Introduction
Fetal gender determination is becoming common practice with expensive horses because it allows professional breeders to predict their budget, plan sales and decide early which stallion to book for the following breeding year. Two ultrasound methods are available for fetal gender determination. The first, described 10 years ago by Curran and Ginther [1, 2], is a reliable method for early diagnosis and uses the transrectal approach. The second, reported more recently by Renaudin, Gillis and Tarantal [3, 4, 5] is extremely accurate during mid gestation and uses a combination of transrectal and transabdominal ultrasonographic approaches. The objective of this paper is to give a practical overview of each of the two ultrasonographic methods currently available for equine fetal gender determination.

Early diagnosis of fetal gender
Anatomic principle - Early determination of fetal gender is based upon the identification and the location of the genital tubercle. The genital tubercle is the embryonic structure that differentiates into the penis in males and into the clitoris in females. During differentiation, the anogenital distance (distance from the anus to the genital tubercle) increases greatly in the male but not in the female. The genital tubercle changes in relative location from its initial position between the rear limbs to the proximity of the umbilical cord in the male and remains near the tail in the female.

Ultrasound equipment - This method requires a high quality real-time B-mode diagnostic scanner equipped with a 5 MHz linear array transducer. Most ultrasound equipment used routinely in equine reproduction is suitable for this procedure.

Method - The mare should be restrained in an examination chute, and the scanner screen placed in close proximity and at eye level for the examiner. Feces are removed from the rectum, and the probe is introduced into the rectum at the level of the uterus. Three sequential views of the fetus can be used to determine its gender:

- cross-sectional views (transverse plane: a plane perpendicular to the median plane). The probe is placed across the fetus and fetal examination should begin at a landmark such as the head (Fig. 1a) or the beating heart (Fig. 1b). The transducer is moved caudally through the area of the umbilical cord abdominal insertion (Fig. 1c). If the fetus is a male, a hyperechoic bilobed structure (male genital tubercle) is immediately imaged behind the umbilical cord abdominal insertion (Fig. 1d). In case of a female, the fetal examination is continued to the rear limbs and tail area. A hyperechoic bilobed structure (female genital tubercle) is seen below the tail (Fig. 2).

- frontal views (a plane perpendicular to both median and transverse planes). In these views, the legs, the umbilical cord, the tail and the genital tubercle are seen in cross section. The umbilical cord, the tail and hind legs have to be identified in the same image. The hyperechoic structure located between the tail and the umbilical cord is the genital tubercle. It is immediately caudal to the umbilical cord in males and under the tail in females.
- *sagittal views* (a plane parallel to median plane). The fetus is seen in a longitudinal section. Fetal landmarks are easily identified. The same principle as above is used to determine fetal gender.

In my experience, cross-sectional views are the easiest ones to get and interpret. Trained examiners just need a few minutes to diagnose fetal gender. However, when one of these three views cannot be obtained within ten minutes, it is wise to stop the examination and try again later when the fetus is better positioned.

Figure 1a. 63-day-old male fetus: cross-sectional views of the head (the nose is to the left and the cranium to the right of the ultrasound image). To view this image in full size go to the IVIS website at www.ivis.org.

Figure 1b. 63-day-old male fetus: cross-sectional views of the cranial thorax showing the cardiac cavity (CC) filled with semi-echoic fluid and one front limb (FL). To view this image in full size go to the IVIS website at www.ivis.org.

Figure 1c. 63-day-old male fetus: cross-sectional views of the abdomen (A) at the level of the umbilical cord abdominal insertion. UC: umbilical cord. To view this image in full size go to the IVIS website at www.ivis.org.

Figure 1d. 63-day-old male fetus: cross-sectional views of the abdomen at a level immediately caudal to the umbilical cord abdominal insertion showing the genital tubercle (GT) and one hind limb (HL). To view this image in full size go to the IVIS website at www.ivis.org.
Results - This method has been shown to be extremely accurate. The authors Curran and Ginther were correct in their fetal gender diagnosis in 97% (138/143) of diagnoses of males and in 100% (92/92) of diagnoses of females when they could locate the genital tubercle relative to the tail and umbilical cord. The errors that occurred were in the first few horses examined and were attributed to operator inexperience. The optimal days for fetal gender determination using this method are between day 59 and day 68 of gestation. During this time, the fetus is most accessible and the genital tubercle identifiable and assignable to a location characteristic of either male or female. Prior to day 53, the genital tubercle cannot be identified primarily due to the small size of the fetus. After day 68, fetuses start to be deeply situated in the mare and are not adequately accessible via transrectal ultrasonography.

Mid-gestation diagnosis of fetal gender

Mare preparation - Mares are placed in stocks in order to comfortably restrain them. This way, sedation is often not necessary. Unless the hair is very short, clipping the ventral abdomen from the mammary glands to the xyphoid and widely on both sides is necessary. Afterwards, the area is cleaned with water and then with alcohol in order to remove dust or debris, which reflect ultrasound and impair image quality. Finally, a good quality ultrasonographic coupling gel is applied onto the cleaned area.

Ultrasound equipment - A good quality real-time, B-mode, diagnostic scanner equipped with a 5 and a 3.5 MHz linear probe is necessary. The 5 MHz linear array transducer is used with fetuses ranging in age from 100 to 160 days gestation. With older fetuses, a 3.5 MHz probe is necessary in order to obtain greater depth penetration. Sector transducers can also be used, but linear arrays are preferable because they provide a larger surface of contact and thus a greater footprint. It is also easier for inexperienced ultrasonographers to understand fetal orientation in relation to the position of the transducer with a linear array transducer.

Method

Locate the fetus - The transducer is first placed cranial to the mammary glands, on the ventral midline. Usually, parts of the fetus and fetal fluids can be readily identified in this location, immediately dorsal to the uterus and the placenta. If none of these structures are imaged, the transducer is moved abaxially from the ventral midline. With young fetuses located high in the abdomen, it is necessary to place the transducer in the inguinal area, just above the mare's mammary glands. Once the fetus is located, the transducer is moved over the abdomen according to the anatomy of the fetus with the goal of locating the hindquarter.

Identify anatomical landmarks - Ribs are usually easy to identify sonographically in either longitudinal or frontal views of the fetus (Fig. 3a) because they have a round hyper echoic appearance. Once the ribs are located, the probe is rotated to obtain a cross-sectional view of the fetus. In the fetal thoracic region, the heart and the lungs are seen. With movement of the probe caudally, the lungs, liver and diaphragm are observed (Fig. 3b). In the cranial abdomen, the stomach is noted to be filled with black, anechoic fluid (Fig. 3c). On the dorsal aspect of the fetal abdomen, the spleen and kidneys are then identified. On the ventral portion of the fetal abdomen, the umbilical cord abdominal insertion (Fig. 3d) and the fetal gonads are located.
Figure 3a. Longitudinal view of a fetal thorax showing the round hyperechoic appearance of the ribs (R). To view this image in full size go to the IVIS website at www.ivis.org.

Figure 3b. Cross-sectional view of a fetal thorax showing the lungs (LU), diaphragm (D) and liver (LI). To view this image in full size go to the IVIS website at www.ivis.org.

Figure 3c. Cross-sectional view of a fetal abdomen at the level of the stomach (S). To view this image in full size go to the IVIS website at www.ivis.org.

Figure 3d. Cross-sectional view of a fetal caudal ventral abdomen at the level of the umbilical cord abdominal insertion. To view this image in full size go to the IVIS website at www.ivis.org.

Identify fetal gender - To identify fetal gender, cross-sectional views are mainly used. Male gender is determined based on the presence of the prepuce or the penis immediately caudal to the umbilical cord abdominal insertion (Fig. 3e). Female gender identification is based on the presence of the mammary glands and teats (Fig. 4a) and/or the characteristic ultrasonographic appearance of the fetal gonads, which contain a circular echo prior to 133 days of gestation (Fig. 4b).
Results - This method is very reliable. There is 100% agreement in gender determination between the results obtained by ultrasonography (n = 98) and those obtained at birth. In males, the penis and/or the prepuce are routinely imaged from 100 days to 220 days gestation. The prepuce or penis on cross-sectional views has a round shape with parallel linear echogenic foci up to approximately 140 days and then appeared triangular. The penis is often pendulous particularly during fetal motion. The penis and prepuce are often difficult to distinguish from each other on the ultrasound image except during a penile erection. The male gonads are oval in shape and measure 2 to 7 cm in length, depending on the stage of gestation. They are situated in the caudal ventral abdomen along the thighs on frontal views. Their echogenicity is similar to that of the fetal liver. They appeared homogeneous with a thin longitudinal straight and central echogenic line that is not consistently present after 125 days. In females, the mammary glands and teats are observed from 118 days to 227 days of gestation. The female gonads are oval and similar in size and location to the male gonads. However, the presence of the circular echo within the fetal ovary allows making a diagnosis of female gender from 100 days to 133 days of gestation. Most gender diagnoses can be performed using the transabdominal approach. However, prior to 183 days of gestation, the transrectal approach is helpful in some cases where fetuses are either located too high in the mare’s abdomen or in a posterior presentation to be readily imaged. The 5 MHz linear transducer, routinely used in equine reproduction, allows gender diagnosis up to 160 days.
gestation in most cases. When the fetus is located too high in the mare's abdomen or when its size exceeds the capacity of the ultrasound equipment, a 3.5 MHz probe has to be used. Both ultrasound techniques described above are very accurate, provided gender identification is made during the optimal window of time, with recognition of the correct anatomical structures. Considerable experience and training in ultrasonography are required for accurate fetal gender determination. Because there is no mare preparation time, the transrectal technique to visualize the genital tubercle has the advantage of being fast. However, the second technique has a much wider optimal window of time (100 to 220 days gestation vs 59 to 68 days gestation) therefore allowing rechecks to confirm or to make a final diagnosis. The transabdominal approach is very safe, the risk of rectal tear is avoided, and it is advantageous for small mares, such as American Miniature horses and ponies when rectal palpation is extremely difficult or even impossible. While determining the sex, one may take the opportunity to perform a complete fetal evaluation and assess fetal well being as well as fetal growth.

References


5. Renaudin CD, Gillis CL, Tarantal AF. Transabdominal ultrasonographic determination of fetal gender in the horse, during mid-gestation. Video tape, UC Davis School of Veterinary Medicine, Office of the Dean, Academic programs 1999. - U.C.Davis -

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