How to Use Anti-Müllerian Hormone Testing to Diagnose Granulosa Cell Tumors in Mares

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Background

Granulosa cell tumors (GCTs) are the most common form of ovarian neoplasm in mares, accounting for more than 85% of all tumors of the reproductive tract in female horses.[1] Histopathologically, GCTs are sex cord-stromal tumors that may be comprised primarily of granulosa cells or a mixture of granulosa and thecal cells. There is no breed or age predilection for GCTs in mares, and although most GCTs are diagnosed in sexually mature mares, they have also been diagnosed in prepuberal fillies. In adult horses, GCTs have been diagnosed in maiden, barren, pregnant and postpartum mares. Although there are exceptions, most GCTs are unilateral, benign tumors associated with cessation of cyclical reproductive activity that is typified by the presence of a small, inactive contralateral ovary. Affected mares generally exhibit one of three behavioral patterns: anestrus, continuous or intermittent estrus (i.e., nymphomania), or stallion-like behavior, with the type of behavior reflecting the specific tumor cell type(s) involved and the attendant steroid hormone production (if any).

Since GCTs are the only ovarian abnormality in the mare characterized by the presence of an enlarged ovary that is accompanied by inactivity of the contralateral ovary, that clinical presentation by itself raises suspicion of a GCT, particularly if it is associated with aberrant behavior.[1] In addition, as the tumor increases in size the ovulation fossa is often obliterated, so the absence of a palpable ovulation fossa becomes an additional finding on physical examination that is consistent with a GCT. Ultrasonographically, the appearance of the affected ovary can be quite variable, ranging from multi-cystic to solid to large solitary cystic masses. Clinical diagnosis of a GCT requires endocrine testing in order to differentiate them from other ovarian tumor types or other causes of ovarian enlargement such as an anovulatory hemorrhagic follicle or ovarian hematoma.[1] Assessment of testosterone and inhibin levels has been the primary endocrine method of detecting GCTs, since when they are used together, testosterone and inhibin are diagnostic for approximately 95% of GCTs.[1,2] Importantly however, mares that display anestrus or persistent estrus usually have normal testosterone levels and inhibin levels may not be elevated in the early stages of GCT formation requiring additional time and expense for repetitive sampling before one or both hormones becomes diagnostically elevated. In addition, testosterone and inhibin are both elevated in pregnant mares, making their interpretation in pregnant mares difficult. Because of these limitations of inhibin and testosterone testing, additional endocrine methods of diagnosing GCTs would be clinically useful.

Assessment of Anti-Müllerian Hormone (AMH) level has recently been validated as an additional diagnostic test for GCTs, as evidenced by the finding that for 44 GCTs verified by histopathology, the sensitivity of detection for AMH was 98%, compared to sensitivities of 80% for inhibin, 48% for testosterone and 84% when

inhibin and testosterone were combined.[3] Based upon immunohistochemistry, AMH is expressed in the granulosa cells of normal equine ovaries as well as GCTs.[4] In a study of circulating AMH levels in normal cycling mares, pregnant mares and mares with histologically confirmed GCTs, AMH concentrations did not differ between normal cycling and pregnant mares, and there were no significant changes in AMH concentrations during the estrous cycle or during pregnancy.[5] In contrast, AMH was elevated (compared to normal cycling and pregnant mares) in all 11 mares with confirmed GCTs and the level of AMH declined after surgical removal of the tumor.[5] Collectively, these studies confirm that systemic levels of AMH are elevated in mares with GCTs, and determination of AMH concentration is a more sensitive method of detecting GCTs compared to the assessment of inhibin and/or testosterone. In addition, unlike inhibin and testosterone, interpretation of AMH level is not confounded by stage of the estrous cycle or pregnancy. Therefore, determination of the AMH level can facilitate the diagnosis of a GCT.

Clinical Use of AMH Testing

A recent clinical case exemplifies the value of determining AMH levels when presented with a presumptive GCT.[6] A 2-year-old Quarter Horse filly was retired from race training in the fall of 2012 and placed under artificial lighting in preparation for breeding in 2013. Transrectal palpation and ultrasonography were performed in January to assess the status of the filly's reproductive tract. Examination revealed the left ovary was small (31 mm L x 12 mm W x 23 mm H), firm and devoid of follicular activity. In contrast, the right ovary was considerably larger (approximately 70 mm in diameter), lacked a palpable ovulation fossa, and was characterized ultrasonographically by a large, thick-walled cavity containing echogenic fluid. The uterus and cervix were flaccid, consistent with a seasonally anovulatory state, and suggestive that the right ovary did not contain functional luteal tissue producing progesterone. A stallion was not available for assessment of the filly's reproductive behavior, but when she was turned out with other mares she was behaviorally normal. Although the findings of the reproductive examination raised suspicion of a tumor of the right ovary, the decision

was made to delay additional diagnostic testing and reexamine the filly in approximately 2 weeks for evidence of any notable change in the status of her reproductive tract and/or behavior. It was anticipated that if the enlargement of the right ovary were due to an ovarian tumor, there would be no appreciable change in the status of either ovary. In contrast, other potential causes of ovarian enlargement such as an anovulatory hemorrhagic follicle would be more likely to exhibit changes in one or both ovaries, such as increased follicular activity of the left ovary and/or evidence of luteinization within the cavity in the right ovary.

The filly was reexamined 17 days later, at which time there was no appreciable change in the status of either ovary (Fig. 1), tubular genitalia or behavior. Therefore, a tentative diagnosis of a
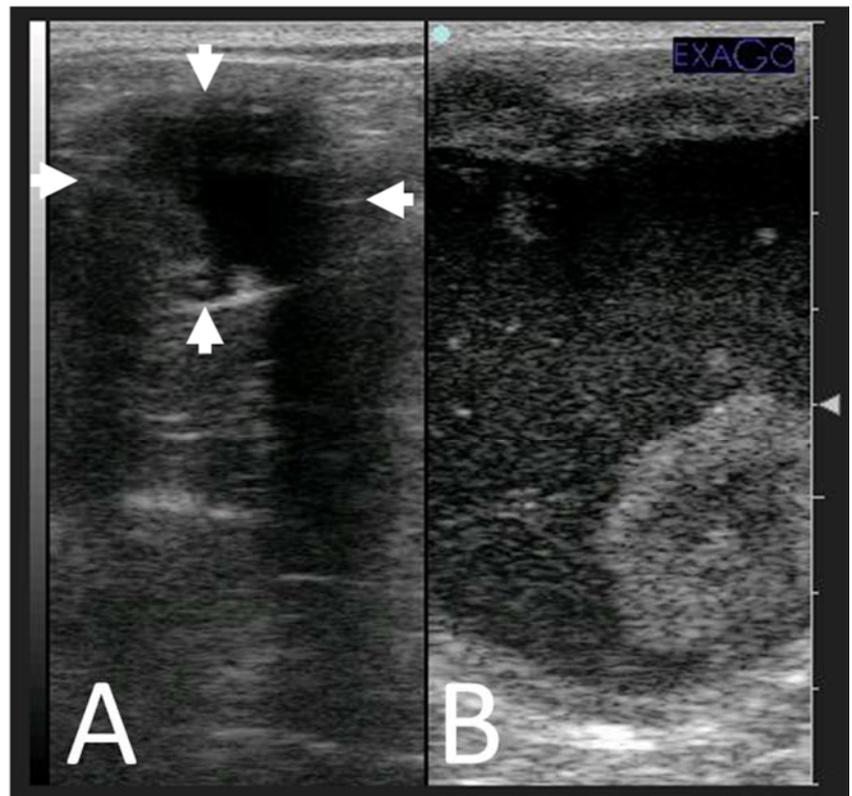

Figure 1. Transrectal ultrasonographic images of the left (A) and right (B) ovaries of a 3-year-old Quarter Horse filly. The left ovary was small, firm and devoid of follicular activity (arrows demarcate outer margins of the ovary). The right ovary was approximately 70 mm in diameter; lacked a palpable ovulation fossa; and consisted of a large, thick-walled cavity containing echogenic fluid. Each gradation along the right side of the image equals 10 mm. (Reproduced from *J Am Vet Med Assoc* 2013;243:791-793, with permission).

GCT of the right ovary was made and a jugular blood sample was collected for serum endocrine testing and submitted to the laboratory that developed the equine AMH assay (Figs. 2 and 3).[a] In addition to analyzing a standard "GCT panel" that includes inhibin, testosterone and progesterone, AMH was analyzed based upon its recent validation for use as a diagnostic marker for GCTs.

CLINICAL ENDOCRINOLOGY LABORATORY
Sample Instructions & Pricing

Instruction for samples:

PLEASE SEND SERUM ONLY, no whole blood or red cells.
Also, do not use Becton-Dickinson serum separator tubes; they will degrade the analyte, particularly progesterone, and invalidate results if the sample is stored in these tubes. Overnight is OK if unable to transfer immediately.

Volume Required:
Testosterone: 2 mL serum each
AMH (equine), Estrone Sulfate, Progesterone or Inhibin: 1 mL serum each. Canine inhibin: 1 ml fasted
AMH Canine/Feline Spaychek: 200 µL serum, fasted, 30 days post- surgery
Cryptorchid Panel: 2 mL serum
Pregnancy Panel: 2 mL serum
Granulosa Cell Tumor Panel: 3 mL serum

Shipping Instructions:
Overnight on an icepack is highly recommended for the Spaychek or inhibin assay or for any panel which includes inhibin. Overnight or second day air in winter without ice is OK to save on shipping costs. Progesterone, estrone sulfate and testosterone are all stable and can be shipped by regular mail, if not in a hurry for results. Fed Ex or UPS will deliver directly to us, while the US Postal Service will go through the University Mail Division and may take up to one week until received in the lab. Please complete the submission form and include with your shipment.

SHIP TO:
ENDOCRINOLOGY LABORATORY
DEPT. of POPULATION HEALTH & REPRODUCTION
4206 VM3A
1 SHIELDS AVENUE
DAVIS, CA 95616-5270

Fig. 2. Sample preparation guidelines and shipping instructions for testing at the Clinical Endocrinology Laboratory at the University of California at Davis. Available at: www.vetmed.ucdavis.edu/phr/endolab/index.cfm. (Reproduced with permission).

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www.vetmed.ucdavis.edu/phr/endolab

□ Pregnancy Panel ($40.00) (Prog / Estrogen Sulfate)	□ Cryptorchid Panel ($75.00) (Testosterone / AMH)	□ GCT Panel ($90.00) (Inhibin / Testo / Prog)
□ Progesterone ($22.00)	□ Testosterone ($29.00)	□ AMH / Inhibin / Testo ($130.00)
□ Estrogen Sulfate ($25.00)	□ Inhibin ($57.00)	□ AMH / Inhibin ($100.00)
□ AMH ($60.00) Feline/Canine Spaychek		□ AMH (Equine) ($60.00)

Client Information (PLEASE print clearly) Customer No: 3-VPHR_____
Clinic/Hospital Name: _____
Address: _____
City: _____   State: _____   Zip Code: _____
Contact: Dr. _____   Phone: _____

Patient Information:
Owner: _____   Animal name: _____
Animal/Reference #:_____   Species: _____   Age: _____
Sex: □ Male □ Female   □ Intact □ Castrated □ Spayed   □ Mini-equine
Date collected: _____   Last breeding date: _____
History:_____
_____
_____

Receive Results:
□ Fax: _____   □ Email: _____

Payment Information
Signature: _____Date:_____
□ MasterCard   □ Visa
Credit Card # _____ - _____ - _____   Expiration Date: _____

Fig. 3. Sample submission form for the Clinical Endocrinology Laboratory at the University of California at Davis, with options for GCT testing highlighted. For the present case, a GCT panel and AMH testing were performed. Form available at: www.vetmed.ucdavis.edu/phr/endolab/index.cfm. (Reproduced with permission.)

The endocrine results from the filly are summarized in Table 1. As noted, inhibin and testosterone levels were borderline elevated and marginally elevated, respectively, precluding a definitive diagnosis based solely upon those results. In contrast, AMH levels were nearly five times above the upper normal limit, providing a definitive clinical diagnosis of a GCT of the right ovary.

Table 1. Endocrine testing results for a Quarter Horse filly with a presumptive GCT of the right ovary.[a]

Hormone	Test result	Reference range	Laboratory designation
Inhibin	0.78 ng/mL	0.1 - 0.7 ng/mL*	Borderline elevated
Testosterone	56.5 pg/mL	20 - 45 pg/mL*	Marginally elevated
AMH	19.0 ng/mL	≤ 4.2 ng/mL	Elevated
Progesterone	0.3 ng/mL	< 0.5 ng/mL**	Absence of luteal tissue

* Normal values for a non-pregnant mare.
** Indicative of the absence of luteal tissue.

Based upon the results of the endocrine testing (i.e., the elevated AMH level), the affected right ovary was surgically removed via colpotomy using a chain ecraseur. On gross examination the ovarian mass was spherical, 7.5 cm in diameter, white, and had a single central cyst filled with viscous yellow fluid (Fig. 4). Histopathologically, the cyst wall consisted of vascularized fibrous tissue in which both granulosa and theca cell components were present, confirming the diagnosis of a GCT.[7]
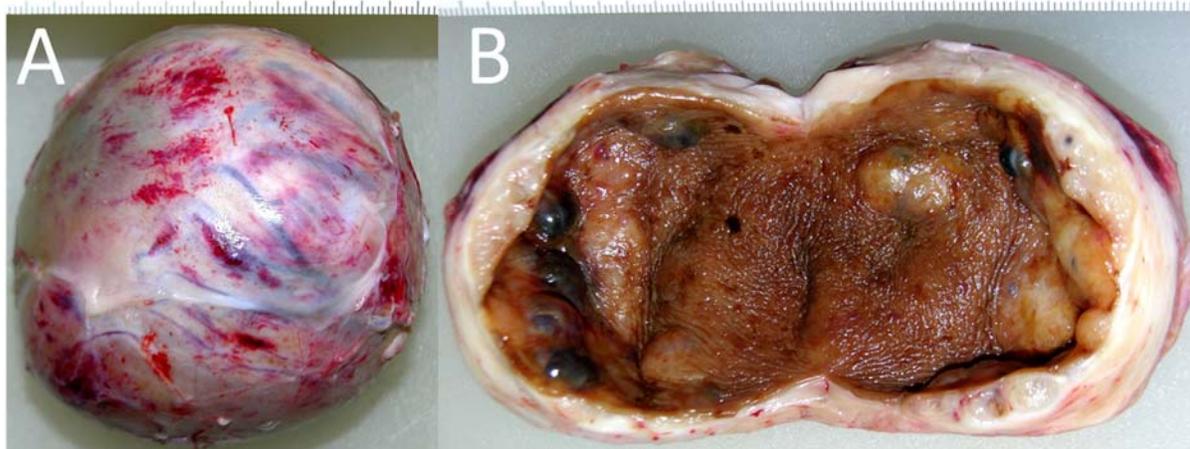

Fig. 4. Photographs of the intact (A) and bisected (B) right ovary of a 3-year-old Quarter Horse filly confirmed to be a GCT. Each gradation along the top edge of both images = 1 mm. (Reproduced from *J Am Vet Med Assoc* 2013;243:791-793, with permission).

The filly recovered uneventfully following the unilateral ovariectomy procedure and was examined with transrectal palpation and ultrasonography 29 days after surgery. Examination revealed the left ovary was still relatively small, but appeared ultrasonographically to contain evidence of luteal tissue. In addition, uterine and cervical tone were consistent with a progestational effect, therefore a jugular blood sample was collected and submitted for serum endocrine testing for progesterone at the same laboratory.[a] Blood progesterone concentration was 10.9 ng/mL, confirming the presence of functional luteal tissue, indicating the filly had ovulated since the initial pre-surgical blood sample was collected. Regarding return to cyclicity of the contralateral ovary after removal of a GCT, in one report,[8] 42 of 57 mares resumed normal estrous cycles an average of 8.5 months after surgery (range of 2 to 16 months), and in another report,[9] 8 of 10 mares resumed normal estrous cycles during the spring after removal of the abnormal ovary. It seems likely the filly in this

report resumed ovulatory activity of the normal ovary so rapidly after removal of the GCT because of the early diagnosis associated with use of the AMH assay, which allowed removal of the affected ovary early enough in the breeding season (in conjunction with artificial lighting) to hasten the return of ovulatory activity. This is in contrast to a "wait and see" approach that would have been indicated had only inhibin and testosterone testing been performed, which would have delayed diagnosis and treatment in this filly.

In summary, this case exemplifies the clinical value of determining AMH levels when presented with a presumptive GCT, since neither the level of inhibin (borderline elevated) or testosterone (marginally elevated) was definitively diagnostic at the stage of tumor development when endocrine testing was performed. In contrast, the level of AMH was unequivocally diagnostic, allowing immediate therapeutic removal of the affected ovary.

Footnotes

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