How to Monitor the Subfertile Mare During the Last Trimester

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1. Introduction
After expending considerable time in establishing pregnancy in the subfertile mare, it is imperative that she be adequately monitored to detect complications in the later part of gestation. The most likely complication to arise during the last third of gestation in the subfertile mare would be placentitis. Timely identification of placentitis is crucial for multimodal therapy to be successful. Other potential reproductive causes of high-risk pregnancy such as utero growth retardation and cord abnormalities might also be identified.

Placentitis has been reported to be responsible for 9.8%, 1 19.4%, 2 24.7%, 3 and 33.5% 4 of abortions, stillbirths, and perinatal losses in horses. Bacterial infections are responsible for 53% of these losses; Streptococcus equi subsp. zooepidemicus (S. zooepidemicus) was isolated in 28% of these cases. 3 Other bacteria frequently identified were Escherichia coli, Leptospira spp., Crossiella equi, Pseudomonas spp., S. equisimilis, Enterobacter spp., Klebsiella spp., α-hemolytic streptococci, Staphylococcus spp., and Actinobacillus spp. 3 Infection was localized to the cervical star in 95% of cases, supporting the argument that ascension of aerobic bacteria through the vagina and cervix is the most frequent route of infection. 5 Clinical signs include vulvar discharge, udder development, prenatal lactation, and premature delivery or stillbirth. Abortions can occur from 75 days to term, 5 although the majority of clinical cases are presented to practitioners during the third trimester. In a survey of 200 fetal membranes with infective placentitis, 36% of those with bacterial infections were recorded to have had premonitory signs, compared with 63% of those with fungal infections. 5

A notable exception to the transcervical ascension of pathogens is focal mucoid placentitis (“nocardioform placentitis”) caused by actinomycetes. The majority of isolates have been C. equi, 6 although other species have been identified, including Amycolatopsis spp., Streptomyces spp., and Cellulosimicrobium cellulans. Although sporadic cases of nocardioform placentitis have been reported in South Africa, Florida, and Italy, 7–9 most reported cases are from Kentucky. Inflammation of the chorion extends from the cranial ventral uterine body, usually at the base of the gravid horn, with an adherent tan to brown exudate. 10 Precocious udder development is occasionally observed with nocardioform placentitis, but vaginal discharge is uncommon. The majority of abortions occur during the 9th and 10th months of gestation. Interestingly, other non-nocardioform bacteria have been isolated.
from lesions that are grossly indistinguishable from nocardioform placentitis,\textsuperscript{11} so perhaps the gross appearance of this form of placentitis is unique to its distribution pattern rather than the causative agent.

2. Materials and Methods

Ultrasonography is a key diagnostic modality for diagnosing disturbances of the feto-placental unit and for monitoring the progression of fetal viability and placental changes. Evaluations are made both transrectally and transabdominally and are best performed when the mare is restrained in stocks in a quiet environment. Mare agitation or anxiety can produce fetal tachycardia without concomitant fetal movements. Sedation is also to be avoided, due to the associated fetal bradycardia. Hormonal profiling also provides crucial information and is complementary to the information gained by ultrasonography. Other modalities that can be used include centesis of fetal fluids and echocardiography. Increasingly, ultrasonography has replaced fetal echocardiography as a means of monitoring fetal heart rate.

Transrectal Ultrasonography

For transrectal examination, a 5.0- to 7.5-MHz linear-array transducer is positioned just cranial to the junction of the cervix and the uterine body. Small lateral movements are made until a vessel on the ventral border of the uterus is located. This vessel is variably described in anatomy texts but most commonly is designated as the uterine branch of the vaginal artery, which is a branch of the internal pudendal artery. Several measurements are taken from the leading edge of the allantoic surface to the leading edge of the blood vessel and averaged. An increase in the combined thickness of the uterus and placenta (CTUP), especially with concomitant accumulation of echogenic fluid between the endometrial and chorionic surfaces (Fig. 1), is characteristic of placentitis. If serial monitoring is anticipated, it is beneficial to consistently measure the CTUP at the same distance from the cervix during each examination. Upper limits of the normal CTUP have been established\textsuperscript{12}, finding increases in the CTUP of >8 mm during the 9th month, >10 mm during the 10th month, and >12 mm after day 330 signals that placental failure and abortion may occur.\textsuperscript{13,14} Certain circumstances can lead to CTUP values being greater than that considered normal in the absence of placentitis. Episodes of high fetal and uterine activity, especially when the fetus becomes positioned in the caudal uterine body, can produce CTUP values that are above the normal range for that stage of gestation. Apposition of the amnion with the allantois produces a slight increase in the CTUP. Allanotic fluid is hypoechoic in comparison to amniotic fluid, with small hyperechoic foci ("vernix") suspended in the anechoic medium. Vernix is also present in the amniotic fluid but is somewhat less obvious due to the overall greater echogenicity of amniotic fluid compared to allantoic fluid. This hypoechoigenicity of the allantoic fluid persists until parturition nears, while the amniotic fluid gradually increases in echogenicity during the last month of gestation.\textsuperscript{12} In-
increased echogenicity of the allantoic fluid coincided with the first day of recovery of bacteria from allantoic fluid with experimental infection.\(^{15}\) Edema of the chorioallantois at term is normal and simply indicates impending parturition. Edema of the chorioallantois, or a discernible difference in the echogenicity of the uterine wall and the chorioallantois at other times, should be considered as an indicator of potential premature delivery. Chorioallantoic edema was seen with experimentally induced placentitis in the 24 hours preceding abortion.\(^{16}\) Fetal presentation is easily confirmed by identifying the presence (anterior) or absence of (posterior) the fetal orbit.

**Transabdominal Ultrasonography**

For transabdominal ultrasonography, a 3.5- to 5.0-MHz sector-array transducer is preferred. In very late gestation or in heavy breed mares, a 2- to 2.5-MHz sector-array transducer may allow for greater access to the fetus. Transabdominal ultrasonography is useful for assessing fetal heart rate (FHR), fetal activity, fetal presentation and position, character and depth of fetal fluids, as well as in cases of placentitis not due to ascension through cervix (e.g., nocardioform placentitis or hematogenous infection). For single transabdominal evaluations, the hair of the ventral abdomen should be cleaned of all dirt and debris, and alcohol and ultrasound coupling gel is applied liberally. In patients for whom serial examinations are anticipated, clipping of the abdomen is recommended, followed by application of alcohol and coupling gel. If the abdomen has been clipped, scanning can then be performed in either a caudal to cranial or cranial to caudal direction. If the belly was not clipped, then scanning in a cranial to caudal direction avoids “ruffing” the hair, which increases air interference and reduces image quality. Scanning should begin at the ventral midline, evaluating in parallel parasagittal planes from the mammary gland to the umbilicus or sternum, depending on the stage of pregnancy.

Baseline FHRs in the last weeks of pregnancy are 60 to 75 bpm, with a range of 40 to 250 bpm. Low or high FHRs are cause for alarm if they are persistent with no accelerations or decelerations or accelerations are unaccompanied by fetal movement.\(^{16}\) Fetal activities (e.g., movement, breathing, swallowing, sucking) should be noted and either scored subjectively or quantified as number of movements in a given time period. Fetal presentation is confirmed on the basis of orientation of the fetal thorax relative to the maternal pelvis. In addition to flexion and extension of the extremities, other more extreme motion patterns can sometimes be observed: rotation on the short axis (spinning) or long axis (rolling) and whole-body shifting vertically or horizontally (translation). The fetus is able to change presentation through the first 8 months of gestation. Since cases of nocardioform do not have vulvar discharge or increases in the CTUP of the caudal uterine seg-

ment, transabdominal ultrasound has the best chance of making a diagnosis. Unfortunately, it can be difficult to locate the area of separation of the chorioallantois from the endometrium; therefore false-negative results are commonly encountered.

Identification of cord abnormalities such as long cord/cord torsion may on occasion be detected with transabdominal ultrasonography, especially with the use of Doppler technology. Unfortunately, only short segments of the cord can usually be imaged at a time, which lessens the likelihood of detecting cord abnormalities before abortion. Likewise, assessment of in utero growth retardation (small for gestational age) can be estimated by measuring the fetal aortic diameter, which is significantly correlated with neonatal foal weight. Fetal aortic diameter is measured during systole from leading edge to leading edge as it emerges from the heart. Fetuses with substantial deviations from the mean aortic diameter for the stage of gestation may be small for gestational age\(^{17}\); however, this may not be a very reliable predictor of in utero growth retardation.

**Allantocentesis/Amniocentesis**

Although amniocentesis is commonly used by physicians to diagnose chorioamnionitis in women and assess fetal maturity, allantocentesis is rarely used in cases of suspected placentitis in the mare either to document infection in the fetal fluid compartments or to ascertain readiness for birth. Lecithin: sphingomyelin ratio (L:S) and lamellar body count in amniotic fluid have been used in human medicine to predict the presence of phosphatidylglycerol, a marker for fetal lung maturity.\(^{18}\) The L:S and lamellar body count in amniotic fluid in healthy equine term neonates have been measured\(^{19}\), however, neither the L:S nor the percentage phosphatidylglycerol were found to provide useful antepartum predictors of fetal maturity from day 292 to term.\(^{20}\) In experimental models of placentitis, prostaglandin (PG) F\(_{2}\alpha\) and PGE\(_{2}\)\(^{15,21,22}\) and cortisol\(^{22}\) were found to be elevated before pre-term delivery. Because PG\(_{2}\alpha\) and PGE\(_{2}\) are labile and require strict and cumbersome sample handling to avoid analyte degradation, measurement of prostaglandins in fetal fluids is not practical. Elevated cortisol concentrations in fetal fluids signal that the fetal hypothalamic-pituitary-adrenal axis has been prematurely activated and that delivery may be impending. Until reliable markers for fetal maturity and for infection are identified, the use of allantocentesis in the diagnosis and management of placentitis should be undertaken with caution. Continued research is needed to identify reliable indicators of equine fetal maturity. In mares with suspected hydromic pregnancy, the concentrations of creatinine and chloride are useful in determining whether hydrions allantois or hydrions amnii is present. During the last 4 months of pregnancy, chloride is significantly higher in amniotic than in allantoic fluid (112.3 to 0.5x and 37.3 ± 0.3x mM, respectively, where x is the days
prepartum), whereas creatinine is significantly lower in amniotic than in allantoic fluid (12.796 ± 0.26x + 0.0008x^2 and 1,000.96 + 2.574 + x + 0.014x^2 mg/dL, respectively, where x is the days prepartum). Calcium was significantly increased in both fetal fluid compartments with impending term parturition; however, calcium was decreased in pregnancies after intrafetal betamethaxone administration, questioning the usefulness of fetal fluid calcium concentration as a determinant for readiness for birth in preterm pregnancies. Hormonal Profiling

Several hormones in the maternal circulation may be useful to monitor during high-risk pregnancies. Total progestins are commonly measured in pregnant mares, although single samples probably are not as informative as serial samples. Total maternal plasma progestins (as measured by cross-reactivity with a progesterone assay) are low (2 to 12 ng/mL) until the last 15 to 21 days of gestation, climb substantially, and then fall abruptly within 24 hours of parturition. Precocious increases in progestins before day 315 may be seen with placitits; acute declines in progestagens are associated with fetal demise and impending abortion. It is crucial that samples be submitted to a laboratory that has established values for progestagens in the late-term mare. Each progesterone assay has different cross-reactivities with the various progestagens, so it is not possible to compare values from one laboratory with ranges established by another laboratory that uses a different assay. Estrone sulfate can be used to monitor fetal well-being but is not sensitive in detecting early stages of placenitis. From day 150 to 310, total estrogens (predominately conjugated estrogens) are greater than 1,000 pg/mL, falling to 500 pg/mL by day 340; mares with total progestins greater than 15 ng/mL and total estrogens less than 500 pg/mL between days 150 and 280 aborted, whereas those with progestins less than 8 ng/mL and total estrogens greater than 1,000 pg/mL during this same time frame delivered live foals. Relaxin is produced by the placenta and has been found to be decreased in mares with placenitis or signs of endophyte-infected fescue toxicosis. Unfortunately, at this time no commercial assay is available for relaxin.

3. Conclusions

Timely identification of abnormalities during the last trimester of the subfertile mare is key to achieving the desirable outcome of a healthy neonate. The success of multimodal therapy for placenitis hinges on early recognition of infection. Unfortunately, the clinical symptoms of placenitis are not consistent, but a combination of serial ultrasonography and maternal hormonal profiling may allow the earliest identification of mares with an abnormal feto-placental unit. Subfertile mares, or those with a history of previous ascending placenitis, should have serial examinations beginning no later than the start of the last trimester. In some cases, monitoring from midgestation onward would be prudent. Further research on biomarkers for identifying infection of the allantoic fluid will aid in improving outcomes of cases with placenitis.

References and Footnote


*Lyle SK. Unpublished observations, 2008.*