



# Theriogenology Question of the Month

A 15-month-old 500-kg (1,100-lb) Holstein heifer housed at the Michigan State University Dairy was evaluated because of a large right ovary. The heifer had no history of estrous behavior and had not been artificially inseminated. The heifer had been selected as a recipient for embryo transfer, and estrus induction was attempted via an injection of 5 mL (25 mg) of prostaglandin  $F_{2\alpha}$ ; the heifer was not observed in estrus by the farm manager following that injection. Ten days after the prostaglandin injection (7 days after presumed induced estrus), transrectal palpation was performed by a veterinarian to determine the presence of a corpus luteum prior to embryo transfer. A large right ovary was detected.

One week later, transrectal ultrasonography of the affected ovary was performed by use of a portable ultrasound machine with a variable-frequency (4.5 to 8.5 MHz) linear transducer.<sup>b</sup> Ultrasonographic examination of the right ovary revealed a markedly large gonad (approx  $10 \times 5$  cm) with irregular architecture (Figure 1). Multiple cystic structures were evident within the ovarian mass, and echogenicity of the mass varied from anechoic to hyper-echoic. The left ovary was barely detectable via ultrasonography or transrectal palpation; it was  $< 2$  cm in diameter with no evidence of follicular activity. On the basis of the ultrasonographic appearance of the large right ovary combined with the inactive left ovary, the primary differential diagnosis was a granulosa theca cell tumor (GTCT).

Thirty days later, the heifer underwent exploratory laparotomy at the Michigan State University Veterinary Medical Center to remove the ovarian mass. At the time of surgery, it was also noted that the heifer had slight precocious udder development. A venous blood sample (6 mL) was collected, and serum was harvested and stored at  $-20^{\circ}\text{C}$  until analysis. Hair was clipped from the right paralumbar fossa, and the skin was surgically prepared in a routine manner for right flank laparotomy. Regional anesthesia was achieved with a proximal paravertebral block (20 mL of 2% lidocaine solution injected into each intervertebral space).

This report was submitted by Jennifer N. Roberts, DVM; Carla L. Carleton, MS, DVM; Alan J. Conley, BVSc, PhD; and Dalen W. Agnew, DVM, PhD; from the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824 (Roberts, Carleton); the Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616 (Conley); and the Department of Pathobiology and Diagnostic Investigation, Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI 48910 (Agnew).

Presented in abstract form at the Annual Conference of the Society for Theriogenology, Portland, Ore, August 2014.

The authors have no conflicts of interest to declare.

Address correspondence to Dr. Roberts (wileyje1@cvm.msu.edu).



Figure 1—Ultrasonographic image of the large right ovary of a 15-month-old Holstein heifer. Dimensions of each box of the grid are  $1 \times 1$  cm.

A 20-cm incision was made in the caudal aspect of the paralumbar fossa. Underlying tissues were transected, and the peritoneum was incised. The right ovary was exteriorized and examined. The ovarian pedicle was large because of the increased diameter of blood vessels supplying the affected ovary. The ovarian pedicle was ligated with transfixation sutures of No. 1 absorbable, braided suture material<sup>c</sup> and then transected. The ovarian pedicle was examined for signs of hemorrhage and subsequently replaced into the abdomen. The tissues and skin were closed in a routine manner. Penicillin G procaine<sup>d</sup> at a dose of 24,000 U/kg (10,909 U/lb) was administered once daily for 5 days postoperatively. The excised ovary was submitted to the Michigan State University Diagnostic Center for Population and Animal Health for histologic and immunohistochemical evaluation.

## Question

What additional endocrinologic tests could be used to confirm the diagnosis of GTCT? *Please turn the page.*

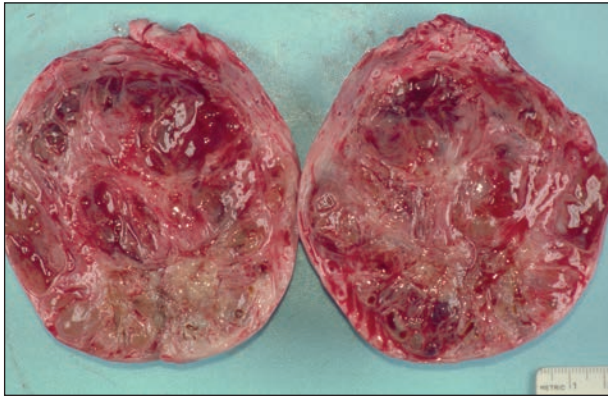


Figure 2—Photograph of cross sections of the ovary removed from the heifer of Figure 1. Notice that the encapsulated ovarian mass (12 cm in diameter) has irregular architecture and contains numerous cystic structures. The gross appearance is consistent with the ultrasonographic images and with a diagnosis of granulosa theca cell tumor (GTCT). Scale marker in the lower right corner is in centimeters.

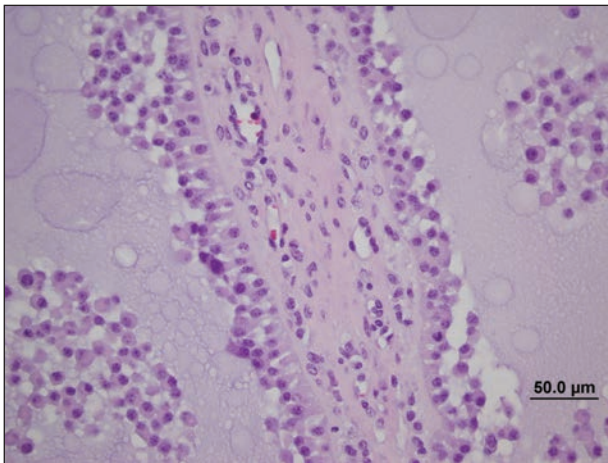


Figure 3—Photomicrograph of a tissue section from a cystic structure in the ovarian mass. The wall of the structure is lined with layered columnar cells that are suggestive of granulosa cells. H&E stain; bar = 50 μm.

## Answer

Measurement of inhibin and anti-Müllerian hormone (AMH) concentrations.

## Results

Gross appearance of the excised ovary was consistent with the ultrasonographic images (Figure 2). Cystic areas were evident throughout the ovarian mass. Microscopic examination revealed that the mass contained multiple, irregularly shaped cystic structures lined with granulosa cells (Figure 3). Cells lining the cystic structures had positive results for inhibin and AMH, as determined by use of immunohistochemical labels for the respective hormones (Figures 4 and 5).

The serum sample obtained prior to surgical removal of the right ovary was submitted to the Endocrinology Laboratory at the University of California-Davis for analysis of AMH and inhibin concentrations. The AMH and inhibin concentrations were 5.37 ng/mL (cutoff of 0.36 ng/mL as reported in the literature<sup>1</sup>) and

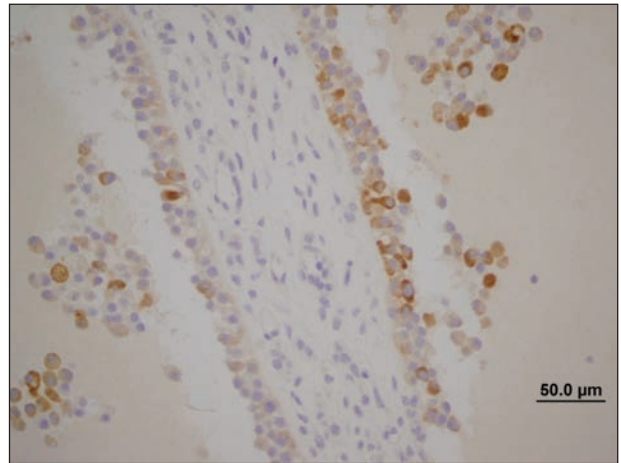


Figure 4—Photomicrograph of a tissue section from a cystic structure in the ovarian mass. Positive staining for inhibin in the area of the granulosa cells is consistent with a diagnosis of GTCT. Notice that immunohistochemical staining for inhibin is intracytoplasmic and variable. Immunohistochemical label with antibody against inhibin; bar = 50 μm.

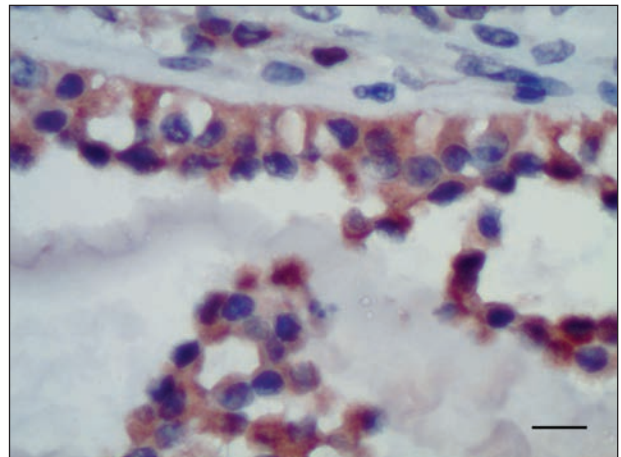


Figure 5—Photomicrograph of a tissue section from a cystic structure in the ovarian mass. Notice that immunohistochemical staining for anti-Müllerian hormone (AMH) is intracytoplasmic and strongly positive. Immuno-histochemical label with antibody against AMH; bar = 10 μm.

1.53 ng/mL (cutoff of 0.69 ng/mL as reported in the literature<sup>1</sup>), respectively.

## Discussion

Ultrasonographic appearance of the large right ovary combined with the inactive left ovary led to a primary differential diagnosis of GTCT. Other differential diagnoses included cystic ovarian disease, ovarian abscess, or another neoplasm such as a teratoma or dysgerminoma. Although rare, GTCTs are the most commonly diagnosed ovarian neoplasm in cattle.<sup>2</sup> Clinical signs of this condition may include anestrus, nymphomania, precocious mammary gland development and lactation in heifers, or bull-like behavior. In the heifer described here, clinical signs were limited to anestrus and slight enlargement of mammary gland tissue. Granulosa theca cell tumors typically are benign and rarely metastasize, but they often result in removal of the animal from the herd because of reproductive inefficiency.

The heifer of the present report had a lack of reproductive cyclicity while under the influence of the ovarian tumor; thus, options for case management were limited to ovariectomy or culling of the heifer from the herd. Successful resumption of ovarian function and pregnancy subsequent to GTCT removal in heifers has been described.<sup>3-5</sup> On the basis of such reports of favorable outcomes following surgical intervention, ovariectomy of the affected ovary was chosen over culling.

Recent studies<sup>1,6</sup> have found that AMH has potential as a biomarker for diagnosis of GTCTs in cattle. Anti-Müllerian hormone is produced by granulosa cells in preantral and antral follicles<sup>7</sup> and is detectable at low concentrations in cows during normal estrous cycles. It has been suggested<sup>1</sup> that a cutoff of 0.36 ng/mL for serum AMH concentration has a sensitivity of 100% and specificity of 99.1% for diagnosis of GTCT. Additionally, the optimal cutoff for inhibin concentrations is 0.69 ng/mL.<sup>1</sup> The presurgical AMH and inhibin concentrations in the heifer described here were 5.37 and 1.53 ng/mL, respectively. Thus, results of endocrinologic testing supported the presumptive diagnosis of GTCT.

Endocrinologic testing, especially for AMH concentrations, of animals suspected to have a GTCT can be a useful aid in diagnosis. Confirming the diagnosis of GTCT allows practitioners to help clients make informed decisions regarding case management. Although the primary focus for the present report was the utility of AMH testing for diagnosis of GTCT in cattle, the inhibin results in this heifer are consistent with those of a published case report<sup>8</sup> describing the use of inhibin for diagnosis of GTCT. Because both inhibin and AMH are reportedly useful for the diagnosis of GTCT, the decision for test choice may depend on cost and availability of the tests. Additional measurement of inhibin and AMH concentrations in cattle with a GTCT would be beneficial for establishing reliable reference ranges for this species.

In cattle with a GTCT, it seems reasonable for practitioners to advocate for unilateral ovariectomy rather than culling if the client desires to maintain the animal in the herd. Although the heifer described here was examined and treated at a veterinary teaching hospital, bovine practitioners possess the skill set to successfully perform ovariectomy in field settings.

## Outcome

The heifer was returned to the Michigan State University dairy farm. Three weeks after surgery, transrectal ultrasonography was performed to evaluate activity

of the remaining left ovary. Follicular activity had resumed, and a 15-mm follicle was detected on the left ovary. A 2-mL dose (100 µg) of gonadotropin-releasing hormone<sup>c</sup> was administered IM to induce ovulation of the follicle. Ultrasonographic examination 7 days later confirmed ovulation and development of a corpus luteum. Sixty days after ovariectomy, the heifer was observed in estrus and was artificially inseminated. The heifer became pregnant as a result of the artificial insemination and calved normally 1 year after the GTCT was initially detected. A serum sample was obtained 18 months postoperatively for follow-up endocrine analysis; AMH and inhibin concentrations were 0.1 and 0.35 ng/mL, respectively. These results, combined with resumption of ovarian cyclicity of the remaining ovary, indicated successful removal of the tumor. The animal has completed its first lactation in the dairy herd, producing 12,104 kg (26,629 lb) of milk over 428 days; the animal is pregnant and due to calve on May 28, 2015.

- 
- a. Lutalyse, Zoetis, Kalamazoo, Mich.
  - b. Easi-Scan, BCF Technology North America, Rochester, Minn.
  - c. Vicryl, Ethicon Inc, Somerville, NJ.
  - d. PenOne Pro, Norbrook Laboratories Ltd, Newry, Northern Ireland.
  - e. Cystorelin, Merial, Duluth, Ga.
- 

## References

1. El-Sheikh Ali H, Kitahara G, Nibe K, et al. Plasma anti-Müllerian hormone as a biomarker for bovine granulosa-theca cell tumors: comparison with immunoreactive inhibin and ovarian steroid concentrations. *Theriogenology* 2013;80:940-949.
2. Peter AT, Levine H, Drost M, et al. Compilation of classical and contemporary terminology used to describe morphological aspects of ovarian dynamics in cattle. *Theriogenology* 2009;71:1343-1357.
3. Leder RR, Lane VM, Barrett DP. Ovariectomy as treatment for granulosa cell tumor in a heifer. *J Am Vet Med Assoc* 1988;192:1299-1300.
4. Hostetler DE, Sprecher DJ, Yamini B, et al. Diagnosis and management of a malignant granulosa cell tumor in a Holstein nulligravida: a case study. *Theriogenology* 1997;48:11-17.
5. Dobson H, Kerby MJ, Chantrey J, et al. Long-term outcome for two heifers with a granulosa-theca cell tumour. *Vet Rec* 2013;172:581.
6. Kitahara G, Nambo Y, El-Sheikh Ali H, et al. Anti-Müllerian hormone profiles as a novel biomarker to diagnose granulosa-theca cell tumors in cattle. *J Reprod Dev* 2012;58:98-104.
7. Rico C, Fabre S, Médigue C, et al. Anti-Müllerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. *Biol Reprod* 2009;80:50-59.
8. Severt MM, Romano JE, Edwards JF, et al. Granulosa cell tumor in a heifer: clinical, ultrasonographic, endocrine, and pathologic findings. *Clin Theriogenol* 2011;3:61-65.