How to Determine Fetal Gender in Early and Advanced Gestation

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Introduction

A wealth of information on equine fetal anatomy and physiology has been acquired in recent years. Ultrasonographic fetal monitoring techniques have documented, in great detail, fetal growth and organ development at various stages of gestation. Ultrasonographic anatomy of fetal sex organs, from the genital tubercle to fully developed organs, has been extensively described in its entire progression.[1–7] Fetal gender determination in the mare provides a useful management tool to breeders by allowing a predelivery estimation of the value of offspring. Knowing fetal gender in advance of delivery allows for commercial strategies to be implemented, as the value of stock at sales time is often determined by the gender of the offspring. Furthermore, it is well established that some stallions have a greater proportion of quality female versus male offspring or just the opposite. Finally, culling of broodmares is easier when fetal gender is known.

Diagnostic Windows for Fetal Gender Determination

First Stage Diagnosis: Early Gestation

There are two different stages when the diagnosis of fetal sex can be determined by ultrasonography. The first stage is between 57 and 70 days gestation and involves the identification of the genital tubercle (Fig. 1) by transrectal ultrasonography.[1] The genital tubercle, the precursor of the penis in the male and the clitoris in the female, appears around day 55 of gestation as a hyperechoic equal sign (=), located between the fetal hindlimbs, at an approximately equal distance between the tail and the umbilicus. As gestation progresses, the genital tubercle migrates towards the tail in the female fetus (Fig. 2) and towards the umbilical cord in the male (Fig. 3). The shape of the genital tubercle may change over time, appearing trilobed (Fig. 4) or conical (Fig. 3). F1 F2 F3 F4

The early stage technique requires good equipment and considerable expertise, allowing consistent fetal sex determination within a small diagnostic window (optimal time days 59–68) and by a single diagnostic parameter. Best imaging will be accomplished when the fetus engages high up into a uterine horn (Fig. 5) and the fetus to transducer distance is greatly reduced. After day 70, the fetus tends to reside more deeply in the mare's uterus/abdomen and is not consistently accessible for imaging by transrectal ultrasonography due to the disproportionate increase in fetal fluid volume compared to fetal body mass, typical of this stage of gestation. After day 100, the fetus can be reliably found within the mare's pelvis. F5

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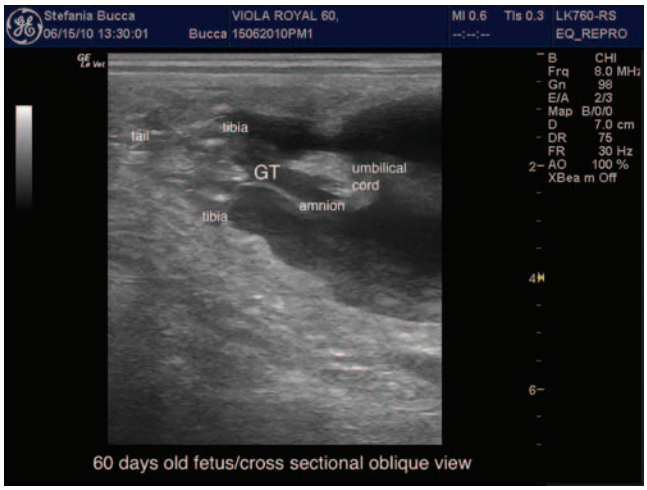

Fig. 1. Transrectal, cross-sectional oblique view of a 60 day-old male fetus (8 MHz linear transducer). The genital tubercle (GT) is seen emerging between the hind limbs and caudal to the umbilical cord.

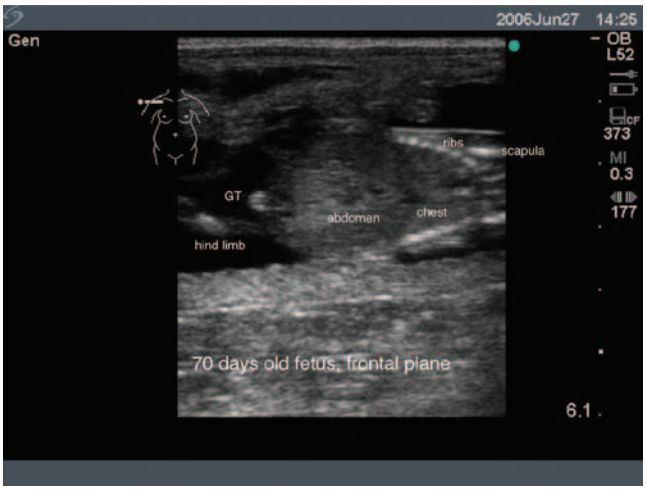

Fig. 3. Frontal plane view of a 70 day-old male fetus by US per rectum (7.5 MHz linear transducer); the GT presents with a conical shape. The chest and scapula are identified cranially (to the right of the sonogram), and the outline of the diaphragm can be barely detected between chest and abdomen.

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Second Stage Diagnosis: Advanced Gestation

The second stage for fetal sex diagnosis avails of a much wider diagnostic window between 100 and 260 days gestation. Multiple parameters can be used to validate diagnosis (fetal primary sex organs) at this stage, but a combination of transrectal and transabdominal ultrasound scanning may be required.

Fetal presentation plays a substantial role in the diagnostic approach in advanced gestation. Establishing fetal orientation should be readily accomplished in the course of the examination, knowing that in the fetus in posterior presentation the hindquarters will be easily accessible per rectum and expedite transrectal diagnosis. The active nature of the equine fetus determines frequent changes in presentation up to 9 months gestation, making transrectal examination potentially diagnostic even in advanced pregnancy. The ability to change presentation decreases as gestation advances,[6,7] as fetal size and the encasement of the hindquarters within the fetal horn prevent further rotations along the short axis. After 9 months, fetal sex can still be determined transrectally in grossly undersized fetuses and when a posterior or transverse presentation persists. A transabdominal approach will be required for diagnosis when the fetal hindquarters are out of reach of the operator's hand per rectum, as it commonly occurs from around 5 months gestation and when the fetus is in anterior presentation (Fig. 6).

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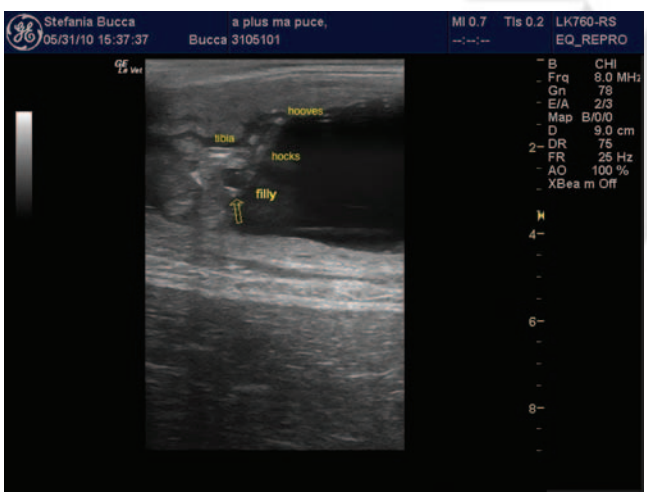

Fig. 2. Cross sectional oblique view of a 60 day-old female fetus, by US per rectum (8 MHz linear transducer). The GT is seen emerging from the buttocks.

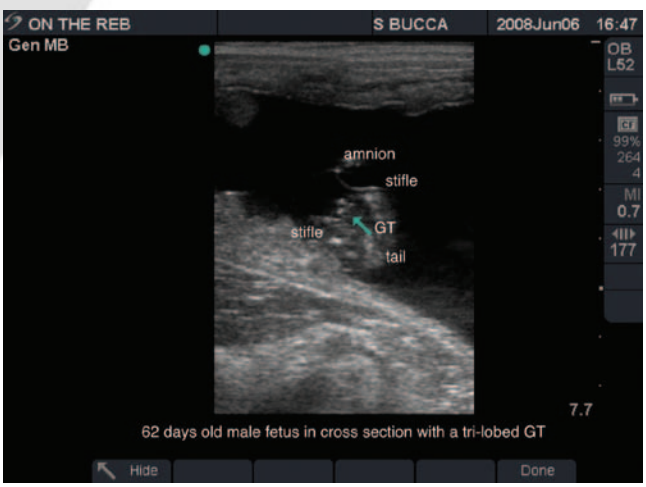

Fig. 4. Cross sectional view of a 62 day-old male fetus with a trilobed GT, US per rectum (7.5 MHz linear transducer).

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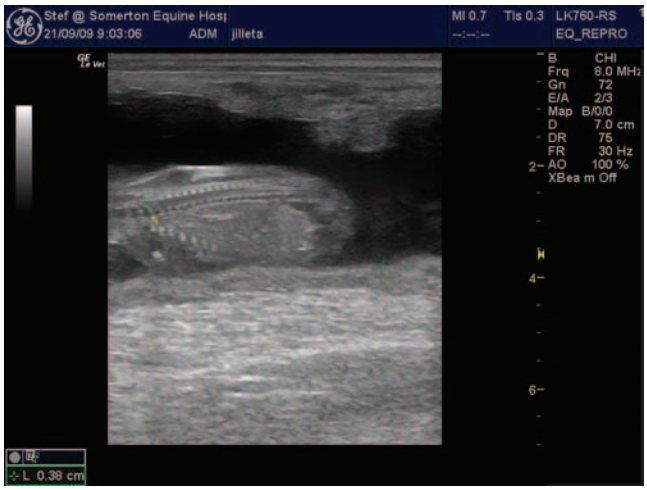

Fig. 5. Detailed sonographic image of a 75 day-old fetus engaged within the uterine horn, in close proximity to the transducer (linear 8 MHz).

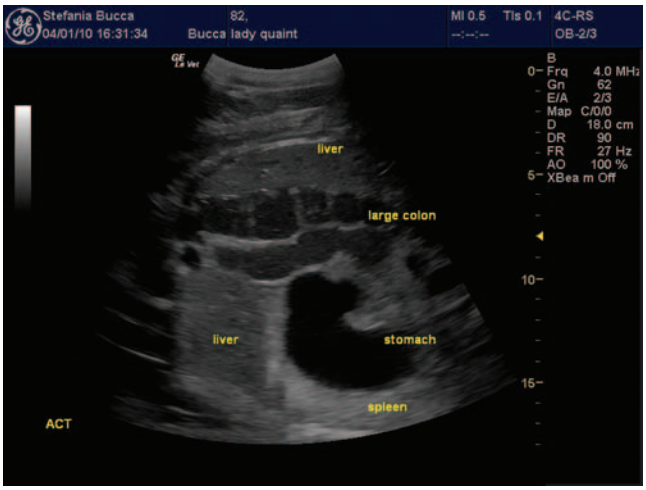

Fig. 7. Transabdominal scan of the abdominal content of a 7 month-old fetus (4 MHz convex transducer).

Finally, fetal gender determination in advanced gestation can be carried out during summer, fall, or early winter, at a more convenient time of the year for the busy equine reproduction clinician.

Equipment

B-mode, real-time portable scanners, equipped with 5 to 7.5 MHz linear-array transducers are commonly employed for assessment per rectum. Linear, sector, or convex 3.5 to 6 MHz transducers can be used transabdominally up to day 260, depending on the size of mare and fetus, the thickness of the mare's ventral abdomen, and the stage of gestation. Occasionally, a 2.5 MHz transducer may be required for adequate visualization of the relevant fetal structures. Doppler technology offers a valuable additional tool in the evaluation of the fetal gonad, both in transrectal and transabdominal scans.[8]

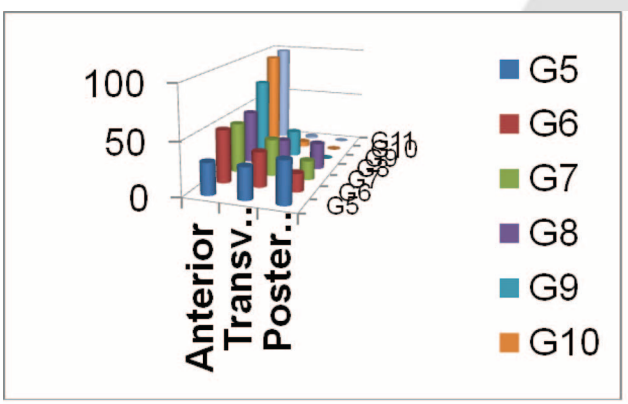

Fig. 6. Incidence of fetal presentation throughout gestation; on x-axis month of gestation, and on y-axis percentage of observed fetal presentation.[7]

Techniques

Transrectal sonographic viewing of the equine fetus requires standard rectal palpation skills as per routine ultrasound (US) examination of the mare's reproductive tract. Thorough cleansing of the mare's abdomen is necessary for diagnostic percutaneous US evaluation. Mares are best examined in stocks and although not usually required, sedation of the mare in advanced gestation reduces fetal activity and lowers the fetus towards the ventral abdomen, enhancing transabdominal imaging. Sedation is contraindicated when a trans-rectal approach is adopted. Clipping, shaving, alcohol, and/or coupling gel application can optimize percutaneous imaging. During the summer months, excellent imaging can be obtained by simply sponging alcohol over the unclipped hair of the ventral abdomen.

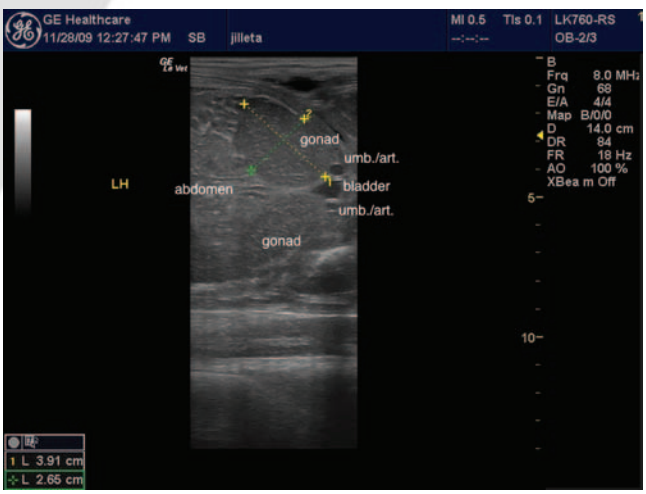

Fig. 8. Transrectal view of a 108 day-old female fetus in posterior presentation: right of the sonogram mare's caudal (8 MHz linear transducer).

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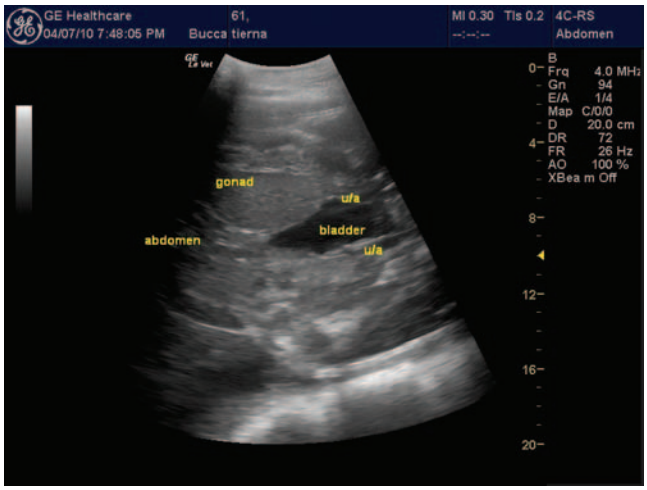

Fig. 9. Transabdominal frontal view of a 7 month-old fetus, showing the gonad in close proximity to the bladder and the associated umbilical artery (4 MHz convex transducer).

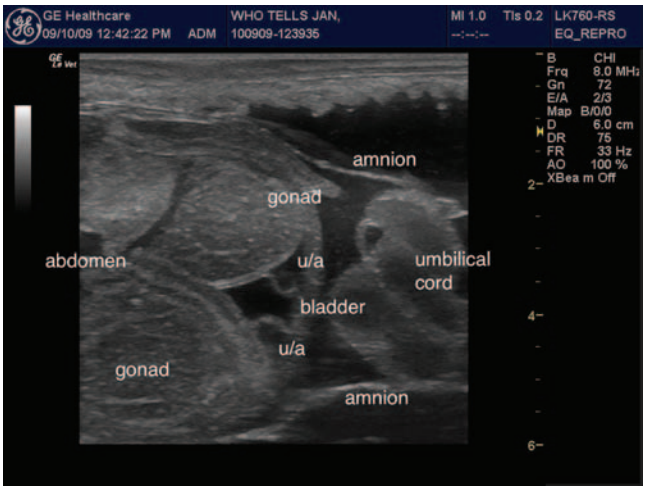

Fig. 11. Transrectal scan of a 110 day-old filly fetus in transverse presentation, where both gonads can be seen in cross sectional view (8 MHz linear transducer).

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Orientation of the sonogram should be initially established in order to identify the scanning direction on the screen of the ultrasound unit. The transducer is then advanced along the sagittal plane within the rectum or over the ventral abdominal wall until fetal parts are recognized. The fetal heart and chest will be located first, as they are easily identified; cranial and caudal orientation of the fetus will be determined next. Fetal head and neck indicate cranial while the bean-shaped, echolucent stomach silhouette is used as a landmark for caudal orientation (Fig. 7). Dorsal and ventral orientation is established by identifying the spinal cord and, opposite to that, the umbilicus. Fetal presentation and position will subsequently be defined. As the equine fetus tends to be a rather dynamic entity, temporary obstruction of the view will intermittently occur due to superimposing umbilical cord loops or fetal bony structures (mostly pelvic limbs) casting acoustic shadows over the areas of interest. Quite often, during early stage diagnosis, the fetus will just disappear from the acoustic field. Considerable time and patience are required during the learning process curve, but an experienced operator will accomplish diagnosis in a short frame of time, ranging between 30 s and 5 min.

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Gender determination is made by scanning of the caudal fetal abdomen, hindquarters, and buttocks to identify the position of the genital tubercle or the anatomical structure of primary sex organs. Frontal, cross-sectional and oblique scanning planes may all be required to obtain adequate visualization of

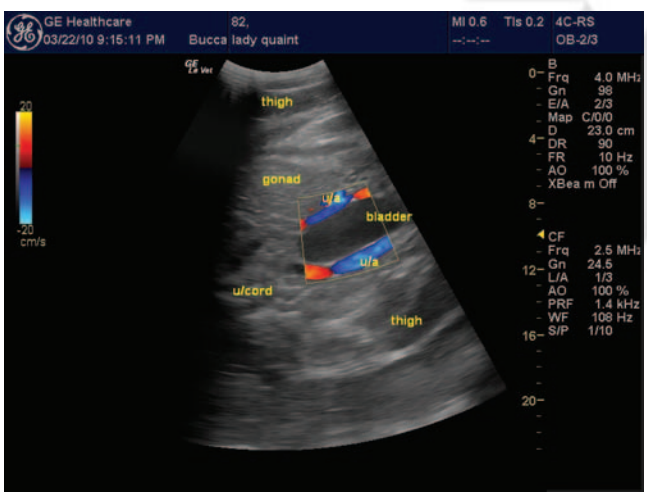

Fig. 10. Transabdominal frontal view of the caudal end of a 7 month-old filly fetus (4 MHz convex transducer). Doppler color flow identifies the two umbilical arteries adjacent to the bladder.

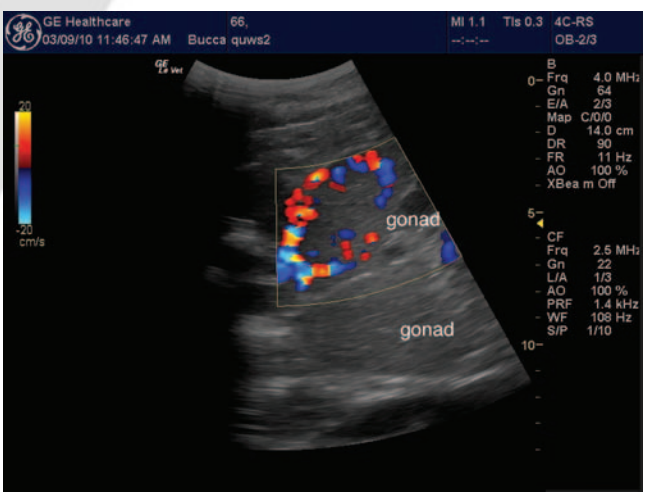

Fig. 12. Strong color Doppler signal over the peripheral layer (ovarian medulla) of an 8 month-old female fetus (transabdominal examination, 4 MHz convex transducer).

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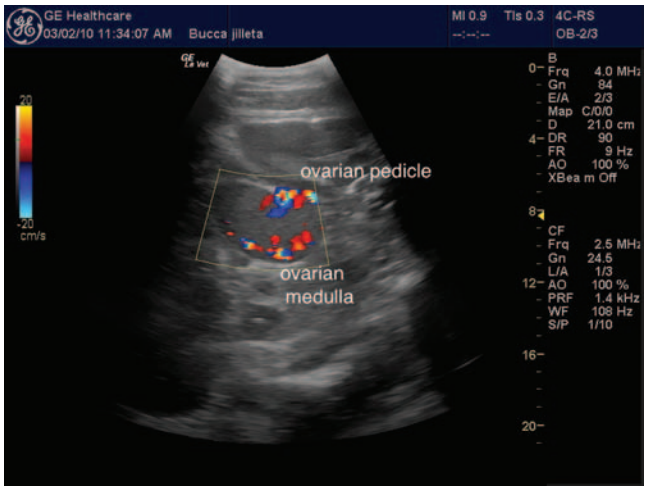

Fig. 13. A strong color Doppler signal is detected over the ovarian pedicle and partially on the peripheral layer of this 7 month-old filly fetus (transabdominal examination, 4 MHz convex transducer).

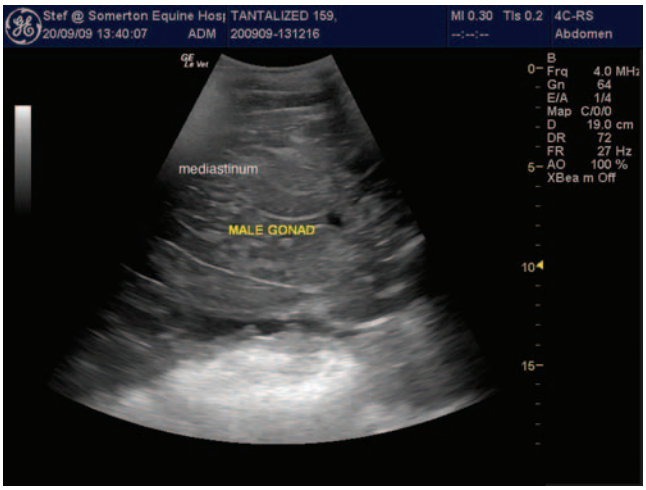

Fig. 15. Transabdominal scan of a 5 month-old fetus. The gonad shows a typical male structure, with a hyper-echoic, longitudinal, central line (mediastinum) (4 MHz convex transducer).

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diagnostic parameters, particularly during early fetal gender determination.

Diagnostic Parameters in Advanced Gestation

Fetal gonads are easily identified within the caudal abdomen as two symmetrical oval structures, ventral to the kidneys, with an oblique orientation of their long axis, converging caudally towards the pelvic inlet (Fig. 8). The caudal poles of the gonads are adjacent to the bladder and in close contact with the abdominal tract of the umbilical arteries (Fig. 9, 10). The fetal gonads represent an excellent landmark within the caudal abdomen and show a distinctive echotexture that differs from male to female,[8] adding great diagnostic value when assessed during the course of a fetal sexing examination. A marked diversity in echotexture can be appreciated in the female gonad between cortex and medulla (Fig. 11), with intense color Doppler signal over the outer band of tissue (Fig. 12), demonstrating high vascularization of the peripheral area. An additional area of intense color Doppler signal can be visualized on the outer lateral portion of the female gonad, corresponding to the ovarian pedicle (Fig. 13). Male gonads appear uniformly echodense, with a small outer dotted area (Fig. 14) and a hyperechoic longitudinal, central line (mediastinum) (Fig. 15). Intense color Doppler signal is detected in these two areas, as they correspond respectively

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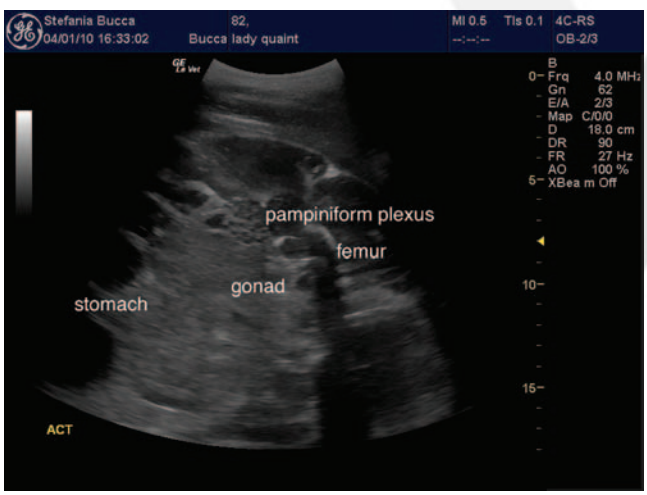

Fig. 14. Transabdominal scan of an 8 month-old male fetus. An outer dotted area can be identified over the lateral aspect of the gonad, corresponding to the pampiniform plexus (4 MHz convex transducer).

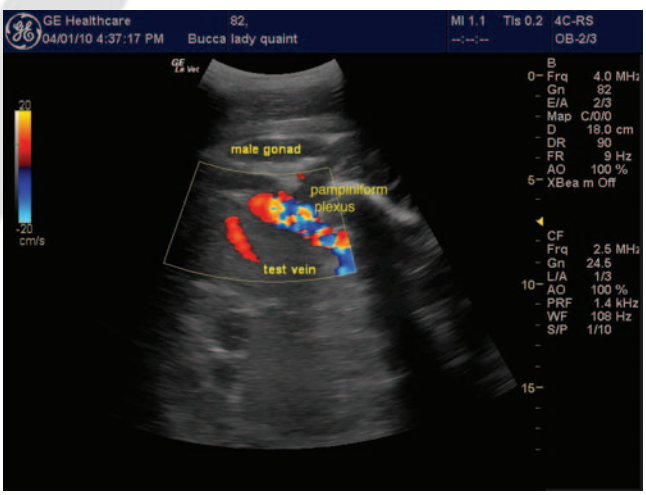

Fig. 16. Same fetus as in Fig. 14 when color Doppler technology was applied to the ventral pelvic area. A strong color signal identifies the location of the pampiniform plexus and, within the gonad, the testicular vein (4 MHz convex transducer).

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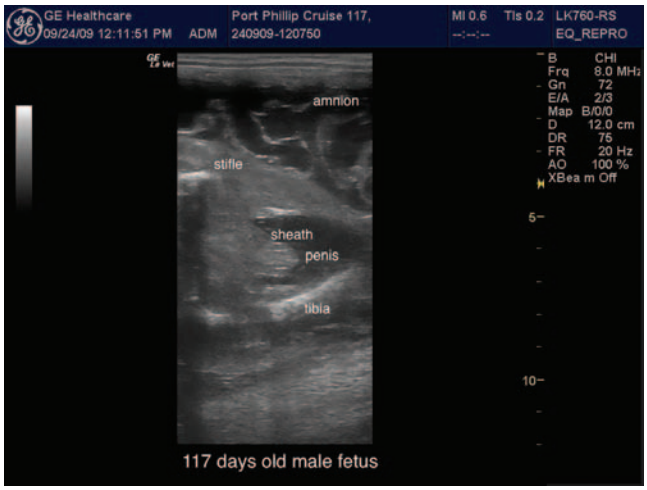

Fig. 17. Transrectal scan of the pelvic area of a 117 day-old male fetus in transverse presentation (8 MHz, linear transducer). The penis appears encased within the sheath.

Fig. 19. Transabdominal view of the pelvic area of an 8 month-old male fetus. The urethra can be seen running over the full length of the flaccid penis, as a hyperechoic, linear structure (4 MHz convex transducer).

to the pampiniform plexus and the testicular vein (Fig. 16). The pampiniform plexus appears larger than the ovarian pedicle and displays a more intense color Doppler signal.

The fetal primary sex organs may be clearly identified on ultrasound as early as 100 days gestation. In the male fetus, a fully comprehensive gender diagnosis will include the identification of: penis and prepuce (Fig. 17, 18, 19), scrotum/testicular compartments (Fig. 20; 21), urethra (Fig. 22), and gonads. The penis is visualized in the ventro-caudal abdomen, just behind the umbilicus. Sometimes the umbilical cord's strong pulsatile activity causes a passive bouncing motion to the penile shaft that is resting over the umbilical arteries (Fig. 23) The penis may be partially or completely encased within the prepuce but can often appear fully extended and occasionally erect. In the erect penis, the urethra is visualized in cross section as a distinct circular hyperechoic structure (Fig. 24). The urethra can be easily visualized along the ventral shaft of the flaccid or erect penis as a double hyperechoic line. Longitudinal and cross-sectional images of the urethra can be obtained over the male perineum. The fetal scrotum displays a composite echodensity, as the scrotal compartments appear as two symmetrical, oval, less echodense areas (Fig. 20). The hypoechoic appearance of each scrotal compartment relates to the presence of the adjacent gubernaculum testis. The male fetal gonads appear uniformly echodense with a hyperechoic cen-

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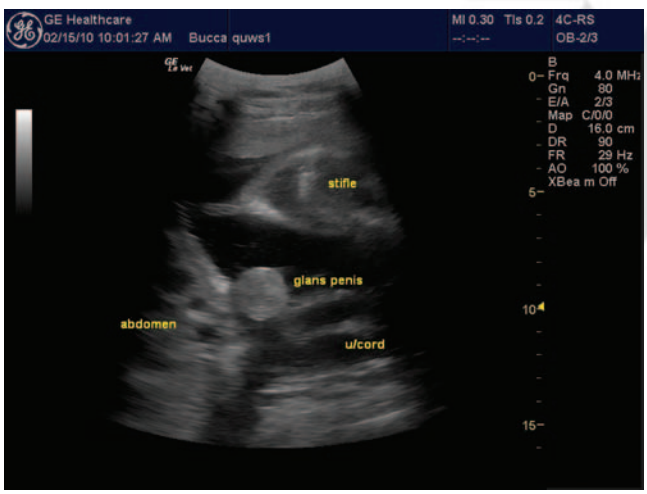

Fig. 18. Transabdominal view of the pelvic area of a 7 month-old male fetus. Note the close association of the erected penis in cross section over the umbilical cord (4 MHz convex transducer).

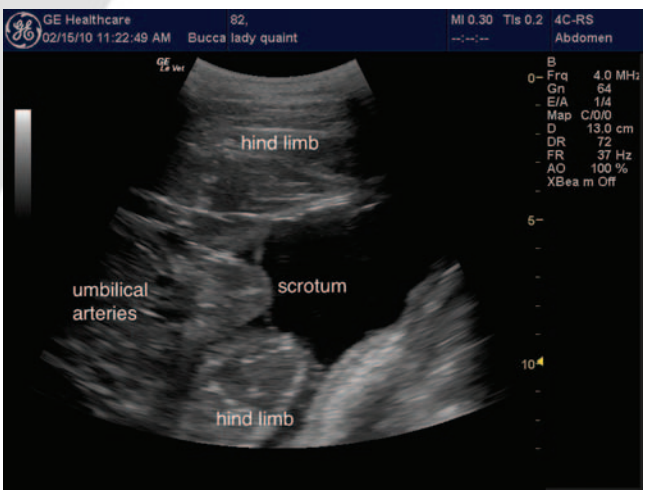

Fig. 20. Transabdominal view of the pelvic area of a 6 month-old male fetus. The scrotum bulges out ventrally with a prominent midline raphe (4 MHz convex transducer).

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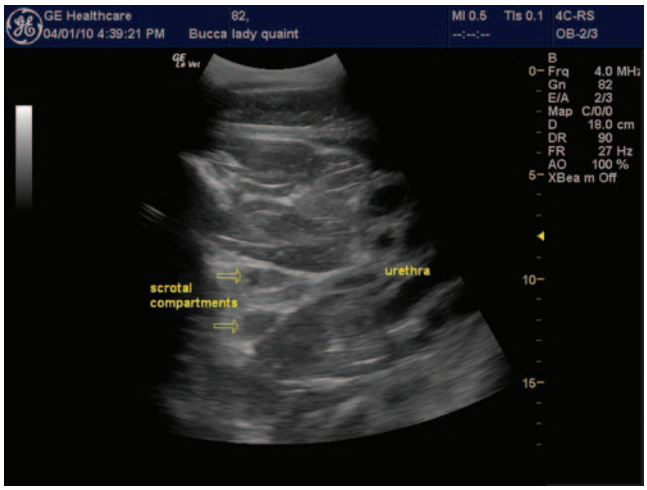

Fig. 21. Transabdominal view of the pelvic area of a 5 month-old male fetus. The perineal urethra merges into the testicular compartments (4 MHz convex transducer).

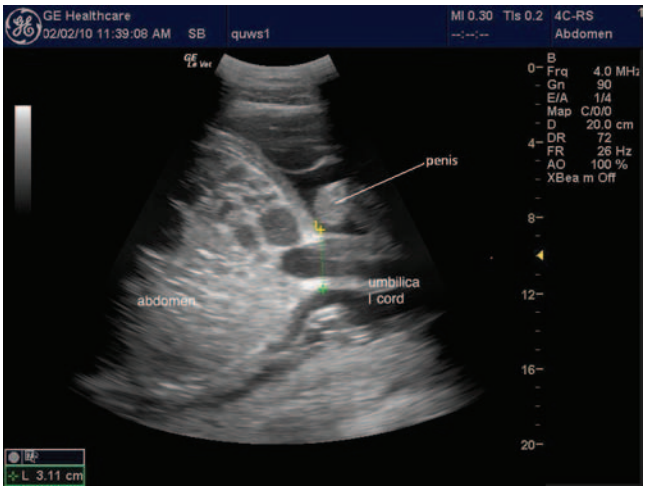

Fig. 23. Transabdominal view of the pelvic area of a 7 month-old male fetus. The penis rests over the umbilical cord (4 MHz convex transducer).

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tral line, correspondent to the testicular vein, as described above.

In the female fetus, the primary sex organs to be visualized to reach diagnosis include: mammary gland, nipples, vulva/clitoris, and gonads. The fetal mammary gland can be visualized in the pubic region and appears triangular (Fig. 25, 26) or trapezoidal (Fig. 27) in shape and uniformly echodense. The nipples emerge from the ventral border of the mammary gland as relatively large hyper-echodense areas (Fig. 28). No relevant structures can be visualized over the ventral perineum (Fig. 26), as opposed to the male fetus, where the urethra runs the entire length up to the anus (Fig. 22). The fetal clitoris is a hyperechoic structure that bulges out of the buttocks (Fig. 29). It is positioned high up in the perineum but should not be confused with the anus, adjacent to the tailhead. The vulvar commissure can be seen coursing between the anus and the clitoris in a cross-oblique section of the fetal buttocks (Fig. 30).

Diagnosis

Diagnosis by a single exam per rectum is rapidly attained when the fetus is in posterior presentation, even up to 8 months gestation. The rate of positive diagnosis per rectum reaches 100% between 110 and 130 days gestation, with an estimated time of less than 150 s.[9] In transverse presentation, gender determination per rectum is easily accomplished

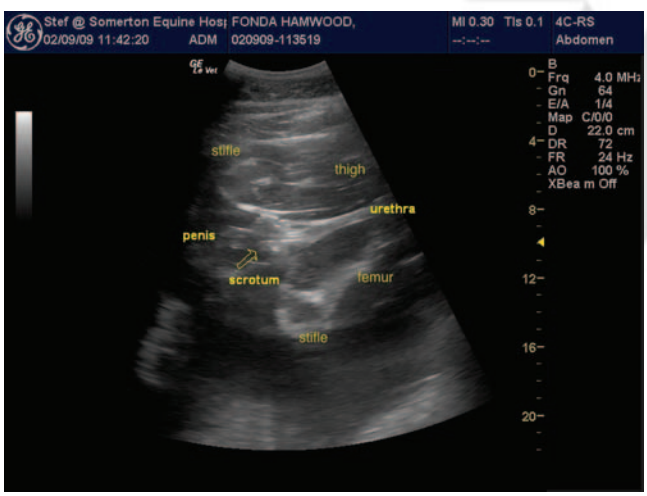

Fig. 22. Transabdominal view of the perineum of a 5 month old male fetus. The urethra fills out the perineal area as a double line structure, in longitudinal section (4 MHz convex transducer).

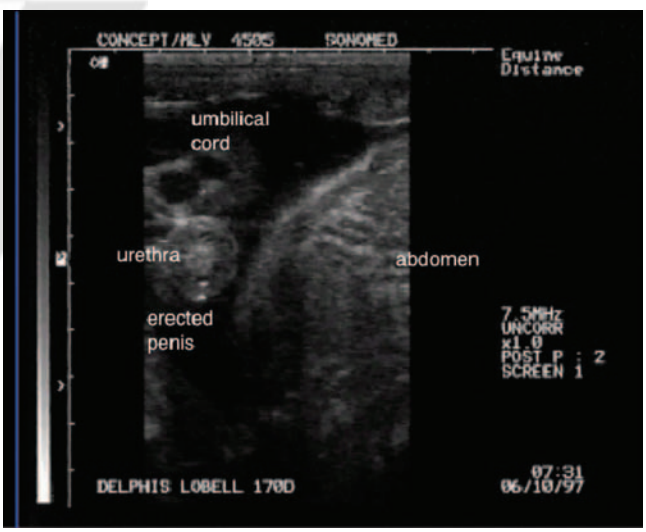

Fig. 24. Transrectal scan of a 170 day-old male fetus in transverse presentation. The urethra can be viewed as a hyperechoic circle in a cross-sectional view of the erected penis (7.5 MHz linear transducer).

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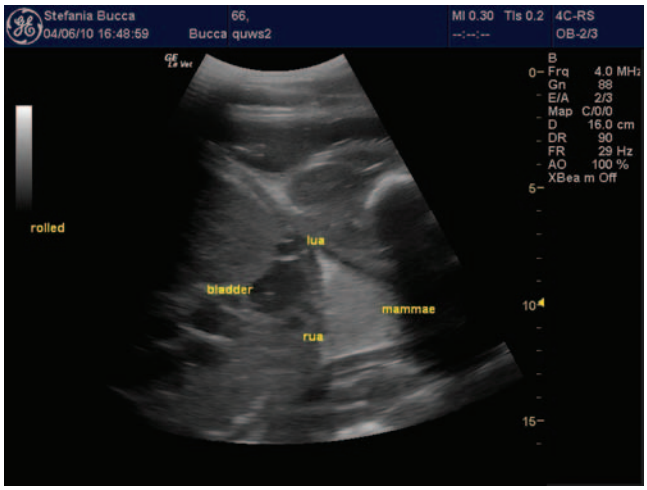

Fig. 25. Transabdominal view of the pelvic area of an 8.5 month-old female fetus. The mammary gland appears as a hyperechoic triangular structure (4 MHz convex transducer).

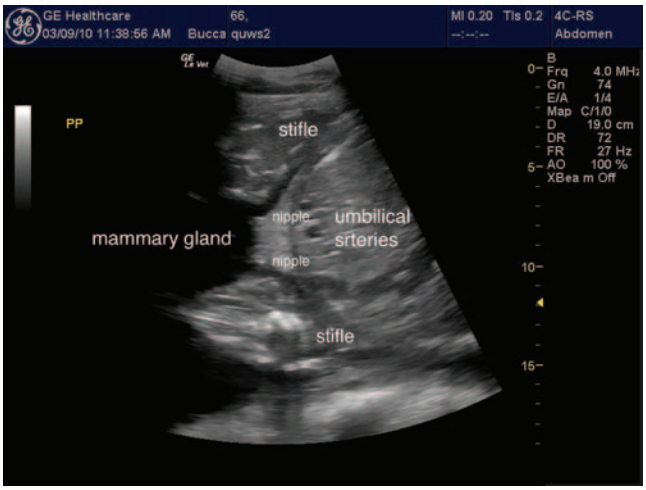

Fig. 27. Transabdominal view of the pelvic area of an 6.5 month-old female fetus. The mammary gland looks like a trapezoidal shaped structure and the nipples emerge as hyperechoic projections of its ventral border (4 MHz convex transducer).

when the fetus assumes a ventrocaudal position within the mare's pelvis. In anterior presentation, the fetal hindquarters can be visualized transrectally up to 5 months gestation according to fetal size and location within the uterus. Rotation of the fetus over the long and short axis is commonly observed up to 8 months of pregnancy, and frequent changes of presentation occur around 5 to 6 months. At this time, repeating the exam 5 to 10 min later may find the fetus in a more advantageous position for diagnosis. A transabdominal approach is usually necessary for gender determination over 5 months of gestation, when the fetus lies in anterior presentation.

Good knowledge of fetal anatomy and rapid identification of fetal parts are essential for ease of diagnosis. Diagnosis in advanced gestation should be based on at least three identified parameters. The time required to conduct a diagnostic examination is 1 to 5 min for the experienced examiner. Variability depends on the difficulty encountered in visualizing the area of diagnostic interest, and a very active fetus generally makes a poor candidate for a rapid diagnosis. Video recording of scans provides opportunities for further studies and detailed evaluations.

Finally, proper identification of the mare at the time of examination and the provision of a signed certificate of fetal gender diagnosis should be an integral part of the service offered.

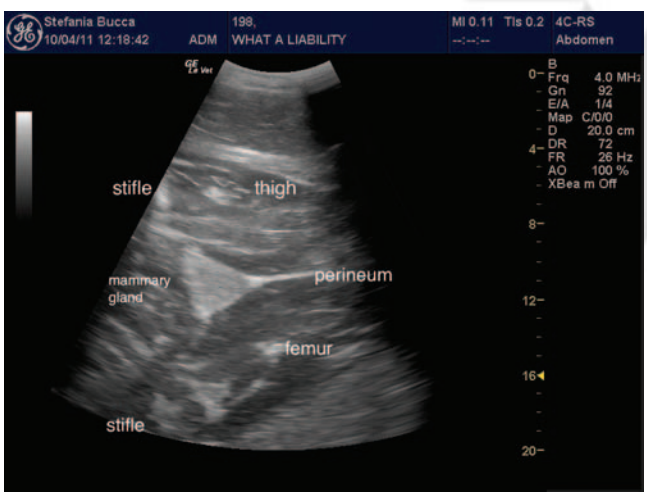

Fig. 26. Transabdominal view of the pelvic area of an 198 day-old female fetus, showing the mammary gland and the perineum (4 MHz convex transducer).

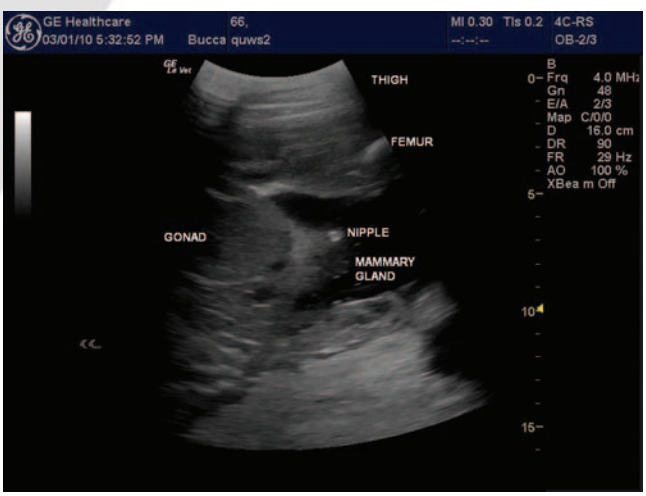

Fig. 28. Transabdominal view of the pelvic area of a 6 month-old female fetus showing the mammary gland and one nipple (4 MHz convex transducer).

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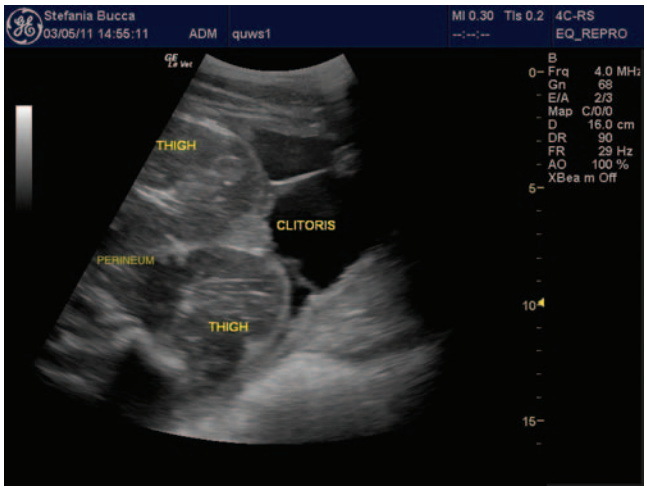
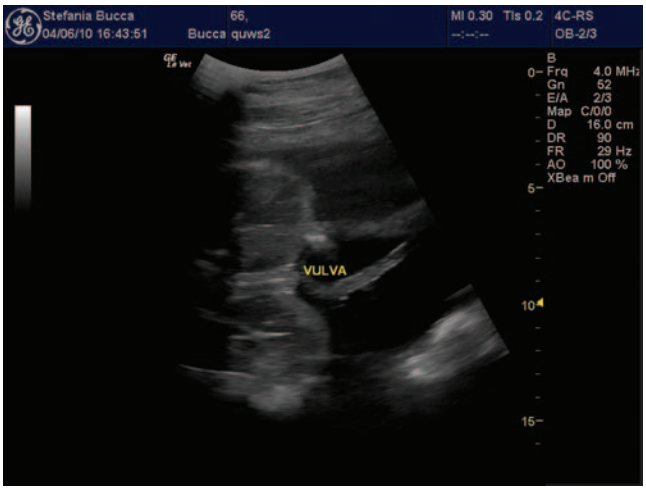


Fig. 29. Transabdominal view of the perineal area of an 5.5 month-old female fetus. The clitoris bulges out of the buttocks (4 MHz convex transducer).



COLOR

Fig. 30. Transabdominal view of the perineal area of an 7 month-old female fetus, displaying the vulvar lips and the clitoris (4 MHz convex transducer).

References

1. Curran S, Ginther OJ. Ultrasonic diagnosis of equine fetal sex by location of the genital tubercle. *J Vet Sci* 1989;9:77–83.
2. Curran S, Ginther OJ. Ultrasonic fetal gender diagnosis during months 5 to 11 in mares. *Theriogenology* 1993;40: 1127–1135.
3. Renaudin CD, Gillis CL, Tarantal AF, ~~Coleman DA~~. Ultrasonographic evaluation of equine fetal growth from 100 days gestation to parturition. In: *Proceedings*. 7th Int Symp Equine Reprod 1998;171–172.
4. Renaudin CD, Gillis CL, Tarantal AF. Transabdominal ultrasonographic determination of fetal gender in the horse, during mid-gestation. *Equine Vet J* 1999;6:483–487.
5. Holder RD. Fetal sex determination in the mare between 55 and 150 days gestation. ~~In: *Proceedings.*~~ Am Assoc Equine Pract 2001;321–324.
6. Ginther OJ. Equine pregnancy, physical interaction between the uterus and conceptus. ~~In: *Proceedings.*~~ Am Assoc Equine Pract 1998;44:73–103.
7. Bucca S. Equine fetal gender determination from mid to advanced gestation by ultrasound. *Theriogenology* 2005;64: 558–563.
8. Bucca S. Fetal gender determination from mid gestation to term. In: AO McKinnon, EL Squires, WE Vaala and DD Varner, eds. *Equine Reproduction*. 2nd ed. New York, NY: Blackwell Publishing Ltd; 2011:2094–2098.
9. Livini M. Determination of fetal gender by transrectal ultrasound examination: Field's experience. ~~In: *Proceedings.*~~ Am Assoc Equine Pract~~,~~ 2010:323–327.

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES **1**

1—Author: Table 1 was converted to Figure 6 and inserted here. The remaining figures were renumbered consecutively. Please confirm this placement. OK

2—Author: Please check: Did you mean "mediastinum" here?
Yes. Mediastinum testis is the anatomical term for the fibrous connective layer that traverses the testicle through its center