# Fetal sexing in early and advanced gestation: more than just a genital tubercle

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# Introduction

Fetal gender determination in the mare provides a useful management tool to breeders, by allowing a pre-delivery 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.

# Diagnostic Windows for Fetal Gender Determination

# First Stage Diagnosis: Early Gestation

There are two different stages when fetal sex diagnosis can be made by ultrasonography. The first stage is between 57 and 70 days gestation and involves the identification of the genital tubercle by trans-rectal ultrasonography. The genital tubercle is the precursor of the penis in the male and the clitoris in the female and, around day 55 gestation, appears 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 and towards the umbilical cord in the male. The shape of the genital tubercle may change in time into tri-lobed or conical

Disadvantages: Small diagnostic window, a single diagnostic parameter and service required usually at peak time in the breeding season.

#### 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 and multiple parameters to validate diagnosis (fetal primary sex organs), but may require a combination of trans-rectal and transabdominal ultrasound scanning. Furthermore, 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.

#### Techniques

Trans-rectal sonographic viewing of the equine fetus requires standard rectal palpation skills, as per routine ultrasound (US) examination of the mare's reproductive tract. Thorough skin preparation of the mare's abdomen is necessary for diagnostic percutaneous US evaluation. Mares are best examined in stocks and although tranquillisation is not usually required, sedation of the mare in advanced gestation reduces fetal activity and lowers the fetus towards the ventral abdomen, enhancing trans-abdominal imaging. Sedation is contraindicated when a trans-rectal approach is adopted.

Cranio-caudal and dorso-ventral orientation of the fetus should be initially established. Gender determination is then made by scanning of the caudal fetal abdomen, hindquarters, and buttocks to identify the position of the genital tubercle or the anatomical structure of the primary sex organs. Frontal, cross-sectional and oblique scanning planes may all be required to obtain adequate visualization of diagnostic parameters, particularly during early fetal gender determination.

# Diagnostic Parameters in Advanced Gestation

Fetal gonads are 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. The fetal gonads show a distinctive echotexture that differs from male to female. A marked diversity in echotexture can be appreciated in the female gonad between cortex and medulla with intense peripheral color Doppler signal. Male gonads appear uniformly echodense, with a small outer dotted area (the pampiniform plexus) and a hyperechoic longitudinal, central line (medianistinum). Intense color Doppler signal is detected in these two areas, as they correspond respectively to the pampiniform plexus and the testicular vein.

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, scrotum/testicular compartments, urethra and gonads. The penis is visualised in the ventro-caudal abdomen, just behind the umbilicus, may be partially/completely encased within the prepuce or appear fully extended and occasionally erect. The urethra can be easily visualised along the ventral shaft of the flaccid or erect penis as a double hyper-echoic line. The fetal scrotum displays a composite echodensity, as the scrotal compartments appear as two symmetrical, oval, less echodense areas. The hypo-echoic appearance of each scrotal compartment relates to the presence of the adjacent gubernaculum testis.

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 visualised in the pubic region and appears triangular or trapezoidal in shape and uniformly echodense. The nipples emerge from the ventral border of the mammary gland as relatively large hyper-echodense areas. No relevant structures can be visualised over the ventral perineum, as opposed to the male fetus, where the urethra runs the entire length, up to the anus. The fetal clitoris is a hyper-echoic structure that bulges out of the buttocks, high up in the perineum. The vulvar commissure can be seen coursing between the anus and the clitoris, in a cross-oblique section of the fetal buttocks.

#### 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 seconds. In transverse presentation gender determination per rectum is easily accomplished when the fetus assumes a ventro-caudal position within the mare's pelvis. In anterior presentation, the fetal hindquarters can be visualised trans-rectally 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 pregnancy and frequent changes of presentation occur around 5-6 months. At this time, repeating the exam 5-10 minutes later may find the fetus in a more advantageous position for diagnosis. A trans-abdominal approach is usually necessary for gender determination over 5 months of gestation, when the fetus lies in anterior presentation. 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.