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Update on placentitis – what has been accomplished in the last 3 years?
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Abstract
Bacterial placentitis is among the leading causes of abortion in the mare. Further, despite intense research and clinical focus, diagnosis for most forms of placentitis remains difficult and clinical treatment success remains limited. As clinical signs are non-specific and may occur late in the course of disease, screening tests have been developed, including ultrasonographic evaluation of the combined thickness of the uterus and placenta, hormonal assays and measurement of acute phase proteins. Mares diagnosed with placentitis are generally treated with a combined regimen including an antibiotic, such as trimethoprim sulfamethoxazole, anti-inflammatory therapy, such as flunixin meglumine or pentoxifylline, and altrenogest. However, despite a large body of research, clinical outcomes remain poor. Key challenges that must be addressed to improve clinical outcomes for mares with placentitis are 1) the development of affordable, sensitive screening tests; 2) identification of effective anti-inflammatory therapy to prevent abortion and fetal compromise; and 3) identification of more effective antibiotic therapy.

Keywords: Equine placentitis, diagnosis, trimethoprim sulfamethoxazole, NSAID, preterm delivery

Introduction
Bacterial placentitis is among the leading causes of abortion in the mare. Further, despite intense research and clinical focus, diagnosis for most forms of placentitis remains difficult and clinical treatment success remains limited.

A series of retrospective studies of equine abortion from the US and Europe document the importance of this disease, and further subcategorize placentitis into subcategories.1-3 The most common type of placentitis is ascending bacterial placentitis, which in turn is most commonly caused by the organisms Streptococcus equi subsp. zooepidemics (S. zooepidemicus) or Escherichia coli. In addition to being the most common cause of equine endometritis and ascending placentitis, S. zooepidemicus has recently gained additional notoriety for its ability to “jump” species.4 It has been reported as an occasional cause of mastitis in cattle and a cause of canine infectious respiratory disease and hemorrhagic streptococcal pneumonia (HSP) in dogs.5,6 There have been numerous reports of disease in people, occasionally associated with mortality.7

Other economically important types of placentitis are nocardioform placentitis, leptospiral placentitis, and the twin conditions equine amnionitis and fetal loss syndrome (EAFLS) and mare reproductive loss syndrome (MRLS). While local outbreaks of each of these diseases has been reported in recent years, the current manuscript will focus on ascending placentitis.

Pathophysiology
Recent reviews by Ousey and Fowden describe the extensive work that has characterized the endocrine control of normal parturition and abortion.8-10 Pregnancy maintenance and parturition are controlled by a complex set of hormonal interactions which serve to first prevent and then promote uterine contractions. In other species, progesterone has been demonstrated to promote “uterine quiescence” at least in part through an upregulation of 15-hydroxy prostaglandin dehydrogenase (PGDH), which inactivates prostaglandins,11 and by preventing prostaglandin-upregulation by cortisol.12 In the mare, this correlation is less well understood. Progesterone is virtually absent from systemic circulation in late pregnancy, and it has further been demonstrated that the uterus of pregnant mares continues to respond to either oxytocin or prostaglandin with increased muscular contractility. However, it is possible that either progesterone or one of the prostaglandins produced by the fetoplacental unit act in a paracrine fashion to diminish uterine contractility in pregnant mares, thus preserving normal pregnancy.8

Mares with experimentally-induced placentitis have been shown to have increased allantoic fluid concentrations of prostaglandin E and prostaglandin F2α. At the time of delivery, proinflammatory cytokines were substantially elevated in placental tissues of mare with intrauterine disease, compared to those that
delivered normally. These findings correspond with work in women, non-human primates, as well as other animal models of preterm labor. Proinflammatory cytokines and prostaglandins both serve to activate the fetal hypothalamic-pituitary-adrenal axis (HPA axis). The fetal adrenal produces both progestins and, once sufficiently mature, cortisol. Fetal cortisol, in turn, enhances placental and uterine prostaglandin production, further enhancing uterine contractility and resulting in fetal delivery.

In addition to its role in causing preterm delivery, recent work in non-human primates further suggests strongly that proinflammatory cytokines have direct detrimental effects on the fetal brain and neurologic system, independent of bacterial damage or anoxia. These new findings have not been confirmed in the horse, but may represent a component of the disease-processes frequently encountered in post-natal foals born to mares with placentitis, even in the absence of detectable bacterial infection or peripartum complications.

**Diagnosis**

Rapid diagnosis and treatment of disease likely play a key role in veterinarians’ ability to affect a positive treatment outcome. However, placentitis often does not result in obvious clinical signs prior to abortion or delivery, and systemic health parameters (temperature, pulse, respiration) or hematologic parameters (complete blood count and serum chemistry values) are generally within normal limits, even in the face of severe intrauterine disease. In an experimental model of induced placentitis, mucoid, purulent or serosanguinous vulvar discharge was the most common clinical sign, however this is infrequently noticed or reported by owners and veterinarians. In contrast, in the author’s experience, the most common presenting complaint in naturally occurring cases of either ascending placentitis or nocardioform placentitis is precocious mammary development prior to day 300-310. The mechanism for such mammary development has not been fully elucidated, but it is presumed to correspond with increasing maternal serum progestin concentrations, as in normal parturition. This would indicate that these changes occur only after substantial fetal compromise and suggest the strong need for more sensitive diagnostic and screening tools.

**Ultrasonography**

Ultrasonographic evaluation of the uterus and placenta during late gestation was first described by Renaudin and Troedsson in 1997, and has since been widely accepted as an effective screening tool. The mean thickness of the uteroplacental unit in nine Thoroughbred and Quarterhorse mares plus two times the standard deviation (95% confidence interval) was less than 7 mm for mares up to 270 days of gestation, less than 8 mm for mares between 271 and 300 days of gestation, less than 10 mm for mares between 301 and 330 days of gestation and less than 12 mm for mares greater than 330 days gestation. Work by da Silva and coworkers in Warmblood mares and independent work in our laboratory in pony mares recently confirmed that these same values can be applied to a wide range of mares. In our laboratory, Doppler ultrasonography did not identify alterations in uterine blood flow in mares with experimentally-induced placentitis. At this time, ultrasonographic examination of the combined thickness of the uterus and placenta represents the most sensitive and specific diagnostic and screening tool and can be recommended for valuable or high-risk patients. In one large farm where mares were routinely screened and treatment based on alterations of the CTUP, pregnancy and neonatal losses were reduced by roughly 50% (Zent W, personal communication, 2008). However, frequent monitoring of mares with no known risk factors for placentitis represents a significant expense and may not be cost-effective or feasible.

**Hormone assays**

Measurement of serum progestins (evaluated via the test-specific cross-reactivity with progesterone) has also been used in research and clinical settings for diagnosis of placentitis. Systemic progestagen concentrations in healthy mares are very low during late gestation and have been shown to rise in conjunction with fetal adrenal maturation after 310 days postovulation, with rapid a decline 24-48 hours prior to parturition. In mares with compromised pregnancies, progestagen levels have been shown to follow one of two patterns: they may either drop precipitously before fetal demise or abortion or they may be prematurely elevated. Premature elevations of maternal serum progestagen concentrations are consistent
with fetal stress and premature activation of the fetal adrenal and these changes may be used as a screening or confirmatory diagnostic tool in mares considered at risk for placentitis. Relative sensitivity of ultrasound and progestagen assays in naturally occurring disease has not been evaluated.

Biochemical and protein markers

In women, biochemical studies have demonstrated promise, with a range of markers detectable in amniotic fluid, serum and cervical mucus. Recent work in mares with experimentally induced placentitis also found detectable differences in the inflammatory acute phase proteins serum amyloid A (SAA) and haptoglobin between mares with placentitis and those without disease. A separate, more comprehensive study compared SAA concentrations in normal mares during late gestation to mares with experimentally induced placentitis. These authors found that SAA remained low until between 120 hours before and 36 hours after parturition in normal mares, at which time a substantial increase in serum concentrations was detectable until about 60 hours after parturition. Infected control mares developed elevated SAA concentrations within 48-144 hours after inoculation. However, in that study, clinical diagnosis preceded the rise in SAA by approximately 24 hours. Treatment was initiated based on clinical signs, and 6/9 treated mares did not experience a rise in SAA. These findings warrant further work in clinical cases of naturally-occurring placentitis to determine whether SAA may be used as an inexpensive, sensitive screening tool for placental function and whether it is influenced by confounding factors, such as extra-uterine disease or obesity.

Rapid diagnosis and screening of high-risk mares may be best performed using a combination of techniques, such as regular biochemical or hormonal screening, combined with intermittent ultrasonographic examination. The cost of such screening represents a major barrier to early diagnosis of placental disease.

Treatment

Based on the pathophysiology of disease and clinical experience, treatment of ascending placentitis, as well as nocardioform placentitis, has relied on a regimen combining antibiotics, anti-inflammatory or immune-modulatory medication, and progestins. Extensive work at the University of Florida has examined the penetration of common equine therapeutics to the allantoic fluid and to placental and fetal tissues. Further, a clinical trial utilizing the commonly used combination of trimethoprim sulfamethoxazole (TMS), pentoxifylline (PTX) and altrenogest (ALT) in experimentally-infected mares resulted in the delivery of 83% viable foals, compared to no viable foals in the control group. However, clinical experience has not been able to confirm similar positive outcomes in naturally infected mares. Furthermore, recent work in our laboratory which delayed treatment onset until CTUP was above published normal values resulted in only 40% viable foals. These findings strongly suggest a need for further research to better understand the mechanistic actions of commonly used drugs at the level of the uterus, placenta and fetus. In this review, we will summarize what is known regarding each of the commonly used therapeutics.

Antibiotic drugs

The first line of defense against placentitis is antimicrobial therapy. Antibiotic agents used to treat placitis in mares include cephalosporins, tetracyclines, sulfonamides, trimethoprim, carboxypenicillins and penicillin plus betalactamase inhibitors. These drugs have good in vitro sensitivity against the most common organisms causing ascending placentitis, including S. zooepidemicus and Escherichia coli. Work by Macpherson and co-workers has further established that gentamicin, penicillin G and TMS each achieve therapeutic concentrations within allantoic fluid. Penicillins are highly effective against S. zooepidemicus, while gentamicin is effective against most gram- organisms. Due to the potential for mixed infections, hospitalized patients are generally treated with a combination of standard doses of penicillin G and gentamicin. However, the need for repeated drug administration and catheter-maintenance makes this combination impractical for prolonged therapy of patients maintained in a farm setting, and TMS is widely used for this purpose. Trimethoprim sulfamethoxazole is a broad-spectrum, bacteriocidal antibiotic with good in vitro activity against common causative organisms of placentitis. Interestingly, despite good clinical outcomes, even prolonged treatment with TMS failed to reliably clear S. zooepidemicus from...
Pentoxifylline is a methylxanthine derivative that is widely used in the treatment of placentitis. In experimental models of placentitis, it was found that administration of PTX and TMS tended to prolong the interval from infection to delivery compared to animals infected and not treated, and that a combination of PTX, TMS and ALT resulted in significantly prolonged intervals between infection and foaling and significantly more viable foals than no treatment. However, its mechanism of action in the treatment of placentitis is not well understood. In models of endotoxemia, pentoxifylline was shown to have anti-inflammatory effects by inhibiting the cytokines TNF and IL-1β and decreasing prostaglandin F2α concentrations. Further work has been suggested to have vasodilatory and rheostatic effects. Longterm administration of pentoxifylline has been confirmed to enhance uterine artery bloodflow in mares and women, however a recent study in our laboratory did not find that short-term administration of pentoxifylline twice daily for three days could increase uterine artery bloodflow. Based on the short interval between diagnosis of placental infection and abortion in untreated, experimentally infected mares, the author concluded that any primary effect of pentoxifylline is most likely not mediated through a rheostatic or vasodilatory effect and would most likely be anti-inflammatory in nature. Further work is needed to establish the role of pentoxifylline in treating mares with placentitis.

Women with preterm labor are frequently administered glucocorticoids, and work in non-human primates with intrauterine infections further demonstrated that glucocorticoids significantly inhibited inflammatory mediators and uterine activity. In mares, work by Ousey and coworkers showed that glucocorticoids therapy could enhance fetal maturation and result in birth of precociously mature viable foals from healthy mares and mares with non-placentitis gestational disease. However, a small trial at the University of Mississippi found that treatment with TMS alone was as effective as dexamethasone and TMS combined. It is known that cortisol and other glucocorticoids are a vital component of fetal maturation prior to term, but also that endogenous or exogenous glucocorticoids enhance placental prostaglandin production. Nonsteroidal anti-inflammatory drugs (NSAIDS)

Flunixin meglumine is commonly used by clinicians as a component of treatment for placentitis, and a retrospective study by Zent and coworkers suggested that a therapeutic regimen including anti-inflammatory medication such as flunixin meglumine could improve foal viability. Further, in mares experimentally infected with endotoxin in early gestation (day 21-35), flunixin meglumine prevented prostaglandin synthesis and subsequent luteolysis, resulting in maintenance of pregnancy. It was not used in recent clinical trials due to the fact that it previously had not been detected in allantoic fluid of normal pregnant mares or mares with experimentally-induced placentitis after fluid collection via microdialysis. Thus, it was not known whether flunixin meglumine could penetrate through the placenta and reach target locations, including fetal fluids and tissues. However, it is possible that the drug was present in allantoic fluid, but not detected due to the nature of the assay, which allowed only small molecules to be collected. Further, recent work in our laboratory utilizing an in vitro model of placental inflammation confirmed that flunixin meglumine effectively inhibits both prostaglandin E and prostaglandin F2α production by chorioallantoic tissue (unpublished data). The use of other NSAIDS, such as aspirin has further been suggested, but limited studies have been performed to support their use at this time.

Other anti-inflammatory drugs

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Further work is needed to determine whether glucocorticoids therapy is efficacious and could improve fetal viability in mares with placentitis.

Tocolytic drugs

Betamimetics and other tocolytic agents, such as oxytocin antagonists and magnesium sulfate are widely used in the treatment of preterm labor in women, but remain controversial.\textsuperscript{59,60} In mares, limited studies have investigated the safety or efficacy of tocolytic agents. Palmer and co-workers investigated the effect of clenbuterol in term mares at multiple doses and were unable to inhibit parturition with this agent.\textsuperscript{61} At this time, only progestins are used routinely for tocolysis in cases of equine placentitis. The rationale for this therapy in preterm labor stems from the role progestins play in inhibiting formation of myometrial gap junctions, which facilitate uterine contractility. These findings were first demonstrated by Garfield and co-workers through \textit{in vitro} studies of ovine endometrium.\textsuperscript{62} More recent \textit{in vitro} studies have shown that progesterone interferes with the binding of oxytocin to its receptor and inhibits prostaglandin secretion.\textsuperscript{63} In women, clinical data clearly demonstrate that progestins are effective at preventing preterm labor,\textsuperscript{64} and at this time, progestins are widely accepted as a key component of therapy for preterm labor in women.\textsuperscript{65,66} Likewise, several \textit{in vivo} trials, utilizing altrenogest in early- and mid-gestation mares, demonstrated that, a synthetic progestin could prevent abortion. In a study by Daels and co-workers, altrenogest was effective at promoting pregnancy maintenance after intravenous infusion of \textit{Salmonella typhimurium} endotoxins to mares between days 21 and 35 of gestation.\textsuperscript{67,68} Mares treated with 44 mg of altrenogest, daily until day 70, maintained gestation to normal term and delivered live foals. In a subsequent study, the same authors also demonstrated that altrenogest prevented cloprostenol-induced abortion at 80-150 days of gestation. McKinnon and co-workers had similar results with altrenogest, but failed to prevent abortion with other progestins after prostaglandin-induced luteolysis in early gestation\textsuperscript{69} while Vanderwall and co-workers were able to prevent abortion in cloprostenol-treated mares with both altrenogest and a compounded injectable progesterone formulation.\textsuperscript{70} Likewise, in an experimental model of placentitis, treatment with a combination of TMS, PTX and ALT improved foal survival, whereas treatment with TMS and PTX was unable to do so.\textsuperscript{20,35} Thus, although the mechanism of action is not well-characterized, the use of a progestin to treat preterm labor and placentitis is strongly supported in both women and mares.

\textbf{Conclusion}

In conclusion, despite substantial research investments into this condition over the past three decades, placentitis remains a significant problem to the equine breeding industry. Further, while recent work from numerous laboratories has made headway in developing a more complete understanding of the pathophysiology of disease and treatment effect, this has not translated to substantial clinical improvements in cases of naturally-occurring placentitis. One key challenge that remains is to develop early, sensitive diagnostic tools that are cost-effective and can be used to screen mares during mid and late gestation. Recent work by da Silva and other groups suggests that SAA may serve this purpose.\textsuperscript{23,32} A second challenge that remains is to identify effective anti-inflammatory therapy. A recent publication by Gravett and coworkers suggests that in human preterm disease, inflammation may result in more fetal damage than infection.\textsuperscript{19} Lastly, achieving bacterial clearance from the fetus, fetal fluids and uterus within a short period of time after diagnosis will likely be a key component of treatment success and the prevention of antimicrobial resistance and secondary infections.

\textbf{References}


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