Placentitis, inflammation or infection of the placenta, has emerged as a leading cause of equine reproductive and economic loss. It has been described as accounting for 20% of reproductive failures including premature births, still births and deaths in the first 24 hours, in central Kentucky during 1988 and 1989 foaling seasons.¹ In addition to inducing premature delivery, chronic placentitis may accelerate foetal maturation, resulting in the birth of precociously mature foals. This is in contrast to the United Kingdom in which only 9.8% of foetal losses were associated with placentitis.² Bacterial placentitis is most commonly caused by *Streptococcus* spp, which can be isolated and identified from the placenta and the aborted foetus. Other organisms identified are; *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Nocardioform Actinomycetes*, and *Leptospirosis* spp. The most common fungus isolated is *aspergillus*. These organisms gain access to the placenta and therefore the foetus by three characteristic mechanisms.

**Ascending placentitis**

An ascending infection occurs when the pathogen enters from the vaginal vault through the cervix causing inflammation and infection of the chorioallantois at the cervical star region. This causes destruction of the microvilli with lesions appearing to spread from the cervical star region moving cranially to the body of the uterus and the foetus. LeBlanc et al. reported the relationships between inflammation of the chorioallantois, increased allantoic fluid prostaglandins, premature parturition and birth of a precociously mature foal in mares with experimentally induced ascending placentitis.³ Concentrations of PGE₂ and PGF₂α rose significantly before premature parturition in infected mares.⁴ They speculated that the source of the increased production of allantoic fluid PGE is the chorioallantois in the region of the cervical star due to release of cytokines by activated macrophages and neutrophils in response to bacterial invasion of the tissue. Infected mares further expressed more mRNA for IL-8 and IL-6 than normal mares.³ The placental disturbances have been correlated to precocious activation of the foetal HPA axis and an early rise in maternal plasma prostagsten concentrations and onset of mammary development prior to day 310 of gestation.⁵ Bacteria move from the chorioallantois to the amniotic space, contaminated amniotic fluid enters the foetus passively through the oropharynx and esophagus, resulting in colonization of the foetal stomach and lungs. Allantoic fluid PG’s rising prior to isolation of bacteria in the allantoic fluid further support the hypothesis that proinflammatory cytokines may be more of a stimulus for the prostaglandins than bacterial concentration. Therefore, premature parturition or abortion associated with placentitis is most likely due to an abnormally regulated cytokine response to infectious stimulus and prostaglandin formation verses later stages, foetal death from septicaemia or placental insufficiency that occurs with the loss of microvilli.³ Differentiation between bacterial and fungal lesions as a cause of ascending placentitis is not possible by visual observation of the placenta.

**Haematogenous infection**

Haematogenous infection occurs when a mare is systemically sick or bacteraemic and the organism becomes seeded within the vasculature of the uterus/ placenta and foetus. *Leptospirosis Spp.*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus*, *Streptococcus and Salmonella abortus equi* are bacteria that can enter the uterus hematomagenously. *Leptospirosis* spp. infections appear to wax and wane from year to year. The most common serovar/serogroup involved in equine placentitis is *Leptospira interrogans* serogroup *Pomona* serovar *kennewicki*, but rarely, other serovars (*Australis,*
Grippotyphosa, Bratislava, Icterohaemorrhagica, Serjoe) have also been isolated. Which serovar identified is usually dependent on the host in a specific area. Leptospiral infection as a cause of equine placentitis and subsequent abortion has been reported as a diagnosis in 3.1% of cases in Hungary, 2.2-3.3% in the USA, in contrast to a 35% prevalence in North Ireland. In Kentucky the source of equine infection is thought to come from the wild animal population and to include raccoons, skunk, deer, opossum, in addition to cattle and swine. The Leptospira ssp. organisms are shed in the infected animal's urine contaminating water and feed which are the probable sources of infection for the equine population. Environmental conditions with low-lying swampy areas and stagnant water such as ponds produce higher incidence of disease. The mare usually displays no premonitory clinical signs before delivery, but often has a high antibody titre against one or more leptospirosis serovars. The mare is exposed, becomes infected and bacteraemic. The organism enters the placenta causing foetal infection with placentitis and funisitis.

Mucoid or nocardioform placentitis

The last means is unidentified and has been attributed to a gram-positive branching bacillus and described as a mucoid or nocardioform placentalitis. Three genera of actinomycetes that cause mucoid placentalitis in horses were discovered. These organisms were named Crossiella equi, Amycolatopsis kentuckiensis, Amycolatopsis lexingtonensis and Amycolatopsis pretoriensis, and Streptomyces atriruber, and Streptomyces silaceus. Very little is known regarding source, route of transmission and pathogenesis of nocardioform placentalitis. The clinical forms of nocardioform placentalitis range from late gestation abortions, stillbirths, prematurity and full term foals that may be small and weak or completely healthy. They produce an extensive and severe exudative placentalitis focused at the most dependent part of the uterus, at the placental body and horns rather than at the cervical star. The sticky brown mucous lies above a well-defined avillus area of the placenta. Cultures taken of these slow growing bacteria should be held for at least 72 hours to provide sufficient time for growth. Unfortunately, how these organisms gain access to the uterus and placenta is not known. One theory is that they enter the uterus when the mare is in estrus possibly at the time of breeding. However, a questionnaire launched by The University of Kentucky Gluck Equine Center, to affected farms during the 1999 breeding season revealed that no predisposing factors could be identified. Further studies were initiated when a second outbreak occurred during the 2011 foaling crop season. Nocardioform placentalitis related abortions occurred mostly between December 2010 and April 2011 happening exclusively in the last trimester. During the 2011 foaling season 76 abortions (11.3% of abortions) were due to gram positive actinomycetes. Crossiella equi spp. (28.9%), and Amycolatopsis spp.,(48.7%) were the two most common organisms identified to produce the characteristic lesions on both bacterial culture and PCR. Breeding time of aborted pregnancies ranged from March 2010 to July 2011, suggesting that if transmission occurred during breeding it was not related to a specific season. A further study was performed on 200 mares, during which 160 samples for PCR were taken prior and post breeding, with all being found negative. Mares infected with Amycolatopsis had an increased likelihood of having a live foal. Further studies in which inoculation of the nocardioform actinomycetes have been done via intra-uterine, intra-nasal, orally and intravenous routes to produce a disease model. Unfortunately this disease has not been able to be reproduced. In the United Kingdom Enterobacter agglomerans has been identified as another organism that can also produce a mucoid placentalitis.

Clinical signs

Clinical signs of bacterial placentalitis include vaginal discharge, premature lactation, and abortion, with ascending infections showing all and hematogenous and mucoid primarily the latter two. If Leptospiriosis ssp. is suspected due to abortion or increased incidence, blood serum titres or urine PCR can be done to establish its presence. However, identification prior to an initial abortion is difficult. Leptospiriosis ssp. can be demonstrated in the foetus, allantochorion, umbilical cord, or kidneys by fluorescent antibody tests (FAT),
silver staining or immunohistochemistry (IHC). Exposure usually occurs 2-4 weeks before abortion therefore the affected mares have high serological titres. Serology in the mare and foetus is based on ELISA and microscopic agglutination tests. Positive diagnosis in mares occurs with serum titres of ≥1:6400. Recently, a validated PCR has been established at the University of Kentucky Livestock Disease Center, which can identify *Leptospira ssp.* in voided urine. The spirochete is shed for long periods of time so retesting via PCR or evidence of a decreasing titre is imperative prior to reintroduction to the herd.

**Ultrasound**

Trans-rectal ultrasonographic evaluation of the caudal reproductive tract has become a routine screening and diagnostic method to monitor the utero-placental unit. Renaudin and co-workers developed the technique for evaluation of the combined uterus and placental thickness (CUPT) and established normal values in light-horse mares throughout gestation. Consistent measurements can be taken 1 - 2 cm cranial ventral to the cervical star measuring between the middle uterine artery ventrally and the allantois dorsally. At least three measurements should be taken and averaged. Normal values for 271 - 300, 301 - 330, and >330 days of gestation are < 8, < 10 and < 12 mm respectively. Trans-rectal ultrasonographic evaluation of the caudal reproductive tract has become an important diagnostic tool for bacterial placentitis. An increase in the CTUP, cervical opening, placental separation and/or edema, the presence of exudate between the chorioallantois and the endometrium, increased amnion thickness and the quantity and echogenicity of the amniotic and allantoic fluids can be identified and evaluated.

Qualitative and quantitative assessment of foetal fluids can be assessed. Hyperechogenicity of foetal fluids is dependent on stage of gestation, foetal movement and inflammation present. Foetal fluids appear hypo-echoic throughout early gestation until > 85 days when increased echogenicity is believed to be due to increased epithelial cells from the foetal membranes and the foetus as well as increased urine production. Fluids that have increased echodensity are likely to have increased cellularity due to infection or inflammation and therefore should be noted. Increased foetal movement can also falsely increase cellularity of the fluid at that period of time due to the stirring affect.

Trans-abdominal ultrasonography is extremely useful in evaluating the presence of multiple foetuses, foetal growth, activity, mobility, presentation, viability as well as placental abnormalities and foetal fluid volume and echogenicity. Normal values for heart rate and rhythm, foetal activity, size, stomach measurements, cervical pole and foetal fluid depth have been determined and therefore comparisons can be made. Although placental thickening is difficult to interpret trans-abdominally, due to the stretching and contracture of the different regions of the pregnant uterus and foetal positioning, separation of the chorioallantois from the endometrium and the presence of exudate as seen with mucoid or hematogenous placentitis can be identified. The foetus and it’s presentation can be identified by finding the ribs and thorax usually mid-line and cranial to the mammary gland. Foetal heart rates can vary depending on activity, ranging from 70-100 bpm with consistently low or high heart rates indicative of foetal stress or distress. Identification and examination of the umbilical cord can sometimes be assessed depending on positioning of the foetus. This is useful when trying to identify abnormalities due to excessive twisting as seen with umbilical cord torsions. An enlarged bladder may be evident if the cord is torsed close to the body wall. A dead foetus can be visualized with no identifiable heartbeat, the organs losing definition and dependent of duration, increased intra-abdominal hyperechogenicity associated with gas bubbles. It is important to always examine both sides of the abdomen completely to eliminate the possibility of twins (a differential for premature mammary gland development). Foetal activity and tone can be determined when monitoring heart rate and reaction to ultrasonography.

**Progesterone**

During pregnancy, progesterone (P4) is synthesized by the ovaries until about 150 days of gestation. From then until 320-360 days, P5 is supplied by the foetus, which is converted
into P4 by the placenta. During the second half of pregnancy little if any P4 is present because it is rapidly metabolized into progestagens. These progestagens increase gradually during the last few weeks prior to parturition (>300 days) but decline within a few days or even hours of delivery. In the first trimester impending abortion is preceded by declining or low P4 levels. However, foetal losses or premature deliveries in late gestation, particularly those caused by placental abnormalities especially placentitis are associated with high concentrations of total progestagens in the maternal plasma. Ousey et al further demonstrated using gas chromatography-mass spectrometry that progestagen profiles for mares with placentitis had increased P5 and/or P4 production by the foetus, probably caused by chronic foetal stress, leading to increased metabolism and elevation of several other pregnanes (5alpha-DHP, P5BB, BB-diol, Baalpha-diol, 20alpha-5P). In mares with avillous placenta or placental oedema, metabolism of many pregnanes is diminished suggesting lack of functional placenta. In mares where there is acute foetal distress (colic, uterine torsion), production of progestagens is dramatically reduced, indicating the importance of a healthy feto-placental unit for progestagen formation. A recent study in which combined thickness of the uterus and placenta (CTUP) and an abbreviated plasma progestagen profile (4 blood samples 48 hours apart) were measured showed that when performing both tests, 20/22 mares were correctly identified with respect to pregnancy outcome. Mares that aborted acutely (within 7 days) from placentitis exhibited a decrease in plasma progestagens whereas mares that maintained their pregnancy for more than 8 days exhibited a rise. When evaluating progestagen levels it is important to know which progestagens cross-react with that particular laboratory assay and what the normal reference ranges are for that specific laboratory. Therefore serum progestagens may provide an effective way for practitioners to identify mares with early placentitis that may have a compromised fetoplacental unit.

**Oestrogen and other hormones and proteins**

Oestrogens in the pregnant mare, are either conjugated to sulfates (example oestrone sulfate) and represent the bound fraction or they are unbound free oestrogens such as oestrone and oestradiol-17B. In general, the predominant oestrogens during pregnancy in mares in order of magnitude are oestrone, oequilin, oequilenin, and oestradiol-17B. These hormones are produced by precursors from the foetus, metabolized by the placenta and act primarily on the maternal uterus. After day 150 of pregnancy, mean concentrations remain above 1,000 pg/ml until approximately day 310 at which time oestrogens fall below this level and are approximately 500 pg/ml at 340 days. Measuring total serum oestrogens should help predict foetal viability.

A recent study performed at Gluck Equine Center at the University of Kentucky looked at a large cohort of mares (n=700) to try to identify early markers signalling placentitis. Serum samples were pulled weekly from the 7th month of gestation until parturition. Retrospectively these samples were run for varying hormones and proteins depending on the pregnancy outcome. Mares that aborted or presented with placental lesions at delivery (n=15) were paired with similar age on the same farm. Oestradiol 17B, alpha-fetoprotein, haptoglobin and serum amyloid A concentrations were assessed. This study concluded that although total oestrogens may be low in placentitis, Oestradiol 17B may be a better marker since serum concentrations were specifically found to be significantly lower in mares that had an abnormal pregnancy. Due to these results, the researchers wanted to determine if supplementation with Oestradiol 17B may be indicated in the treatment of placentitis. Letrozole which inhibits androgen synthesis to oestrogens was administered to mares starting at 8 months of gestation. Maternal serum levels of both Oestradiol 17B and Oestrone Sulfate significantly decreased by 90%. Interestingly there was no change or decrease in the uterine artery hemodynamics. This study concluded that a reduction in peripheral oestrogens in late pregnant mares did not adversely affect pregnancy outcome or neonatal viability although birth weights were reduced by 15%. Therefore reduction of oestrogen concentrations associated with placental disease are likely reflective of disruption of placental function but are not the cause of abortion. A recent study added
Oestradiol Cipionate to the standard placentitis therapy of Trimethoprim Sulfa, Altrenogest and Flunixin meglamine and concluded that in mares with experimentally induced ascending placentitis benefited from oestrogen supplementation, but progestin supplementation did not appear to make a difference in outcome. Obviously this therapy is controversial and studies are not equivocal and further investigation is warranted. Conversely, if placental function is disrupted androgens should increase in the maternal circulation. Unfortunately when DHEA-S was measured prior to abortion in mares with placentitis no significant changes were observed therefore negating the usefulness of androgens as a marker for placentitis. Serum Amyloid A and Haptoglobin are acute phase proteins that become elevated in response to inflammation. They are not normally elevated during pregnancy but are however non-specific. Both become elevated in mares with experimentally induced placentitis. SAA rises more quickly approximately 3 days prior to Haptoglobin. Unfortunately, since these are acute phase proteins their elevation is relative to infection and therefore in clinical placentitis their usefulness has been very limited and unrewarding. Another potential marker for placentitis is Alpha-fetoprotein (AFP). It is present in high concentrations in foetal fluids. Canisso et al showed that maternal AFP was positively associated with abnormal pregnancy outcome. Potential future aids in recognizing utero-placental-foetal deficiencies come in small packages called MicroRNAs. These are small non-coding RNAs produced in the nucleus that are stable in the circulation. Their function is to regulate mRNA degradation or expression and are differentially expressed by tissue type. Most recently a number of mRNA have been identified to be up regulated in association with experimentally induced inflammation of the chorion and endometrium. These mRNA will hopefully be the markers of the future.

**Treatment**

Due to the knowledge above, treatment is directed at resolving microbial invasion, decreasing inflammation and uterine contractions and increasing blood flow to the potentially compromised foetus. Systemic treatment can include antibiotics, exogenous progestagens, anti-inflammatories, tocolytic agents and rheostatic agents. If a vaginal discharge is present and the cervix open, speculum examination and culture of the exudate provides identification and sensitivity of the organism allowing local treatment and appropriate systemic treatment to be initiated.

Factors regulating endometrial quiescence during pregnancy have still not been fully elucidated. Progesterone and it’s metabolites are essential in maintenance of pregnancy. As previously explained, progesterone is reduced to 5 alpha-pregnane and pregnenes within the placenta and possibly within the foetal liver and kidneys. It has been suggested that the 5 alpha pregnanes maintain myometrial quiescence in the horse. Numerous studies have shown that progesterone inhibits oxytocin receptor formation, reducing the sensitivity of the uterus to PGF2α-induced oxytocin secretion. Gap junction formation also is inhibited by progesterone preventing a coordinated uterine contraction during pregnancy. Therefore altrenogest or progesterone in oil can be beneficial in diseases that are associated with excess PGF2α secretion. In a recent study performed by Baily CS, Macpherson ML et al, treatment with oral Trimethoprim Sulfa, Pentoxifylline and a synthetic progestin,(altrenogest), resulted in longer pregnancies and more viable foals in mares with placental infections than untreated mares. This study, compared to a previous study done by their laboratory which showed poor results for pregnancy maintenance and foal viability for both groups with antibiotics and anti-inflammatories or without, attributed the altrenogest as the factor contributing to the improved results.

During the second or third trimester when placentitis is present, an increase in plasma PGFM can produce abnormal myometrial activity and blood flow. Non-steroidal anti-inflammatory drugs such as Flunixin Meglamine or Firocoxib, can inhibit synthesis of PGF2α and ameliorate uteroplacental perfusion by preventing cardiovascular collapse and systemic hypotension.
Pentoxifylline is a rheostatic agent with anti-inflammatory properties that has been used extensively in clinical situations of placentitis, endotoxemia, laminitis and other systemic illnesses to increase perfusion to microvascular. From validated systemic effects, the use of pentoxifylline has been extrapolated to increase blood flow to the microcirculation of the uterus and therefore diminishing placental insufficiency.

Tocolytic drugs such as clenbuterol, isoxsuprine, ritodrine and terbutaline have been used in women in preterm labour with growth retardation. These B-sympathomimetic agents enhance utero-placental blood flow in some placental insufficiencies and inhibit labour. In the horse, clenbuterol has been used both intravenously and orally during dystocias to produce uterine relaxation for manipulation. However, when critically evaluated by Card and Wood, there was a transient increase in maternal and foetal heart rate throughout pregnancy. Early in pregnancy clenbuterol produced decreased uterine tone however later had little to no appreciable effect.

Administration of acetylsalicylic acid (ASA), an anticoagulant, and captopril, a vasodilator, increase both uterine and ovarian perfusion in cycling non-pregnant mares. The increase in uterine and ovarian blood flow has been attributed to the inhibition of platelet cyclooxygenase by ASA; this results in inhibition of the synthesis of thromboxane A\textsubscript{2}, which facilitates platelet aggregation and vasoconstriction. Captopril is an angiotensin-converting enzyme (ACE) inhibitor; ACE promotes synthesis of the vasoconstrictor angiotensin II and the production of bradykinin, which enhances contractility of uterine smooth muscle. Because ACE occurs in the equine endometrium it is believed that captopril acts as a vasodilator in the uterus and inhibits contractility. However in pregnant laboratory animals captopril decreased uterine and uteroplacental circulation. The effects of ASA are more pronounced and consistent than captopril. ASA at 2500 mg twice daily has been shown to improve uterine and ovarian perfusion and increase plasma progesterone concentration, however if this can translate into increased uterine and therefore placental perfusion is yet to be determined. ASA has therefore become a commonly used treatment in placentitis and other high-risk pregnancies with placental insufficiency. Further studies need to be done to ascertain if this in fact is true.

Appropriate antimicrobial therapy is indicated in mares with placentitis. Few studies have elucidated which antimicrobials cross the compromised uteroplacental unit providing protection to the developing foetus. These include, Trimethoprim Sulfamethoxazole (15-30mg/kg PO BID), Penicillin G (22,000IU/kg IM BID), Gentamicin (6.6 mg/kg IV SID), Trimethoprim Sulfamethoxazole has been identified in foetal fluids and determined to control bacterial growth in placentitis. Bailey and Macpherson illustrated that trimethoprim sulfu attenuated bacterial growth when placentitis is induced, but long-term treatment was necessary for foetal viability since Beta Streptococcus was cultured from the uterus post-foaling in both treated and untreated controls. A recent study showed that Enrofloxacin also crosses the placenta and provides MIC levels in the allantoic fluid however further studies are needed to determine dosage and the side effects on foetal growth.

Progress has been made in identifying placentitis or “high risk” mares especially with routine placental ultrasonographic evaluations, in conjunction with foetal viability assessments and serial hormonal evaluations. These techniques allow earlier intervention producing an increase in foal viability. Unfortunately, therapeutic protocols are still mostly derived from extrapolation from human literature, non-pregnant mare research and clinical impressions. Future investigations that directly measure uteroplacental blood flow and a cytokine or hormonal marker panel involved in compromised pregnancies could better elucidate future treatment regimes.

Reference:


Ascending placentitis originating at the cervical star
Nocardioform placentitis with mucoid exudate and avillous area at the bifurcation of the uterus