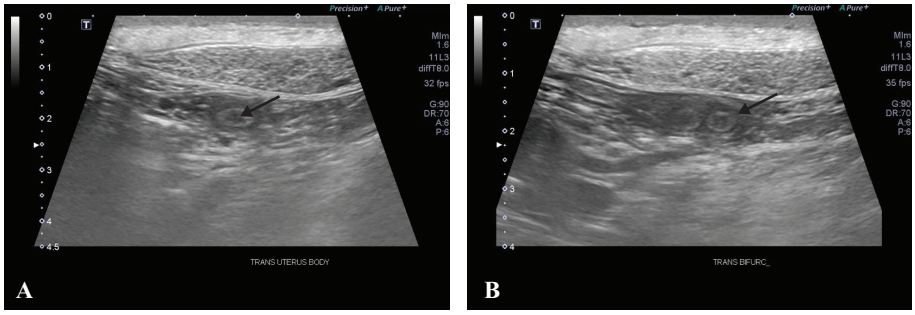

Figure 3. Ultrasonographic images of uterus on day 30: A. transverse view of uterine body and B. uterine horn bifurcation (characteristic of cystic endometrial hyperplasia) cysts; note cysts (arrows) within uterine endometrial lining.

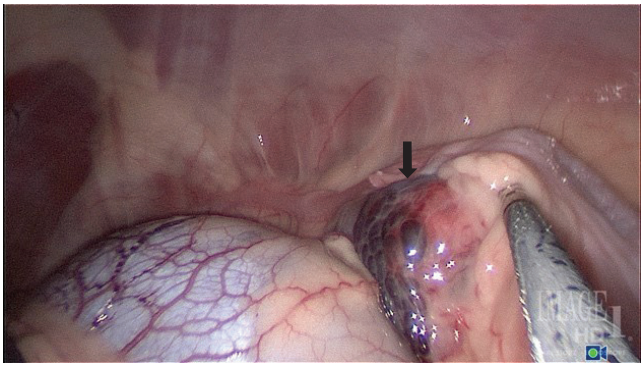

Figure 4. Intraoperative image of left ovary (black arrow) before surgical resection (note abnormal gross appearance ovarian serosal surface with multilobulated darkened parenchyma).

flocculent eosinophilic material. Uterine findings were consistent with CEH.

On day 14 after surgery, serum was collected and submitted (University of California Davis) for measurement of progesterone, inhibin B, and AMH concentrations. Inhibin B concentrations had declined substantially to 8.4 pg/ml; progesterone was baseline (< 0.2 ng/ml), and AMH concentrations remained within normal reference range, consistent with declining concentrations of inhibin after ovariectomy as observed in mares.[22] AMH and inhibin concentrations were not measured during the follow-up period prior to returning to cyclicity.

Dog returned to proestrus 4 months after unilateral ovariectomy. Blood sample was submitted for CBC and chemistry analyses; nothing was remarkable. Importantly, there was complete resolution of nonregenerative anemia, thrombocytopenia, and inflammatory profile. Ultrasonographic examination of uterus demonstrated continued endometrial changes associated with CEH. There was no intrauterine fluid. To maximize chances of pregnancy, breeding management was performed, and the dog was naturally mated (young proven stud dog) on days 4 and 6 after LH0.

Transabdominal ultrasonography was performed on day 30 after LH0; 4 viable amniotic sacs and 1 resorption site (Figure 8) were observed. A single lateral abdominal radiograph was obtained 1 week before calculated

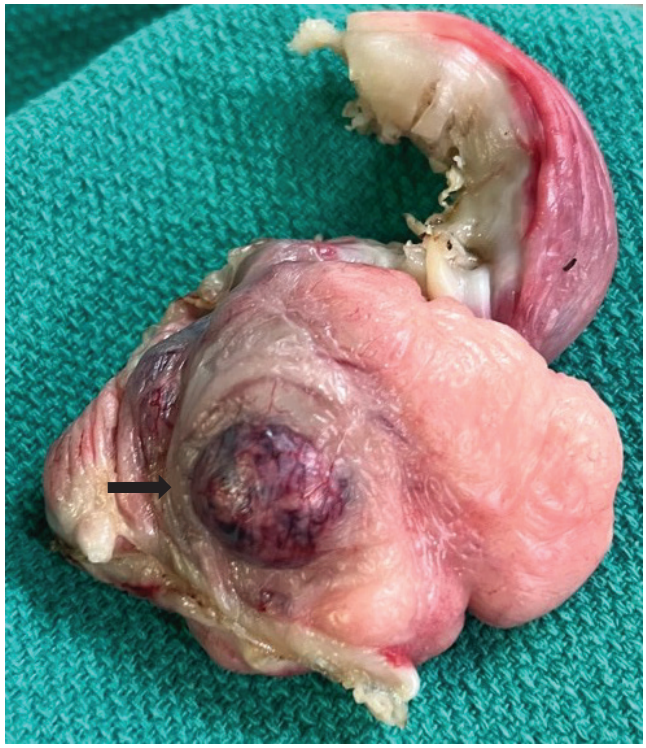

Figure 5. Gross image of left ovary immediately after surgical removal (note irregular nodular mass [black arrow] representing GCT that is clearly visible through ovarian bursa).

whelping date, and 3 fetal skeletons were identified (Figure 9). Due to unexpected complications, cesarian surgery was performed at a local veterinary clinic and deceased pups were removed. Information on presurgical fetal viability (transabdominal ultrasonography) nor gross findings of pups were not provided and necropsy was not performed.

Discussion

Granulosa cell tumors are among the most common ovarian neoplasia identified in female dogs.[42] However, literature regarding diagnosis and treatment options in dogs is limited. This is attributed to most female dogs, especially in North America (gonadectomized early in life), apparently resulting in a low incidence rate of this disease.[15] However, as more female dogs remain intact for extended periods, either for

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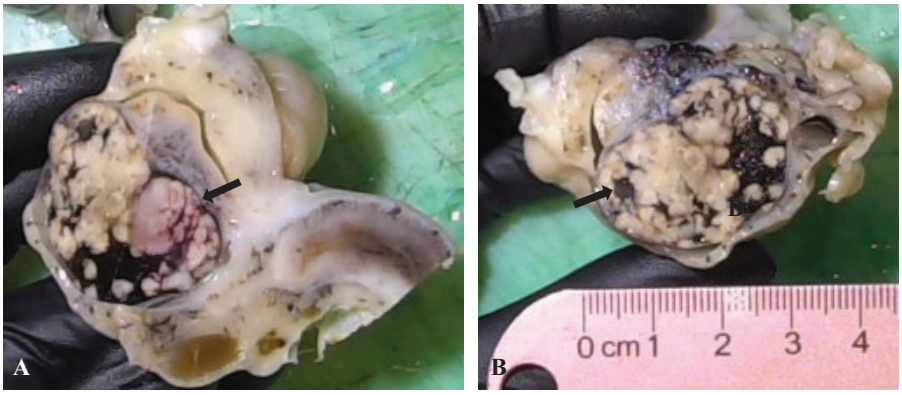

Figure 6. Left ovary after surgical removal; A. GCT along with intact oviduct and uterine horn tip (note cut surface of GCT has well-demarcated white, variably friable, and partially cavitated mass [arrow]) and B. diameter of GCT (~ 3 cm) mass.

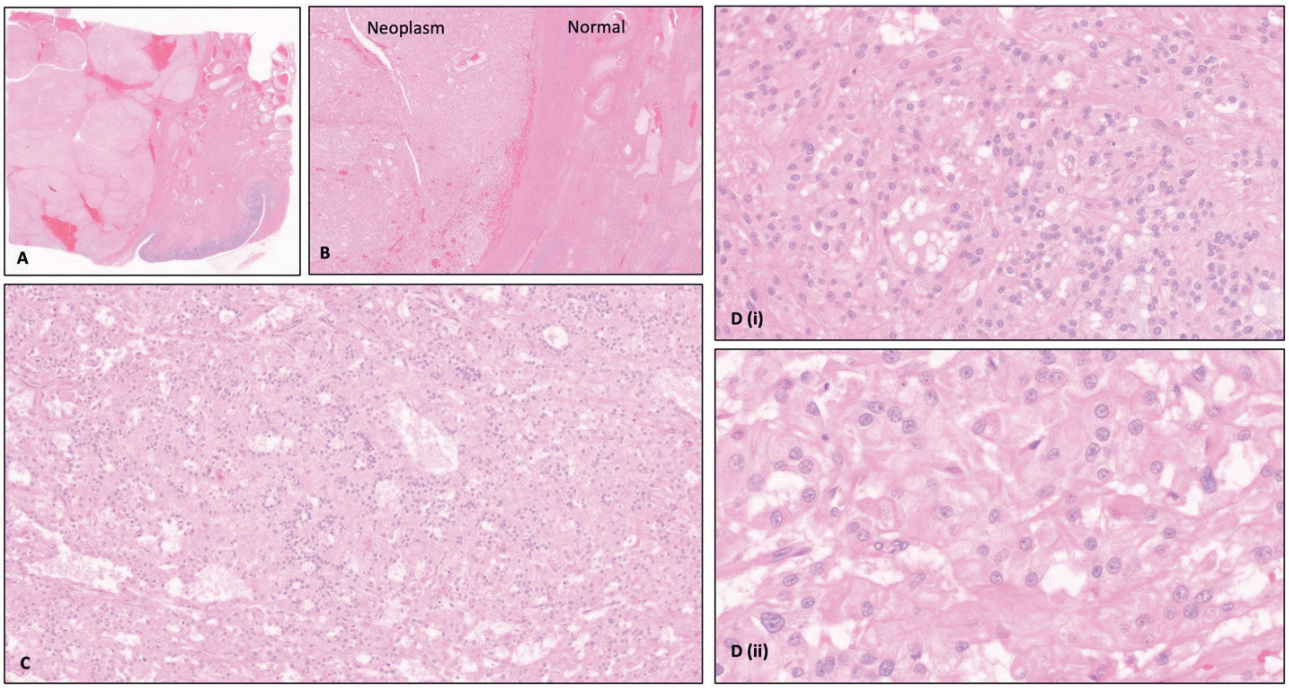

Figure 7. Histopathology of left ovary: A. subgross (10 x magnification); B. tissue margin (20 x magnification); C. neoplastic cells arranged in variably formed glandular structures that contain mucinous eosinophilic material (100 x magnification), and D. neoplastic cells are polygonal to rarely spindled with variably discrete cell borders and foamy eosinophilic cytoplasm, mild to moderate anisokaryosis and anisocytosis (Di 200 x magnification and Dii 400 x magnification).

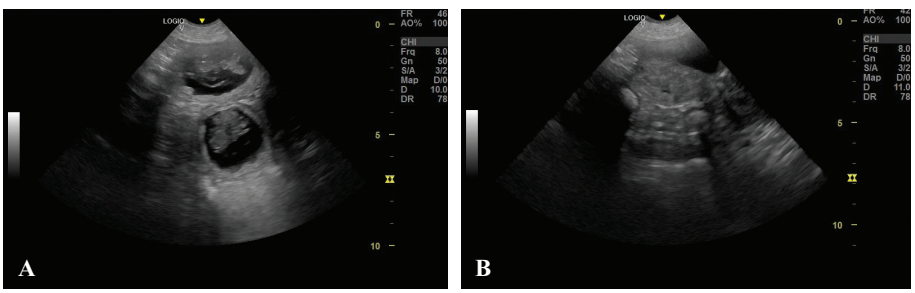

Figure 8. Ultrasonographic image of amniotic sacs (A) and reabsorption site (B)

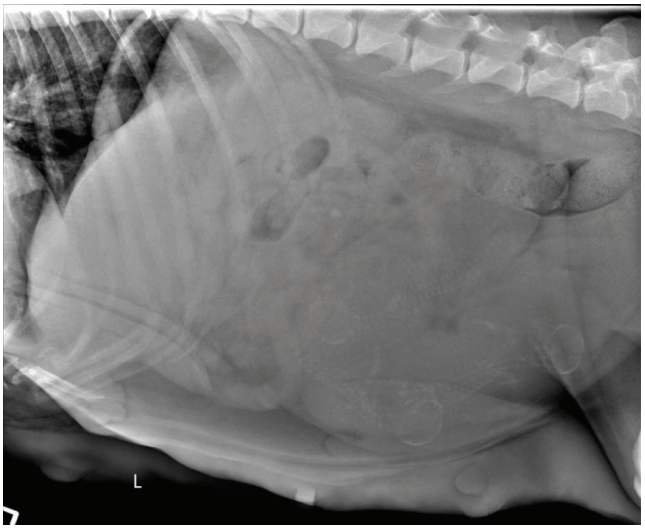

Figure 9. Left lateral radiograph (note: 3 fetal skeletons)

breeding purposes or for potential health benefits, the likelihood of diagnosing an ovarian tumor will become more common.[43,44] Therefore, understanding the current diagnostic tools available and the potential limitations of each method is important for clinicians to recognize when pursuing GCT diagnosis.

In mares, tests available for the diagnosis of a GCT include hormonal bioassays or 'GCT panels' (AMH, inhibin, progesterone, and testosterone), ovarian ultrasonography, transrectal ovarian palpation and overt behavioral changes associated with increased concentrations of estrogen and/or testosterone through stallion-like or aggressive behavior.[20] In dogs, a distinctive behavioral change, similar to stallion-like behavior observed in mares, has not been documented, and diagnosis can be more challenging because there is no pathognomonic finding, no consistent ultrasonographic appearance and no standardized hormone panel with reference ranges specific to dogs. Hormonal findings associated with canine GCTs can be unpredictable, especially when other physiological processes may present concurrently in the early stages of pathology.[20]

Ovarian tumors, particularly GCTs, alter normal ovarian echotexture and have an irregular shape in 69% of reported cases in dogs.[6] However, there can be many variations in appearance; as in our case, only a change in echotexture of the ovarian parenchyma and a slight increase in ovarian size but normal shape was initially detected on ultrasonography. This is most likely attributed to the very early diagnosis of GCT in our case. Human medicine utilizes color Doppler to differentiate between malignant and benign ovarian neoplasms by detecting abnormal changes to the intraovarian vasculature.[45,46] We utilized this technique in our case to demonstrate an increase in the vasculature to and within the abnormal region that when combined with initial changes to a honeycomb-like appearance, aided in the presumptive diagnosis of GCT.

In mares, peripheral AMH and inhibin B concentrations are routinely measured to diagnose GCT.[20] Elevated AMH concentrations ranging from 1.12 to ≥ 23 ng/ml have been reported in dogs diagnosed with GCT.[21] However, reference ranges can be > 12 ng/ml in clinically normal intact dogs.[47] Therefore, supportive clinical signs, vaginal cytological findings (indicative of estrogen), ultrasonography findings and ultimately, histopathology are required for the diagnosis of a GCT in dogs. In this case, circulating AMH concentrations were not high (possibly due to the early diagnosis of a GCT). Another endocrine marker used in large animals to diagnose GCTs is inhibin B that was elevated in this case compared to mare's reference range of 2 to 100 pg/ml.[20] However, there is currently no reference range for inhibin in dogs, though high concentrations of inhibin, in this case, followed by substantial decline after removal of the ovary containing GCT, helped support our other initial clinical findings suggestive of GCT and confirm that a hormonally active tumor had successfully been resected.

Appearance of ovary on ultrasonography with the coinciding lack of contralateral ovarian follicular activity (acquired hypoplasia and suppression of normal positive and negative feedback mechanism of cyclicity) suggested GCT; typical findings in other species with hormonally active GCTs as in mares.[17,48] Lack of follicular activity by the unaffected ovary was hypothesized to be the result of negative feedback due to elevated inhibin secretion by the tumor, causing suppression of follicle-stimulating hormone (FSH).[23,49] Thus, it affects normal follicle recruitment, deviation, growth, and ovulation. Ongoing suppression is likely caused by normal ovary's acquired hypoplasia. We suspect a similar process was occurring in the present case, though data collection at that stage of the investigation was not a priority due to dog's clinical well-being. However, further research is still required to fully understand the pathogenesis of unaffected ovary as there are discrepancies in literature, especially in mares.[17] Reports confirmed low and no differences in FSH concentrations in mares with GCTs.[49,50]

Definitive diagnosis of a GCT was confirmed by histopathology. Resection of part of uterine horn with laparoscopic laparotomy also allowed confirmation of CEH. This information allowed us to give the owner a more informed prognosis of future fertility. Although GCT, in this case, was easily identified by our pathologist through gross and histologic assessment, it is important to note that cytological features can be variable regarding cellularity and mitotic indices that ultimately reflects the wide variability in the functionality of GCTs across species.[20,51]

Unilateral ovariectomy is a standard treatment option for GCTs in mares.[52–54] However, to authors' knowledge, there was no reported cases of pregnancy after unilateral ovariectomy via laparoscopy for GCT treatment in dogs. Laparoscopic approach is minimally invasive and has a lower postoperative complication rate and a shorter recovery time than traditional laparotomy methods.[55] A minimally invasive surgery was particularly important in this case, as the patient had hematological clinical signs associated with bone marrow suppression due to prolonged endogenous estrogen exposure caused by GCT secretions.

Although the objective of our treatment methodology was aimed to preserve dog's reproductive potential, there were several concerns regarding reproductive performance after surgery that included: a) fewer oocytes would be ovulated with only 1 remaining ovary; and b) uterine CEH changes. However, remaining ovary reported to have compensated by undergoing hypertrophy and ovulated similar number of oocytes despite contralateral ovary removal.[56] In this case, a possible slight

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compensatory effect was appreciated with 5 amniotic sacs detected via ultrasonography on day 35 after LH0. Many ovarian pathologies such as GCTs and ovarian cysts, especially those processes that are hormonally active, are associated with concurrent uterine changes, specifically, CEH.[9,36] Repeated exposure of the endometrium to cycles of estrogen priming followed by 2 months of luteal/nonpregnant progesterone is a prominent contributing factor to the development of CEH especially in older, maiden dogs.[6,57–59] CEH is associated with infertility in dogs as the changes induced can result in interference with embryonic attachment and increase the risk of pyometra.[54–58] Endometrial changes associated with CEH include hyperplasia of endometrial glands that become enlarged and cystic in appearance and result in delayed uterine clearance of ascending bacteria during estrus and dead sperm and extenders after intrauterine insemination. Furthermore, the cystic endometrial environment is not conducive to implantation and normal placentation required for embryonic development.[18,59] In mares, endometrial biopsies are graded using the Kenny-Doig system. This helps predict the potential likelihood of pregnancy, maintenance of pregnancy and ultimately, delivery of a live foal based on microscopic features of luminal and glandular epithelium, stratum compactum and spongiosum.[60] Unfortunately, we currently do not have biopsy 'grades' for dogs; furthermore, biopsies are obtained in diestrus (time for it to be diagnostic) can result in pyometra and loss of an estrous cycle due to the unique feature in dogs (no luteolytic mechanism if not pregnant). Larger studies are required in dogs for the development of cytological and endometrial ultrasonographic grading parameters of CEH that are correlated to reproductive performance and allow prediction of the likelihood of fertility/pregnancy success after breeding, as in mares. In this case, despite dog's young age, excessive exposure of endometrium to estrogen and progesterone produced by GCT resulted in CEH development and was most likely the underlying cause of embryonic and fetal loss (detected via ultrasonography and radiography).

Ultrasonography is valuable not only to assist in GCT diagnosis but also to assess any damage to uterus caused by chronic sex steroid hormone exposure. It is important to take into consideration uterine 'health' when offering treatment that also preserves fertility. Loss of at-term fetuses in this case is unknown. Despite loss of pups during whelping, this case had positive outcomes (successful treatment of GCT and maintenance of breeding potential on a young and genetically valuable female dog).

We discussed diagnostic tools and surgical techniques that are available to facilitate the diagnosis and treatment of GCTs to allow a genetically valuable young dog to maintain fertility potential. We also highlighted the importance of assessing secondary uterine changes caused by excessive and constant hormone stimulation to predict future fertility after removal of GCT.

Learning points

- GCT in dogs (unlike mares) have a wide range of undefined ultrasonographic appearances
- Measurement of serum concentrations of Inhibin B and AMH are a beneficial additional diagnostic tool to diagnose canine GCT cases before surgical intervention
- Unilateral ovariectomy is an effective treatment for young, genetically valuable dogs, to maintain their potential fertility
- Uterine changes (e.g. CEH) caused by prolonged exposure to estrogen and progesterone exposure in GCTs, should be considered when selecting dogs for future breeding

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Conflict of interest and funding

Alexandria Horner's residency program is funded by the American Kennel Club and the Canine Health Foundation. Authors report no conflict of interest.

Author contributions

AH wrote most of the manuscript, and MH performed and wrote pathology and histopathology analysis/descriptions. FH, JS, and GB reviewed it, and all authors approved the submission.

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