Techniques to Assess Fetal Well-Being

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ABSTRACT: Indications for fetal examination can include prolonged gestation, placentitis, suspected twin pregnancy or fetal death, and maternal illness. Prolonged gestation in the mare (>350 days) may be caused by ingestion of endophyte-infected tall fescue or by certain physiologic factors, including foaling in the winter or early spring, the sex of the foal, the breed of horse, nutrition, and certain stallion effects. Fetal viability in late gestation may be assessed using transrectal or transabdominal ultrasonography. Ultrasound examination allows the determination of fetal heart rate and measurement of uteroplacental thickness. Measurement of mammary secretion electrolyte (calcium, sodium, and potassium) concentrations aids in predicting fetal maturity and is useful in determining when mares should be observed for impending parturition.

Equine veterinarians are frequently asked to evaluate pregnant mares that are approaching late gestation or that remain pregnant past their expected foaling date. Other indications for fetal examination are placentitis, suspected twin pregnancy or fetal death, and problems with the mare, such as pelvic fracture or severe illness. Horse owners should be very carefully asked about the definitiveness of the mare’s breeding date, the length of the mare’s previous gestations (if available), and whether the mare has access to endophyte-infected tall fescue pasture or hay. The endophytic fungus Neotyphodium coenophialum, which affects certain varieties of tall fescue, produces dopaminergic ergopeptide alkaloid toxins that result in several reproductive difficulties, including prolonged gestation, placental abnormalities, agalactia, and reduced foal survival.²³

A thorough physical examination and rectal palpation of the fetus and reproductive tract should be performed. A vaginal speculum examination may be useful in some mares if placentitis is suspected or to assess cervical relaxation. Several researchers²³⁴ have described ultrasonographic criteria that may be used to determine an equine biophysical profile that may predict the birth of a live, healthy foal. Ultrasonography is a useful and readily available diagnostic tool to assess the general health of the fetus and intrauterine environment.
DETERMINATION OF GESTATIONAL LENGTH

The average duration of gestation in the mare is generally cited as 335 to 342 days.5–7 The point at which gestation is considered prolonged varies and has been reported to range from more than 350 days8 to more than 360 days.6 Pregnancies of up to 400 days have resulted in normal foals.5,7,8 It is estimated that approximately 1% of equine pregnancies may be considered prolonged (lasting more than 350 days).8

Many physiologic and management factors have been found to influence gestational length in otherwise normal pregnancies. Colts tend to be carried an average of 2 to 3 days longer than fillies.5,7,8 Draft horses tend to have longer pregnancies than light horses.7,8 There are conflicting reports of individual stallion7,9 or mare8 influences on gestational length. In a study of 500 Standardbred pregnancies, gestation was consistently shorter than 340 days for foals by certain sires and consistently longer than 350 days for foals by other sires.7 The exact stallion effects that influence the length of gestation are unknown but may be related to fetal size. Gestation in mares of fifth or greater parity tended to be 2 days longer in one study.10 Another study that evaluated excessively prolonged gestation in 21 mares8 reported that twin pregnancies carried to term tended to be prolonged by an average of 9 days.

Mares that foal in the winter and early spring tend to have gestational lengths approximately 10 days longer than those of mares that foal in the summer.5,8,10 Mares bred early in the breeding season tend to have longer pregnancies.7 Gestation decreased by approximately 2.5 days per month in Standardbred mares that were bred during the physiologic breeding season (April through June) and foaled March through May.7

Ideally, broodmares should be maintained at a body condition score of 5/9 (the ribs are easily palpable but not visible). Severe undernutrition in the latter half of gestation increases gestation by an average of 4 to 10 days. Overfeeding mares in later pregnancy may reduce gestational length by a few days.8 Mares with higher body condition scores averaged a 4-day shorter pregnancy in one study.10 To avoid toxicity, pregnant mares should be removed from endophyte-infected tall fescue pastures and hay during the last trimester of gestation and no later than the last 30 days of gestation.1

Embryonic diapause (delayed embryonic development) is thought to occur in the mare.8 It is believed to occur before 35 days’ gestation and the attachment of embryonic villi to the endometrial crypts. Delays in embryonic development that corresponded with delayed endometrial cup formation have been reported, as has the detection of pregnant mare serum gonadotropin in mares with arrested embryonic development.8 The period of diapause lasts approximately 3 to 4 weeks. It is unknown whether a shortened embryonic diapause of only a few days is common in horses.

TRANSABDOMINAL ULTRASONOGRAPHY

Ultrasonography of the fetus may be conducted transrectally or transabdominally. During mid to late gestation, the fetal heart rate can be determined by transabdominal ultrasonography. A 2.5- to 3.5-MHz sector transducer or curved linear-array probe is preferable, but a 5-MHz linear-array probe may provide good visualization during late gestation. Examination should begin just cranial to the udder and progress cranioventrally. The fetus is often best visualized off to one side of the midline. The most useful landmark to identify the fetal thorax is the characteristic appearance of the fetal ribs along with their corresponding anechoic shadows (Figures 1 and 2).

After the fetal thorax has been identified, the contractions of the fetal heart can be visualized. Normal fetal heart rates determined by transabdominal ultrasonography have been reported to be 75 ± 7 bpm3 to 76 ± 8 bpm.4 The fetal heart rate tends to decrease as gestation advances. Mean fetal heart rates have been reported to decrease from 180 bpm at 100 days’ gestation to 60 to 80 bpm less than 2 weeks before parturition.11 Increased fetal activity may raise the fetal heart rate by 25 to 40 bpm for approximately 30 seconds.24 Intermittent fetal tachycardia (up to 200 bpm) of short duration (<1 minute) is a frequent and apparently normal finding in fetuses after 150 days’ gestation.12,13 Significant pro-
longed tachycardia may be an indicator of fetal stress. In a study of 30 pregnancies, five fetuses were found to have abnormal heart rates (either tachycardia or bradycardia); only one of the five foals was normal at birth. It has been suggested that a lack of heart rate responsiveness to fetal movement may be a more sensitive indicator of fetal distress than simple changes in baseline heart rate or range. Bradycardia or a lack of heart rate variability suggests depression of the fetal central nervous system and possible impending fetal death. We have observed that, as a very general rule, normal fetal heart rates range from 60 to 120 bpm, with most foals averaging around 80 bpm in late gestation.

Fetal movements are usually observed during transabdominal ultrasound examinations, especially during later gestation. However, periods of fetal quiescence are common. If a fetus appears to have decreased activity, it should be reexamined several times before drawing definitive conclusions. A previous study reported that inactive fetuses were abnormal foals that were affected with neonatal maladjustment syndrome or sepsis at birth. The echogenicity of fetal fluids normally increases throughout gestation due to the accumulation of vernix caseosa (desquamated epithelial cells and sebaceous secretions). Excessive echogenicity of fetal fluids can indicate placentitis (Figure 3) or fetal sepsis and has been found in early gestation in cases of mare reproductive loss syndrome.

**FETAL ELECTROCARDIOGRAPHY**

Electrocardiography to determine fetal heart rate, fetal death, or the presence of twins has been largely replaced by ultrasonography (Figure 4). Using fetal electrocardiography, the normal range of the fetal heart rate has been reported as 109 ± 15.5 bpm (gestational age: 180 to 330 days) and as 60 to 160 bpm. Parkes and Colles additionally reported that the normal fetal heart rate averages 100 bpm at 5 months’ gestation and is just above the maternal heart rate at term. Buss and colleagues obtained fetal electrocardiograms from 45 pregnancies and found that a fetal QRS complex could be detected in at least one lead in all mares; however, a definitive fetal QRS complex could not be detected in every lead examined.

**COMBINED UTEROPLACENTAL THICKNESS**

Measurement of the combined thickness of the uterus and placenta (CTUP) is important in the examination of mares suspected to have ingested endophyte-infected tall fescue or to have placentitis, intrauterine hemorrhage, placental insufficiency, or premature placental separation. Transabdominal and transrectal methods
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To measure CTUP have been described. For transabdominal measurement of CTUP, a 2.5- to 5-MHz probe is used and applied to the ventral midline cranial to the udder. The CTUP is measured in several areas where there is no contact between the fetus and the uteroplacental wall16 (Figure 5). The mean normal transabdominal CTUP has been reported to be 1.26 ± 0.33 cm4 or 11.50 ± 2.35 to 13.83 ± 2.29 mm.3 Small anechoic spaces between the uterus and placenta can be a normal finding in some mares and must be differentiated from uterine or placental vessels.3 Increased placental thickness may not be observed in mares grazing endophyte-infected tall fescue pastures until 8 hours before parturition.1 This increased thickness is due to placental edema and may accompany premature allantochorial separation.

Renaudin and colleagues16 compared measurements of CTUP obtained transabdominally and transrectally and found no statistical correlation between the two methods. They reported normal transabdominal CTUP measurements of up to 6 mm and found transrectal measurement of CTUP to be of greater clinical value than transabdominal examination.16 To determine transrectal CTUP, a 5-MHz linear array probe is placed over the cervicoplacental junction and the CTUP measured from the ventral aspect of the uterine body (Figure 6). Measurements of the ventral CTUP are more consistent and accurate than

Figure 3. Transabdominal ultrasound image of a late-term Thoroughbred mare with placentitis. Placental exudate and separation are indicated (arrow). The top of the figure is ventral, and the image was obtained with a curved 2.5-MHz linear-array probe. (Image courtesy of Dr. Peter Morresey.)

Figure 4. A fetal electrocardiogram obtained in the last month of gestation using the method of Colles and Parkes.12,13 A three-lead electrocardiogram from the mare is shown at the bottom of the figure. The first several fetal QRS complexes are marked (arrowheads).

Figure 5. Transabdominal ultrasound image of the uterus and placenta in the last month of gestation in a quarter horse mare. The CTUP (+ marks) is normal (10.10 mm) for this stage of gestation. The top of the figure is ventral, and the image was obtained with a curved 3.5-MHz linear-array probe.

Figure 6. Transrectal ultrasound image of the uterus and bladder in the last month of gestation in a quarter horse mare. The CTUP (+ marks) is normal (6.82 mm) for this stage of gestation. The top of the figure is dorsal, and the image was obtained with a 5-MHz linear-array probe.
those of the dorsal CTUP. Some apparently normal mares may have dorsal placental edema during the last month of gestation. Renaudin and colleagues observed normal transrectal ventral CTUP to be 3.50 to 4.69 mm from 4 to 9 months’ gestation and 5.53 to 11.77 mm from 10 to 12 months’ gestation. CTUP did not change between 4 and 8 months’ gestation but increased significantly each month from 10 to 12 months’ gestation. Other authors have suggested positioning the ultrasound probe 1 to 2 inches cranial to the cervix, then moving laterally to visualize the middle branch of the uterine artery. The CTUP is then measured between the middle branch of the uterine artery and the allantoic fluid. Transrectal examination should improve the diagnosis of early placental abnormalities or placentitis because most cases begin as ascending infections from the cervix. An increased transrectal CTUP, placental detachment, or increased echogenicity of allantoic or amniotic fluids may indicate placental insufficiency.

**AMNIOCENTESIS**

Amniocentesis in late-gestation mares has been described, but normal values have not been established in equids. Bacterial culture of amniotic fluid in mares is not useful to determine prenatal infection because positive cultures have been obtained from mares that delivered healthy foals. However, equine herpesvirus type 1 has been isolated from amniotic fluid taken from experimentally infected mares during the ninth month of gestation. Creatinine, phospholipid, and cortisol concentrations in equine amniotic fluid have not proven to be reliable indicators of renal or pulmonary maturity or fetal stress. In mares, spontaneous abortion rates as high as 25% have been reported after amniocentesis when multiple samplings were attempted. Chorioallantoic tenting and subsequent localized placental separation is a problem in mares because of the structure of the equine placenta, which is diffuse compared with the disk-shaped, more localized human placenta. Currently, routine amniocentesis in mares is not advised in most situations.
MAMMARY SECRETION ELECTROLYTE CONCENTRATIONS

Measurement of mammary secretion electrolyte concentrations (calcium, sodium, and potassium) is useful during the peripartum period to assess fetal readiness for birth and to predict impending foaling. If the mare’s breeding date is known, sampling may begin 10 to 14 days before the expected foaling date. In mares with unknown breeding dates, sampling may begin as soon as udder enlargement is noted and mammary secretions can be easily obtained. Initially, prepartum mammary secretions have a high sodium concentration and low potassium and calcium concentrations. As foaling approaches, the concentrations of calcium and potassium increase and the sodium concentration decreases. Commercially available point-of-care tests or water hardness kits are used to measure the concentration of calcium (as calcium carbonate). Measuring mammary secretion concentrations of calcium, potassium, and sodium may allow a more precise assessment of readiness for foaling than measurement of calcium carbonate only. Foaling predictions based on mammary secretion electrolyte concentrations may be more accurate when electrolytes are measured in the late afternoon to early evening because most mares foal during the night.

Rook and colleagues investigated changes in mammary secretion electrolyte concentrations in 18 periparturient Arabian broodmares. The mean sodium concentration decreased from approximately 87 mEq/L at 3 days prepartum to 32 mEq/L on the day of foaling. The mean potassium concentration gradually increased from less than 12 mEq/L at 11 days prepartum to more than 47 mEq/L on the day before foaling. The mean calcium concentration increased significantly from 20 mg/dl at 8 to 3 days prepartum to more than 40 mg/dl on the day of foaling. Mammary secretion calcium concentrations may increase prematurely in pregnancies with placental abnormalities. In cases of placentitis, mammary secretion calcium concentrations may rise, but the inversion of sodium and potassium concentrations does not seem to occur.

Ousey and colleagues proposed an ionic scoring system for mammary secretions to predict whether a fetus is full term and the induction of labor is likely to be safe. In this scoring system, the maximum number of points is assigned if the calcium concentration is 40 mg/dl or greater, the potassium concentration is 35 mEq/L or greater, and the sodium concentration is 30 mEq/L or less. Several studies have reported that a mammary secretion calcium concentration of 40 mg/dl or greater is associated with impending parturition. Foals from induced parturitions had poor chances of survival if the mammary secretion calcium concentration was less than 12 mg/dl and gestational age was less than 320 days.

Ley reported that most mares foal within a short period of achieving mammary secretion calcium carbonate concentrations of 300 to 500 ppm (30 to 50 mg/dl). In a study of 59 Thoroughbred and Warmblood mares in the last 2 weeks of gestation, Ley et al found that 51.4% of mares with mammary secretion calcium carbonate concentrations of 200 ppm (20 mg/dl) or greater foaled within 24 hours and 99.6% of mares with mammary secretion calcium carbonate concentrations of less than 200 ppm did...
not foal within 24 hours. Therefore, this determination is more useful to predict when a mare will not foal within 24 hours than when a mare will foal. However, 97.2% of late-gestation mares are expected to foal within 72 hours of achieving a mammary secretion calcium carbonate concentration of 200 ppm or greater.\textsuperscript{30}

**ENDOCRINE TESTING**

Together, the fetus and placenta produce progestins and estrogens during the second half of gestation.\textsuperscript{32} For this reason, measurement of estrogen and progesterone levels has been suggested as a method of determining fetal health or monitoring for impending abortion. Comparison of published absolute normal ranges of hormone concentrations with normal ranges used by different laboratories is difficult because of varying techniques and assays.

Serial monitoring of progestin concentrations is more reliable than individual point sampling.\textsuperscript{32} In an experimental model of placentitis, progestin levels were considered abnormal if levels in three samples obtained 48 to 72 hours after a baseline sample increased or decreased more than 50% from the baseline. Clinically, fetal stress may be suspected if three serial progestin concentrations are not within normal laboratory limits. Measurement of progestins after 310 days’ gestation is only useful to determine whether concentrations are decreasing, indicating impending abortion. Decreased total estrogen concentration before day 300 of gestation indicates a stressed, severely compromised, or dead fetus.\textsuperscript{33}

**CONCLUSION**

Assessment of fetal well-being, maturity, and proximity to foaling can be very difficult. After analyzing test results, clinicians are confronted with the decision of how to intervene in an at-risk pregnancy. In most situations, the fetus benefits by remaining in the uterus even if the environment is somewhat hostile because premature induction of parturition can be disastrous. No single diagnostic test is completely reliable, and the best estimation is made using most or all of the diagnostics described above and the clinician’s experience. Often, clinicians must rely on the ultimate outcome of a live, viable foal before they can truly assess their decisions. We recommend frequent and repeated monitoring during at-risk pregnancies, using ultrasonography, mammary secretion electrolyte concentrations, and endocrine testing when appropriate to make the best determination of fetal health and the need for therapy or obstetric intervention.

**REFERENCES**

3. Mares foaling in the summer have shorter pregnancies than mares foaling in the
   a. winter.
   b. fall.
   c. late spring.
   d. southern hemisphere.

4. In late gestation, the fetal heart rate tends to be approximately ___ bpm.
   a. 60  c. 80
   b. 70  d. 90

5. Which of the following may be an indicator of impending fetal death?
   a. persistent fetal bradycardia
   b. fetal tachycardia in response to fetal movement
   c. absence of the fetal P wave on fetal electrocardiogram
   d. elevated mammary secretion calcium concentration

6. Transrectal measurement of CTUP may be more clinically useful than transabdominal measurement because
   a. there is normally no contact between the foal and placenta in this area.
   b. most utero-placental infections ascend from the cervix.
   c. transrectal measurement of CTUP is consistently correlated with transabdominal CTUP.
   d. all of the above

7. Amniocentesis in the mare has been used to detect experimental intrauterine infections with
   a. equine arteritis virus.
   b. Leptospira sp.
   c. equine herpesvirus type 1.
   d. equine infectious anemia virus.

8. In late gestation, mammary secretion concentration of sodium ___ and the concentration of potassium ___.
   a. increases; increases
   b. decreases; increases
   c. increases; decreases
   d. decreases; decreases

9. Tests that measure mammary secretion calcium carbonate concentration are more useful to predict when a mare will
   a. foal within 24 hours.
   b. not foal within 24 hours.
   c. foal within 12 hours.
   d. not foal within 12 hours.

10. Placental abnormalities may cause premature increases in mammary secretion concentration of
   a. potassium.  c. calcium.
   b. sodium.       d. all of the above