

Granulosa cell tumor in a heifer: clinical, ultrasonographic, endocrine and pathologic findings

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Summary

An 18-month-old 652-kg black Limousin heifer was evaluated because of an enlarged left ovary and infertility following herd pregnancy examination. The heifer had been treated for cystic ovarian disease, however the left ovary remained enlarged. Palpation per rectum revealed a firm, multilobulated mass in the region of the left ovary. Transrectal ultrasonography confirmed a large, hypo- to anechoic polycystic left ovary. Hormone assays showed an increase in estrogens and inhibin, normal or slightly elevated concentration of testosterone, and a low concentration of progesterone. A left ovariectomy via ventral midline celiotomy was performed. Gross and histopathologic evaluation of the mass confirmed a granulosa cell tumor (GCT). Six months after surgery, the heifer was found dead in the pasture due to causes unrelated to surgery. Necropsy revealed the remaining right ovary had grown in size, suggesting the beginning of follicular activity.

Background

Granulosa cell tumors appear to be the most common ovarian neoplasm affecting cattle.¹ Affected cattle can display altered behavior and decreased fertility due to hormones secreted by the tumor. In particular, inhibin is a glycoprotein hormone secreted by granulosa cells that suppresses follicle stimulating hormone (FSH) secretion from the anterior lobe of the pituitary gland. Therefore, inhibin is one of the hormones responsible for infertility due to suppression of FSH and inactivity of the contralateral ovary.² Alterations in the hormone profile of cattle during the estrous cycle can result in conception failure, which represent an economic loss to the producer. Therefore, identifying cattle with ovarian abnormalities such as GCTs is important to the productivity of a herd. If the female is of sufficient value to the producer, the GCT can be surgically removed. Once the hormone-producing tumor, and thus the source of excess inhibin is removed, the contralateral ovary may regain normal function.

Case presentation

An 18-month-old 652-kg black Limousin heifer was referred to the Texas A&M Large Animal Hospital for evaluation of an enlarged left ovary. The heifer was the only non-pregnant heifer in a group that had been pastured with a bull during the previous summer. An enlarged left ovary was identified on palpation per rectum by the referring veterinarian and the heifer was treated with gonadotropin releasing hormone for cystic ovarian disease. After the next breeding, the heifer still did not become pregnant and the ovary remained enlarged. Blood was collected and submitted for measurement of progesterone and estradiol 17- β concentrations. The progesterone concentration was <0.20 ng/ml indicating that the heifer did not have a functional corpus luteum (CL) on either ovary. Estradiol 17- β concentration was 0.00 pg/ml. The heifer was then referred for further evaluation of the enlarged left ovary.

Initial physical examination revealed a temperature of 101.4 F, pulse of 80 bpm, and a respiratory rate of 40 bpm. Body condition of the heifer was scored 8/9. The heifer was noted to have a masculinized appearance due to well developed neck musculature. The ranch manager stated that the heifer had not displayed any bull-like behavior. There were no other significant findings.

Vaginoscopy was performed and revealed a normal vagina with a closed cervix. Palpation per rectum revealed a 'basketball' sized, firm, multilobulated mass at the distal end of the left uterine horn. The uterine horns were flaccid and the entirety of the mass could not be appreciated due to inability to retract the uterus because of the size and weight of the mass. Transrectal ultrasonography with a 7.5 MHz

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linear transducer revealed a large, multilobulated, hypo- to anechoic polycystic ovary (Figure 1). Many vessels were observed suggesting high vascularization of the mass.

Differential diagnosis

A presumptive diagnosis of a left ovarian GCT was made. Differential diagnoses included atypical cystic ovarian degeneration, abscess, lipomatosis, and lymphoma.

Treatment

The owner elected surgical removal of the left ovary. A preoperative hemogram revealed a mild stress leukogram and no clinically significant abnormalities of serum chemistry. Blood was also submitted for measurement of progesterone, estrogens, testosterone, and inhibin concentrations (Table).

Food and water were withheld for 48 hours in preparation for a left ovariectomy via ventral midline celiotomy. A catheter was placed in the left jugular vein. Ceftiofur sodium (3.0 mg/kg IV and SQ), flunixin meglumine (2.2 mg/kg IV), and tetanus toxoid were administered prior to surgery. In the event of excessive blood loss during surgery, eight L of fresh, whole blood was collected from a donor cow.

Table. Serum concentrations of progesterone, estradiol 17- β , testosterone and inhibin.

Progesterone	0.3 ng/ml
Estradiol 17- β	21.5 ng/ml
Testosterone	111.6 pg/ml
Inhibin	4.56 ng/ml

The patient was anesthetized and positioned in dorsal recumbency on a padded surgical table. The ventral abdomen was clipped and prepared for aseptic surgery. A 30 cm incision was made on the ventral midline beginning 10 cm cranial to the umbilicus and ending 20 cm caudal to the umbilicus. The left uterine horn was approximately 5 cm in diameter. Multiple vessels estimated to be 5-10 mm in diameter were palpable in the left ovarian pedicle. The left ovary was not adhered to other structures in the abdomen, but could not be completely exteriorized (Figure 2). The right uterine horn was approximately 1 cm in diameter and the right ovary was approximately 2 mm in diameter and 5 mm in length. An unsuccessful attempt was made to reduce the size of the mass using a 14-gauge needle. Only a small amount of viscid, black material could be removed by suction. Using hemostats, openings were created in the mesovarium on either side of the vessels supplying the ovarian mass. Ligation was accomplished with sterile umbilical tape. A vessel sealing device (LigaSure™, Covidien, Mansfield, MA) was used to create a path of sealed tissue between the ligatures and the base of the ovary. The ovarian mass was removed by cutting along the path of the sealed tissue with surgical scissors. The ovarian mass was separated from the uterine horn without entering the lumen, however a large vessel was encountered in the mesovarium which bled profusely. A whole blood transfusion was initiated as attempts to identify and ligate the vessels were made. As the serosal surface of the left uterine horn was removed with the ovary, the tip of the left horn was oversewn with a single layer of size 1 polyglactin 910 in a Lembert pattern. The linea alba, subcutaneous space and skin were closed in a routine fashion.

Outcome

Postoperatively, the heifer appeared anxious and was tachypneic (80 bpm). Additional butorphanol tartrate (0.1 mg/kg, SQ) was administered every six hours for analgesia. The heifer appeared comfortable and grain and coastal hay were offered. The third day postoperatively the heifer showed signs of colic and became anorexic. Butorphanol tartrate and flunixin meglumine were administered. A hemogram and serum chemistry panel were performed and the results were within reference ranges. Signs of colic subsided following treatment. The heifer's appetite remained reduced, and two days later the heifer's rectal temperature was 103.0° F. Differential diagnoses included septic peritonitis, incisional infection, aspiration pneumonia or pleuritis, and endogenous pyrogen release from tissue destruction

associated with surgery. The heifer continued to pass normal amounts of feces and lung sounds were normal. The incision was not painful, warm, and did not have any drainage. Edema of the ventral midline continued to subside. The heifer was again treated with a single dose of flunixin meglumine and tulathromycin. Over the next two days the rectal temperature steadily declined to within normal range and the heifer's appetite improved. The heifer remained hospitalized for 10 days postoperatively. The heifer was discharged with instructions to keep her confined to a small paddock separate from other cattle for an additional 90 days to allow her incision to fully heal.

The left ovary weighed 3.6 kg and measured 28 x 27 x 18 cm with multiple dark red to tan 6-8 cm nodules (Figure 3). Sectioning of the mass revealed two (2 cm and 4 cm) cysts filled with clear fluid. Numerous nodules of dry, necrotic tissue were surrounded by hemorrhage.

Histological evaluation revealed a GCT with viable ovarian tissue mixed with areas of subacute, coagulative necrosis and pools of fresh hemorrhage. Several vessels contained thrombi. Proliferating, cuboidal epithelial cells were piled 2-3 cells deep on a fine fibrovascular stroma (Figure 4). The cells had round, hyperchromatic nuclei of variable diameter (anisokaryosis), coarsely clumped chromatin, and ample, purple cytoplasm. Groups of cells were subtended by thick, connective tissue bands. Mitoses were rare, and necrosis occupied 60% of the sections examined.

Discussion

Various ovarian neoplasms have been described in cattle, and although rare, GCTs appear to be the most common.¹ One of the authors (JFE) routinely encounters GCTs in two to three per 1000 mature beef cows at slaughter in Texas. From 20,913 reproductive examinations in dairy cattle from Minnesota and Wisconsin, 0.1% were diagnosed with GCTs.³ The prevalence of GCTs in cattle varies, with a recent report of 11 GCTs in 1,489 (0.74%) of slaughtered cattle.⁴ Although most authors report that GCTs in all species are rarely malignant, one study⁵ found nine of 13 bovine GCTs had implanted in the abdomen and metastasized and in another case⁶ a malignant GCT in an Angus cow with peritoneal implantation and distant metastasis was reported.

Granulosa cell tumors are most commonly characterized by unilateral ovarian enlargement. The enlarged ovary may be smooth or coarsely lobulated and the contralateral ovary may be small. Behavior of affected cows can range from anestrus, to nymphomania, to bull-like behavior. Affected cattle can have a masculinized appearance. Udder development and lactation may occur in affected heifers with hyperestrogenism.⁷ Occasionally, GCTs are found in pregnant animals.⁸ A presumptive diagnosis is made by palpation per rectum coupled with behavioral abnormalities. Transrectal ultrasonography can provide additional evidence for the diagnosis of GCTs.⁹ Ultrasonic examination of the mass often reveals a multicystic or honeycombed structure. In the present case, transrectal ultrasonography revealed a large, lobulated ovary with multiple hypo- to anechoic regions within the neoplasm (Figure 1). Hormone profiles can provide supporting evidence for the diagnosis of GCTs. In horses these tumors are hormonally active and assays for the detection of a GCT include measurements of inhibin, testosterone, and progesterone.^{19,20} Estrogen, testosterone, and progesterone concentrations in cattle affected by GCTs have been reported.^{9,21-23} However, a review of the literature did not reveal published concentrations of inhibin in cattle affected by GCTs.

The hormone profile in the present case showed a significant increase in the concentrations of estrogens and inhibin, a normal to slightly elevated concentration of testosterone, and a low concentration of progesterone. The concentration of estrogen in the present case was higher than those reported in females during the estrous cycle^{10,11} or cows with follicular ovarian cysts.¹² The concentration of testosterone in blood was similar to¹¹ or slightly higher than those reported for cows during the estrous cycle,¹³ but lower than the values reported for bulls.^{14,15} A slight increase in testosterone can exert anabolic effects such as an increase in neck musculature as seen in this heifer. The concentration of progesterone indicated an absence of luteal tissue.¹⁰ The plasma concentration of inhibin was more than ten times higher than basal levels in healthy cattle reported previously.^{16,17} Inhibin is secreted mainly by the granulosa cells of antral follicles and has the ability to suppress FSH secretion in mammals.¹⁸ Estrogens are also produced by the granulosa cells.¹⁰ The increased plasma concentrations of estrogen

and inhibin found in this case suggest an up-regulation of granulosa cellular function. Unfortunately, no other published reports of the concentrations of inhibin in cattle with GCTs were found.

Inhibin is elevated in approximately 90% of mares with GCT.¹⁹ Inhibin produced by the GCT is thought to be responsible for the inactivity of the contralateral ovary by suppressing FSH release. Serum testosterone is elevated in approximately 50-60% of mares and is associated with stallion-like behavior.²⁰ Progesterone concentrations are low, usually less than 1 ng/ml, since ovulation and corpus luteum formation do not occur.²⁰ One report²¹ described elevated plasma estrogen and testosterone concentrations and a low progesterone concentration in a cow with a GCT. After surgical removal of the affected ovary, estrogen and testosterone levels fell and estrous cycles were re-established three weeks after surgery. Another author⁹ reported elevated testosterone and progesterone concentrations in a heifer with a malignant GCT.

Definitive diagnosis of GCT requires histopathologic confirmation. Sections of the tumor often reveal neoplastic changes such as anisokaryosis, as evident in this case, along with variable amounts of thrombosis and necrosis. Much of the neoplasm in this case was undergoing ischemic necrosis, presumably due to compromise of the blood supply.²²

Though most bovine GCTs are found at slaughter, surgical removal of the affected ovary is a viable treatment option. Due to the genetic value of the female of this report, a unilateral ovariectomy was recommended. This advice was based on previously published information that showed that animals can resume estrous cycles and can become pregnant between two and 12 months after surgery.^{9,21,23,25-28} Unfortunately, in the present case the animal was found dead but a necropsy showed that the remnant ovary began to show signs of follicular activity six months after surgery.

Ovariectomy via a ventral midline approach as a treatment for a GCT in a 15-month-old Holstein heifer has been described.²⁶ The heifer was bred and delivered a healthy heifer calf approximately one year after surgery. Surgical approaches for ovariectomy in cases of GCTs include colpotomy, flank laparotomy, and ventral midline celiotomy^{9,25-27,29}. Due to the size of this heifer, position of the mass, and inability to palpate the full extent of the ovary per rectum, a ventral midline approach was selected in this case. During surgery, the tumor could not be completely exteriorized preventing full visualization of the left ovarian pedicle. In some cases including the present case, GCTs are highly vascular. The risk of hemorrhage is high,²⁸ and in this case, there was hemorrhage from the left ovarian pedicle, necessitating a blood transfusion. The heifer in this case was hospitalized for a total of ten days postoperatively and was discharged with instructions to keep her separate from other cattle for a total of 90 days to allow her incision to fully heal. A subsequent report from the ranch manager indicated that the heifer was noted to stand for service by a bull.

In summary, GCTs are a common ovarian neoplasm in multiple species, including cattle. This report describes a clinical case of a GCT in a heifer in which hormone assays coupled with palpation per rectum and ultrasonography supported the histopathological diagnosis. Though concentrations of inhibin have not been reported previously in cases of GCTs in cattle, this information provided valuable supporting evidence for the diagnosis in this case and should be considered when selecting diagnostic tests. More work is needed to develop reference ranges for inhibin cattle. As demonstrated by this case and others reported previously, treatment of GCTs by surgical removal is a viable option in cattle.

Learning points

- This is the first report describing the measurement of inhibin concentration determination to aid in the diagnosis of GCTs in cattle.
- Future work is needed to develop bovine-specific reference ranges for hormone concentrations associated with GCTs.
- Surgical removal of the affected ovary remains a viable treatment option in cattle with GCTs.

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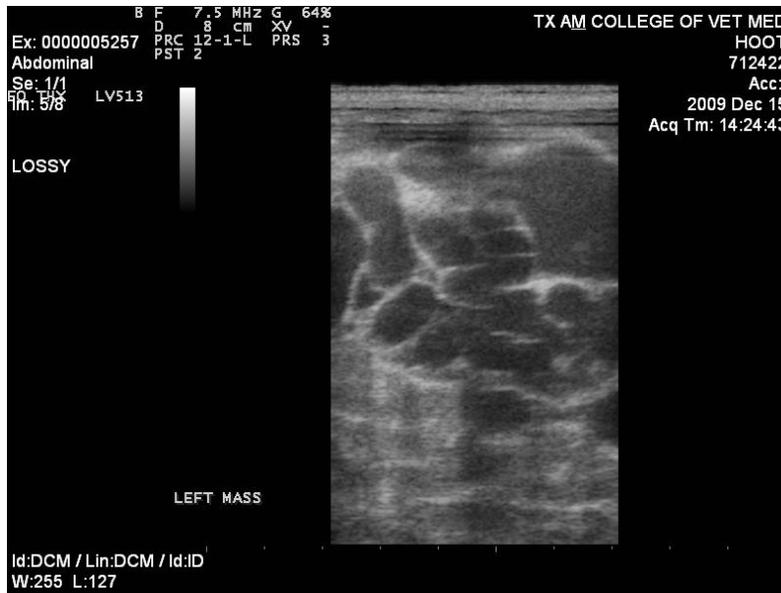


Figure 1. Transrectal ultrasonography with a 7.5 MHz linear transducer revealed a large, multilobulated mass with multiple hypo- to anechoic cystic areas.

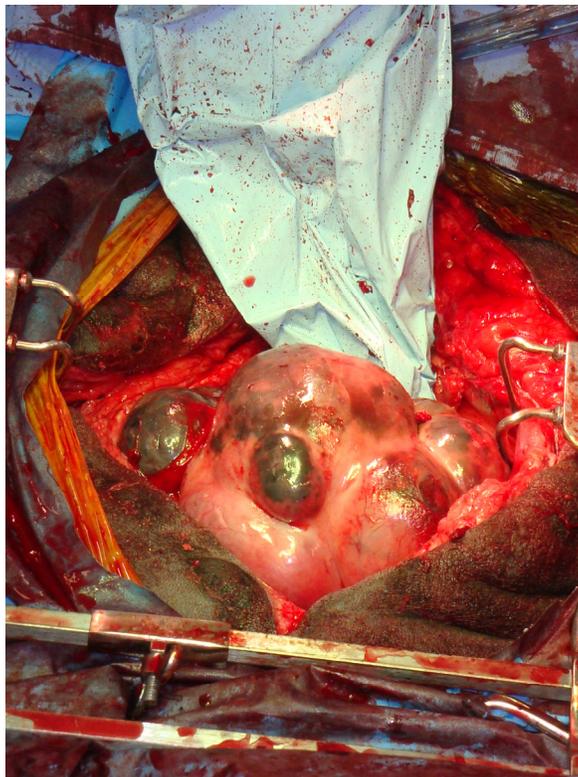


Figure 2. The left ovary during surgery prior to removal. The ovary was not adhered to other structures in the abdomen, but could not be completely exteriorized. The ovary is supported by the surgeon's arm.

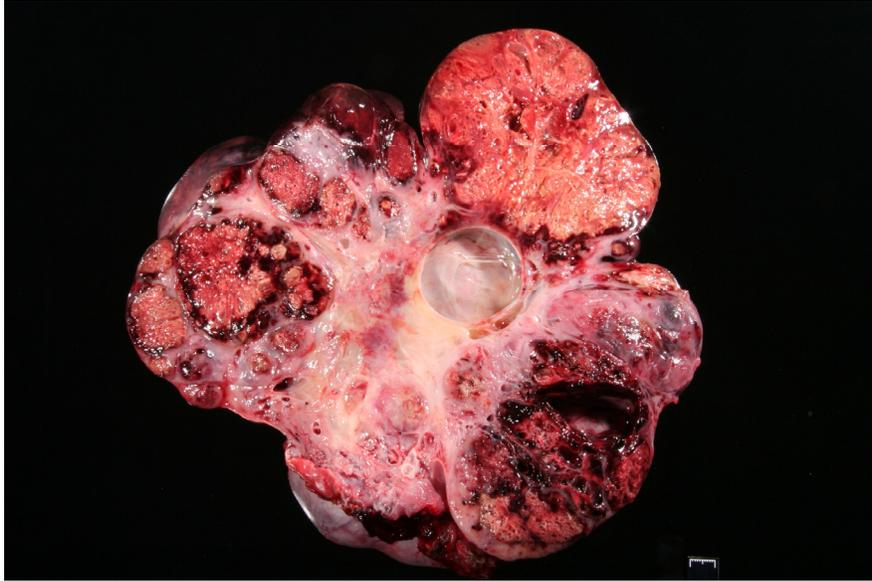


Figure 3. Gross appearance of a left ovarian mass. The 3.6 kg neoplasm measured 28 x 27 x 18 cm with multiple dark red to tan, 6-8 cm nodules.

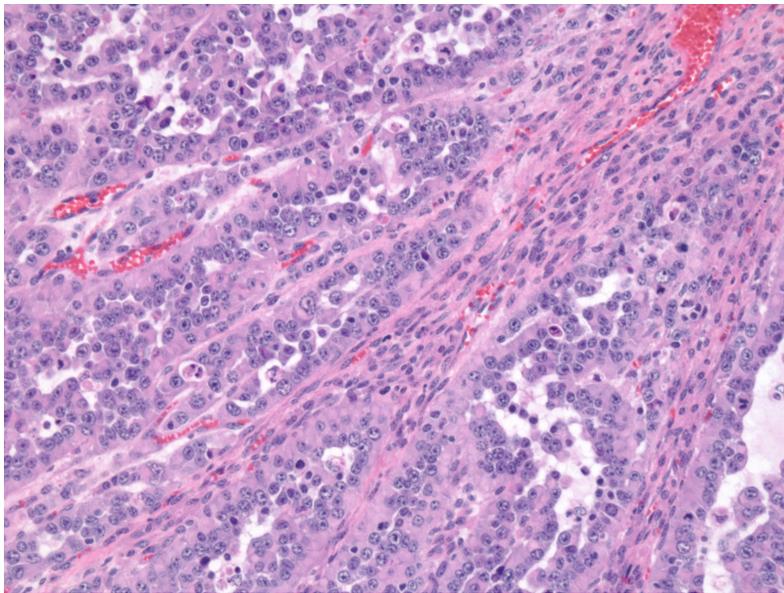


Figure 4. Photomicrograph of a section of the viable tissue within the GCT at 200x magnification. Areas of proliferating, cuboidal epithelial cells piled 2-3 cells deep on a fine fibrovascular stroma.

