

Treatment strategies for mares with placentitis

Margo L. Macpherson *

*College of Veterinary Medicine, Department of Large Animal Clinical Sciences, University of Florida,
P.O. Box 100136, Gainesville, FL 32610, USA*

Abstract

Equine placentitis, and resultant preterm labor, are important sources of fetal and neonatal loss. The primary cause of equine placentitis is infection of the placenta with *Streptococcus equi* subspecies *zooepidemicus*, which ascends through the caudal reproductive tract. Current treatment protocols for mares affected with placentitis are empirical. This paper reviews treatment approaches for resolving placentitis and preterm labor in both equine and non-equine species. Specific therapies reviewed include antimicrobial, anti-inflammatory, tocolytic, and progestin agents.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Mare; Pregnancy; Placentitis; Preterm labor; Infection

1. Introduction

Treatment strategies for mares with ascending placentitis are currently ill-defined. Little information is available regarding penetration of drugs through the equine placenta, and efficacy of those drugs in combating the inflammatory processes that contribute to preterm labor in mares. Many treatment regimens have been extrapolated from other species, such as humans. Treatment efforts are directed at several factors, including combating infection, reducing inflammation, and controlling myometrial activity.

* Tel.: +1 352 392 4700; fax: +1 352 392 8289.

E-mail address: macphersonm@mail.vetmed.ufl.edu.

2. Antimicrobial therapy

The majority of placental infections are caused by opportunistic bacteria migrating into the uterus from the caudal reproductive tract. The most commonly isolated bacteria in equine placentitis/abortion include *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and nocardioform species [1]. Fungal and viral organisms can also infect the placenta of mares; however, these organisms typically cause abortion earlier in gestation. Therefore, treatment modalities are aimed at broad-spectrum coverage to combat infections with both gram-positive and gram-negative organisms.

Little is known about placental penetration of antibiotics in horses. Sertich and Vaala [2] administered commonly used antibiotics to 11 reproductively normal mares, in late gestation, to evaluate the efficacy of the drugs in penetrating fetal membranes. Mares were treated with: (1) potassium penicillin G and gentamicin sulfate; (2) trimethoprim sulfadiazine; (3) gentamicin sulfate or (4) potassium penicillin G. Antibiotics were administered to mares daily until parturition. Samples were obtained from allantoic and amniotic fluid, when possible, and serum. Penicillin and gentamicin were detected in the mares' serum at normal concentrations but not in amniotic fluid or foal serum. Penicillin was detected in the allantoic fluid of one mare. Trimethoprim sulfadiazine was recovered from both amniotic ($n = 2$) and allantoic ($n = 4$) fluid and was detectable in neonatal serum of two foals. The authors concluded that penicillin and gentamicin might have passed through the fetal membranes but the assays were not sensitive enough to reliably detect the drugs. They felt that penicillin and gentamicin would not be in concentrations consistent with efficacious therapy for a fetal infection, but would also not pose a risk to the developing fetus (gentamicin). The authors also concluded that concentrations of trimethoprim sulfadiazine detected in fetal fluids were consistent with levels necessary to combat most bacteria that were sensitive to the drug.

Santschi and Papich [3] monitored the pharmacokinetics of gentamicin in late pregnancy and early lactation. Seven mares were treated with one dose of gentamicin sulfate 1–4 weeks prior to parturition, again 1–4 weeks after parturition, and pharmacokinetic analysis was performed on serum samples. The authors also studied the pharmacokinetics of gentamicin sulfate in a subpopulation of foaling mares ($n = 3$). Parturition was induced (with oxytocin) in three mares within 60 min of gentamicin administration. Amniotic fluid was collected from one mare. Serum samples were collected from foals at 10, 20, 40, 60, and 120 min after delivery. Gentamicin concentrations in serum were measured with a fluorescence polarization immunoassay. Gentamicin was detected in all of the mare serum samples at expected concentrations. Gentamicin was not detected in foal serum or in the amniotic fluid sample. The authors concluded that their inability to detect gentamicin in foal serum or amniotic fluid indicated that gentamicin did not readily pass the equine placenta. However, the authors also suggested that the time between drug administration and sample collection might have been too short for sufficient drug distribution into fetal fluids and foal serum.

Recently, workers from the University of Florida [4] used in vivo microdialysis to detect concentrations of commonly used drugs in allantoic fluid of pregnant pony mares. This sensitive technique provided continuous measurement of drug concentrations in serum and

allantoic fluid after drug administration. In the first study, five reproductively normal mares (Days 269 to 271 of gestation) had microdialysis probes placed in the allantoic cavity and jugular vein. Mares were treated intravenously with potassium penicillin G (22,000 IU/kg, q 6 h), gentamicin (6.6 mg/kg, q 24 h) and flunixin meglumine (1 mg/kg, q 12 h). Allantoic fluid and blood were collected for 24 h. Analysis of microdialysate samples demonstrated that both antibiotics were present in allantoic fluid, albeit at lower concentrations than present in serum. Concentrations of penicillin in allantoic fluid achieved the minimum inhibitory concentration (MIC) against *S. equi* subspecies *zooepidemicus*. Gentamicin concentrations in allantoic fluid were adequate to be effective against *E. coli* or *K. pneumoniae*. Unfortunately, the group was unable to determine the pharmacokinetics of flunixin meglumine because the protein-bound drug did not penetrate the microdialysis membrane pores.

In the same study, placentitis was induced in two of the reproductively normal mares by placing an inoculum of *S. equi* subspecies *zooepidemicus* into the cervix on Days 279 and 283 of gestation, respectively. Microdialysis studies were performed as described above. Penicillin and gentamicin were detected in allantoic fluid of the two infected mares after drug administration. However, the pharmacokinetics and efficacy of the drugs could not be determined due to the limited number of mares.

In a second investigation at the University of Florida (S.A. Rebello, personal communication), the pharmacokinetics of trimethoprim sulfamethoxazole and pentoxifylline in allantoic fluid of pregnant mares were studied. Advantages of using trimethoprim sulfa in mares with placentitis include oral bioavailability and good uterine penetration. Pentoxifylline was evaluated because it appears to down-regulate pro-inflammatory cytokines [5,6]. Ten pregnant pony mares were used for the study. Five mares (placentitis group) were inoculated, intracervically, with *S. equi* subspecies *zooepidemicus* 5 days before drug administration and measurement of drug concentrations by microdialysis. Five mares served as uninfected controls. All mares were treated with trimethoprim sulfa (30 mg/kg, BID) and pentoxifylline (8.5 mg/kg, BID), orally, for 14 days. Both drugs penetrated fetal membranes and were detected in allantoic fluid of treated and control mares. In this study, four of the five infected mares aborted. Three mares aborted after termination of drug therapy (10, 17, and 19 days after the last day of treatment), one mare aborted on the 13th day of drug therapy and one mare carried a normal foal to term (40 days after cessation of drug therapy). All control mares carried pregnancies to term and delivered healthy foals. These data suggest that trimethoprim sulfa and pentoxifylline may delay preterm delivery in mares with placentitis.

Initial work from a large clinical trial (Troedsson and Zent, personal communication) revealed interesting results regarding treatment of mares diagnosed with placentitis. The investigators examined records of 477 mares over 6 years. Fifteen mares were diagnosed with placentitis. Criteria for treatment included increased thickness of the uteroplacental unit using transrectal ultrasound, placental separation and/or vulvar discharge and udder development. The average gestational age at diagnosis was 8.6 months. Mares were treated with a combination of systemic antibiotics (trimethoprim sulfa, ceftiofur or penicillin and gentamicin), pentoxifylline, altrenogest, and non-steroidal anti-inflammatory agents. Mares were treated until abortion or delivery of a foal. Twelve of fifteen (84%) treated mares carried their foals to term and 11 of 15 (73%) delivered live foals. Birth weights of surviving foals from mares treated for placentitis were similar to foals from non-affected

mares. Data from these two studies suggest that long-term antibiotic and anti-inflammatory treatment may positively impact pregnancy outcome in mares with placentitis. Further studies to examine the effect of individual drugs and/or length of treatment on neonatal outcome are necessary to effectively direct therapy in these mares.

3. Anti-inflammatory therapy

Inflammation has recently been identified as an inciting factor in preterm labor. Several studies in humans and non-human primates provide evidence that pro-inflammatory cytokines play a key role in the pathogenesis of infection-associated preterm delivery [7]. Specifically, interleukin 1 β (IL-1 β), interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor α (TNF- α) have been implicated in preterm labor. Bacteria or bacterial products in the fetal membranes stimulate cell-mediated immune mechanisms with subsequent release of pro-inflammatory cytokines from macrophages and the decidua. In turn, pro-inflammatory cytokines stimulate release of prostaglandins E₂ (PGE₂) and F₂ α (PGF₂ α) from the endometrium. Prostaglandins then initiate uterine contractions.

Recent research in non-human primates has been directed at identifying therapies that are effective for treating this multifactorial disease. Specifically, a potent cyclooxygenase inhibitor, indomethacin, has been shown to significantly inhibit prostaglandin secretion and uterine contractility in monkeys with experimentally induced preterm labor [8]. Immunomodulators, such as dexamethasone or interleukin-10, have also been effective in reducing amniotic prostaglandin synthesis in monkeys with experimentally induced preterm labor [9]. Monkeys treated with dexamethasone did not deliver fetuses prematurely, while all other monkeys delivered prematurely.

More recent work [10] compared the effects of antibiotics alone (ampicillin), and antibiotic therapy in combination with dexamethasone and indomethacin, for delaying experimentally induced preterm labor in monkeys. Results from this study showed that ampicillin, alone, was effective in eradicating bacteria from the amniotic fluid of infected animals. However, elevations of amniotic fluid cytokines, prostaglandins, and uterine contractions persisted in spite of maternal antibiotic treatment. Amniotic fluid cytokines and prostaglandins were suppressed in animals treated with ampicillin, dexamethasone, and indomethacin. Encouraging results from this study indicate that combined therapy is necessary to stem the bacterial infection as well as to suppress the subsequent inflammatory response.

The inflammatory mechanisms initiating premature labor in horses are not well known. LeBlanc et al. [11] identified elevated concentrations of prostaglandins (PGE₂) and (PGF₂ α) in allantoic fluid from mares with experimentally induced placentitis. Allantoic concentrations of cytokines (IL-1, IL-6, TNF α) were not different between infected and control mares; however, concentrations of IL-6 and IL-8 were elevated in the placentas of infected mares. The effectiveness of therapies directed against the inflammatory cascade is equally elusive. Murchie et al. [4] attempted to determine if the potent anti-prostaglandin agent, flunixin meglumine, penetrated the placenta in normal and experimentally infected mares. Using microdialysis methodology, flunixin meglumine was undetectable in both allantoic and serum samples. The authors attributed their inability to detect flunixin meglumine to the highly protein-bound nature of the drug. Large proteins are unable to

pass through microdialysis pores, thus preventing passage of the drug into the allantoic space. Florida workers (S.A. Rebello, personal communication) also used microdialysis to detect concentrations of pentoxifylline in the allantoic space of both normal and experimentally infected pregnant pony mares. Pentoxifylline, a xanthine derivative, is thought to exert anti-inflammatory/cytokine effects [12,13]. Concentrations of the drug were detected in the allantoic fluid of both normal and infected mares. Pentoxifylline, in combination with trimethoprim sulfa, appeared to prolong time to abortion in treated mares with experimentally induced placentitis.

4. Tocolytics

The goal of tocolytic therapy is to prevent, or disrupt, uterine contractions and premature labor. Tocolytic agents are commonly employed in women with clinical signs of preterm labor. A variety of agents have been used including: magnesium sulfate, β -sympathomimetic agents (ritodrine, terbutaline), prostaglandin synthesis inhibitors (indomethacin, sulindac, ibuprofen, aspirin), calcium channel blockers (nifedipine), and oxytocin antagonists (atosiban) [14]. The ability of these agents to prevent active labor is limited. Tocolytic agents have not been shown to significantly prolong pregnancy or improve neonatal outcome when used alone. Historically, tocolytics prolong pregnancy for up to 48 h during which time glucocorticoids can be administered to the mother in an effort to expedite fetal maturation. Side effects from tocolytic agents can be significant and include: cardiac/respiratory arrest (magnesium sulfate); cardiac arrhythmia, pulmonary edema and myocardial ischemia (β -sympathomimetics); hypotension (nifedipine); gastrointestinal disturbance and oligohydroamnios (indomethacin) [15].

Clenbuterol, a β -sympathomimetic agent, is used in clinical equine practice. The effects of clenbuterol administration on uterine tone and maternal and fetal heart rates were examined by Card and Wood [16]. Clenbuterol was administered intravenously (300 μ g) to four pregnant mares at 30, 40, 50, and 60 days of gestation and once monthly until parturition. The final dose was administered when the mare was thought to be close to parturition as indicated by concentration of calcium and magnesium (120 ppm) measured using water-hardness test strips. Fetal heart rate, maternal heart rate and uterine tone (measured by palpation) were recorded after drug administration. Mares and fetuses experienced transient tachycardia after drug administration at all time points. Resting uterine tone changed significantly after clenbuterol administration to mares early in gestation. Uterine relaxation after clenbuterol administration was less profound later in gestation when uterine tone was decreasing naturally. Uterine relaxation occurred within 3 min of drug administration and persisted up to 120 min. The authors concluded that clenbuterol was effective in causing uterine relaxation throughout gestation, and that the side effects were minimal and transient.

A more recent study [17] reported the effects of clenbuterol when administered to mares late in gestation. Twenty-nine pregnant pony mares with similar breeding dates were enrolled in the study. Beginning Day 320 of gestation, mammary secretion electrolyte changes were monitored using a calcium strip test. Treatment started when calcium levels reached a maximum level of 13 mM (four squares reacted on the strip test). Mares were

treated with varying doses of clenbuterol, i.v.: 0.6 mg ($n = 6$); 1 mg ($n = 5$); and 1.5 mg ($n = 4$). Fifteen control mares were treated with saline. All mares were treated once daily, at 22:00 h, until parturition. No differences were detected between groups for length of gestation, number of treatments or time to foaling after the last dose was administered. Treatment dose did not affect outcomes. Mares in the low-dose treatment groups (0.6 and 1 mg) showed no side effects, while mares treated with 1.5 mg showed transient signs of abdominal distress and sweating. All foals were clinically normal except one foal from the treatment group that died after dystocia. The authors concluded that clenbuterol was not effective in preventing the onset of myometrial contractions in normal foaling mares at term. Treated mares in this study actually foaled earlier in the evening than untreated mares. The authors speculated that the relaxant effects of clenbuterol might have promoted cervical relaxation and subsequent parturition. Based on side effects detected when clenbuterol is administered to pregnant mares [16,17], and lack of effect for delaying normal parturition, the authors suggest that this agent has limited usefulness in horses.

Progestin therapy is currently being implemented in humans to halt preterm labor. A recent double-blind, placebo-controlled study [18] showed a beneficial effect when women with a documented history of spontaneous preterm delivery were treated with progesterone. The incidence of recurring spontaneous preterm delivery was significantly lower in women treated with 17 alpha-hydroxyprogesterone than in untreated women (36.3% versus 54.9%, respectively). In addition, babies from progesterone-treated women required less oxygen therapy and had fewer cases of necrotizing enterocolitis and intraventricular hemorrhage than babies delivered from untreated mothers. Whether progesterone plays a role in inhibiting formation of gap junctions, which facilitate myometrial contractions [19], or it interferes with prostaglandin-induced myometrial contractions stimulated by pro-inflammatory cytokines is unknown. However, results from these studies strongly support the use of progestin therapy in mares at risk for preterm labor.

Treatment with progestins has long been advocated to promote uterine quiescence in mares with uterine pathology. The actual rationale for progestin use in late pregnancy is not clear. Presumably, the anti-prostaglandin effect of progestins would contribute to reduced myometrial activity by interfering with upregulation of prostaglandin and oxytocin receptors [19]. Without receptor formation, gap junction formation would be inhibited and uterine contractility prevented. Daels et al. [20] tested the effects of progesterone and altrenogest, a synthetic progestin, on pregnancy maintenance in mares treated with the prostaglandin analog, cloprostenol. Sixteen mares with pregnancies ranging from 93 to 153 days of gestation were included in the study. Cloprostenol (250 μ g) was administered to all mares, intramuscularly, for five consecutive days. Progesterone (300 mg, SID) was administered to eight mares, intramuscularly, beginning 18 h after cloprostenol treatment and discontinued 18 h after the last cloprostenol treatment. Altrenogest (44 mg, SID) was administered to eight mares, orally, beginning 12 h after cloprostenol and discontinuing 12 h after the last cloprostenol treatment. Cloprostenol-treated control mares were extrapolated from a previous study. Five of eight mares in the progesterone-treated group maintained pregnancies after cloprostenol treatment, while three mares aborted during treatment. All eight mares treated with altrenogest maintained pregnancies. Control mares all aborted after cloprostenol treatment. Administration of exogenous progestins to mares treated with cloprostenol was associated with an endogenous decrease in prostaglandin

metabolite concentrations. This important study demonstrated that progestin supplementation was able to prevent prostaglandin-induced abortion in most cases. These findings support progestin supplementation in mares at risk for preterm labor.

Effective treatments for placentitis in mares are still elusive. Data from studies involving humans and non-human primates indicate that combined therapies with antibiotics and anti-inflammatory agents show the most promise for interrupting preterm labor. Preliminary data in horses support this concept.

References

- [1] Giles RC, Donahue JM, Hong CB, Tuttle PA, Petritesmurphy MB, Poonacha KB, et al. Causes of abortion, stillbirth and perinatal death in horses – 3,527 cases. *J Am Vet Med Assoc* 1993;203:1170–5.
- [2] Sertich PL, Vaala WE. Concentrations of antibiotics in mares, foals and fetal fluids after antibiotic administration in late pregnancy. *Proc Am Assoc Equine Pract* 1992;38:727–36.
- [3] Santschi EM, Papich MG. Pharmacokinetics of gentamicin in mares in late pregnancy and early lactation. *J Vet Pharmacol Ther* 2000;23:359–63.
- [4] Murchie TA, Macpherson ML, Leblanc MM, Luznar SL, Vickroy TW. A microdialysis model to detect drugs in the allantoic fluid of pregnant pony mares. *Am Assoc Equine Pract* 2003;49:118–9.
- [5] Lauterbach R, Pawlik D, Zembala M, Szymura-Oleksiak J, Lisowska-Miszczuk I, Kowalczyk D, et al. Pentoxifylline in and prevention and treatment of chronic lung disease. *Acta Paediatr* 2004;93:20–2.
- [6] Lauterbach R, Zembala M. Pentoxifylline reduces plasma tumour necrosis factor-alpha concentration in premature infants with sepsis. *Eur J Pediatr* 1996;155:404–9.
- [7] Dudley DJ, Trautman MS. Infection, inflammation, and contractions – the role of cytokines in the pathophysiology of preterm labor. *Sem Reprod Endocrinol* 1994;2:263–72.
- [8] Sadowsky DW, Haluska GJ, Gravett MG, Witkin SS, Novy MJ. Indomethacin blocks interleukin 1beta-induced myometrial contractions in pregnant rhesus monkeys. *Am J Obstet Gynecol* 2000;183:173–80.
- [9] Sadowsky DW, Novy MJ, Witkin SS, Gravett MG. Dexamethasone or interleukin-10 blocks interleukin-1 beta-induced uterine contractions in pregnant rhesus monkeys. *Am J Obstet Gynecol* 2003;188:252–63.
- [10] Gravett M, Sadowsky D, Witkin S, Novy M. Immunomodulators plus antibiotics to prevent preterm delivery in experimental intra-amniotic infection (IAI). *Am J Obstet Gynecol* 2003;89:56.
- [11] Leblanc MM, Giguere S, Brauer K, Paccamonti DL, Horohov DW, Lester GD, et al. Premature delivery in ascending placentitis is associated with increased expression of placental cytokines and allantoic fluid prostaglandins E-2 and F-2 alpha. *Theriogenology* 2002;58:841–4.
- [12] Lauterbach R, Pawlik D, Zembala M, Szymura-Oleksiak J, Lisowska-Miszczuk I, Kowalczyk D, et al. Pentoxifylline in and prevention and treatment of chronic lung disease. *Acta Paediatr* 2004;93:20–2.
- [13] Lauterbach R, Zembala M. Pentoxifylline reduces plasma tumour necrosis factor-alpha concentration in premature infants with sepsis. *Eur J Pediatr* 1996;155:404–9.
- [14] Ramsey PS, Rouse DJ. Therapies administered to mothers at risk for preterm birth and neurodevelopmental outcome in their infants. *Clin Perinatol* 2002;29:725.
- [15] Goldenberg RL. The management of preterm labor. *Obstet Gynecol* 2002;100:1020–37.
- [16] Card CE, Wood MR. Effects of acute administration of clenbuterol on uterine tone and equine fetal and maternal heart rates. *Biol Reprod Mono* 1995;1(1):7–11.
- [17] Palmer E, Chavette-Palmer P, Duchamp G, Levy I. Lack of effect of clenbuterol for delaying parturition in late pregnant mares. *Theriogenology* 2002;58:797–9.
- [18] Meis PJ, Klebanoff M, Thom E, Dombrowski MP, Sibai B, Moawad AH, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *N Engl J Med* 2003;348:2379–85.
- [19] Garfield RE, Kannan MS, Daniel ME. Gap junction formation in the myometrium: control by estrogens, progesterone and prostaglandins. *Am J Physiol* 1980;238:C81–9.
- [20] Daels PF, Besognet B, Hansen B, Mohammed H, Odensvik K, Kindahl H. Effect of progesterone on prostaglandin F-2 alpha secretion and outcome of pregnancy during cloprostenol-induced abortion in mares. *Am J Vet Res* 1996;57:1331–7.