INTRODUCTION

Ascending placentitis in the mare is the most common non-infectious cause of premature birth, abortion, and early foal death (Hong et al, 1993; Giles et al, 1993). Timely diagnosis can be difficult, as many mares show subtle clinical signs or only show clinical signs late in the disease process. Although this disease has been studied for many years, the best treatment approach remains somewhat elusive. This review will include recent diagnostic approaches and current treatment protocols.

REVIEW OF PREGNANCY MAINTENANCE AND PLACENTAL FUNCTION IN THE MARE

The key to understanding the mechanism of pregnancy loss and the rationale of subsequent veterinary intervention is a basic understanding of the establishment and maintenance of pregnancy in the mare. Five to six days after fertilization, the conceptus enters the uterus and migrates through the horns and body until fixation at the base of one horn around day sixteen. This migration is considered to be an integral part of maternal recognition of pregnancy and is influenced by the hormone estradiol. During this time, the primary corpus luteum (CL) provides a continued high level of progesterone that is essential for pregnancy maintenance. The chorionic girdle, a milky colored band on the embryo that contains invasive trophoblast cells, begins to develop between days 25 and 35 of pregnancy. These cells invade the adjacent uterine epithelium between days 36 to 38 and establish endometrial cups. The endometrial cups secrete equine chorionic gonadotropin (eCG), a luteotropic hormone that leads to increased function of the primary CL and the formation of secondary CL's. The cups reach maximal size around day 60-70, and then begin to degenerate. They are typically sloughed completely by day 130. Both the primary and secondary CL's remain functional until approximately day 180 of pregnancy.

The placental trophoblast cells that cover the majority of the developing embryo ultimately form the microcotyledons of the equine placenta. Primary villi, the precursor of the microcotyledon, are clearly present after day 60 of pregnancy. The eventual formation of the microcotyledon allows for rapid interchange of small molecules between the fetus and the dam. Aside from this important exchange of molecules, the placenta ultimately becomes the only source of pregnancy maintenance in the mare, but the exact time this occurs has not been determined.

Progesterone (P4) is present only through approximately the first 150-180 days of pregnancy. After this time, the predominant progestins are 5α-pregnanes produced via the fetoplacental unit. The fetus produces large amounts of pregnenolone (P5) within the adrenal gland, which is converted by the uteroplacental unit into other progestins (including 5α-pregnanes). After the appearance of 5α-pregnanes within the maternal circulation between days 30-60, their levels gradually increase until day 300 of pregnancy where they stabilize. A rapid, dramatic increase is then seen 20-30 days pre-partum and a like-wise dramatic drop in their levels is seen within 24 hours of foaling.

Estrogens, while not essential for the maintenance of pregnancy in late gestation, are important in active labor and can be indicative of the developmental status of the fetus. Eight estrogens have been isolated from pregnant mare urine, the most prominent being estrone, equilin, and equilenin. The production of estrogens in the pregnant mare also relies on the fetoplacental unit. The estrogen precursors are produced by the enlarged fetal gonads, and are subsequently aromatized by the placenta to form estrogens. Total estrogen concentrations increase at
approximately 100 days of pregnancy and remain high until a gradual decline that occurs in the two months prior to parturition.

Other important hormones involved in the pregnant mare include oxytocin, relaxin, and prostaglandins. Relaxin is important in the maintenance of uterine quiescence and is produced mainly by the placenta. Concentrations of this hormone increase at day 80 of pregnancy and remain high for the remainder of gestation. Oxytocin levels remain at very low levels throughout gestation and only increase significantly during parturition. Prostaglandins, PGF$_{2\alpha}$ and PGE$_2$, are synthesized by the uteroplacental tissues, but are rapidly inactivated by an enzyme present in the endometrium. Consequently, prostaglandin levels remain low within the maternal circulation, but are present in higher concentrations within the fetal fluids.

CLINICAL EVALUATION OF THE LATE-TERM PREGNANT MARE

Early clinical signs of ascending placentitis are often subtle and can easily be missed. Premature udder development is the most common presenting complaint. It is important to remember that the presence of twins will also cause precocious udder development in the mare and must be ruled out in all cases. Vulvar discharge is likely present in most cases of ascending placentitis, but is often slight and can be difficult to detect. Affected mares are frequently multiparous with poor perineal conformation (Macpherson & Bailey, 2008).

When asked to evaluate the clinical status of a late-term pregnant mare and her fetus, the veterinarian can be presented with a diagnostic challenge as current diagnostic techniques are lacking in specificity and sensitivity. Relying on a combination of several different modalities allows for the best possible outcome in obtaining the “whole picture” in evaluating for feto-placental compromise. A comprehensive examination should include general physical examination, rectal examination, possible vaginal examination, both transabdominal and transrectal ultrasonography, and hormone analysis.

Ultrasonography is an important tool in the assessment of the mare with feto-placental compromise. It allows for detection of multiple fetuses (if fetal parts are accessible), assessment of fetal fluids, assessment of the placenta, and assessment of fetal growth, activity, mobility, and presentation. The use of a 5 MHz probe is generally sufficient for examination via both transrectal and transabdominal approaches. Other useful probe frequencies include 2.5 MHz for complete imaging of the late-gestation fetus, and 6-7.5 MHz for detailed images of the placenta.

Perhaps the most useful or common measurement performed via transrectal ultrasound in the late-term pregnant mare is the combined thickness of the uterus and placenta (CTUP) at the placentocervical junction. This area is most often affected by ascending placentitis. The value is easily obtained and can provide valuable information concerning the status of the placenta. The ultrasound probe is placed 2.5-5 cm cranial to internal cervical os and the ventral aspect of the combined uterus and placenta are measured. Caution should be taken in interpreting a single measurement as the positioning of the foal within the mare’s pelvis can erroneously increase or decrease the value. Abnormalities in this measurement, both increases and decreases, are associated with poor fetal outcomes and are most commonly associated with placentitis, placental edema, and/or premature placental separation. As a general guideline, CTUP values are as follows.

<table>
<thead>
<tr>
<th>CTUP Value</th>
<th>Stage of Gestation</th>
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<tbody>
<tr>
<td>6 mm</td>
<td>210 days</td>
</tr>
<tr>
<td>&lt; 8 mm</td>
<td>271-300 days</td>
</tr>
<tr>
<td>&lt; 10 mm</td>
<td>301-330 days</td>
</tr>
<tr>
<td>&lt;12 mm</td>
<td>&gt; 330 days</td>
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These normal values were developed in Quarter Horse mares; therefore, values can be expected to be slightly higher in Warmblood or draft mares and slightly lower in pony mares. Generally speaking, a value of greater than 12 mm at 11 months gestation and greater than 15 mm at 12 months is strongly suggestive of a placental abnormality.

A visual analysis of fetal fluids can be performed with both transrectal and transabdominal ultrasonography. While direct measurements of fluid depth are not useful, the echogenicity of fetal fluids can easily be determined. Using a systematic approach, the echogenicity can be graded from level I (white; hyperechoic) to level IV (black; anechoic). Normal allantoic fluid is a level III (dark gray with few hyperechoic suspended foci) from approximately four months gestation to term. The echogenicity of amniotic fluid will gradually increase over time as gestation progresses and is consistently a level II (light grey; semi-hyperechoic) by the last month of gestation. Sudden increases in amniotic echogenicity may be associated with passage of meconium suggesting fetal stress, hemorrhage suggesting placental detachment, or inflammatory debris suggesting infection.

Various fetal measurements can be made with ultrasonography to evaluate fetal growth, development, and viability. Fetal heart rate is easily attained with transabdominal ultrasonography in later pregnancy. A wide range of normal rates is found in the equine fetus, but both bradycardia and tachycardia have been associated with negative outcomes. The following table includes information from a large study involving 150 normal pregnancies from 80 mares over a three year time period (Bucca et al., 2005). As a general trend, fetal heart rate gradually decreases and maternal heart rate gradually increases as parturition approaches.

<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>HR – Activity</th>
<th>Max</th>
<th>Min</th>
<th>HR – Rest</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months</td>
<td>130</td>
<td>146</td>
<td>113</td>
<td>113</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>7 months</td>
<td>140</td>
<td>176</td>
<td>115</td>
<td>112</td>
<td>125</td>
<td>95</td>
</tr>
<tr>
<td>8 months</td>
<td>124</td>
<td>143</td>
<td>111</td>
<td>106</td>
<td>115</td>
<td>95</td>
</tr>
<tr>
<td>9 months</td>
<td>106.6</td>
<td>120</td>
<td>90</td>
<td>91</td>
<td>102</td>
<td>79</td>
</tr>
<tr>
<td>10 months</td>
<td>110</td>
<td>136</td>
<td>92</td>
<td>86.6</td>
<td>98</td>
<td>82</td>
</tr>
<tr>
<td>11 months</td>
<td>90.7</td>
<td>140</td>
<td>70</td>
<td>72.5</td>
<td>86</td>
<td>55</td>
</tr>
<tr>
<td>12 months</td>
<td>86</td>
<td>105</td>
<td>71</td>
<td>66.4</td>
<td>77</td>
<td>56</td>
</tr>
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As shown, average heart rate will change depending on fetal activity level and a single measurement is not a good instantaneous measure of vitality. A 30-minute period of monitoring provides the examiner with a more accurate picture. Periods of dormancy in the equine fetus typically last less than 10 minutes in the healthy fetus, but periods of up to 60 minutes are occasionally detected.

Another common and useful diagnostic tool in monitoring equine pregnancy is hormonal profiling of the maternal plasma. Any condition that directly affects the placenta or fetus is likely to have disruptive effects on the endocrine capacity of the fetoplacental unit, and will manifest as abnormalities in progestins and estrogens in the maternal plasma. Up to 310 days gestation, progestins are very useful in predicting fetal health, as placental pathologic findings are strongly correlated with maternal plasma levels. Generally, progesterone assays (RIAs or ELISAs) that cross-react with many of the progestins are used. Caution should be used in interpreting these results directly as the degree of cross-reactivity will vary between assays. Individual laboratories should provide normal values associated with their particular test. While maternal plasma progesterone concentrations can fluctuate significantly in early pregnancy, progestin concentrations in late pregnancy do no fluctuate making single level interpretations more meaningful. Premature rises in plasma progesterone can be seen secondary to increased pregnenolone production from stressed fetal adrenal glands occurring from chronic conditions (e.g. placentitis, twins, or chronic laminitis). Rapidly declining or extremely low values of these hormones represent the worst case scenario and generally indicate fetal death and/or imminent

Adapted from Bucca et al. 2005
fetal expulsion. Such cases tend to occur with severe, acute conditions such as uterine torsion or colic. Maternal plasma estrogens are also useful in predicting fetal viability until approximately 300 days gestation. They are not a good indicator of fetal stress as estrogen precursors are produced by the fetal gonads versus the fetal adrenal glands. Between days 150 and 300 gestation, values above 1000 pg/mL are considered normal. A value of less than 500 pg/mL is commonly associated with a severely compromised or dead fetus and a value between 500-800 pg/mL indicates a compromised fetus. During late pregnancy when levels are normally declining, estrogens do not provide an accurate indicator of fetal viability. Most recently, maternal relaxin levels have been evaluated for the detection of placental insufficiency and have shown promise as a means of identifying compromised pregnancies (Ryan et al, 2009; Stewart et al, 1992; Ryan et al, 2002). To the author’s knowledge, the assay is not currently commercially available, but may become an important means of monitoring the high-risk mare in the future.

TREATMENT OF THE HIGH-RISK MARE

Current interventions available for the treatment of a compromised equine pregnancy are less than ideal and there are limited controlled studies to guide veterinarians. Currently, the most common pharmaceutical interventions include the use of progestins, anti-inflammatories, anti-cytokines, and antibiotics. The basis for progestin treatment is their ability to maintain uterine quiescence – presumably due the reduction of myometrial activity via interference of up-regulation of prostaglandin and oxytocin receptors. However, their true ability to accomplish the goal of prolonging gestation has not been proven and the underlying principle of treatment is unclear when progestins are typically high in mares showing signs of premature labor. Regardless of this argument, supplemental progestins have been shown to prevent prostaglandin-induced abortion in late-term pregnant mares and show improved outcomes in mares affected with placentitis as part of a multi-drug treatment strategy.

Antibiotic therapy is a staple of treatment for cases of suspected or confirmed placentitis. Bacteria most commonly responsible for ascending placentitis include: Streptococcus zooepidemicus, Excherichia coli, Pseudomonas aeruginosa, and klebsiella pneumonia (Acland, 1993). The goal of anti-microbial therapy is to provide broad spectrum coverage for both gram-positive and gram-negative bacteria with a drug that readily crosses the placental barrier and does not negatively impact the developing fetus. Studies have shown that potassium penicillin G, gentamicin sulfate, and trimethoprim sulfamethoxazole penetrate to the allantoic cavity in therapeutic concentrations (Murchie et al, 2006; Rebello et al, 2006). Further, trimethoprim sulfamethoxazole has been shown to provide therapeutic levels within the foal itself (Graczyk et al, 2006).

While bacterial invasion initiates placentitis, it is the resulting associated inflammation that results in abortion (LeBlanc et al, 2002). Therefore, anti-inflammatory medications are an important component of treatment of the high-risk pregnant mare. Flunixin meglumine is commonly administered, but due to its large degree of protein binding, microdialysis technology has not been able to detect the drug within the allantoic fluid. Whether the drug is unable to cross the placenta in detectable levels due to this protein-binding or is simply not detectable with current methods remains unclear. Pentoxifylline has recently been studied in the treatment of compromised pregnant mares due to its anti-inflammatory and anti-cytokine effects. (Cytokines have been shown to play a role in the inflammatory component of placental infection.) The drug crosses the placental barrier as it has been detected within the allantoic fluid of pregnant mares. At a dose of 8.5 mg/kg twice daily, pentoxifylline has recently been evaluated as a component of combined drug therapy with encouraging results (Bailey et al, 2007).

Tocolytics, such as clenbuterol, have been previously evaluated for their ability to prevent or disrupt uterine contractions and premature labor in the mare. Unfortunately, data available in normal foaling mares treated with clenbuterol has not shown differences between length of
gestation, the time to foaling, or the fetal outcome. The use of these medications in the high-risk mare is currently of questionable effectiveness.

It has long been understood that the control of parturition lies in the developing fetus. The maturation of the fetal hypothalmo-pituitary-adrenal axis in preparation of impending parturition is of critical importance for neonatal survival. In contrast to other domestic animals, fetal cortisol levels increase only in the two to three days prior to parturition in the horse. This delay means there is a significant threat to the health of the equine neonate in the case of preterm delivery. Foals delivered prior to the rise in fetal cortisol show significant signs of prematurity and typically die of multiple organ failure. Because of these circumstances, hastening the maturation of the hypothalmo-pituitary-adrenal axis has potential clinical application in cases where pre-term delivery is eminent or induction of labor is necessary. There is evidence that improved outcomes can occur with either fetal or maternal treatment with glucocorticoids or corticotropin. Unfortunately, fetal treatment carries significant risk of abortion and maternal treatment has provided inconsistent results as cortisol does not readily cross the placental barrier. Further study is needed in this area.

CONCLUSION

After the institution of treatment, it is important to continue monitoring the mare closely. Traditionally, treatment has been continued to term although in some cases it can be stopped when evidence of disease has reversed. While the identification and treatment of high-risk pregnant mares remains challenging, there are tools available to the veterinarian. Judicious interpretation of clinical findings and utilizing a combined therapy approach yield the best chance of positive results for both fetus and dam. Continued study of the equine model will be necessary to minimize the risks of an unfavorable outcome.

FURTHER READING