When and how to perform a transvaginal ovarian biopsy in a mare

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Abstract

Transvaginal ovarian biopsy (TVOB) is a clinical tool to confirm the presence of an ovarian abnormality and additionally may be used in research to obtain serial ovarian samples to study physiologic functions without negative effects on mare health and fertility. Circumstances where the TVOB is useful as a clinical diagnostic or research tool include: discordant ovarian tumor biomarker results and clinical findings; biomarkers are elevated but it is unclear which ovary is abnormal or both ovaries are abnormal; or serial biopsies are desired. Details regarding how to perform the TVOB procedure are described and case examples included to illustrate its clinical application.

Keywords: Transvaginal ovary biopsy, mare, ovarian tumor

Introduction

Investigators have developed a variety of transvaginal ultrasound guided clinical techniques that have been applied to the equine ovary, e.g. follicle aspiration for oocyte collection (TUGA), trans-follicular oocyte transfer, luteal biopsy (TVLB) and ovarian biopsy (TVOB).[1-3] Transvaginal ultrasound guided aspiration (TUGA) of follicles in the mare was reported in 1988.[1] The TUGA procedure is repeatedly performed in mares to recover oocytes; ovaries of mares that had undergone many TUGA had fibrosis in the ovarian stroma, but there were no reported effects of repeated TUGA on fertility.[4] Luteal biopsies in the mare and cow were described by Slough et al. and Aerts et al.[5,6] Health and fertility effects of transvaginal ultrasound guided luteal biopsy (TVLB) along with transvaginal ultrasound guided ovarian biopsy (TVOB) in mares was reported, with fertility unaffected.[3] In previous studies, experiments were done on abbatoir-derived equine ovaries. TVLB /TVOB allows serial sampling of the ovary which has the positive benefit of reducing the number of horses required for research, while enabling study of various physiologic functions. The TVOB was developed to assist clinical decision making by obtaining biopsy information from abnormal areas of an ovary or ovaries.[3]

Review of ovarian abnormalities

In most clinical settings, an abnormal ovary is identified though transrectal palpation of the ovary followed by a transrectal ultrasonographic examination. A mare's history may include concern with the mare's behavior such as: anestrus, stallion-like behavior or frequent estrus. Abnormal transrectal findings may be present and include: abnormal ovarian texture, abnormal ovarian size, inability to discern the ovulation fossa, abnormal mobility of the ovary, adhesions to the ovary, and presence of static structures on the ovary. These findings may be associated with neoplastic and nonneoplastic processes.[7] Neoplastic processes include tumors, e.g. granulosa theca cell tumor (GTCT), the most common ovarian tumor in the mare and arises from gonadostromal cells.[7] Other gonadostromal tumors (thecoma), germ cell tumors (teratoma, dysgerminoma), epithelial tumors (cystadenoma), mesenchymal tumors (e.g. leiomyoma), and metastatic tumors have been reported.[3] The GTCT has been reported in mares with a wide age range, from fetuses to aged mares, and may be unilateral or bilateral. The classic presentation of a GTCT is an enlarged ovary with a "cluster of grapes" appearance, along with a small, inactive contralateral ovary. Mares with GTCT may exhibit anestrus, stallion-like behavior, persistent estrus or short interestrus intervals. The GTCT are associated with unusual echotextural features; however, small tumor masses maybe be present in ovaries that appear to have a normal echotextural appearance.[7] A few mares with GTCT continue to have estrous cycles, may become pregnant and foal. Reproductive activity may continue for a number of years, but in most cases, there is negative feedback on the pituitary from tumor products from the abnormal ovary, resulting in a small, static contralateral ovary. Epithelial tumors and GTCT in mares may present with a relatively normal echotextural appearance.[8]

As not all ovarian abnormalities arise from neoplastic tissue, a clinician should be aware that other abnormalities have been reported, including ovarian abscesses, foreign bodies, torsions, large follicular cystic structures, cystic embryonic remnants and other physiologic structures that may or may not enlarge the ovary, including persistent anovulatory follicles, epidermal inclusion cysts, supplementary corporal lutea and hematomas.[9]

Ultrasonography of the ovary may identify both normal tissue such as stromal, luteal, and follicular tissues, as well as abnormal tissue, including tumors. Other imaging modalities to visualize the equine ovary, as magnetic resonance imaging (MRI), computerized tomography (CT), and diffraction enhanced radiography, are not performed due to the large size of mares, deep abdominal position of their ovaries, cost and availability. In some cases, ancillary tests such as a CBC, serum chemistry, serum amyloid A, and abdominocentesis may be helpful to identify ovarian inflammation, e.g. when a foreign body or abscess is present in the ovary.[9]

When an ovarian abnormality, such as a tumor is suspected, usually a serum sample is obtained for ovarian tumor biomarker analysis, including inhibin, testosterone and anti müllerian hormone (AMH).[10,11] Analysis of serum concentrations of AMH has been shown to have a high sensitivity and specificity for detection of GTCT in mares, when compared to measurement of inhibin or testosterone. Androgen concentrations may be elevated in mares with ovarian cystadenomas, although most other ovarian tumors do not produce reliable biomarkers.[10,11] It is noteworthy that biomarker analysis for ovarian neoplasia indicates that certain types of tumor tissue are present, but the analysis does not confirm if one or both ovaries are affected, nor does it detect all types of ovarian neoplasia.

Prior to development of the TVOB, a laparoscopic approach was used to examine the gross appearance of ovaries suspected to be neoplastic.[3] Ovariectomy was then performed to provide a definitive diagnosis and allow a return to reproductive function. A less expensive option than laparoscopy is a targeted TVOB to obtain ovarian tissue for histopathology, which may be utilized in advance of surgery to identify the ovary or ovaries with neoplasia.[7]

When to perform a TVOB

The TVOB is predominately utilized in a clinical setting when there is a need to confirm the presence of an ovarian tumor and when biomarker analysis (inhibin, testosterone and AMH) are discordant with clinical findings. Criteria for the use of a TVOB include: abnormal ovarian texture or consistency, inconclusive hormonal findings, questionable or abnormal ultrasonographic echotexture of the ovary, undiagnosed behavioral or estrous cycle irregularities, difficulty accessing biomarker laboratories, need to rule out ovarian neoplasia as a cause of behavioral problems, and investigation of possible bilateral tumor involvement. Health and fertility of mares undergoing TVLB or TVOB of neoplastic ovarian tissue have been described, with fertility not affected by TVOB.[3]

How to perform a TVOB

Transrectal ultrasonography is performed using a linear-array transrectal transducer to confirm the ovary to be biopsied and area(s) on each ovary targeted for biopsy. A TUGA transducer (7.5 - 10 mHz) (Minitube®, Ingersoll, Ontario, Canada) is utilized, or a vaginal transducer housing is disinfected with cold sterilant, rinsed with distilled water and a microconvex 7.5 - 10 mHz ultrasound transducer disinfected with 70% alcohol, is secured into the probe housing. A sterile insemination pipette or similar sterile plastic tube may be inserted into the needle track and secured in place, if desired. The TVOB device is a modified, 14-gauge, 20 cm, single-action semiautomatic spring-loaded biopsy needle (Supercore®, Argon Medical, Frisco, TX), modified to be 65 cm long, with the tip beveled to a taper point. The TVOB device may be sterilized with either gas or ultraviolet light, or the handle and needle portion wiped with alcohol or disinfectant wipes and the distal portion of the needle immersed in a cold sterilization media (Cidex®) for at least 10 minutes. Two TVOB devices should be prepared for each procedure, so a different device may be used per ovary if both ovaries are biopsied, and in order to have a spare device if a malfunction occurs. If cold sterilization media is used, it should be rinsed off the end of

the biopsy device with sterile water or saline, with the notch in the biopsy needle exposed. The instrument is then placed on a sterile field.

Experienced individuals require < 10 minutes after sedation in most mares to perform TVOB. Depending on mare temperament, a combination of sedatives and analgesics are given, such as: acepromazine (Atravet® Boehringer Ingleheim, Ridgefield, CT) 0.04 mg/kg 0.01 - 0.02 mg/kg IV; butorphanol tartrate (Torbugesic®, Zoetis, Kalamazoo, MI), 0.01 - 0.02 mg/kg IV; detomidine hydrochloride (Dormosedan®, Zoetis) 0.04 - 0.08 mg/ kg IV; or xylazine (Rompun®, Bayer, Mississauga, Ontario, Canada) 0.2 - 0.5 mg/kg IV. The mare's head should be positioned to prevent airway obstruction as her head drops due to sedation. Hyoscine butylbromide (Buscopan®, Boehringer Ingelheim) 0.1 mg/ kg IV is then administered to relax the rectum. The perineum is cleansed as for artificial insemination. The vaginal probe is covered, lubricated, and inserted into the vagina using a sterile gloved hand and arm and positioned lateral to the cervix, ipsilateral to the ovary to be biopsied. Operator then removes their hand from the vagina, inserts their arm in rectum, removes any manure and manipulates the target ovary cranial into the abdomen and then up and over the broad ligament so the ovary is positioned adjacent to the transducer. The entry point to the needle channel on the vaginal transducer should be kept covered either by a sterile plastic cover (Minitube®, Delavan, WI), or sterile towel during this manipulation to prevent contamination of the biopsy needle channel port. The ovary is carefully examined using transvaginal ultrasonography. Transducer and ovary are manipulated so the region of the ovary to biopsied is easily visualized and there is no bowel or large blood vessel visible between the ovary and transducer.

Biopsy instrument should be handled using sterile gloves and is cocked by pulling back on the tip of the handle, resulting in an audible click. Biopsy needle tip and side of the needle are then lightly lubricated using a small quantity of a sterile lubricant (Minitube®). The biopsy needle is inserted carefully into the needle channel of the transducer and advanced until it meets resistance in the front of the vagina. At this time, the veterinarian supporting the ovary through the rectum against the vaginal wall must hold the ovary steady and firmly against the vaginal transducer. An on-screen biopsy needle track (guide) may or may not be used. A TVOB needle positioned for obtaining a biopsy within an ovary is shown (Figure 1). If a needle track guide is used, the ovary must be positioned so the region to be biopsied is accessible, with the track bisecting the area of interest. The depth needed to reach the target area to be biopsied in the ovary is assessed by evaluating centimeter marks on the monitor. The biopsy needle is grasped at the desired distance to be advanced into the ovary and the ovary entered using a controlled stabbing motion under real-time visualization. The entrance of the biopsy device into the ovary of the mare may elicit some motion in some mares. If the mare moves excessively during the procedure, additional sedation should be administered. For safety reasons, a biopsy sample should not be captured when the mare is moving. The authors recommend that only the side of the needle should be used for advancing the needle for positioning, as manipulation of the device's handle may result in accidentally firing the instrument. An unplanned firing of the biopsy instrument may puncture the mare's rectum or the veterinarian's hand in the rectum. The biopsy needle is guided into or near the target area by visualizing the bright echo produced by reflections generated from the diamond scoring on the needle tip. Solid areas of suspect ovarian tissue should be targeted for biopsy, as better samples are obtained compared to cystic areas.

A 2 step biopsy method involves the veterinarian visualizing the area of interest in the ovary and advancing the biopsy needle tip under direct visualization to just behind the biopsy location. The needle tip is then advanced to the first stop into the exact location to be biopsied, by applying gentle pressure on the tip of handle and then the needle is moved slightly to fill the notch on the biopsy needle. The biopsy instrument is then fired by firmly pushing on the end of the instrument to reach the second stop and an audible click will be heard. Using this 2 step method, the biopsy needle tip does not advance when firing, rather the cutting sheath moves and closes over the notched portion of the needle to capture the sample. Alternatively, the other choice of technique is called the 1 step procedure. For this, the biopsy needle tip is advanced to the perimeter of the tissue of interest, accounting for fact the needle will advance 2 cm during firing. The biopsy needle should not be held during firing with either method and only the handle

of the device should be manipulated during firing, as holding the needle prevents the cutting sheath from advancing and may cause the device to malfunction.

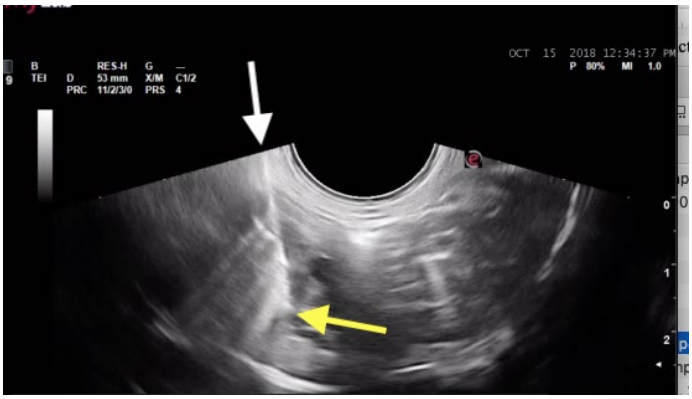


Figure 1. Transvaginal ultrasound image of a biopsy needle in position to capture a sample using transvaginal ovarian biopsy technique. Needle is visible as a white line casting an acoustic shadow. White arrow indicates where the needle enters the ovary, and horizontal arrow indicates needle tip.

The biopsy needle is then removed from the biopsy channel and placed flat on the sterile field. The captured sample is accessed by pulling back on the tip of the handle to re-cock the instrument (an audible click is heard), then advancing the needle to the first stop by gentle pressure on the end of the instrument to expose the captured biopsy core sample (Figure 2). Needles (22 gauge) are often used to remove the sample from the needle notch. Care should be taken when removing biopsies, as they are thread like and easily stretched. Biopsy cores are placed in a small amount of fixative (10% buffered formalin). The biopsy needle may be rinsed with sterile saline or wiped with sterile gauze and the procedure repeated to capture at least three core samples in the area(s) of interest. Captured ovarian fluid may be dropped onto sterile glass slides, dried and processed for cytology. Following completion of the sample collections, the vaginal probe is removed. A small amount of blood may be present on the tip of the probe from the biopsy needle puncturing the cranial vaginal wall. Fixed tissues for histological examination are processed in a routine manner and stained using hematoxylin and eosin (Figure 2). It may also be possible to perform a TVOB procedure through an ultrasound-guided flank approach with a non-modified device without the extension of the biopsy needle, by pushing the ovary against the body wall. However, heavy, very enlarged ovaries are somewhat cumbersome to hold in position with either technique.

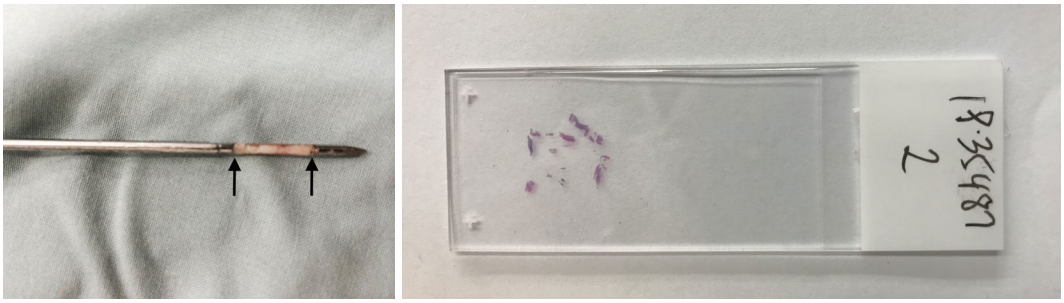


Figure 2. Image of an ovarian biopsy sample (left panel) in the notch of 14 gauge needle of transvaginal ovarian biopsy device (black arrows) and histology slide (right panel) containing ovarian samples obtained during transvaginal ovarian biopsy.

Undesired outcomes and complications

Mares with a solitary large cystic structure or multi-cystic ovarian tissue may be problematic to biopsy, as there is little tissue to capture. In these cases, more solid areas of the mass should also be targeted for biopsy. Occasionally non-diagnostic samples are obtained that have been obtained off target. An instrument may malfunction. Gastrointestinal or rectal puncture may occur during the procedure due to: bowel present between the transducer and ovary; mare movement; over zealous insertion of the biopsy needle; failure to identify the location of the biopsy needle before firing; positioning errors in areas difficult to biopsy; operator error, especially when learning the technique; or inadvertently firing the biopsy device. The veterinarian's hand may also be penetrated by the biopsy needle, in which case the biopsy needle will have to be retracted through the rectum and ovary. The mare's rectum may be injured during manipulation. The possibility of biopsy needle puncture through the rectum should be disclosed to the client. During the TVOB's we have performed, a few of these rectal and hand punctures have occurred; mares were treated with NSAIDs, antibiotics and rectal temperatures were measured for 3 days, but no complications occurred in the mares. First aid should be available for the operator. Ovarian abscess or infection may occur if the rectum is punctured or if inadvertent needle contamination occurs during the procedure. A biopsy instrument that punctures the rectum should be considered contaminated and should be either cold sterilized or gas sterilized before it is used again. Ideally, ovarian biopsy instruments are single use. A rectal injury, such as minor bleeding from the rectum or a small laceration of the mucosa, may occur with this type of ovarian manipulation, and should be investigated when discovered and treated accordingly. We have not detected neoplastic tissue seeding other locations in the abdomen as a result of the TVOB procedure for GTCT.

Aftercare

Aftercare includes monitoring the mare's physical examination parameters (temperature, pulse, respiration), appetite and demeanor for 3 days after TVOB. Generally, mares are not treated with NSAIDs or antibiotics before or after the TVOB procedure, except when rectal bleeding is detected and appropriate measures are taken, or an inadvertent rectal puncture has occurred, then antibiotics and NSAIDs should be administered.

Case 1

History

August 2018. Initial examination by RDVM included the following history: Mare was reported to have aggressive, out-of-character behavior since spring of 2018. There was a thick-walled structure with a hyperechoic center and appearance of a corpus hemorrhagicum on the right ovary. There were multiple small follicles on the left ovary and grade 1 uterine edema. The mare was given $PGF_{2\alpha}$ 14 days before referral.

Biomarker analysis

Progesterone was 2.6 ng/ml, consistent with active luteal tissue; inhibin (32.3 pg/ml) was normal (reference range: 92 - 100 ng/ml); AMH (12.9 ng/ml) was elevated (reference range: 0.1 - 6.9 ng/ml); and testosterone (45.8 pg/ml) marginally elevated (reference range: 12 - 45 pg/ml for a non-pregnant mare). Ovariectomy was recommended based on biomarkers. In November 2018, mare (Figure 3) was presented for ovariectomy. Ovaries were normal (Figure 3). Hormonal analysis was repeated, with testosterone < 0.2 pg/ml and AMH within reference ranges. A TVOB was performed and histology of the ovarian tissue had normal stromal and luteal tissue (Figure 4). The owner elected to take the mare home and no surgery was performed.

Figure 3. Case 1 - picture of a quarter horse mare (left) panel, and ultrasound images of the mare's left ovary (center panel) and right ovary (right panel) in November 2018.

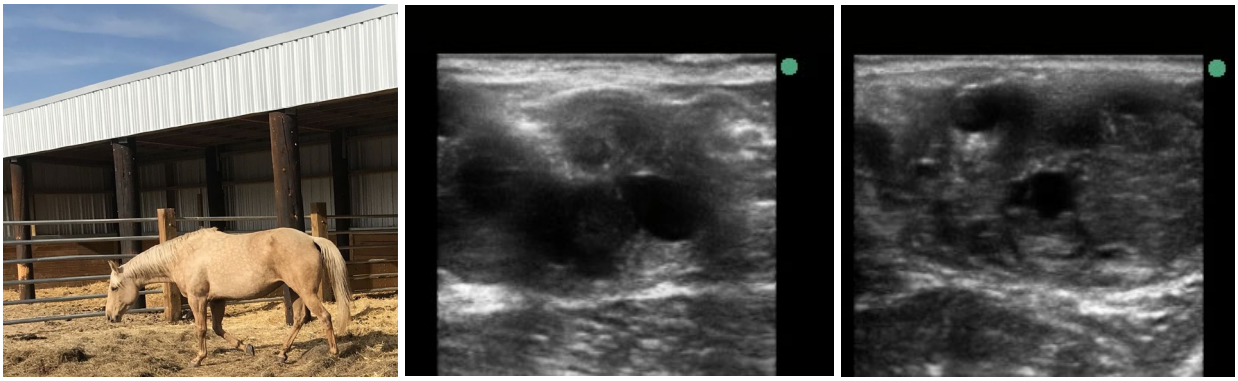
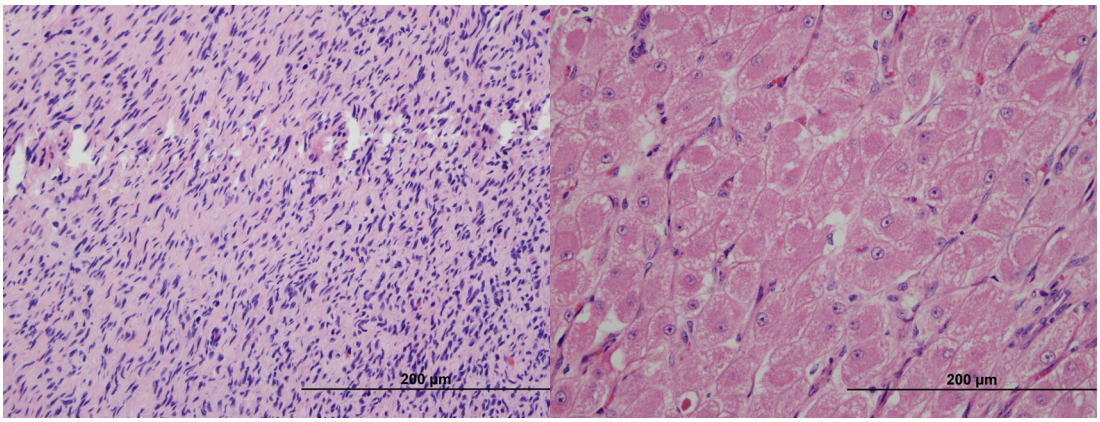


Figure 4. Case 1 - Histologic image of stromal (left panel) and luteal (right panel) tissue obtained using a transvaginal ovarian biopsy from the mare's ovaries.



Diagnosis

Transiently elevated biomarkers, ovarian tissue within reference range.

Case 2

History

An AQHA 10 year old broodmare was presented with a history of signs of aggression and persistently enlarged ovaries. Mare was owned by a veterinarian. Owner reported that the mare had unexplained infertility with no inflammation on endometrial cytology and a grade IIB endometrial biopsy. Upon referral, based on transrectal palpation and ultrasonography, ovaries were within normal limits in consistency, size and echotextural features.

Biomarker analysis

Testosterone and inhibin concentrations were within normal ranges, and due to cost, AMH was not analyzed. Karyotype analysis was WNL. Owner requested a biopsy of both ovaries.

Diagnosis

Histology of biopsies obtained using TVOB indicated healthy ovarian tissue. Owner reported the mare was inseminated twice in 2017 with cooled semen, but failed to become pregnant. In 2018, she was inseminated with cooled semen and became pregnant, but experienced early embryonic loss by 30 days.

Case 3

History

A 12 year old Warmblood mare was presented with a history of repeated estrus. In July 2018, she had an enlarged right ovary containing luteal tissue and a small left ovary. At a repeat evaluation in February 2019, there was an enlarged (> 10 cm) firm ovary and a very small left ovary. Uterus and cervix were moderately soft and there was grade 2 -3 uterine edema and scant grade 0 intrauterine fluid. The mare was considered a valuable brood mare and the owners did not want to go ahead with ovariectomy until confirmation of ovarian neoplasia was obtained.

Biomarkers

At the July examination, concentrations of all ovarian tumor biomarkers were within normal limits. A follow up transrectal and ultrasonographic examination confirmed the large right ovary and small left ovary. A TVOB was performed in February, but did not yield a diagnostic sample. Biomarker analysis and repeat TVOB were scheduled (Figure 6).

Ultrasonographic appearance of a GTCT may be quite variable. Three ultrasonograms that illustrate the variable appearance of GTCT are shown (Figure 5). A TVOB is useful in many cases to determine if both ovaries contain GTCT; note the GTCT tissue obtained from a TVOB (Figure 6) and compared it to tissue in Figure 4.

Figure 5. Ultrasound appearance of three ovaries that each contain a granulosa theca cell tumor (GTCT). The enlarged ovary in left panel has a classic appearing (GTCT), center panel contains an ovary with a GTCT that appears within normal limits, and right panel contains a GTCT that appears as a large cystic structure.

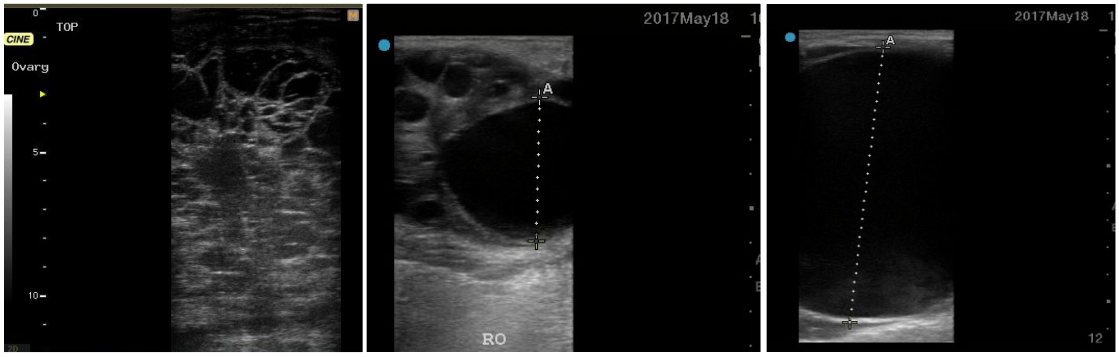
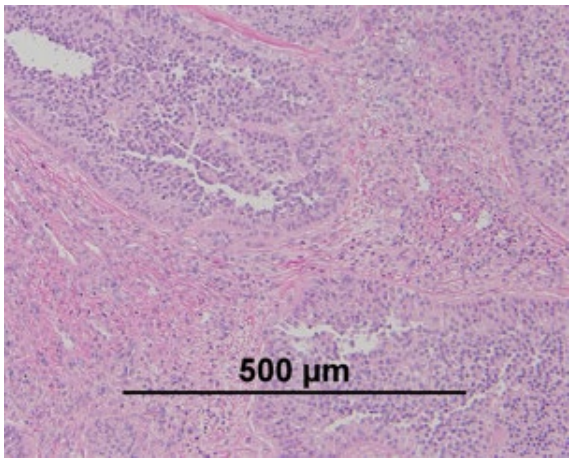


Figure 6. Histologic appearance of granulosa theca cell tumor tissue obtained using a transvaginal ovarian biopsy.



Discussion

While there have been some extensive studies regarding serum concentrations of AMH and inhibin in mares with GTCT, reference ranges provided by laboratories should still be considered a guide to be interpreted and applied in the context of other diagnostic tests. The clinician should integrate results of transrectal palpation and ultrasonography, ovarian tumor biomarker analysis and ovarian biopsy in cases where the diagnosis is not straight forward.

Case 1 illustrates a mare where 2 ovarian tumor biomarkers were initially increased and then returned to within normal limits. In our equine practice, we have identified occasional mares with this same AMH profile; that is, elevated AMH concentrations that then decrease to the reference range. Case 2 is an example of a mare where it was unlikely that a GTCT was present, however not all tumors produce elevated ovarian tumor biomarkers, or are visible using ultrasonography. The owner wanted to rule out neoplasia as a factor in the mare's poor fertility using TVOB as a modality. Case 3 represents a mare that is presented with an abnormal ovary detected with ultrasonography, without an elevation in ovarian tumor biomarkers. This may arise as a result of the tumor being predominately comprised of thecal tissue, i.e. a thecoma, another type of tumor (teratoma, cystadenoma, secondary metastasis) or it might accompany an error in sample handling or laboratory analysis. The history of the contralateral small ovary and persistently enlarged right ovary were highly suggestive of a GTCT tumor; however other ovarian abnormalities are also associated with 1 small ovary.[12] Evaluation of the AMH reference ranges for GTCT versus other ovarian abnormalities may result in changes in the reported reference range.[12] In conclusion, TVOB has specific applications for cases where the nature of an ovarian abnormality is not diagnosed, or to determine if 1 or both ovaries are affected by neoplasia. The TVOB procedure has not been associated with impairment of fertility and may provide useful diagnostic information.

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Conflict of interest

Author claims no direct or indirect affiliation with any of the manufacture's listed in the text. Information regarding the equipment and various manufacturers is solely based on experience and is for readers' reference only.

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