In cooperation with

Theriogenology Question of the Month

History

A 12-year-old 443-kg (975-lb) Quarter Horse mare, intended to be used for barrel racing, was referred to the theriogenology service of the veterinary teaching hospital at the University of California-Davis for a reproductive examination to assess fetal viability. Fifteen days prior to admission for the reproductive examination, clinicians at the same veterinary teaching hospital had examined and treated a laceration and subsequent septic condition involving the right metacarpophalangeal joint of the horse. At the time of admission, a solution (1 g of amikacin in 40 mL of isotonic saline [0.9% NaCl] solution) was perfused into the distal aspect of the affected limb via the cephalic vein; the horse also received 1 g of phenylbutazone, PO, every 24 hours, as needed, for analgesia of the laceration and septic joint.

The mare had been purchased by the current owners 5 months before the admission for the reproductive examination. At the time of purchase, the mare was housed in a pasture with a stallion; reproductive history of the mare was not known. Transrectal palpation during a prepurchase examination performed by the referring veterinarian revealed a fetus estimated to be at 3 months of gestation. Transrectal ultrasonography was not performed during the prepurchase examination. During the intervening 5 months, the owners reported that signs of estrus were not observed, but the mare displayed aggressive, stallion-like behavior.

A complete physical examination was performed at the time of admission to the veterinary teaching hospital. The horse was bright, alert, and responsive; had a body condition score of 4.5 (scale of 1 to 9); and had decreased loudness of borborgymi in both the left dorsal and left ventral abdominal quadrants. Mammary glands were consistent with those of a nonlactating multiparous mare. No major abnormalities were identified during physical examination, except for local abnormalities associated with the right metacarpophalangeal joint. There was no lameness evident during walking.

Transrectal palpation revealed an enlarged and dilated cervix, a flaccid uterus, and a mass consistent in size, but not texture, with a pregnancy of 3 to 4 months' gestation. The mass was located approximately 5 cm left of the uterine bifurcation; uterine boundaries in the region of the mass could not be ascertained during transrectal palpation. Transrectal ultrasonography revealed an echogenic, round-to-oval mass with a mixed heterogeneous pattern of hyperechoic and hypoechoic areas and an anechoic, round-to-oval structure that was 1 cm in diameter and consistent with the size of the orbit of a fetus of 3 to 4 months' gestation[1] (**Figure 1**). No fetal fluids, fetal movement, or fetal organs were detected during transrectal ultrasonography. Uterine boundaries in the area of the mass could not be ascertained during transrectal ultrasonography. The right ovary was small with tiny (< 5 mm in diameter) follicles.

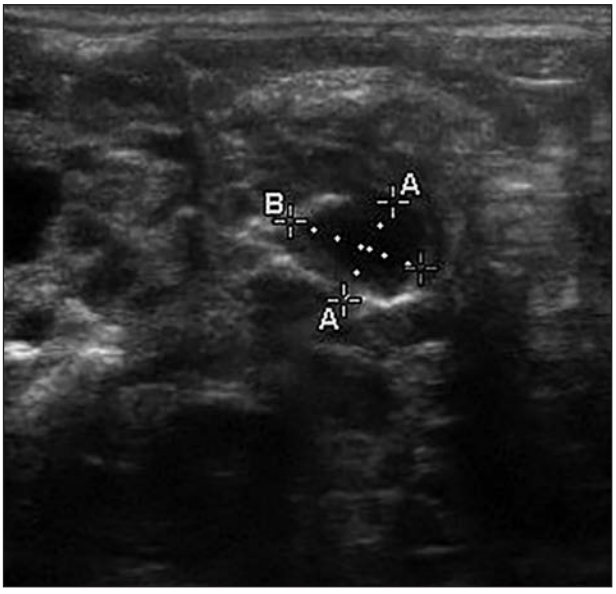

Figure 1—Transrectal ultrasonographic image of the reproductive tract of a 12-year-old Quarter Horse mare. A mass contained a heterogenous structure approximately 1 cm in diameter that resembled the orbit of a fetus of 3 to 4 months' gestation. Distance between cursors was as follows: A = 0.93 cm and B = 1.18 cm.

Transabdominal ultrasonography with a 5-MHz curvilinear transducer revealed an oval heterogeneous mass in the right caudoventral aspect of the abdomen located immediately cranial to the apex of the bladder. The mass appeared to contain multiple anechoic pockets of fluid and a few hyperechoic structures that cast echogenic shadows. The mass was approximately 13.6 × 16.6 cm, but it was partially obscured by overlying large intestine. No fetal heartbeat or other recognizable fetal structures were seen. No placenta or portion of the uterus was associated with the mass. At this time, the clinicians suspected the mass was not associated with the uterus and likely was the caudoventrally displaced left ovary.

Question

What is the next test that should be performed to yield a diagnosis for the mass in the caudoventral aspect of the abdomen of this mare? ***Please turn the page.***

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Answer

Submit serum for endocrinologic analysis of inhibin, testosterone, progesterone, and anti-Müllerian hormone (AMH) concentrations to determine the likelihood that the mass is a granulosa-theca cell tumor (GTCT).

Results

Serum was placed in a sterile evacuated glass tube with no additives and submitted for analysis of serum concentrations of inhibin, testosterone, progesterone, and AMH. Results were as follows: inhibin, 11.4 ng/mL (reference range for nonpregnant mares, 0.1 to 0.7 ng/mL); testosterone, 66.1 pg/mL (reference range for nonpregnant mares, 20 to 45 pg/mL); progesterone, 0.5 ng/mL (reference range for mares with absence of active luteal tissue, < 1 ng/mL); and AMH, 14.2 ng/mL (reference range for nonpregnant mares, 0.1 to 3.8 ng/mL). On the basis of these results and results of the aforementioned diagnostic imaging, the caudal abdominal mass was presumed to be a GTCT. The horse was transferred to the equine surgery service at the veterinary teaching hospital for unilateral ovariectomy.

A CBC was performed. The only abnormality was mature neutrophilia, which was likely attributable to inflammation associated with the laceration and septic condition of the horse's right metacarpophalangeal joint. Another possibility was a stress response. Food was withheld from the horse overnight.

The next morning, a 14-gauge catheter was aseptically inserted in the right jugular vein and secured with 2-0 monofilament nonabsorbable suture.[a] The horse received 9.6 million U of penicillin G procaine IM in the left side of the neck. Subsequently, 2.9 g of gentamicin and 450 mg of flunixin meglumine were administered IV via the jugular vein catheter.

Because of the size of the mass (> 15 cm), it was decided that the ovary should not be removed via a flank incision. Laparoscopic ligation of the blood supply to the ovary and ovarian pedicle followed by ventral midline celiotomy was selected as the preferred surgical approach.

The horse was sedated by IV administration of 10 mg of acepromazine maleate and 75 mg of xylazine hydrochloride. The horse was maintained sedated in a standing position by a continuous rate infusion of xylazine. A caudal epidural was performed with 45 mg of morphine in isotonic saline solution. The skin and subcutaneous tissues in the left paralumbar area were desensitized with 10 mL of a 2% solution of lidocaine hydrochloride, and laparoscope and instrument portals were created. The mesovarium and mesometrium were desensitized with 40 mL of a 2% solution of lidocaine hydrochloride, which was applied with laparoscopic guidance. The left ovarian pedicle was ligated with a laparoscopic electrocautery system[b] and staples.[c]

Anesthesia was then induced by the IV administration of 900 mg of ketamine hydrochloride, 15 mg of midazolam, and 17.5 g of guaifenesin; anesthesia was maintained with isoflurane in oxygen. The 2.2-kg (4.84-lb) mass (dimensions, 18 × 17 × 11 cm) was removed via ventral midline celiotomy. The abdominal incision was closed in a routine manner, and the horse recovered from anesthesia without incident.

The horse received trimethoprim-sulfamethoxazole (12.5 g, PO, q 12 h for 5 days) and flunixin meglumine (250 mg, PO, q 12 h for 5 days, then 250 mg, PO, q 24 h for 3 additional days) after surgery. The horse was discharged to the owners 2 days after surgery. The horse was scheduled to return to the veterinary teaching hospital 2 weeks after the day of surgery for suture removal and a follow-up examination.

The mass was cut in cross section for gross examination (**Figure 2**). Histologic examination of tissue sections of the mass revealed that the normal architecture was completely replaced by moderately cellular, multinodular, often cystic structures that extended to all edges of the mass. The mass was composed of variably discrete and confluent nests and papillary trabeculae of polygonal to cuboidal to vaguely columnar cells separated by thin to thick bands of dense fibrovascular connective tissue. Cells had variably defined cell borders; moderate amounts of finely granular to vacuolated, eosinophilic cytoplasm; and a large, oval, slightly eccentric nucleus with up to 3 distinct basophilic nucleoli. Cells lining cavitary structures often were columnar, palisading, and 1 to 10 layers deep and had basilar nuclei (**Figure 3**). There was moderate anisocytosis and anisokaryosis, and 10 mitotic figures were detected in 10 hpfs (400×). Individual necrotic cells were occasionally seen. Small foci of fibrillar to amorphous eosinophilic material were evident within the cavitations formed by nested spindle cells (Call-Exner bodies). These findings were consistent with a GTCT.

Discussion

Granulosa-theca cell tumors comprise approximately 2.5% of all neoplasms reported in horses and are the most commonly diagnosed tumor of the reproductive tract in mares (85% of all reproductive tract tumors in mares).[2] In a mare with a caudal abdominal mass of unknown etiology, a GTCT must be considered as a differential diagnosis. Other differential diagnoses, depending on location of the mass and the clinical signs, should include pregnancy, a mummified or resorbing fetus, leiomyoma, uterine lipoma, gastrointestinal stromal tumor, teratoma, dysgerminoma, ovarian hematoma, or other neoplastic ovarian structure (eg, cystadenoma). Large ovaries may be attributable to persistent anovulatory follicles or cystic structures within the ovulatory fossa

Figure 2—Photograph of the cross section of the caudal abdominal mass removed from a 12-year-old Quarter Horse mare. Dimensions of the mass were 18 X 17 X 11 cm.

or oviduct. There is physiologic ovarian enlargement during pregnancy in mares.[3]

The key to successful diagnosis of a GTCT is correlation of the clinical signs, findings for transrectal palpation and ultrasonography, and results of endocrinologic analysis of serum. A thorough medical history and physical examination are cornerstones of the diagnostic process.

Although there are many exceptions, several clinical signs are commonly associated with GTCTs and appreciable during physical examination. Detection of these signs should warrant further diagnostic evaluations to rule out a possible GTCT. The most common clinical signs in mares with GTCTs are persistent anestrus, aggressive or stallion-like behavior, or persistent estrus. Colic, lameness, weight loss, and a myriad of other clinical signs have been reported[3]; however, these are less common. The authors are aware of no breed predisposition associated with the likelihood of developing a GTCT. The mean age of mares with GTCTs is 10 to 11 years[4]; however, GTCTs have been reported in juvenile (< 1-year-old) mares.[3] A crested neck, large clitoris, and increase in muscle mass may be evident in mares with GTCTs; these are clinical signs associated with increased testosterone concentrations attributable to a GTCT.[3]

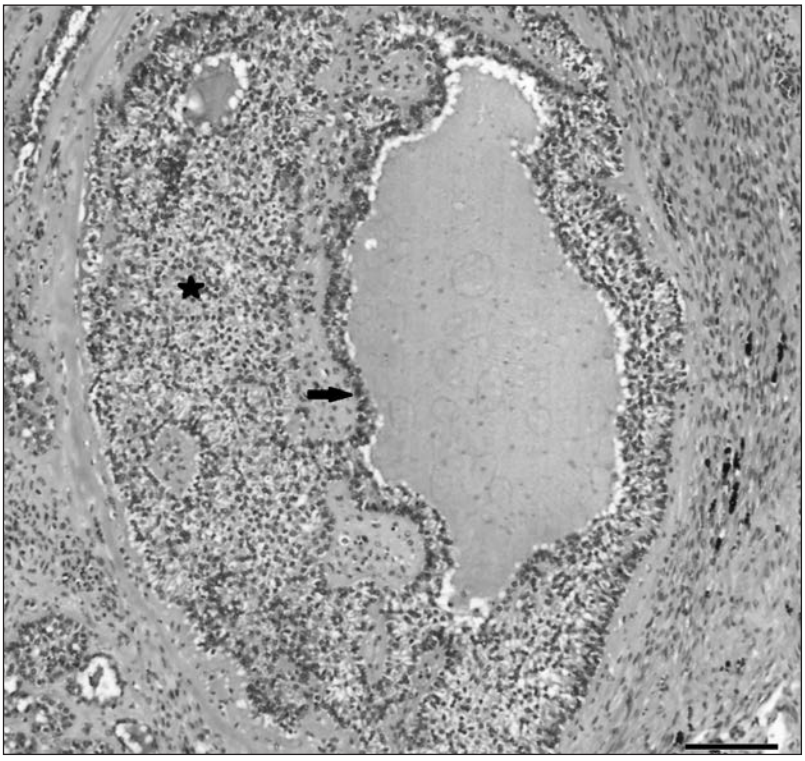

Figure 3—Photomicrograph of a tissue section of the caudal abdominal mass removed from a 12-year-old Quarter Horse mare. The mass is composed of variably discrete and confluent nests and papillary trabeculae of polygonal to cuboidal to vaguely columnar cells (star) and cysts lined with palisading columnar cells (arrow). These features are consistent with a GTCT. H&E stain; bar = 200 µm.

A few common, distinct features of GTCTs may be apparent during transrectal palpation. Most mares with GTCTs will have asymmetric ovaries. The GTCT-affected ovary typically is between 10 and 20 cm in diameter, is extremely firm, and lacks a palpable ovulation fossa.[4] The contralateral ovary typically is extremely small. A GTCT-affected ovary often is found in an abnormal location, as was evident for the mare of the present report in which the GTCT was located near the bifurcation of the uterus. These anatomic considerations can help differentiate GTCTs from other suspected ovarian abnormalities during transrectal palpation. Ultrasonography can often be used in mares with an abnormal ovarian position to better define the proximity and relationship of the ovary to surrounding abdominal structures.

Ultrasonography can be extremely useful in the diagnosis of ovarian pathological conditions; however, GTCTs can have a variable ultrasonographic appearance. An affected ovary can appear ultrasonographically as a single solid mass, fluid-filled structure, heterogeneous multicystic mass, or any combination of these[2] (**Figure 4**). The tunica albuginea surrounding the ovary can often appear thickened.[5] Follicular activity should be ultrasonographically evaluated in the contralateral ovary because mature GTCTs will often suppress activity in the contralateral ovary through inhibin secretion. However, some mares may continue to have estrous cycles, and ovarian asymmetry in these mares may be minimal or inapparent.

Submission of serum for measurement of inhibin, testosterone, and progesterone concentrations has traditionally been the preferred choice for diagnosis of GTCTs. Inhibin, a glycoprotein responsible for suppressing the release of follicle-stimulating hormone from the anterior pituitary,

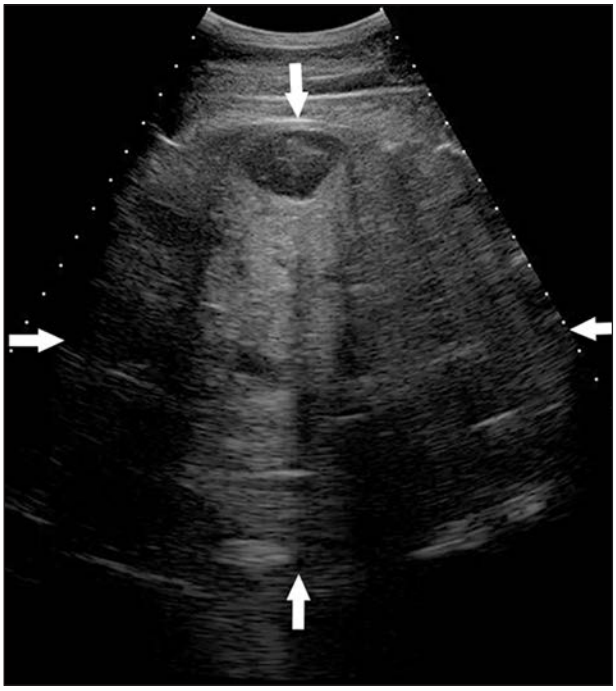

Figure 4—Transabdominal ultrasonographic image of the heterogeneous mass identified in the caudoventral aspect of the abdomen of the mare in Figure 1. The image was obtained with a 5-MHz curvilinear transducer at a depth of 19 cm. Boundaries of the mass are indicated (arrows). Tick marks on the side are at intervals of 1 cm.

was found to have a sensitivity of 80% for the diagnosis of histologically confirmed GTCTs.[6] An elevated inhibin concentration is responsible for the cessation of follicular activity in the contralateral ovary because of inhibin's suppressive effects on the release of follicle-stimulating hormone. Testosterone has been used as a diagnostic marker for ovarian tumors for several years. When used alone as a diagnostic tool, evaluation of testosterone concentration has a sensitivity of approximately 50%.[6] High testosterone concentrations are associated with a large number of theca cells within the interstitial tissue of tumors, such as in GTCTs.[3] However, there are substantial day-to-day variations in testosterone concentration during the estrous cycle of clinically normal mares.[3] Although a testosterone concentration may be highly suggestive of a GTCT, evaluation of serum testosterone concentration alone is not specific enough for a definitive diagnosis of GTCTs in mares. Combining elevated serum concentrations of inhibin with results of serum testosterone assays yields a sensitivity of 84% for diagnosis of GTCTs in mares.

Serum progesterone concentrations usually are determined in conjunction with inhibin and testosterone concentrations. Because of the suppression of ovulation associated with most mature GTCTs, affected mares may have serum progesterone concentrations < 1 ng/mL.[3] However, a progesterone concentration < 1 ng/mL would also be consistent with a clinically normal mare during estrus. Mares with GTCTs that still have estrous cycles could have elevated progesterone concentrations because a corpus luteum could be present on the contralateral ovary. Mares with GTCTs can still have estrous cycles when the GTCTs are not secreting high amounts of inhibin that lead to down-regulation of follicular activity and the estrous cycle.

Until recently, measurement of inhibin, testosterone, and progesterone concentrations was the triad of endocrinologic analyses available for the serologic diagnosis of GTCTs. However, elevated AMH concentrations have been identified as diagnostic for GTCTs.[6–8] Anti-Müllerian hormone is a homodimeric glycoprotein hormone that is secreted by sustentacular (Sertoli) cells during sexual differentiation[8] in male fetuses and causes regression of the paramesonephric (Müllerian) ducts in males.[6] In neonatal and sexually mature females, granulosa cells of preantral and small antral follicles produce AMH.[6,8] Most importantly, AMH is also expressed by GTCTs, and circulating AMH concentrations have been used for the diagnosis of GTCTs in women.[6]

Serum AMH concentrations > 4.0 ng/mL appear to be specific for mares affected with GTCTs and have a sensitivity of 98% for detection of histologically confirmed GTCTs.[6,8] Additionally, the concentration of AMH does not differ substantially during the estrous cycle or pregnancy, which provides further diagnostic applicability when evaluating AMH concentrations in patients.[7] This diagnostic serum test has added to the ability of clinicians to diagnose GTCTs.

The use of serologic hormone concentrations, ultrasonographic results, and findings during transrectal palpation, when combined with a thorough physical examination and medical history, provides a reliable means of diagnosing GTCTs in mares. It must be emphasized that every case has its own set of diagnostic hurdles and complicating clinical signs. Combining diagnostic tools can aid in the early diagnosis and subsequent surgical treatment of GTCTs. In mares intended for use as broodmares, there is a positive prognosis for return to fertility after removal of a GTCT. It has been reported[3] that 80% of mares resumed normal estrous cyclicity the spring after surgical removal of a GTCT.

Because of the size of the mass in the mare of the present report, ventral midline celiotomy was necessary to facilitate removal. To minimize the amount of time the horse would be anesthetized and positioned in dorsal recumbency, the decision was made to sedate the horse, restrain the horse in a standing position, and perform laparoscopy to surgically ligate blood vessels and free the mass. Then, anesthesia would be induced, the horse would be positioned in dorsal recumbency, and the mass would be removed via ventral midline celiotomy. Use of this surgical approach resulted in the need for the anesthetized mare to be positioned in dorsal recumbency for only 45 minutes.

Many large ovarian tumors can be removed through a flank incision if they have fluid-filled structures or multiple cystic cavities that can be drained. However, tumors > 20 cm in diameter and that are composed of a fibrous stroma will require transection into multiple smaller pieces and use of a catch bag or removal by use of a tissue morselizer. Resecting a mass within the abdomen is challenging and has the potential for spreading tumor cells if the tumor pieces are not safely confined in a catch bag. Tissue morselizers are expensive, and there is also the potential for seeding the abdomen with tumor cells when morselizers are not used correctly. Therefore, on the basis of the size and structure of the mass in the horse of the present report, ventral midline celiotomy was more efficient and effective for tumor removal without spreading tumor cells within the abdomen. Although recovery from anesthesia poses risks, the percentage of catastrophic complications is low.

Outcome

Recovery after surgery was without complications. The clients reported that the mare was less aggressive and noticeably more amenable to handling and training. Because the mare was not intended as a broodmare, follow-up evaluation of the contralateral ovary was not performed.

a. Ethilon, Ethicon Inc, Somerville, NJ.
b. Caiman Lektrafuse system, Aesculap, Center Valley, Pa.
c. Endo GIA autosuture 60 mm × 4.8 mm, Covidien, Mansfield, Mass.

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