Proceedings of a Workshop on

UTERINE INFECTION IN MARES
AND WOMEN: A COMPARATIVE
STUDY II

9th – 13th November 2005
South Carolina, USA

Editors: M. M. LeBlanc, J. F. Wade and L. Foster
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Being given the opportunity to host a Havemeyer Foundation Workshop is an honor and a challenge. It is an honor because one is given funds to design a scientific workshop in a venue of their choosing and invite prominent scientists and their graduate students to discuss a significant problem in the horse industry for three days. It is a challenge because participation is limited to 30 individuals in an attempt to foster animated discussion and collaborations.

The second workshop on infections in late gestation: comparisons between women and horses brought old and new faces to Hilton Head South Carolina for three sunny beautiful days in November 2005. It was a huge success in regards to science, camaraderie and the formation of new collaborations. The group included three human perinatologists and a basic scientist who gladly shared their clinical expertise in human medicine and their scientific acumen on animal models with veterinary clinicians, reproductive physiologists and pathologists. Members of three veterinary colleges presented work from their models of placentitis while equine neonatologists discussed neonatal outcome. And our reproductive pathologists reminded us how infection gravely affects reproductive biology and neonatal outcome. Discussions far outlasted the time allotted them. Three topics that will require future study were outlined on the last day.

- There is a need for uniformity in the models of equine placentitis. There are currently three models of infection in the USA. Experimental numbers are small in each model and there is lack of uniformity between models. It was decided that the three groups would identify an inoculum that would be used in all models to enable future comparisons.

- Treatment studies in human medicine, laboratory animal models and in the mare indicate that pregnancy can be prolonged in the face of infection with drugs (systemic antibiotics, non-steroidal anti-inflammatory drugs and tocolytics), however, neonatal outcome is not necessarily improved. Preliminary data on administration of dexamethazone to the dam during infection in laboratory models appears to improve neonatal survival. Its use needs to be investigated in the horse in an attempt to improve neonatal outcome.

- The cervix is ‘sacred’. If it is damaged or loses its seal, ascending infection can seed the fetal membranes. Our knowledge of how the cervix functions at the molecular level during pregnancy or even during the estrous cycle is limited. Nor do we have a clear understanding of how hormonal interactions are changed during infection.

The monograph that follows includes a review article presented at the Annual Convention of the American Association of Equine Practitioners followed by long and short abstracts submitted by participants. The length of the abstracts varies as some work presented at the workshop has not been published. Current restrictions on abstract length have prohibited some investigators from including extended abstracts.

As always Gene, thank you. The Foundation has given me some of the most memorable days in my life. I would have never dreamed that a prominent human perinatologist would share similar concerns over the fragility of the cervix in regards to pregnancy maintenance until ‘A mighty fortress is the cervix’ was sung to me at dinner and there was more than one verse.

Michelle LeBlanc
### HAVEMEYER SCIENTIFIC WORKSHOPS

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<th>Organisers</th>
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<td>October</td>
<td>New York City, USA</td>
<td>Dr. D. F. Antczak</td>
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<tr>
<td>1982</td>
<td>Second International Workshop on Lymphocyte Alloantigens of the Horse</td>
<td>October</td>
<td>Cornell University, Ithaca, New York, USA</td>
<td>Dr. D. F. Antczak</td>
</tr>
<tr>
<td>1983</td>
<td>Third International Workshop on Lymphocyte Alloantigens of the Horse</td>
<td>April</td>
<td>New Bolton Center, University of Pennsylvania, USA</td>
<td>Dr. D. F. Antczak</td>
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<td>1984</td>
<td>First International Symposium on Equine Embryo Transfer</td>
<td>October</td>
<td>Cornell University, Ithaca, New York, USA</td>
<td>Drs D. F. Antczak and W. R. Allen</td>
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<td>1985</td>
<td>Fourth International Workshop on Lymphocyte Alloantigens of the Horse</td>
<td>October</td>
<td>University of Kentucky, USA</td>
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<td>University of Guelph, Canada</td>
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<td>April</td>
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<td>Dr. D. F. Antczak and Professor S. Lazary</td>
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Third International Symposium on Equine Embryo Transfer  
February - Buenos Aires, Argentina  
*Organisers: Drs D. F. Antczak, W. R. Allen, J. G. Oriol and R. Pashen*

1995

Equine Perinatology  
July - Cambridge, England  
*Organiser: Dr P. D. Rossdale*

Second International Equine Leucocyte Antigen Workshop  
July - Lake Tahoe, California, USA  
*Organisers: Drs D. F. Antczak, P. Lunn and M. Holmes*

First International Workshop on Equine Gene Mapping  
October - Lexington, Kentucky, USA  
*Organisers: Drs D. F. Antczak and E. Bailey*

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October - Mount Joy, Pennsylvania, USA  
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October - Concord, Massachusetts, USA  
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1997

Second International Workshop on Equine Gene Mapping  
October - San Diego, California, USA  
*Organisers: Drs D. F. Antczak and E. Bailey*

Maternal Recognition of Pregnancy in the Mare  
January - Dominican Republic  
*Organisers: Drs W. R. Allen and T. A. E. Stout*

Uterine Clearance  
March - Gainesville, Florida, USA  
*Organiser: Dr M. M. LeBlanc*

Trophoblast Differentiation  
September - Edinburgh, Scotland  
*Organisers: Drs D. F. Antczak and F. Stewart*

1998

Third International Genome Workshop  
January - San Diego, California, USA  
*Organisers: Drs D. F. Antczak and E. Bailey*
Third International Workshop on Perinatology: Genesis and Post Natal Consequences of Abnormal Intra-uterine Developments: Comparative Aspects
February - Sydney, Australia
Organiser: Dr P. D. Rossdale

Horse Genomics and the Genetic Factors Affecting Race Horse Performance
March - Banbury Center, Cold Spring Harbor, New York, USA
Organisers: Drs D. F. Antczak, E. Bailey and J. Witkowski

Allergic Diseases of the Horse
April - Lipica, Slovenia
Organisers: Drs D. F. Antczak, S. Lazary and E. Marti

Equine Placentitis Workshop
October - Lexington, Kentucky, USA
Organisers: Drs D. F. Antczak, W. R. Allen and W. Zent

Septicemia II Workshop
November - Boston, Massachusetts, USA
Organiser: Dr M. R. Paradis

1999
Equine Genome Project
January - San Diego, California, USA
Organisers: Drs D. F. Antczak and E. Bailey

Third International Equine Genome Workshop
June - Uppsala, Sweden
Organisers: Drs D. F. Antczak, E. Bailey and K. Sandberg

Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza
August - Miami, Florida, USA
Organiser: Dr J. Mumford

European Equine Gamete Workshop
September - Lopuszna, Poland
Organisers: Drs W. R. Allen and M. Tischner

Fetomaternal Control of Pregnancy
November - Barbados, West Indies
Organisers: Drs T. Stout and W. R. Allen

2000
Equine Genome Project
January - San Diego, California, USA
Organisers: Drs D. F. Antczak and E. Bailey

Uterine Infections in Mares and Women: A Comparative Study
March - Naples, Florida, USA
Organiser: Dr M. M. LeBlanc
5th International Symposium on Equine Embryo Transfer  
July - Saari, Finland  
*Organiser: Dr T. Katila*

**2001**

**USDA International Plant & Animal Genome Conference**  
January - San Diego, California

**Equine Immunology in 2001**  
January - Santa Fe, New Mexico  
*Organiser: Dr D. P. Lunn*

**Asthma and Allergies II**  
April - Hungary  
*Organisers: S. Lazary and E. Marti*

**From Elephants to Aids**  
June - Port Douglas, Australia  
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**International Equine Gene Mapping**  
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*Organiser: K. Bell*

**Second Meeting of the European Gamete Group (EEGG)**  
September - Loosdrecht, The Netherlands  
*Organiser: Dr T. A. E. Stout*

**Foal Septicemia III**  
October - Tufts University European Center, Talloires, France  
*Organiser: M. R. Paradis*

**Infectious Disease Programme for the Equine Industry and Veterinary Practitioners**  
October - Marilyn duPont Scott Medical Center, Morvan Park, Virginia, USA  
*Organisers: Drs J. A. Mumford and F. Fregin*

**From Epididymis to Embryo**  
October - Fairmont Hotel, New Orleans, USA  
*Organiser: Dr L. H-A. Morris*

**2002**

**USDA International Plant & Animal Genome Conference**  
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*Organiser: P. Sibbons*
Stallion Behaviour IV
June - Reykjavik, Iceland
Organisers: S. McDonell and D. Miller

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July - Pullman, Washington
Organiser: J. Prescott

Equine Orthopaedic Infection
August - Dublin, Ireland
Organiser: E. Santschi

Inflammatory Airway Disease
September - Boston, USA
Organiser: Dr E. Robinson

2003

USDA International Plant and Animal Genome Conference
January - San Diego, California

Embryonic and Fetal Nutrition
May - Ravello, Italy
Organiser: S. Wilsher

Genomics and the Equine Immunity System
June - Ithaca, New York
Organiser: D. F. Antczak

Fifth International Gene Mapping Workshop
August - Kreuger Park, South Africa
Organiser: E. Baily and E. Vandyke

Equine Recurrent Laryngeal Neuropathy
September - Stratford-upon-Avon, UK
Organisers: P. Dixon and E. Robinson

Transporting Gametes and Embryos
October - Brewster, Massachusetts
Organiser: E. Squires

Third Meeting of the European Gamete Group (EEGG)
October - Pardubice, Czech Republic
Organiser: J. and Z. Müller

Nosocomial Infections and Biosecurity in Equine Hospitals
October - Lexington, USA
Organiser: F. Bain and J. Taub-Dargatz
2004

**USDA International Plant and Animal Genome Conference**  
January - San Diego, California

**Equine Viral Herpesvirus Workshop**  
June/July - Tuscany, Italy  
*Organiser: P. Lunn*

**Equine Embryo Transfer VI Workshop**  
August - Rio de Janiero, Brazil  
*Organiser: M. Alvarenga*

**Sporting Injuries in Horses and Man: A Comparative Approach**  
September - Lexington, USA  
*Organiser: E. J. L. Soulsby*

**Maternal Recognition of Pregnancy in the Mare III**  
November - Barbados, West Indies  
*Organiser: T. A. E. Stout*

2005

**USDA International Plant and Animal Genome Conference**  
January - San Diego, California  
*Organiser: J. Mickelson*

**Comparative Placentology**  
April - Victoria, Canada  
*Organiser: P. Sibbons*

**Sixth International Gene Mapping**  
July - Dublin, Ireland  
*Organisers: E. Bailey and J. Flynn*

**World Equine Airway Symposium**  
July - Ithaca, USA  

**Genetic Relatedness Between Different Breeds of Horses using Molecular Markers**  
August - Poland  
*Organisers: M. Binns, G. Lothran and B. Graiak*

**International Equine Gamete Group**  
September - Kühlungsborn, Germany  
*Organisers: H. Alm, H. Torner, K. Hinrichs and E. Squires*

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October/November - Colorado, USA  
*Organiser: W. McIlwraith*
Equine Influenza and Cross Species Transmission
November - Florida, USA
Organiser: J. Mumford

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November - South Carolina, USA
Organiser: M. M. LeBlanc

2006
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SESSION I:

Review of the problem

Chairman:
M. M. LeBlanc
REPRODUCTIVE PHYSIOLOGY OF THE LATE GESTATION MARE

D. Paccamonti

Department of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, Louisiana 70803 USA

The scope of this paper is not to review thoroughly our knowledge of the physiology of the late gestation mare. Rather it is a brief overview of some aspects of late gestation in the mare with an attempt to highlight some of the similarities and differences between mares and women. The goal in horse breeding is to raise an athlete, whether a body builder (ie halter class Quarter Horse) or track star (ie Thoroughbred). Successful therapeutic options for the compromised fetus or neonate must achieve this goal. In the case of a premature human infant, an economic value is not assigned and no financial limit is put on treatment options. However, with a foal, economics are often a limiting factor and mere survival is not usually considered a satisfactory outcome.

The human neonatal Intensive Care Unit (ICU) model for treatment of prematurely delivered infants may not serve the equine industry as well. Because of the advances in human neonatal medicine, infants can be delivered evermore prematurely, with a good chance of survival. Nevertheless, it is well known that intrauterine growth retardation and premature delivery may have ramifications much later in life (Barker et al. 1989). For example, it has been reported that low birth weight resulting from intra-uterine growth restriction was associated with decreased brain weight and increased adiposity at maturity in sheep (Louey et al. 2005). How the effects of pre-term delivery might manifest themselves in the equine adult is unknown.

While great strides have been made in human neonatal intensive care, enabling earlier and earlier delivery of premature infants, the same cannot be said for equine neonatology. The ability to deliver an at-risk human fetus, provide treatment and care for it outside the womb is a luxury that is not an option in veterinary medicine. We are often unable to support a severely compromised premature foal outside the mare’s uterus, and we lack a clear time point in gestation after which we can expect a favourable outcome to treatment. Because of the poor prognosis for survival after delivery of a premature foal, treatment directed at maintaining the pregnancy or stimulating precocious maturation rather than inducing delivery currently offers the best hope for success. If an equine neonate can be maintained in utero for a time after diagnosis of placental disease, precocious development may occur and the foal can be born early, seemingly mature and with a good chance of survival, after a gestation period usually considered too short for normal fetal maturity.

Understanding the pathogenesis of pre-term delivery is critical in developing effective treatment modalities. Should treatment be aimed at directly inhibiting myometrial activity, preventing the switch from contractures to contractions; or at controlling possible infection and the associated inflammatory process, thereby promoting myometrial quiescence? The current working hypothesis of pre-term delivery due to placentitis attributes fetal loss to a rise in prostaglandins resulting from an increase in pro-inflammatory cytokines (LeBlanc et al. 2004). If this hypothesis is supported by future studies, treatment modalities must be directed at controlling the bacterial infection, preventing the rise in pro-inflammatory cytokines and prostaglandins and inhibiting myometrial contractions (LeBlanc et al. 2004). In support of this concept, pre-term delivery in sheep initiated with RU486 was successfully prevented by the PGF\textsubscript{2\alpha} receptor antagonist THG113.31 (Hirst et al. 2005).

Typical therapy for placentitis includes systemic antibiotics, most commonly trimethoprim sulfa because of its oral availability. Pentoxyfilline is often administered because of its anti-
inflammatory properties and ability to improve tissue perfusion. Studies examining concentrations of antibiotics such as trimethoprim sulfa and pentoxyfilline in allantoic fluid have shown that these commonly used therapeutic drugs do achieve appreciable levels in the fetal compartment (LeBlanc et al. 2004). Exogenous progestogen, usually altenogest at double the recommended dose, is given to maintain myometrial quiescence.

Although treatment of the equine fetus in utero is usually via systemic treatment of the dam, direct injection into the allantoic cavity may be an option to consider. A report by Stefos et al. (2005) described treatment of anhydramnios by serial infusion of normal saline into the amniotic cavity. In the cases described, one woman received 3 infusions and the other 4 infusions of 100 or 200 ml saline at 4 or 5 week intervals. Both women gave birth to normal infants. In an earlier report, transcervical amnioinfusion of amphotericin was used successfully to treat an intra-amniotic Candida albicans infection, resulting in the birth of a normal infant (Shalev et al. 1994).

Urinary trypsin inhibitor (UTI) is an anti-inflammatory substance that is also a potent inhibitor of myometrial contractions (Kanayama et al. 1995a; Pugia and Lott 2005). Normally present in human amniotic fluid, UTI has been reported to be capable of preventing pre-term delivery induced by lipopolysaccharide (LPS) through suppression of cytokine production (Kaga et al. 1996). Urinary trypsin inhibitor inhibits in vitro production of PGE₂ by myometrial cells stimulated with IL-1 and LPS (el Maradny et al. 1996), suppressed IL-1, IL-6 and TNFα after LPS administration in mice (Kaga et al. 1996), and inhibited cervical softening and dilation induced by IL-8 in rabbits (Kanayama et al. 1995b). In Japan, UTI has been shown to be clinically effective in inhibiting uterine contractions and has been used to prevent premature delivery in women (Kanayama et al. 1996). Whether UTI plays a role in equine pregnancy, or may be of therapeutic value, is unknown at this time.

Various aspects of late gestation in the mare make horses unique among the domestic species. Gestation length is extremely variable, with the range for normal gestation length in horses encompassing a 6-week period. In no other species is the concept of ‘readiness for birth’ so apparent. Fetal maturation is important in timing the onset of parturition in most species but variability is expressed in days, not weeks. Increasing oestrogens and declining progestogens at the end of gestation are characteristic of many domestic species. In horses, however, oestrogens decline and progestogens rise in the last weeks of gestation, followed by a rapid decline in progestogens in the last days before delivery. Abnormal progestogen concentrations may signal placental disease. A precocious rise in progestogens is often observed with placentitis. Recently, Ousey et al. (2005) reported that increased progestogens were found in mares with placentitis compared to mares with normal pregnancies, indicating increased fetal production or increased utero-placental metabolism in response to the chronic stress. Stawicki et al. (2002) and Leblanc et al. (2004) reported similar findings in a model of experimental placentitis. Conversely, an early decline in progestogens is usually associated with acute stress and abortion (LeBlanc et al. 2004). As with many other species, fetal adrenocortical hormones increase near term but, in the horse, this change only occurs within a few days of parturition. The rise in fetal cortisol is associated with a number of changes associated with fetal maturation and readiness for birth, such as an increase in thyroid hormone, an increase in the neutrophil: lymphocyte ratio, and maturation of the fetal lung and gut. Foals delivered pre-term with precocious changes in progestogens often have concurrent precocious adrenocortical activity and have improved chances of survival.

In comparative studies of gestational disease in mares and women, the presence of 2 fetal compartments (amniotic and allantoic) in the mare, in comparison to a single compartment in humans, is an important difference. The allantoic and amniotic compartments are dissimilar in origin, components and dynamics, with many of the constituents changing over time. Amniotic fluid is derived primarily from the respiratory and gastrointestinal systems. Allantoic fluid is in large part fetal urine; however, because of the fusion of the allantois with the chorion, and its relationship to the maternal endometrium, changes in the constituents of allantoic fluid may reflect placental function. How do the allantoic and amniotic compartments behave in relation to fetal health and placental function? Often viewed as a waste receptacle, should the allantoic compartment be viewed as more of a dynamic component in fetal wellbeing? Further studies of
the fluids during normal and abnormal gestations may shed light on the pathophysiology of disease and may lead to more successful treatment modalities.

REFERENCES

Uterine infections are common in both pregnant mares and women. Microbial invaders gain access to the outer fetal membranes by either traversing the cervical canal (‘ascending’ route), or by crossing from the endometrium. Data from large retrospective studies, done both in Europe and the US reveal that placentitis is found commonly in aborting mares. Placentitis was present in 9.8% of 1,252 equine abortions in the United Kingdom (Smith 2003) and placentitis was detected in 24.7% of placentas examined from aborted, stillborn or premature foals (236 of 954 placentas) in a study from Kentucky (Hong 1993).

Ascending bacterial infections typically produce visibly prominent, severe, subacute to chronic locally extensive necro-suppurative placentitis involving the chorioallantois adjacent to the inner os of the mare’s cervix. This area of the choriallantois is known as the ‘cervical star’. Bacteria most commonly responsible for ascending infection in the mare include Streptococcus zooepidemicus, Escherichia coli, Pseudomonas spp., etc. In marked contrast, placentitis that develops by extension of infection from the endometrium typically produces a ‘diffuse’ lesion pattern, which, when found, suggests either Leptospira spp. bacterial or Candida yeast infection. A third and very unusual, (and hence ‘diagnostic’) pattern of placental infection, is produced by ‘nocardioform’ (Amycolaptosis, spp., Crosiella eq. sp. nov.) infections resulting in locally extensive chronic placentitis which typically is limited to areas of chorioallantois that occupy the base of the horns and cranial-ventral uterine body.

Common features of inflamed chorioallantois detected by histopathology include: oedema, vascular congestion, tissue necrosis, inflammatory cell exudation, neovascularisation, fibrosis, and reactive hyperplasia of the allantoic epithelium. Severe infections can result in locally extensive areas of chorioallantoic infarction. Acute bacterial infections induce neutrophilic cell infiltration while more chronic infections are associated with macrophage and, less commonly, lymphocytic inflammation.

Most placental infections in mares are subacute to chronic, but inflamed and necrotic tissues quickly release cytokines and other mediators that have broad-ranging effects. Placental separation, compromise of vascular perfusion and decrease of the exchange area quickly combine to depress placental function. Expanded use of ultrasonography has enabled veterinary clinicians to routinely diagnose and treat placentitis in their equine patients. Exciting new opportunities exist for development of innovative methods and new model systems for study of the pathogenesis of equine bacterial placentitis, such as those provided by use of chronically instrumented mares and their fetuses. Better understanding of the pathogenesis of bacterial placentitis and its associated fetal compromise will provide guidance for the development of more rapid and accurate diagnostic tests, and clinical detection and treatment regimes.

REFERENCES


Disruption of the intra-uterine environment will not only result in fetal distress, but may be a major cause of neonatal diseases recognised in foals. Conditions of neonatal foals which may be directly associated with placentitis include prematurity, intrauterine growth restriction (IURG) and precocious maturation. Other conditions that may be associated with placentitis include the complex of Neonatal Encephalopathy (NE), Neonatal Nephropathy (NN), Neonatal Gastroenteropathy (NG) and maladaptation of other systems.

Prematurity in the foal has been defined as birth before 320 days gestation. The term 'dysmature' has been used to describe foals with physical characteristics of prematurity in a foal born after a gestation more than 320 days. These definitions are problematic because of the wide variation in normal gestational length in mares. This has lead to confusion in diagnosing prematurity, dysmaturity and IUGR. In the author’s opinion it is more helpful to define prematurity based on the mare’s normal gestational length, use IUGR for foals small for gestational age (which are not constitutionally small) and avoid using the term dysmaturity.

There is a neonatal multisystem maladaptation syndrome which includes neurological, renal, gastrointestinal and other system malfunctions which may be secondary to placental disease. The most prominent signs are neurological abnormalities traditionally referred to as Neonatal Maladjustment Syndrome (NMS). Research findings in other species early in the 1990s led many equine neonatologists to speculate about a hypoxic ischaemic or asphyxial origin for this syndrome. The term Hypoxic Ischaemic Encephalopathy (HIE) largely replaced NMS. It is clear, however, that despite attractive experimental models showing many similarities, often this disease syndrome occurs in the absence of a detectable hypoxic ischaemic insult. Because of this the author prefers to use the terms, such as Neonatal Encephalopathy, that do not specify an aetiology or pathogenesis but only the organ dysfunction and age group. Most recently there has been speculation that inflammatory mediators secondary to placentitis may (possibly by initiating a hypoxic ischaemic insult) be responsible for the multiorgan dysfunction.

Foals suffering from NE may show changes in responsiveness, muscle tone, behavior, evidence of brain stem damage or seizure-like behaviour. Changes in responsiveness include hyperesthesia, hyper-responsiveness, hyperexcitability, hypo-responsiveness, periods of somnolence or unresponsiveness. Often the foals go through a period of increased responsiveness followed by a period of decreased responsiveness. Changes in muscle tone include increased extensor tonus, hypotonia and other less common changes such as neurogenic myotonia or failure to protract front legs. Changes in behaviour are very common and include loss of suckle response, loss of tongue curl, loss of tongue co-ordination, disorientation especially relative to the udder, aimless wandering, loss of affinity for the dam and abnormal vocalisation. Foals with NE commonly have changes in respiratory patterns with central tachypnoea, apneusis, apnoea, cluster breathing, ataxic breathing, Cheyne-Stokes breathing or central hypercapnia. Other signs of brain stem damage include loss of thermoregulatory control, generalised weakness, anisocoria, pupillary dilation, pinpoint pupils, central hypotension, decreased responsiveness, difficult to arouse, loss of consciousness, vestibular signs (circling, head tilt), facial nerve paresis and a variety of other signs. Foals with NE have a wide variety of signs
and degrees of severity. More than 90% of affected foals are normal within 10 days.

Foals may also develop renal maladaptation referred to as Neonatal Nephropathy (NN). There is a wide spectrum of disease seen in cases of NN including incomplete transition from fetal renal physiology, water/sodium retention, mild tubular dysfunction (sodium wasting), abnormal excretion of drugs (eg high amikacin trough levels), acute tubular necrosis or decreased glomerular filtration rate (GFR). Often the signs of dysfunction are subtle and easily overlooked unless anticipated. Although almost always transient, on occasion significant acute damage may lead to chronic renal disease. These foals often have a decreased GFR as reflected by a slow decrease birth creatinine or decreased creatinine clearance, delayed water excretion with oedema formation and weight gain and slow response to fluid challenges.

Neonatal Gastroenteropathy (NG) can be manifested by a wide spectrum of signs ranging from mild indigestion with dysmotility and enema dependence to moderate disease with ileus, diapedesis of blood into the lumen and mucosal oedema to severe disease with epithelial necrosis, intussusceptions, structures, haemorrhagic gastritis/enteritis/colitis, and pneumatosisis intestinalis. Even mild forms predispose to sepsis and systemic inflammatory response system (SIRS) with increased likelihood of translocation of bacteria. Like NN, often the signs of dysfunction are subtle and easily overlooked unless anticipated. The most common manifestation is dysmotility with meconium retention and lack of faecal passage for days (range 2–30 days). Despite faecal retention, an important aspect is lack of discomfort. Classically, foals with dysmotility will not return enema fluid or strain associated with rectal distension.

Often, affected foals have the triad of NE, NN and NG. Other problems seen include metabolic maladaptation, autonomic failure and other systemic problems. Foals born from an environment of placentitis commonly have a generalised inflammatory response as reflected by systemic and haematological reactions. The activation of inflammatory and anti-inflammatory cascades may support precocious maturation of many body systems and may, in fact, impart some protection from systemic infections.

Prematurity and IURG are easily confused. Clinically prematurity is marked by low birth weight, small frame, thin, poor muscle development, periarticular laxity and general flexibility. IUGR is marked by apparent cachexia and disproportional growth. Either may be a direct result of placentitis and it is common for both to be present concurrently.
SESSION 2:

Animal models

Chairman:

M. Macpherson
Pre-term birth continues to pose a significant clinical dilemma and contributes to both acute and long-term neonatal morbidity. Despite efforts, the incidence of pre-term birth has not decreased, partly because of the lack of understanding of the mechanisms that trigger parturition. Animal models are essential research tools for investigating the pathways that promote pre-term parturition and for testing therapeutic interventions. In the last decade, a growing body of evidence demonstrates that infection or inflammation is a significant contributor to pre-term birth. Consequently, many investigators have created animal models that reflect these findings. Current animal models of pre-term parturition include several species, varying means of inducing an inflammatory or infectious state, and different routes of administration. Although each of these models can advance our knowledge, it is important to understand their advantages, disadvantages and unique characteristics. An understanding of such models will hopefully promote continued research that will ultimately lead to a decrease in pre-term birth and an improvement in neonatal outcome.
A model of ascending placentitis was developed in the mare to characterise myoelectrical and endocrine patterns in late gestation and determine how ascending placentitis altered these patterns.

Thirty ponies were grouped into controls (n=8), experimentally infected (n=4), instrumented control mares (n=7) and instrumented infected mares (n=11). Pony mares were instrumented with myometrial electrodes and allantoic fluid catheters (n=4) 7–10 days before experimental mares received an intra-cervical inoculation with *Streptococcus equi* subspecies *zooepidemicus* (Day 285–293 of gestation). Myometrial electrical activity was analysed during the early morning, late morning and evening hours until delivery. Blood and allantoic fluid were collected to measure progestins and prostaglandin F$_{2\alpha}$ and E$_2$.

Three of 15 inoculated mares exhibited no outward signs of infection. Plasma progestins either rose (n=8) or fell after inoculation (n=7) in inoculated mares, whereas levels remained at baseline in control mares until approximately 21 days before delivery when they began a steady rise. Progestin levels declined in all mares in the 24 h preceding delivery. All inoculated mares delivered prematurely, 5–27 days after inoculation. Thirteen of 15 foals were aborted and 2 were viable. Control mares foaled after dGA 320. Control mares exhibited 2 types of myometrial activity, low amplitude epochs of activity that lasted for one min or more (large spike bursts) and high amplitude epochs of activity that were less than 30 s (small spike bursts). The duration and number of large spike clusters varied little as gestation progressed whereas the number of small spike bursts began to increase at night in the last 6 days of gestation and continued to increase at night until parturition. Mares with experimentally induced placentitis did not exhibit a rise in small burst clusters in the last week of gestation as did control mares. They exhibited an increase in the duration and intensity of the large spike bursts in the 4 days preceding parturition. Concentrations of prostaglandins (PGE$_2$) and (PGF$_{2\alpha}$) in allantoic fluid samples collected within 48 h of abortion or delivery in mares with experimentally-induced placentitis rose significantly and were higher than that of control mares.

These findings indicate that ascending placentitis due to *Streptococcus equi* subspecies *zooepidemicus* is associated with a rise in allantoic fluid prostaglandins, premature delivery and in some cases, accelerated maturation of the foal. Myoelectrical activity pattern in infected mares differs from that of non-infected mares that deliver at term.
EXPERIMENTALLY-INDUCED PLACENTITIS IN LATE GESTATION EWES AND MARES: EVALUATION OF PATHOGEN PROGRESSION USING LUX-MODIFIED BACTERIA AND BIOLUMINESCENCE IMAGING

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Placental infection due to opportunistic pathogens such as *Escherichia coli* (*E. coli*) is the single most common cause of abortion, stillbirth and premature delivery in women and domestic animal species. The consequence of placentitis during late pregnancy increases the incidence of abortion, pre-term delivery or stillbirth. Moreover, increasing evidence demonstrates that pathogen progression during placentitis may involve invasion of fetal tissues, including the brain, leading to increased pro-inflammatory cytokine expression resulting in onset of premature delivery and/or fetal neurological damage. Thus, the objectives of this pilot study were 1) to develop a model for pre-term delivery using late term pregnant ewes; and 2) to determine pathogen progression and invasion of the fetal environment by experimentally-induced uterine infection with a *lux* gene-modified *E. coli* using real time biophotonics imaging technology in the ewe and mare.

In Experiment 1, 30 pregnant ewes (~124 days gestation) were assigned to one of 3 experimental groups. Following surgical scrub of the abdomen, ewes were inoculated trans-abdominally (ultrasound-guided either intra-cotyledon or intra-amniotic fluid) with 1 ml sterile broth alone (Control), or with either a low (Low, 1.2–4.0 × 10^6 CFU) or high (High; 5.6–20 × 10^6 CFU) dose of *E. coli* transformed with the pAK1-*lux* plasmid (*E. coli-lux*). The plasmid (11,904 bp) used is a broad-host-range cloning vector with numerous plasmid replicons. A maximum of 30% of bacteria ejected the *lux* plasmid within the first 24 h post transfection, while 70% retained and maintained the plasmid over an 8-day test period. Following inoculation, ewes were monitored continuously for signs of pre-term delivery. Trans-abdominal ultrasonography was performed every 24 h for confirmation of fetal viability over a 7-day interval post inoculation. Rectal temperatures were recorded twice daily and blood collected by venipuncture daily for cortisol (C) and progesterone (P4) analysis. Lambs from ewes that pre-term delivered within the 7-day interval were imaged immediately. If ewes did not pre-term deliver within the 7-day interval, euthanasia was performed on the 7th day and lambs recovered. Following euthanasia of ewes, the uterus and lambs or pre-term lambs were subjected to biophotonic imaging using a Berthold/NightOwl camera for detection of *lux*-expressing bacteria ( photon emission) over a 5 min period and single frame accumulation to determine pathogen tissue localisation. Subsequent to intact uteri and whole animal imaging, lambs were dissected and specific organs imaged including heart, lungs, liver, gastrointestinal (GI) tract and brain. Uterine and fetal fluids and fluids from GI tract, stomach and bladder were imaged for presence of emitting bacteria. All fluid samples were analysed to determine total bacteria counts (CFU/ml). Pre-term delivery in ewes occurred between 48 and 120 h post inoculation. Of the 10 control ewes, 2 (20%) pre-term delivered and 8 carried normal pregnancies to Day 7 post inoculation (sterile broth) when lambs were recovered. While a mixed bacterial growth was found in fetal fluid from one of the pre-term fetuses, no photon-emitting bacteria were detected in any tissue or fluids from control lambs. Of the Low and High infected ewes, 6 (60%) and 7 (70%) of the ewes pre-term delivered, respectively. Imaging revealed in most lambs that pre-term delivered photon-emitting bacteria were observed in the lungs, stomach, GI
tract, bladder, and in uterine fluids, but not in the heart or liver. No photon-emitting bacteria were detected in brain tissue from any of the lambs delivered or recovered from *E. coli-lux*-infected ewes. Cultures of fetal stomach, bladder and uterine fluids confirmed presence of photon-emitting bacteria.

In Experiment 2, 2 pony mares (late gestation) were inoculated trans-amniotically with *E. coli-lux* (1 $\times$ 10^6 CFU) to determine pathogen progression and localisation in fetal foal tissues using the same imaging approach as in Experiment 1. Fetuses were collected 24 and 40 h post inoculation for imaging. *Lux*-expressing bacteria were found at 40 h post inoculation in the lungs, GI tract, nares and sinuses but not in the brain, heart or liver. These data clearly demonstrate the power of bioluminescence imaging technology in detecting the progression and localisation of pathogens involved in placentitis and pre-term delivery in domestic species.

In conclusion, these studies demonstrate that biophotonics and real time imaging provide a novel but valuable means of understanding the pathogenesis of bacteria associated with placentitis and pre-term birth in living mammalian species. Development of the pregnant ewe or mare as a model for monitoring pathogen progression during placentitis in women has real potential.

**ACKNOWLEDGEMENTS**

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ALLANTOIC CATHETERISATION OF THE PREGNANT MARE: DEVELOPMENT OF THE TECHNIQUE AND PRELIMINARY FINDINGS

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Inflammation of the equine chorioallantois is proposed to cause increases in pro-inflammatory cytokines. Increased mRNA expression of IL-1, IL-6, and IL-8 in the chorioallantois and higher concentrations of PGF\(_2\alpha\) and PGE\(_2\) in allantoic fluid were observed in mares transcervically inoculated with Streptococcus equi subsp. zooepidemicus compared to non-inoculated mares; however, no differences were detected in the concentrations of soluble TNF-\(\alpha\), IL-1 and IL-6 between groups. Placental infection causes elevations of pro-inflammatory cytokines in fetal fluids in other animal models (rhesus monkey, rabbit and mouse) and women. This unexpected discrepancy emphasises the need for a non-invasive method of allantoic catheterisation for reliable sampling to study equine feto-placental physiology and pathology. The short-term goal of the present study was to identify a catheter that could be laparoscopically placed into pregnant mares and would maintain patency for a minimum of 7 days. The long-term goal is to use this catheter system to investigate the pathophysiology of fetal demise for a variety of gestational diseases. Mares were not fasted and received only sedation and regional local anesthesia pre-operatively.

During the first year of the study, 4 catheter designs were tested. Four mares were catheterised successfully with a commercially available human nephrostomy catheter, although biochemical confirmation that the fluid composition was allantoic was only performed in 3 mares. Catheters were patent for 5.6 days, with a range of 5–6 days; however, contamination of the allantoic catheter usually occurred by Day 5.

During the second year of the study several refinements were made to improve patient comfort and reduce the risk of contamination, and 12 mares were successfully catheterised during 30 laparoscopies. These objectives were optimised by incorporating a subcutaneous access port system with an indwelling Huber needle, and clipping and aseptically preparing the flank 24 h before surgery and again immediately prior to surgery. Fluid samples were obtained at surgery, 4, 8, 12, 16, 20, 24, 28, 36, 42, 50 h post-operatively, and then daily until fetal delivery. Biochemical and cytological analysis of each sample verified the nature of the fluid as allantoic, amniotic, peritoneal, or an admixture of allantoic and amniotic fluid. For all mares, catheters were indwelling for a mean of 9.6 days and were patent for 8.3 days. Of a potential 188 samples, in only 16 instances could no fluid sample be obtained. The last 4 mares instrumented were induced to deliver at Day 8 following surgery; samples were obtained from all from all of these mares at each of the specified time points. Fluid from 2 of these mares was purely of allantoic origin, while the other 2 mares showed evidence of admixture of the allantoic and amniotic compartments. The latest refinement of this procedure was the application of laparoscopic ultrasound to identify the allantoic compartment, thereby avoiding amniotic puncture. Ultrasound-guided, laparoscopic catheterisation of the allantoic space will provide a reliable means of investigating the pathophysiology of various diseases of equine pregnancy.
TROPHOBLAST CELL PHAGOCYTOSIS: AN IN VIVO STUDY OF EARLY EVENTS IN ESTABLISHMENT OF EQUINE BACTERIAL PLACENTITIS


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INTRODUCTION

Bacterial placentitis is a common cause of equine abortion. Before bacteria can establish placental infection, they first must breach a layer of trophoblast cells that line the outer surface of the chorioallantoic membrane. Careful microscopic examination of placental tissues from equine abortuses reveals that bacteria are occasionally found within the cytoplasm of trophoblast cells in areas where allantoic invasion and secondary inflammation has not yet occurred, suggesting that trophoblast cells had phagocytosed bacteria to which they are exposed. These observations led to the working hypothesis that one of the earliest events associated with establishment of bacterial placentitis is phagocytosis of bacteria (or other microbes) present on the endometrial surface. Once bacteria have gained entry into placental tissues, infection then quickly spreads, inciting tissue damage and inflammation that lead to fetal compromise and abortion.

MATERIALS AND METHODS

The hypothesis that trophoblast cells phagocytise material present on the surface of the chorioallantois was tested by placement of fluorescent microspheres in small chambers created surgically by separation of the endometrium and placenta. Three mares in their last trimester of gestation were used. Mares were anaesthetised and a suspension containing $3 \times 10^9$ 1 µm fluorescent carboxylate-modified microspheres (Molecular Probes, Eugene, OR) was injected through a small catheter temporarily placed in a small chamber, approximately 2 cm², that had been surgically created as described above. Uterine and placental tissues were collected when mares were euthanised 4 h, 4 days, and 36 days later; samples were either fixed in formalin or snap frozen. Frozen sections were examined by ultraviolet microscopy. Fixed tissues were paraffin embedded, stained with H+E and examined by light microscopy.

RESULTS

Microspheres were easily detected in the space between the endometrium and chorioallantois in samples collected at 4 h and 4 days. In the 4 day samples, large numbers of spheres were present in macrophages around suture material and along the incision line in the uterine wall. Large numbers of microspheres were found within the cytoplasm of trophoblast cells but only rarely were spheres found within endometrial epithelial cells lining the chambers. Trophoblast cells had much greater phagocytic activity than did endometrial epithelial cells.

CONCLUSIONS

Trophoblast cells lining the equine placenta readily phagocytise particulate material. This experimental evidence suggests that trophoblast cells play a role in the earliest stages of transplacental infections. This experimental approach holds promise for more detailed, sequential, quantitative studies of mechanisms associated with movement of particulate material into trophoblast cells and transmission of microbial agents from mothers, across their placentas to their fetuses.
IDENTIFICATION OF NOVEL BIOMARKERS OF INTRA-AMNIOTIC INFECTION BY PROTEOMIC PROFILING

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Despite improvements in obstetrical care, pre-term birth remains the major obstetrical problem in developed countries. Sub-clinical or occult intra-amniotic infection (IAI) is a major cause of pre-term birth, responsible for more than 50% of extreme pre-term birth, where the highest proportion of neonatal deaths and serious complications occur. Unfortunately, most women in pre-term labour associated with occult intra-amniotic infection are refractory to standard tocolytic therapy. Moreover, antibiotic therapy has not prevented pre-term delivery in most studies, possibly because the patient subgroup with early IAI that might benefit from therapy is identified too late or not at all. Recent advances in proteomics present a new opportunity to examine the global, or differential, expression of proteins in tissues or fluids. The proteins or peptides that are differentially expressed in a disease or pathological state are well suited for the development of convenient, rapid, sensitive, and specific diagnostic assays. In this study proteomic profiling methods were used to discover novel biomarkers for sub-clinical IAI in an experimental non-human primate model of IAI and in a cohort of pregnant women in pre-term labour.

Surface-enhanced laser desorption-ionisation/time-of-flight mass spectrometry (SELDI-TOF), gel electrophoresis, and tandem mass spectrometry (MS/MS) were used to characterise amniotic fluid peptides in 19 chronically instrumented pregnant rhesus monkeys before and after experimental IAI. Candidate biomarkers were identified by liquid chromatography MS/MS and confirmed by Western blot. Amniotic fluid peptide profiles identified in experimental IAI were subsequently tested in a cohort of 33 women in pre-term labour and delivery associated with sub-clinical IAI (n=11), pre-term labour and delivery without IAI (n=11), and pre-term contractions with subsequent term delivery (n=11).

Protein expression profiles in amniotic fluid showed unique signatures of overexpression of polypeptides in the 3- to 5-kDa and 10- to 12-kDa molecular weight ranges in all animals after infection and in no animal prior to infection. In women, the 10- to 12-kDa signature was identified in all 11 patients with subclinical IAI, in 2 of 11 with pre-term delivery without IAI, and in 0 of 11 with pre-term labour and term delivery without infection (P<0.001). Peptide fragment analysis of the diagnostic peak in amniotic fluid identified calgranulin B and a unique fragment of insulin growth factor binding protein 1, which were also expressed in maternal serum. Mapping of other amniotic fluid proteins differentially expressed in IAI identified several immunoregulators not previously described in amniotic fluid.

This proteomics-based characterisation of the differential expression of amniotic fluid proteins in IAI identified a distinct proteomic profile in an experimental primate chorioamnionitis model that detected subclinical IAI in a human cohort with pre-term labour. This diagnostic protein expression signature, complemented by immunodetection of specific biomarkers in amniotic fluid and in maternal serum, has application in the early detection of IAI. This may allow for the timely diagnosis and treatment of pre-term labour associated with subclinical IAI.

ACKNOWLEDGEMENTS

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Pre-term premature rupture of membranes (PPROM) and pre-term labour (PTL) are the leading causes of perinatal morbidity and mortality. Although the aetiology of PPROM and PTL are often uncertain, they have been frequently linked to sub-clinical or clinical infection in the reproductive tract. During normal pregnancy, the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α is suppressed. In the presence of infection, however, microorganisms increase the production of pro-inflammatory cytokines at the maternal-fetal interface. These cytokines, in turn may promote PPROM and PTL by increasing apoptosis in placental cells, increasing the production of matrix metalloproteinases or increasing prostaglandin secretion.

Much of the work with regard to how intra-uterine infection may cause PTL has been limited to models that make use of lipopolysaccharide (LPS). Although valuable, they represent only infections caused by Gram-negative bacteria such as Escherichia coli. The majority of intra-uterine infections, however, are caused by the genital mycoplasmas Ureaplasma urealyticum and Mycoplasma hominis. These small organisms, unlike E. coli, lack cell walls and hence LPS as well. Therefore, models that use LPS poorly represent infections caused by genital mycoplasmas that must have different virulence factors.

Therefore, we have focused our research on purifying and characterising the pro-inflammatory components of these organisms. We have found that Triton X-114 detergent extracts from M. hominis and U. urealyticum are potent stimulators of TNF-α production by human macrophages. Extracts from M. hominis activated Toll-like receptor (TLR)-2 but not TLR-4, the pattern recognition receptor for the lipid A component of LPS. The pro-inflammatory activity of M. hominis was stable to heating, partially reduced by proteinase K digestion, and completely abrogated by alkaline hydrolysis. Enrichment of the activity with SDS-PAGE and reverse-phase chromatography led to the isolation of a 29 kDa protein. Detergent extracts from U. urealyticum, however, were able to interact with both TLR-2 and TLR-4. The pro-inflammatory activity of U. urealyticum was partially sensitive to heating but completely removed by alkaline hydrolysis or proteinase K digestion and the activity was associated with proteins at 40 and 66 kDa. These studies suggest that the activity of genital mycoplasmas contain a factor(s) that may be similar to the Macrophage-activating lipoprotein-2 (MALP-2) or its precursor (MALP-404) that was previously isolated from Mycoplasma fermentans. Further work is needed to purify the activity to homogeneity and to test its ability to cause pre-term birth in an animal model.
THE USE OF BETAMETHASONE TO ADVANCE FETAL MATURATION IN THE EQUINE

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An important adjunct to the treatment of placentitis is the attempt to induce precocious fetal maturation to ensure fetal viability in the event of a potential pre-term delivery. In women, betamethasone sodium phosphate (Celestone) or dexamethasone is commonly used to advance fetal maturation in high-risk pregnancies. Various attempts to induce precocious maturation of the equine fetus have met with mixed results. Intratetral injections of adrenocorticotropic hormone (ACTH), betamethasone (BMS), and dexamethasone (DEX) have been shown to advance equine fetal maturation, but are associated with a high (~30%) incidence of spontaneous abortion. Currently, although DEX is thought to be effective only when given very late in gestation, practitioners utilise high doses of DEX (100 mg) given to the dam 4 days consecutively in an attempt to initiate equine fetal maturation. Therefore, the aim of this pilot study was to ascertain whether fetal maturation of the foal could be induced precociously by maternal injection with BMS. Thirteen Quarter Horse mares received either saline (SAL, n=5), or BMS injections (im) at 12 mg (n=3), 24 mg (n=3) or 30 mg (n=2) on Day 305, 306 and 307 of gestation. Delivery was induced (20 IU oxytocin injections) on Day 320 in 5 mares (2 x 12 mg, 2 x 24 mg and 1 x 30 mg). Other BMS-treated mares were not induced due to a poor response to treatment. Foal blood samples were collected at 0, 24 and 48 h and analysed for cortisol (C), progesterone (P4), thyroxine (T4) and triiodothyronine (T3) concentrations. Blood cell counts at 0 h were greater in T-I than T-NI (P<0.05) and SAL (P<0.1) foals with a neutrophil:lymphocyte ratio of 4:7, 7:2 and 4:1, respectively. SAL and T-NI treated mares foaled at term without complications, with BMS advancing delivery by 7–14 days in T-NI mares.

In summary, while BMS did not accelerate fetal maturation adequately to successfully induce pre-term delivery of foals, maternal BMS treatment appeared to advance delivery date in non-induced mares. As an advanced delivery date would likely indicate an effect on fetal maturation, a second study was undertaken to determine if this observation was significant.

Sixteen Quarter Horse type mares were paired by gestation length. Mares were injected on Days 305, 306, and 307 of gestation with either saline or
24 mg BMS and allowed to foal. Serum was collected from mares on Day 305 and then 3 times weekly until foaling for P4 analysis. Whole blood and serum was collected from the foals at birth for a CBC and P4 analysis and serum was collected at 24 h of age for additional P4 analysis and evaluation of passive transfer. All foals were clinically mature at birth; however, although not statistically significant, there was a trend toward decreasing gestation length in the treated mares versus the control mares (339 ± 3.8 vs. 345 ± 12.2 days respectively). Further studies need to be conducted to determine if increased dose or prolonged treatment may enhance this effect as well as to ascertain any differences which may occur in the presence of placentitis.

**ACKNOWLEDGEMENTS**

Supported by Mississippi Agriculture and Forestry Experiment Station.
SESSION 3:

Pathological findings

Chairman:
C. Sanchez
EQUINE PLACENTAL PATHOLOGY: THE COMMON AND THE NOT SO COMMON


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INTRODUCTION

The University of Kentucky Livestock Disease Diagnostic Centre (LDDC) is located in the heart of the central Kentucky bluegrass region with its numerous horse breeding operations. Each year the centre receives several hundred fetuses, placentas, and stillborn foals for necropsy examination, making it one of the most valuable sites for prospective/retrospective studies of naturally occurring equine feto-placental disease and pathology.

MATERIALS AND METHODS

A computer-based search of the LDDC case records from the past 3 foaling seasons: 2003, 2004, and 2005 was conducted. All equine cases with a standard diagnosis of placentitis were identified. The case reports were reviewed and the number of cases of placentitis, the time of occurrence of each case, the pattern of the gross pathology, the bacteria isolated and the sites where the bacteria were isolated were investigated. The cases were grouped by total yearly cases, time of occurrence, pathology, and bacterial isolates.

RESULTS

The most common diagnosis in fetuses and placentas submitted to the LDDC is placentitis. The majority of the cases result from bacterial infection of the placental membranes, often with concurrent fetal infection. Over the 3 foaling seasons, 619 cases of placentitis were identified. There were 372 cases in foaling year 2003, 127 cases in 2004, and 120 cases in 2005. Few cases of placentitis were diagnosed between the months of June through September. The number of cases began to increase in October and reached a peak during the early months of the year, followed by a tapering toward the end of the foaling season (Fig 1). This pattern of gradual increase and decline was consistent for the seasons that were studied.

The case review revealed that, of the 619 cases, 302 had gross lesions on the placenta suggestive of placentitis. Based on the narrative description, 52 cases were determined to represent ascending placentitis. The typical gross lesions consisted of areas of discoloration and thickening of the chorion with variable surface exudate present. Of the 619 cases, 205 were diagnosed as having placentitis by finding inflammation within the placental tissues on microscopic examination with no suggestive gross changes. These cases typically had low numbers of neutrophils and mononuclear cells in the chorionic villi and sub-villous stroma with degeneration of the chorionic epithelium. Cases with gross changes suggestive of placentitis usually had more severe inflammation and degeneration of the chorionic cells, often with congestion and haemorrhage.

Bacteriological cultures were typically performed on the placenta (chorion) and fetal lung, liver, and stomach fluid. Of the 619 placentitis cases, significant bacteria were isolated in 364 cases (59%). Bacteria were also cultured from sites in addition to the placenta in 139 cases (38% of the cases in which bacteria were isolated). Cultures in 118 cases were overgrown by saprophytic microorganisms and 136 cases had bacteria considered to be nonpathogenic. There were 28 cases of mycotic placentitis diagnosed. These were typically ascending placentitis cases and the fungus was usually classified as Aspergillus sp.
The most commonly isolated bacteria were the gram-positive branching filamentous bacilli. These partially classified bacteria are placed in the broad group nocardioform actinomycetes. These are the bacteria associated with nocardioform placentitis, a common diagnosis in central Kentucky but only rarely reported elsewhere.

The next most common group of bacteria was the streptococci, followed by a variety of other organisms, including the recently reported (Bolin et al. 2004). Cellulosimicrobium cellulans (Table 1). Rare and uncommon isolates included single cases of *Aeromonas hydrophila*, *Dermatophilus congolensis*, *Salmonella paratyphi*, and *Rhodococcus equi* infection.

Nocardioform placentitis is an important cause of fetal loss in central Kentucky. It is characterised by inflammation of the chorion in the area of the base of the placental horns or anterior body of the placenta, with accumulation of thick mucoid material on the surface (Donahue and Williams 2000). This form of placentitis was first recognised in Kentucky in 1986. The yearly caseload since 1991 typically averages from 20 to
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40 cases (Fig 2). However, several years have had few cases and other years have produced many more cases.

Nocardioform placentitis does not involve the cervical star area. It appears to begin as a single focus of infection that expands and enlarges over time. The central area is characterised by low-level chronic inflammation (mononuclear) and destruction of villi, while the periphery has more abundant active inflammation and cellular degeneration. Bacteria are scarce in the centre of the lesion but are typically numerous in the periphery. The bacteria do not appear to be invasive and are not cultured from the fetal fluids or tissues (other than the chorion). Nocardioform placentitis appears to injure the fetus by causing placental insufficiency. The time of entry and the route of infection are not known.

Nocardioform placentitis usually results in no outward manifestation of infection and placentitis in the mare; however, some mares exhibit premature mammary gland development and lactation. There are 4 possible outcomes to nocardioform placentitis: the mare may abort, the mare may carry the fetus to term but produce a stillborn foal, the foal may be born alive (premature or full term) but weak and compromised, or the mare may deliver a normal foal. Following delivery the mare usually clears the infection rapidly, rebreeds normally, and does not appear to be at increased risk for subsequent abortion. Occurrence on a particular farm is sporadic.

Several strains of the nocardioform actinomycetes causing nocardioform placentitis have been classified. These include Crossiella equi, Amycolatopsis kentuckyensis, Amycolatopsis lexingtonensis, and Amycolatopsis pretoriensis (Donahue and Williams 2002; Labeda et al. 2003).

Cases of placentitis indistinguishable grossly from nocardioform placentitis, but associated with other types of bacteria, have been identified. Over the 3 foaling seasons, 32 such cases were identified. Cultures and histopathology were negative for nocardioform-type bacteria and, in all cases tested, PCR tests for C. equi and Amycolatopsis were negative. Bacteria isolated from these cases included Pantoea agglomerans, Cellulosimicrobium cellulosum, Pseudomonas spp., Enterobacter spp., Enterococcus spp., and Staphylococcus spp. In addition to the placenta, these bacteria were also sometimes cultured from the fetal tissues.

Unusual cases of chronic amnionitis resulting in thickening and contraction of the amnion were diagnosed occasionally. There were 21 cases in 2003, 4 in 2004, and one in 2005. Some cases also had allantochorion and cord lesions; however, most involved only the amnion. The cause is usually not known but bacteria were isolated in about 25% of the cases, suggesting possible chronic bacterial infection. Some cases were associated with premature meconium passage.

Mare Reproductive Loss Syndrome (MRLS) resulted in numerous early gestation and late term abortions in 2001 and 2002. Funisitis, rarely seen

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**TABLE 1: Bacterial isolates from placentitis cases**

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>111 (26%)</td>
<td>Gram-positive branching filamentous bacteria</td>
</tr>
<tr>
<td>81 (19%)</td>
<td><em>Streptococcus</em> (zooepidemicus, equisimilis, spp.)</td>
</tr>
<tr>
<td>68 (16%)</td>
<td><em>Leptospira</em> spp.</td>
</tr>
<tr>
<td>45 (11%)</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>27 (6%)</td>
<td><em>Pantoea</em> (Enterobacter) agglomerans</td>
</tr>
<tr>
<td>20 (5%)</td>
<td><em>Cellulosimicrobium</em> (Oerskovia) cellulosum</td>
</tr>
<tr>
<td>15 (4%)</td>
<td><em>Pseudomonas</em> spp.</td>
</tr>
<tr>
<td>12 (3%)</td>
<td><em>Enterobacter cloacae</em></td>
</tr>
<tr>
<td>11 (3%)</td>
<td><em>Enterococcus</em> spp.</td>
</tr>
<tr>
<td>7 (2%)</td>
<td><em>Stenotrophomonas</em> (Pseudomonas) maltophilia</td>
</tr>
<tr>
<td>7 (2%)</td>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td>5 (1%)</td>
<td><em>Actinobacillus equuli</em></td>
</tr>
<tr>
<td>4 (1%)</td>
<td><em>Citrobacter freundii</em></td>
</tr>
<tr>
<td>2</td>
<td><em>Corynebacterium</em> spp.</td>
</tr>
<tr>
<td>1</td>
<td><em>Aeromonas hydrophilia</em></td>
</tr>
<tr>
<td>1</td>
<td><em>Dermatophilus congolensis</em></td>
</tr>
<tr>
<td>1</td>
<td><em>Salmonella paratyphi</em></td>
</tr>
<tr>
<td>1</td>
<td><em>Rhodococcus equi</em></td>
</tr>
</tbody>
</table>
with other causes of equine abortion and placentitis, was a characteristic feature of aborted late term fetuses. The affected cords were oedematous with dull gray discoloration and roughening of the surface. Only the amniotic segment was affected, changes were not noted in the allantoic portion of the cord. Microscopically, stromal oedema, haemorrhages, inflammatory cell infiltrates, and colonisation of the surface by bacteria were observed. Placentitis (chorionitis) was diagnosed in some cases but was much less common than funisitis (Williams et al. 2003). Placentitis was not typically suspected on gross examination and, microscopically, the inflammation was mild. Chorionic inflammation or bacterial colonisation was not observed. The inflammation was predominately neutrophilic and located in the stroma, often within the extra embryonic coelom.

**DISCUSSION**

Other studies have found that most infections of the equine feto-placental unit are bacterial in origin with *Streptococcus zooepidemicus* being the most commonly isolated pathogen (Giles et al. 1993; Hong et al. 1993). In this report the group of nocardioform actinomycetes was most commonly isolated. The streptococcal bacteria were the second largest group. Isolates of *Streptococcus zooepidemicus* and *Streptococcus equisimilis* were approximately equal in number, however. In agreement with a previous report (Hong et al. 1993) that found that bacteria were isolated in 68% of placentitis cases, the present study found bacteria in 59% of cases.

This study found that approximately 60% of placentitis cases had gross lesions on the placenta suggesting placentitis. The finding that approximately 40% of the placentitis cases were diagnosed microscopically without gross lesions suggests that many cases are the result of acute, rapidly progressing infections.

Placentitis cases that on gross examination appeared to be nocardioform placentitis but were actually the result of infection by other bacteria were occasionally seen. This indicates that the development of this particular form of placentitis is not uniquely a property of nocardioform bacteria, but bacteria that also cause more classical placentitis can, under certain unknown conditions, produce placentitis morphologically identical to nocardioform placentitis. Therefore, naming this form of placentitis based on the bacteria is probably inaccurate and a morphologic name without specification of causative agent may be more appropriate, i.e., focal mucoid placentitis.

**REFERENCES**


From 1999–2005, placentas were examined from 36 pony mares with experimentally induced bacterial placentitis and one pony mare with bacterial placentitis contracted after experimental surgical intervention. Tissues from 32 foals which were born dead or euthanised due to poor health shortly after birth were examined at the same time. (5 foals lived). The experimentally infected mares had been inoculated with $10^7$ or $10^8$ colony forming units of *Streptococcus equi* subspecies *zooepidemicus* between 270 and 295 days of gestation. Sixteen of the mares had been treated with various antibiotics and anti-inflammatory drugs for varied periods of time after inoculation.

Pathology samples from the placentas included cervical star region, body, gravid horn, and nongravid horn of the chorioallantois, amnion, and umbilical cord. Foal tissues included lung, liver, kidney, spleen, and adrenal. The most common lesion was necrotising placentitis in the cervical star region of the chorioallantois with spread to the uterine body region, seen in 34 cases. Bacteria were recognised in the necrotic tissue in 30 cases and in one additional case in which necrotising placentitis developed only at a catheter entrance site. Funiculitis of the umbilical cord was recognised in 19 cases. Bacteria were found on or in the peripheral layers of the umbilical cord in 14 cases. Localised amnionitis was recognised in 10 cases. In 20/32 cases bacteria were found in airways and alveoli of the foals’ lungs. The lung tissue exhibited early pneumonia in 7 cases.

Cultures collected at the time of parturition correlated well with the bacteria seen in the tissues histologically – *Streptococcus equi* subspecies *zooepidemicus* in 11 cases, with a gram negative rod (*Escherichia coli, Klebsiella*) as a dual infection with *Streptococcus* in 3 cases, a gram negative rod alone in 5 cases, and *Nocardia* in one case. Bacterial septicemia was never recognised in a foal. All organs except lung were histologically uninfamed and uninfected. The authors suspect the organisms reached the lung by traversing the chorioallantois (in the cervical star region of the placenta in all but one case) and travelling to the amniotic fluid along the umbilical cord, to be aspirated by the foal.
SESSION 4:

Treatment strategies for infection in late gestation

Chairman:  
M. M. LeBlanc
INNATE IMMUNITY AND MICROBIOLOGICALLY INFORMED APPROACHES TO PREVENTION AND TREATMENT OF INTRAUTERINE INFECTION DURING PREGNANCY

J. A. McGregor, O. Equils, M. Wilson and S. Witkin

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AIMS

1) This study aimed to analyse systems biology (molecular-clinical) approaches to prevent and/or treat intra-uterine infection/inflammation during pregnancy; 2) Highlight clinical and research opportunities to optimise reproduction outcomes impaired by infection/inflammation.

MATERIALS AND METHODS

Selected current research and ‘best’ clinical care practices were reviewed regarding periconceptional, embryonic, and perinatal immune, microbiological, and inflammation interactions affecting pregnancy and neonatal/childhood outcomes (pre-term birth, low birth weight, pre-term premature rupture of membranes and perinatal organ injury).

RESULTS

Selected ‘key’ pathophysiological areas presented included: 1) mechanisms of intra-uterine infection/inflammation; 2) evidence of intra-uterine microbes and adverse fetal/maternal effects (pre-term labour and birth, rupture of membranes, impaired growth and development and organ damage; 3) inter-relationships between maternal, fetal, and paternal biologic relationships; 4) evidence of improved reproductive outcomes with appropriate detection and treatment of abnormal host microbial interactions, especially during early gestation; 5) exploration of ‘best’ antimicrobial and other treatments to optimise prevention and treatment strategies for preventing infection-inflammation caused prematurity and perinatal organ system damage; 6) use of biomarkers to detect and monitor intra-uterine infection/inflammation.

CONCLUSIONS

Comparative and systems-biology informed approaches were presented to guide and inform research and clinical care in order to optimise reproduction outcomes.
NOVEL TREATMENT STRATEGIES FOR INFECTION-INDUCED PRE-TERM BIRTH: A NON-HUMAN PRIMATE MODEL

M. G. Gravett

Department of Obstetrics & Gynecology, Oregon Health & Science University, Portland, Oregon and Division of Reproductive Sciences, Oregon National Primate Research Center, Beaverton, Oregon, USA

Pre-term birth and its consequences account for 80% of perinatal deaths not attributed to congenital malformations. Intra-amniotic infections (IAI) are an important and potentially preventable cause of extremely premature births at 24–30 weeks of gestation, where perinatal mortality and morbidity are high. Antibiotics alone, however, are not effective in preventing infection-induced pre-term birth. Pro-inflammatory cytokines and prostaglandins participate in the pathogenesis of infection-associated pre-term births. The mechanisms, however, by which infection and the cytokine/prostaglandin cascade lead to prematurity remain speculative and treatment strategies largely untested. It has been demonstrated previously in non-human primates, in the absence of infection, that intra-amniotic infusion of the pro-inflammatory cytokine IL-1β results in increases in uterine contractility and pre-term delivery and that this contractility can be blocked by administration of cytokine immunosuppressants (dexamethasone) or prostaglandin synthesis inhibitors (indomethacin). Chronically instrumented pregnant rhesus macaques were therefore used to investigate the hypothesis that down-regulation of the inflammatory cascade together with antibiotic treatment would prolong gestation in the setting of infection.

Fifteen chronically instrumented pregnant rhesus monkeys with timed gestations received experimental IAI by intra-amniotic inoculation of 10^6 Group B streptococci (GBS) at 134–141 days of gestation (term is 167 days). Amniotic fluid (AF) cytokines (IL-1β, IL-6, IL-8, TNF-α), prostaglandins (PGE_2 and PGF_2α), matrix metalloproteinases (MMP-2, MMP-9), and uterine contractility (hourly contraction area, or HCA, in mmHg s/h) were serially measured before and after infection and/or intervention. Following infection and increases in uterine contractility, 4 animals received no treatment, 4 animals received maternal ampicillin (IV 30 mg/kg every 6 h), and 5 animals received ampicillin plus maternal oral indomethacin (50 mg bid) plus maternal dexamethasone (IV 1 mg/kg every 6 h).

Following intra-amniotic infection with Group B streptococci without treatment, there were sequential increases in AF pro-inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF), prostaglandins (PGE_2 and PGF_2α), matrix metalloproteinases (MMP-9), and uterine activity. Uterine activity increases occurred from pre-inoculation HCA levels of 0–100 mmHg s/h to peak levels of 10,000 to 20,000 mmHg s/h after inoculation and led to delivery at an average of 37 + 9 h after infection.

Animals treated with antibiotics plus immunosuppressants had significant prolongation of pregnancy following infection (213 + 50 h), when compared to those treated with antibiotics alone (81 + 28 h), or those receiving no treatment (37 + 9 h; P<0.01, ANOVA).

These data support the central role of the pro-inflammatory response in infection-induced pre-term birth and suggest that novel immunomodulators in combination with antibiotics may be more effective than antibiotic therapy alone in the prevention of infection-induced pre-term birth. The chronically instrumented pregnant rhesus monkey model provides insights into the pathophysiology of infection-induced pre-term labour, and will facilitate development of rationale and effective medical strategies to prevent prematurity.

ACKNOWLEDGEMENTS

Supported by NIH AI42490.
DETECTION OF GENTAMICIN AND PENICILLIN CONCENTRATIONS IN ALLANTOIC FLUID OF PREGNANT PONY MARES BY IN VIVO MICRODIALYSIS

T. A. Murchie, M. L. Macpherson, M. M. LeBlanc, S. Luznar and T. W. Vickroy

College of Veterinary Medicine, Western University of Health Sciences, 309 E. Second Street, Pomona, California 91766-1854, USA

OBJECTIVES

Current treatment protocols for equine placentitis inconsistently prevent premature parturition. Little is known about the transfer of drugs across the equine placenta, and treatment strategies are largely empirical. The primary objective of this study was to develop an in vivo microdialysis technique for monitoring drug concentrations in allantoic fluid of pregnant pony mares following systemic drug administration.

MATERIALS AND METHODS

The study was conducted on 5 pony mares in late gestation.

Microdialysis probes were inserted into the allantoic fluid using transabdominal ultrasound guided allantocentesis. Penicillin G, gentamicin and flunixin were given iv and dialysate samples were collected continuously over a 24 h period. In a separate study, samples were collected from 2 pony mares following intracervical infection with an inoculum of Streptococcus equi subspecies zooepidemicus. Drug concentrations in dialysate fractions and serum samples were determined using high performance liquid chromatography (penicillin G, flunixin) or an enzyme-linked immunosorbent assay (gentamicin). Descriptive data analysis was performed, all results reported as means ± standard error, and minimum inhibitory concentrations (MIC) indicated for the 2 infected mares.

RESULTS

Penicillin G and gentamicin were detected readily in allantoic fluid of all 5 non-infected mares, with peak concentrations of 9.8 ± 2.2 mg/ml and 8.5 ± 3.1 mg/ml, respectively. When compared to drug concentrations in serum, penicillin G remained detectable in allantoic fluid for approximately twice as long. Gentamicin exhibited similar elimination profiles from serum and allantoic fluid. In addition, penicillin G achieved similar concentrations in the allantoic fluid of the 2 infected mares (11.2 mg/ml) as non-infected mares whereas gentamicin was present at much lower concentrations in infected mares (3.9 mg/ml). Furthermore, penicillin G concentrations in the allantoic fluid of the 2 infected mares exceeded the MIC appropriate for Streptococcus equi subspecies zooepidemicus (β-haemolytic streptococci; MIC ≤ 0.12 mg/ml) tested in this study. Gentamicin allantoic concentrations did not reach the reported MIC values for certain Gram negative organisms (Klebsiella spp., MIC ≤ 10 mg/ml).

Flunixin was undetectable throughout the entire sampling period in allantoic fluid of all subjects.

CONCLUSIONS

Microdialysis is a useful tool for short-term continuous monitoring of drug concentrations in equine allantoic fluid. The results demonstrate that penicillin G and gentamicin undergo effective placental transfer in non-infected mares although the pharmacokinetic profiles of individual drugs are notably different between serum and allantoic fluid. Therapeutic concentrations of penicillin G were observed in allantoic fluid of the 2 inoculated mares.
PLACENTAL PENETRATION OF TRIMETHOPRIM SULFAMETHOXAZOLE AND PENTOXIFYLLINE IN MARES WITH PLACENTITIS

S. Rebello, M. L. Macpherson, T. Murchie†, M. M. LeBlanc* and T. W. Vickroy††

Department of Large Animal Clinical Sciences and ††Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida; †Western University of Health Sciences, Pomona, California; *Rood and Riddle Equine Hospital, Lexington, Kentucky, USA

Placentitis is the most prevalent cause of equine abortion, and remains a large source of economic loss to the breeding industry. Current treatment modalities have been inconsistent in preventing infection-associated premature labour. The primary objective of this study was to determine the ability of selected drugs to pass through the blood-placental barrier in normal mares and mares with experimentally-induced placentitis. The authors hypothesised that placentitis would not alter the pharmacokinetic profiles or the free concentrations of selected therapeutic agents in allantoic fluid.

Ten late-gestational mares (276–300 days of gestation) were used for this study. Placentitis was induced in 5 mares using an intracervical inoculation of Streptococcus equi subspecies zooepidemicus (10^7 CFU) prior to sample collection. Five mares served as uninfected controls. Allantoic fluid drug concentrations were determined for all mares by in vivo microdialysis. Prior to drug treatments, a microdialysis probe was implanted in the allantoic cavity using transabdominal ultrasound guidance. Trimethoprim-sulfamethoxazole (TMP-SMZ, 30 mg/kg, q 12 h, PO) and pentoxifylline (PTX, 8.5 mg/kg, q 12 h, PO) were administered until abortion or parturition for a maximum period of 2 weeks. Microdialysate samples were collected continuously for a period of 36 h in conscious, free-standing mares. Drug concentrations were analysed by reverse-phase high performance liquid chromatography with ultraviolet spectroscopy. Peak drug concentrations, time-to-peak intervals, and total drug concentrations (reported as area-under-the-curve) were recorded as means ± standard error. Control versus infected groups were statistically compared using a non-parametric Wilcoxon Rank-Sum Test (P<0.05). Mean TMP and SMZ levels were compared to the minimum inhibitory concentration (MIC) reported for in vitro control of S. equi zooepidemicus.

All drugs exhibited placental transfer and were detectable by microdialysis sampling. No significant differences (P>0.05) were detected between control and infected mares with regard to peak levels of TMP, SMZ, or PTX in allantoic fluid. Based upon reported MIC values against S. equi zooepidemicus, the levels of TMP and SMZ in allantoic fluid should be sufficient to elicit an antibiotic effect for up to 4 h following each treatment in control and infected mares. It is unclear whether allantoic levels of PTX are sufficient to elicit any therapeutic action in vivo. Together, these data demonstrate that passage of the selected drugs through the blood-placental barrier is unchanged in mares with experimentally-induced placentitis, and reveals non-linear relationships between drug levels in plasma and allantoic fluid.
Placental infection due to opportunistic pathogens (ie Streptococcus equi subspecies zooepidemicus (S. equi)) is the single most common cause of abortion, stillbirth and premature delivery in horses. The consequence of placentitis and pre-term delivery results in severe financial loss to the equine industry. Moreover, increasing evidence demonstrates that placentitis increases pro-inflammatory cytokine expression leading to premature delivery. The authors’ working hypothesis is that inhibition or blocking of the pro-inflammatory cytokine responses with the use of a combinatorial drug therapeutic strategy that includes antibiotics and known anti-inflammatory cytokine agents will reduce the incidence of pre-term labour in pregnant mares with uterine infections. Thus, the objective of this pilot study was to induce ascending placentitis and evaluate two therapeutic strategies to prevent cytokine-induced pre-term birth in late gestation mares.

To this end, in Experiment 1, 6 ponies (~290 days gestation) were infected intracervically with ~2 x 10^6 colony forming units (CFU) of a clinical strain of S. equi and assigned (n=3/group) to receive either trimethoprim sulfamethoxazole (TMS; 30 mg/kg BW, q12h) alone or in combination with Regumate (altrenogest; TMS+R; 2.0 mg/50 kg BW, q24h). Drug sensitivity tests confirmed that this strain of S. equi was sensitive to TMS.

In Experiment 2, 12 late term-pregnant mares were assigned to one of 3 experimental groups. Eight mares were inoculated transcervically S. equi (~2 x 10^6 CFU) as in Experiment 1 and were assigned to receive either antibiotics alone (TMS; 30 mg/kg BW, q12h) or in combination with dexamethazone (TMS+DEX) while 4 mares served as non-infected, non-medicated controls (CON). DEX was administered daily over a 6 day period with decreasing doses every 2 days from 40, 35 to 25 mg, respectively. Blood samples were collected prior to infection and at 12, 24, 48, 72 h post infection and 3x/week thereafter until delivery for macrophage cytokine mRNA, relaxin and progesterone (P4) analysis. Fetal and placental well-being was evaluated daily by transrectal ultrasonography. Treatment (TMS, TMS+R or TMS+DEX) commenced upon initial signs of vaginal discharge and/or placental changes. TMS was maintained through to delivery and for 7 days post partum. Blood was collected from foals at 0 and 24 h post partum for complete blood count (CBC) (neutrophil: lymphocyte ratio), immunoglobulin G (IgG) and P4 analysis as indices of maturity. Placentaes and fetuses were submitted for necropsy and histopathology.

In Experiment 1 all 6 mares showed signs of vaginal discharge and/or placental changes within 36 h of inoculation. Three aborted 11, 12 and 14 days post inoculation, 2 from the TMS+R group and one from the TMS group; the remaining 3 mares carried to near-term delivering viable foals. Mean birthweight of TMS and TMS+R foals was 19.55 ± 1.82 and 17.55 ± 1.71 kg, respectively. Placental thickening increased (P< 0.001) from 0.77 ± 0.04 cm at pre-inoculation to 1.17 ± 0.06 cm at 48 h post inoculation. Pathology confirmed ascending necrosuppurative placentitis and bronchopneumonia in aborted fetuses. Culture of stomach contents of aborted fetuses revealed heavy growth of S. equi.
In Experiment 2 the mean gestational stage at time of *S. equi* inoculation was 297 ± 2.6 for infected vs 307 ± 3.4 days for control mares. The average stage of gestation at time of delivery for CON, TMS and TMS+DEX mares was 340 ± 7.6, 319 ± 6.4 and 293 ± 3.7 days, respectively. All CON mares delivered normal viable foals while 2 of the inoculated mares aborted dead fetuses, one from each treatment group. Of the remaining infected mares, 6 produced live pre-term foals (3 from TMS, 3 from TMS+DEX) 5 of which were viable while one from the TMS+DEX group was euthanised due to poor viability indices. Mean days at which pre-term delivery occurred for CON, TMS and TMS+DEX mares were -0.25 ± 7.6, 20.8 ± 6.3 and 37.3 ± 3.2 days, respectively. Mean birthweight of CON, TMS, TMS+DEX foals was 47.2 ± 1.8, 41.0 ± 3.6 and 34.9 ± 2.3 kg, respectively. Pathology of placentae from infected mares showed varying degrees of lesions and oedema consistent with ascending placentitis. In conclusion, due to low numbers, data is inconclusive as to whether the combinatorial therapy of TMS+R or TMS+DEX is more effective in the prevention of pre-term delivery than TMS alone. Cytokine and blood hormone analyses are pending.

**ACKNOWLEDGEMENTS**

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PROGESTINS AND PRE-TERM BIRTH: WHAT IS THE EVIDENCE AND DOES IT MAKE SENSE?

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Over half a century ago, progestins were used to prevent pre-term birth. In 1990, a meta-analysis suggested that these agents may be of benefit but further research was warranted. In 2003, a randomised control trial demonstrated that a progestational agent, 17-α hydroxyprogesterone (17-P), significantly decreased the rate of pre-term birth in high-risk patients. However, since progesterone levels in pregnancy are already quite high, it remains unclear by what mechanisms progestational agents can prevent pre-term birth in human pregnancy. Animal studies have begun to elucidate the mechanisms by which progestins may decrease pre-term birth. These studies suggest that progestatinal agents may prevent pre-term birth through non-traditional progesterone-receptor mediated events. A better understanding these mechanisms will allow for more appropriate use of these drugs in human pre-term birth and thus, ultimately help to decrease pre-term birth without incurring undue fetal or maternal harm.
THE ROLE OF ENDOGENOUS AND EXOGENOUS PROGESTAGENS IN PREGNANT MARES

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Progesterone (P4) and related compounds (progestagens) are necessary for maintenance of pregnancy in the mare. Before about 100 days gestation, progestagens are produced by the ovaries, removal of which causes a decline in circulating concentrations and abortion. After this time, progestagens are produced increasingly by the feto-placental tissues, and concentrations only decline, in healthy mares, at parturition (term 320 and 360 days). P4 is one of about 10 progestagens which have been quantified in maternal and fetal plasma using gas chromatography-mass spectrometry (Holtan et al. 1991). The progestagen metabolic pathway and enzymes involved are shown in Figure 1. Because progestagens are synthesised within the uterus, umbilical concentrations of most progestagens are substantially higher than in the maternal circulation. The fetus produces large quantities (3.6 increasing to 9.9 mmol/min in late gestation) of pregnenolone (P5) probably from the adrenals, although whether the adrenal glands possess sufficient P450scc enzyme activity to produce all this P5 remains unknown. The P5 is metabolised by the utero-placental (UP) tissues into P4, 5α-dihydroprogesterone (5α-DHP) and 20α-hydroxy-5-pregnan-3-one (20α-5P). Small quantities (<0.5 mmol/min) of P4 are excreted exclusively into the umbilical circulation so consequently P4 is rarely found in the maternal circulation. In contrast, excretion of 5α-DHP and 20α-5P by the UP tissues, is divided between the umbilical and uterine circulation. From mid-gestation, 5α-DHP excretion into the uterine circulation predominates but, during the last month of pregnancy, a significantly greater proportion of 5α-DHP is excreted into the umbilical than uterine blood (Ousey et al. 2003). Whether this redirection of 5α-DHP has any relevance to parturition remains unclear. Any 5α-DHP returned to the fetus is metabolised into other progestagens which are excreted back to the UP tissues. There is no net transfer of progestagens from the mare across the UP tissues into the fetus.

Because little P4 is present in maternal plasma, measurement of progesterone by ELISA or RIA tends to reflect the total progestagen concentrations and clearly values vary according to the specificity of the progesterone antibody and type of assay system used. Concentrations also vary between Thoroughbreds and Ponies although the individual progestagens detected in both breeds are similar (Rossdale et al. 1991). Therefore, it is important to establish normal values for healthy pregnant mares for a given assay system. Both fetal and maternal plasma total progestagen concentrations increase gradually over gestation with a large rise occurring during the last few weeks pre-partum, associated with increased production of P5 by the fetus. In the last 24–48 h prior to delivery, fetal (and maternal) progestagen concentrations decline concurrent with a rise in fetal plasma cortisol. This indicates a switch from progestagen to glucocorticoid production due to activation of the enzyme, 17α-hydroxylase, in the fetal adrenal.

Damage to the placenta whether through placentitis or other types of placental disease, stimulates a precocious rise in total progestagen concentrations in maternal plasma (Fig 2). A similar increase usually can be induced in healthy mares by experimental manipulation of the placenta causing placental damage and/or infection (Rossdale et al. 1991; Stawicki et al. 2002). This precocious rise in total progestagens probably occurs through activation of the fetal HPA axis in response to the chronic placental insult, because total progestagens in maternal
**Cholesterol**

![Diagram of cholesterol metabolism]

**Abreviations**

P₅: Pregnenolone  
P₅ββ: 5-pregnene-3β,20β-diol  
P₄: Progesterone  
20αOH-P₄: 20α-hydroxyprogesterone  
20βOH-P₄: 20β-hydroxyprogesterone  
5αDHP: 5α-pregnane-3,20-dione  
3β-5P: 3β-hydroxy-5α-pregnan-20-one  
20α5P: 20α-hydroxy-5-pregnan-3-one  
α-diol: 5α-pregnane-3β,20α-diol  
β-diol: 5α-pregnane-3β,20β-diol

**Enzymes**

1. P₄₅₀ₛₛ, sidechain cleavage (F&P)
2. 3β-HSD, 3β-hydroxysteroid dehydrogenase (F&P)
3. 5α-reductase (E&F)
4. 3β-oxidoreductase (P)
5. 3-oxidase (P)
6. 20β-reductase (F)
7. 20α-reductase (E)

*P = Placenta  
E = Endometrium  
F = Fetus*

**Fig 1:** Progestagen synthesis in the pregnant mare and fetus.

**Fig 2:** Maternal plasma progestagen concentrations in healthy, pregnant Thoroughbred mares (n=19, mean ± sem) and in 4 pregnant Thoroughbred mares with clinical problems.
plasma can rise in response to intra-fetal injection with adrenocorticotrophic hormone (ACTH). In contrast, when pregnant mares have more acute clinical problems, not involving the placenta directly, eg colic or uterine rupture, maternal plasma progestagen concentrations usually decline associated with rapid fetal demise and before activation of the fetal HPA axis (Fig 2). GC-MS analysis of the progestogens present in maternal plasma from clinical cases with placentitis (n=7) has revealed elevated concentrations of almost all of the progestagens including P4 and/or P5 over the weeks prior to abortion or premature delivery (Ousey et al. 2005). These results indicate increased production of P5 by the fetus and increased metabolism of P5/P4 into their metabolites by the UP tissues, despite placental infection and possible fetal compromise. In contrast, in 2 clinical cases of mares aborting with extensive villous poverty and placental oedema, respectively, total progestagens were raised associated with increased plasma P4 but concentrations of most progestagen metabolites, including 5α-DHP, were either normal or even reduced. In these cases it appears that damage to the UP tissues was such that metabolism of P4 into other progestagens was compromised, leading to transfer of P4 into the maternal circulation.

Exogenous progestagen therapy is often prescribed for mares at risk from abortion. For mares with placentitis this therapy appears unnecessary because their progestagen concentrations are already elevated. However, administration of synthetic P4 (altrenogest) combined with antibiotic and anti-inflammatory treatments, has extended gestational length and improved foal viability (Troedsson and Zent 2004), although at present there appears to be no scientific rationale for this treatment. Indeed other progestagens, for example 5α-DHP, may be more biologically important in maintaining quiescence of the equine myometrium. 5α-DHP binds more strongly than P4 to the uterine P4 receptor and is present in higher concentrations than P4 in the umbilical circulation (Chavatte-Palmer et al. 2000). However, studies, in vitro, using myometrial tissue from pregnant mares has demonstrated that neither P4 nor 5α-DHP can prevent or alter the frequency of myometrial contractions induced with oxytocin (Ousey et al. 2000). But how these in vitro analyses relate to the situation in vivo, particularly in mares with clinical problems, remains unclear. Few studies have investigated the effects of exogenous progesterone or its synthetic counterpart, altrenogest, on endogenous progestagens in late gestation mares. Administration of P4 to healthy mares at 318 days gestation significantly shortened gestational length compared with control mares, but no such effect was observed in mares receiving either progesterone or altrenogest for 10 days from 300 days gestation (Alm et al. 1975; unpublished observations); there was an increase in P4 following progesterone treatment only, but all other progestagens remained unchanged following either treatment, compared with controls. Differences between these 2 studies may relate to basal progestagen concentrations at the time of treatment. Administration of altrenogest to 2 mares with placentitis did not appear to alter progestagen profiles, (or outcome) compared with 5 mares with placentitis that received no altrenogest (Ousey et al. 2005).

It has been suggested that P4 may alter myometrial contractility by regulating UP synthesis of prostaglandins (PG) in the mare, as occurs in other species. Local production of P4 from P5, via 3β-hydroxysteroid dehydrogenase (3β-HSD) in the fetal trophoblast, may act in a paracrine fashion to regulate production of prostaglandin dehydrogenase (PGDH) located in the adjacent maternal microcotyledons (Han et al. 1995). This may be one pathway whereby exogenous P4 could control PG production and myometrial contractility. However, progestagens at similar concentrations to those found at the end of pregnancy inhibit placental 3β-HSD in vitro and blockade of 3β-HSD activity in vivo and in vitro, causes a transient decline in P4 and other progestagens although parturition was not induced and 5α-DHP concentrations recovered within 1.5 h (Fowden and Silver 1987; Chavatte et al. 1997). Therefore, these results indicate that high concentrations of progestagens paradoxically could lead to a decline in circulating levels and possibly to early delivery. Moreover, they do not appear to support the case for progesterone therapy, particularly in mares that may already have elevated endogenous progestagen concentrations. Clearly, more information is needed about effects of progestagen therapy on steroid metabolism within the fetoplacental unit in healthy and sick pregnant mares.
REFERENCES


OMEGA-3 (N-3) FATTY ACID NUTRITION: OPTIMISING REPRODUCTIVE AND EARLY LIFE NEUROLOGICAL PERINATAL OUTCOMES

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AIMS

This study aimed to analyse aspects of n-3 polyunsaturated fatty acid (PAFAs) nutrition and pregnancy performance and selected offspring neurological outcomes.

MATERIALS AND METHODS

Information was analysed regarding Docosahexanoic acid (DHA), eicosapentaenoic acid (EPA) and related substances obtained in food or diet supplementation which affects reproductive performance and/or growth, development, and neurological functioning in offspring.

RESULTS

Systems-based research demonstrates multiple, protean biologic effects n-3 polyunsaturated fatty acids (PUFA) nutrition and metabolism as well as interacting substances including n-6 PUFAs, trans fats, and n-6 FAs. ‘Nutrigenomic’ studies show altered epigenetic and patterning functioning of multiple genes (ras, others) affecting form, and function of maternal and fetal organ systems. ‘Proteinomic’ and physiological analyses demonstrate multiple effects of n-3 nutrition, including cell membrane fluidity, receptor activity (insulin, IGF axis), and CNS functions. Pregnancy evaluation in both animals and humans show alteration of gestational length (DHA, EPA). Developmental investigations show improved functioning of CNS and special sense (vision) performance parameters. Increasing evidence demonstrates n-3 efficacy in treatment and possible prevention of post partum and other types of depression.

CONCLUSIONS

Rapidly accumulating information justifies further basic and clinical research into improving pregnancy and offspring outcomes by means of enhanced fatty acid nutrition during pregnancy and lactation.
SESSION 5:

Effects of the neonate

Chairman:

D. Paccamonti
Disruption of the intra-uterine environment may be the initiator of many serious neonatal diseases. The inflammatory response may directly affect placental sufficiency or the inflammatory cascade accompanying the placentitis may have secondary adverse consequences for the fetus. The authors report on a preliminary retrospective study of the relationship between placentitis and neonatal diseases and the effect of therapy.

Clinician notes from foals and their mares from 2000 through 2005 were reviewed. Cases with complete fetal membrane evaluation by the second author (PLS) and foal evaluation by the first author (JEP) were included. Data was analysed through logistic regression. A p-value of 5% was used to separate chance from factor driven differences in outcomes, and a p-value interval of 5% and 10% was used to highlight the presence of trends.

One hundred and eight (108) cases were identified. When all cases were considered, there was no association of Neonatal Encephalitis (NE), Neonatal Nephropathy (NN) or Neonatal Gastroenteropathy (NG) with placentitis or pre-partum treatment regardless of the occurrence of placentitis. However, when treatment of placentitis was considered, NE, NN and NG were significantly more likely to occur in foals born to mares with untreated placentitis. There was a trend for bacteremia to be associated with placentitis and it was also more likely in foals with untreated placentitis. Treatment of mares with any combination of antimicrobials, NSAIDs or progestins significantly protected foals against the development of NE, NN and NG. No significant benefit of treatment was seen in preventing sepsis or bacteremia. Mares with placentitis were more likely to have a normal foal if treated with antibiotics, NSAIDs or progestins.

There is a strong association of placentitis and neonatal diseases (NE, NN and NG) but only in untreated cases. This strong association supports the hypothesis that placentitis is the cause of these diseases. In addition pre-partum treatment of the mare for placentitis appears to strongly protect against development of these diseases. Commonly utilised therapy seemed to contribute to this protective affect. Surprisingly, treatment, independent of the presence of placentitis, showed a trend to protect against sepsis suggesting that something other than placentitis which responded to treatment could pre-dispose the foal to sepsis. Alternately, treatment of the mare might decrease the exposure of the neonatal foal to factors that predispose to sepsis. These trends will be explored further as more cases are added to this data set. Although bacteremia was more likely in foals from mares with untreated placentitis, pre-partum treatment of the mares did not protect from bacteremia. Treatment of mares with placentitis significantly increased the odds of having a foal without any of the neonatal problems. Mares with suspect placentitis should be treated pre-partum to prevent development of common neonatal diseases.
The association of sepsis, inflammation and coagulation derangement is well documented in veterinary and human patients (Short 2004). In the initial stages of sepsis, inflammatory cytokines activate tissue factor (TF) while TF inhibitors may be decreased. This leads to unregulated coagulation and complications such as disseminated intravascular coagulation (DIC) which may be an important contributor to Multiorgan Dysfunction Syndrome (MODS).

Although perinatal coagulopathy has been associated with congenital infections, there is scant information in the literature regarding coagulation in critically ill neonatal foals or neonatal foals born to mares with placentitis (Barton et al. 1995; Barton et al. 1998; Bentz et al. 2002). This study explores the association of abnormal coagulation parameters and the occurrence of placentitis accessed through fetal membrane examination.

Prospective evaluation of coagulation parameters in 68 neonatal foals admitted to our NICU has been previously reported. A subgroup of 14 foals born to mares with gross and histological placental examinations is reported here. Eight mares were treated with anti-inflammatory, antimicrobial and/or progestin therapy. Five of these mares had no significant findings on placental examination and 3 were classified as placentitis.

Parturition was attended and blood collected by direct venipuncture at birth, 24 h and 48 h after delivery for platelet count, prothrombin time (PT), partial thromboplastin time (PTT), fibrin degradation products (FDPs), fibrinogen and antithrombin. DIC was defined as 3/6 parameters outside normal range during one sampling period. Placentas were collected immediately after parturition and evaluated by gross and histological examination.

Foals were categorised into 4 groups: septic shock, sepsis, other diseases or healthy (Bone et al. 1992; Abraham et al. 2000). Outcome was defined as survival to discharge or death prior to discharge. Coagulation parameters, clinical diagnosis and outcome were analysed in relationship to presence or absence of placentitis.

Fetal membranes of 6/14 foals were classified as abnormal with 4/14 being retained, 1/14 having gross placentitis only, 1/14 having histological placentitis only (as well as being retained) and 1/14 with both gross and histological placentitis. Foals born to these mares were classified clinically as follows: 2/6 suffering septic shock, 2/6 having sepsis, 1/6 with other problems and 1/6 healthy. Foals born with normal fetal membranes were classified as follows: none having septic shock, 2/8 having sepsis, 3/8 having other problems and 3/8 being healthy. There was one non-survivor from the non-placentitis group. No foal exhibited clinical evidence of bleeding; however, 3 foals born to mares with normal placental examination were classified in DIC. 13/14 foals survived to discharge.

Analysis of coagulation parameters in relationship to placental examination and maternal therapeutic intervention was performed using logistic regression. Differences between groups and/or sampling intervals were evaluated using the Kruskal-Wallis test. Significant coagulation results for the foals included decreased PT and platelet count if mares were treated. Additional investigations are needed.

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Equine neonatal sepsis has been implicated as the major cause of morbidity and mortality in the young foal. Both prenatal and post natal conditions have been implicated as predisposing factors for the development of sepsis. Prenatal factors, such as placentitis, dystocia, and premature placental separation, were reported in 24% of bacteremic foals in a recent study (Stewart et al. 2002).

The purpose of the current study was to evaluate short- and long-term survival rates, and identify changes in microbial isolates and their antimicrobial susceptibility patterns over the last 22 years. All available medical records from equine neonates (30 days or less at first admission) admitted to the Hofmann Equine Neonatal Intensive Care Unit at the University of Florida between 1982 and 2004 were used and data were entered into a computerised database format. From these records, information regarding foals diagnosed with sepsis was obtained, including clinical data, clinicopathological data, and short-term outcome. Race records for Thoroughbred foals and their 3 closest maternal siblings were collected. Information regarding the microbial isolates, including source, timing relative to admission, and antimicrobial sensitivity patterns was also recorded. For purposes of this study, septic foals were considered those from which a positive blood culture was obtained. Foals were grouped by decade (1982–1989; 1990–1999; 2000–2004) for some analyses. This study was expanded from data reported previously (Sanchez and Lester 2000) to include foals from 2000–2004.

Positive blood cultures were recorded from 393 foals during the study period. Overall, 467 microbial isolates were obtained from foals at admission, and 41 isolates were obtained after admission. Throughout the study period, E. coli was the predominant organism isolated. Over 50% of all isolates were categorised as enteric gram-negative rods, although this percentage decreased slightly each decade. Gram positive organisms accounted for approximately 20–25% of all isolates throughout the study. Interestingly, the percentage of gram-positives increased in the 2000-2004 group when considering isolates obtained after admission only. Overall short-term survival was 57%, with the survival rate increasing steadily by decade. Long-term outcome, as measured by the percentage of starters and winners, of short-term survivors compared favourably with that of their maternal siblings.

REFERENCES
THE OUTCOME OF FOALS BORN TO MARES TREATED FOR PLACENTITIS

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Equine placentitis is the most common cause of abortion and stillbirth in the mare (Giles et al. 1993). Pre-term birth or the birth of a weak and compromised foal are also the sequelae of placentitis. Any of these scenarios result in economic losses to the equine owner. Pre-term birth can result in complex complications in the equine species because fetal maturation occurs within the last few days of gestation. Thus, the foal is often born alive, but may require extensive hospitalisation or maybe small in size and less likely to become an athlete. Clinical signs of equine placentitis include premature lactation and vaginal discharge. If these clinical signs are observed, diagnostics are performed to assess the lower and upper reproductive tract and the viability of the fetus. Diagnostics include transrectal sonogram, transabdominal sonogram and vaginal speculum examination. The mare is most frequently treated with antimicrobials, non-steroidal anti-inflammatories and tocolytics (Macpherson 2005). If the foal is born pre-term or term, but weak and compromised the prognosis is assumed to be poor. The purpose of the study reported here was to determine the short-term and long-term outcome of foals born to mares treated for placentitis. Medical records at Rood and Riddle Equine Hospital from 1986–2005 were evaluated to identify those cases in which placentitis was the diagnosis based on pathology report of the placenta and that information was available on the mare and foal. Information that was obtained from the mare’s record included history or reason for evaluation (premature lactation, vaginal discharge), diagnostics performed (transabdominal ultrasound, transrectal ultrasound and vaginal speculum examination) and treatment (type and length). Information obtained from the foal’s record included viability of the foal at birth, gestational length, physical characteristics (body size), health of the foal, length of treatment if the foal required hospitalisation and diagnosis.

Thirty mares/foals were identified that met the inclusion criteria. Twenty-eight of the 30 cases were Thoroughbreds, one was a Standardbred and the other was an American Saddlebred. The most common reason for admission and evaluation of the mare included premature lactation or vaginal discharge (22/30 cases). Five of the 30 mares were admitted with a history of placentitis, definitive clinical signs were unable to be obtained from the records. Two mares were admitted with a compromised neonate that was less than 24 h old. One mare was admitted with a history of abnormal stage one labour. A total of 15 mares had a transabdominal ultrasound performed, 10 of the 15 were noted to be abnormal. Twenty-seven of the mares had been treated for placentitis prior to parturition, whereas 3 had not. Medication used for treatment included: antimicrobials (trimethoprim/sulfa, penicillin, gentamicin, metronidazole), anti-fungals (diflucan), anti-inflammatories (flunixin meglamine, phenylbutazone), and tocolytics (regumate, isoxxsuprine, clenbuteral). The trimethoprim-sulfa and regumate combination was the most commonly administered treatment. Only 13 of the 30 placentas submitted for pathological evaluation resulted in growth of a microorganism. The most common isolated organism was Noradioform (8/13) (gram-positive bacillus) with other organisms cultured including Aspergillus, Pantoea (Enterobacter) agglomerans, Streptococcus zooepidemicus and E. coli.

Twenty-nine of the 30 foals were born alive, whereas one was dead. Of the 29 born alive, 23 lived to discharge and 6 foals died or were
euthanised. The physical characteristics were based on the foal having a small or average body size as noted by the clinician in the record. Average size was considered to be approximately 110 pounds for a Thoroughbred foal. Twenty-three of the 30 foals were noted to be small in size and seven were noted as being average size. Sixteen of the 29 foals were born healthy (not requiring any treatment or extensive hospitalisation). The healthy foals ranged from 311–347 days of gestation. There were 13/29 unhealthy/compromised foals that required treatment and hospitalisation. The gestational age of the unhealthy foals ranged from 299–355 days of gestation. Clinical diagnosis of the unhealthy/compromised foals included: prematurity (4), septicemia (2), neonatal encephalopathy (1), pneumonia (1), failure of passive transfer (1), congenital abnormalities (1), neonatal encephalopathy/septicemia (1), prematurity/septicemia (1) and prematurity/failure of passive transfer (1). Four of these foals died and 2 were euthanised, the rest were alive at discharge. The length of hospitalisation for the foal ranged from 2–14 days with a cost estimated cost of $2,000 to >$10,000. Twenty foals were of racing age. Race records were obtained through the Bloodstock Research Information Services or US Trotting Association. Fifteen of the 20 foals had one start, with the range of starts 1–26. The earnings ranged from $0–$35,949 with the average $13,367.

Nocardioform placentitis is found commonly in Central Kentucky, but rarely reported elsewhere. This is caused by gram-positive filamentous branching bacteria (Donahue and Williams 2000). Eight mares were diagnosed with nocardioform placentitis and all foals were viable at birth. Seven of the 8 were alive at discharge and one was euthanised due to economic reasons. The range of gestation for these foals was 311–355 days. Seven of the 8 foals were born with a small body size, whereas one was of average size. Three of the 7 were born healthy, but 3 were premature and one was septic. The 4 compromised foals required extensive treatment and hospitalisation. Each of these 8 mares had been treated for placentitis prior to parturition. All but one was treated with the following combination: trimethoprim/sulfa, regumate and flunixin meglamine. The other mare was treated with penicillin, gentamicin and regumate. Seven of these foals were of racing age. Four of the 7 started but only one won a race.

Overall this retrospective study identified that a majority of the foals were viable at birth but small in size. If the foal was born healthy, but small in size, the foal was discharged from the hospital. If the foal was born unhealthy and small in size only 45% were discharged. Out of the population of small foals that are of racing age, 9 out of 14 raced in at least one race, whereas 6 of the 7 average size foals started. Looking at the group as a whole, these foals did not earn much as a racehorse, which confirms our assumptions that these foals do not excel as a racehorse. Some of these foals may have gone on to be riding horses and may have excelled in that capacity, but this information is not known. This study reveals some preliminary information about foals born to mares treated for placentitis, but further data needs to be obtained to comfortably give a prognosis on the outcome of these foals.

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APPENDIX
Ascending Placentitis: What We Know About Pathophysiology, Diagnosis, and Treatment

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1. Introduction
Premature delivery of a weak foal is devastating to horse owners. Even if they receive the best neonatal care, most of these foals, if they live, never have productive performance careers. The single most important cause of premature delivery in the United States is placentitis. It accounts for nearly one-third of late-term abortions and fetal mortality in the first day of life.1 Placentitis is most commonly caused by bacteria that ascend through the vagina2-4 and breach the cervical barrier. Streptococcus equi (subspecies zooepidemicus) is isolated in the greatest number of cases.5 Information on the pathophysiology of placentitis in the mare has been generated primarily from gross and histological observations made in clinical cases (Figs. 1-3). Typically, placental pathology is localized to the area of the cervical star.6 Grossly, there is thickening and separation of the chorionallantois from the endometrium. Histologically, there is a supplicative, necrotic, focally extensive placentitis that corresponds to the grossly affected area.1-3

Ascending placentitis may be identified clinically in some mares by observing a vaginal discharge or premature udder development in a pregnant mare. Management of these mares is directed at prolonging pregnancy, because chronic placentitis has been associated with accelerated fetal maturation. If premature birth can be delayed for a few weeks after clinical signs of placentitis develop, a foal may be born significantly premature but survive with limited neonatal care. However, treatment protocols currently used are empirical, because little is known about the pathogenesis of the disease. Inducing precocious maturation of the equine fetus with corticosteroids is not a reliable option in mares because of the risk of premature birth.6,8 Administration of physiological concentrations of corticosteroids to the mare, which is routinely performed in women prone to premature labor, is not associated with maturation of the equine fetus, because steroids do not readily cross the equine placenta. Additionally, few veterinary clinicians have the option of inducing labor prematurely and delivering a mature foal. The equine fetus becomes “viable” only in the last 5 days of gestation when fetal cortisol begins to rise. Because normal gestation length in the mare varies from 320 to 365 days, determination of the day of
Uterine Infection in Mares & Women: A Comparative Study II

IN-DEPTH: PLACENTITIS

Fig. 1. Premature separation of the choriosallantois is common in mares with placentitis. Note that the cervical star region has brown discoloration.

delivery is difficult at best. Removing the fetus from its dam's uterus before the narrow window of final maturation will result in a premature foal that will probably not survive, even in the best of neonatal units. Thus, current research in this area is aimed at understanding the mechanisms involved in the birth process and how placentitis affects those parameters. This information can be used to develop better diagnostic indicators and improve therapeutic strategies. This paper will review the physiology of late gestation in the mare, present what we know about the pathophysiology of placentitis, discuss diagnostic methods and their shortcomings, and present treatment strategies. Current theories on how infection disrupts pregnancy in other species will be discussed, because data indicate that there are similarities between mares with ascending placentitis and primates with chorioamnionitis.

Much of the data presented here was obtained from two experimental models of ascending placentitis created at the University of Florida. These models were developed to gain an understanding of how placentitis affects pregnancy in the

Fig. 2. Not all mares with histological evidence of ascending placentitis exhibit gross placentitcal lesions. The placenta on the left is from a mare with experimentally induced ascending placentitis. This mare aborted 4 days after bacterial inoculation of the cervix. The placenta on the right is from a mare that also had experimentally induced ascending placentitis. This mare aborted 15 days after cervical inoculation. Both had histological lesions.

Fig. 3. Amnionitis may be seen with ascending placentitis.
mare and to evaluate drug transfer across the equine placenta. Temporal relationships between infection, the inflammatory response, endocrine profiles, myoelectrical activity, and premature delivery were characterized in the first model. In addition, physical changes induced by placentitis including ultrasonographic appearance of the placenta, mammary development, and vaginal discharge were compared with qualitative changes in maternal plasma progestins to improve diagnostic efficiency. In the second model, a microdialysis system was designed to evaluate transfer of drugs across the normal and diseased placenta.

2. Endocrinology: Estrogens, Progestins, and Cortisol in Late Gestation

The equine uteroplacental tissues form part of a fetoplacental steroidogenic unit that produces both progestins and estrogens during the second half of gestation. This steroidogenic unit involves the endometrium, placenta, and several other fetal tissues including the adrenals, gonads, and liver. The fetal tissues provide precursors that are taken up by the uteroplacental tissues and metabolized further before release into the uterine circulation, umbilical circulation, or both. The term “fetoplacental unit” derives from the discovery in the 1960s that the placenta and the fetus in human pregnancies each lack a complete system of enzymes for the biosynthesis of estrogens. Only when their activities are combined do estrogens result. The fetal component in humans is the adrenal gland that becomes increasingly larger as the pregnancy progresses. In the horse, the fetal gonads provide the precursors for estrogen formation by the placenta. The size of the gonads increases greatly in mid-gestation, only to decrease in size during the last 3 mo. Circulating concentrations of estrogens in mare serum parallel an increase and decrease in the size of the gonads (Fig. 4). Peak concentrations of total estrogens are seen around 210 days of gestation. Concentrations begin to fall gradually after 280 days of gestation. The estrogens produced by the fetoplacental unit are not essential for the maintenance of pregnancy. The removal of the fetal gonads does not affect the length of the gestation in the mare. However, labor is prolonged, and fetuses are growth retarded in these circumstances. This suggests that estrogens may affect uterine contractility and blood flow in the horse similar to other species. Total estrogens have been measured between 150 and 280 days of gestation as an indicator of fetal well being. A concentration >1000 ng/ml is considered to be normal. Levels <1000 ng/ml are considered as indicative of fetal stress. Before 300 days of gestation, total estrogens <500 ng/ml are commonly associated with a severely compromised or dead fetus, whereas levels between 500 and 800 ng/ml indicate a compromised fetus.

Progestosterone and/or its metabolites (progestins) are considered to be the hormone(s) that maintain uterine quiescence during pregnancy. The progestin profile in the mare during mid- to late gestation is unique and differs greatly from that seen in other domestic species. In the cow and small ruminant, progesterone remains high during mid-gestation and begins to decrease a few weeks before delivery. In the mare, progesterone is non-detectable in the maternal circulation from about 180 days of gestation until the 10 days preceding labor. Beginning at about 60 days of gestation, the fetoplacental unit begins to produce progestins. Concentrations of progestins remain relatively low in maternal plasma until 15–21 days before parturition. Then, levels rise dramatically, only to fall precipitously 24 h before foaling (Fig. 4). Progestins are believed to be synthesized by fetoplacental tissues and by endometrium from the precursor pregnenolone (P5) derived from the fetal adrenal. In vitro studies have shown that ACTH stimulation of the fetal equine adrenal leads to P5 production, even when the mare is close to term, and that the equine endometrium can produce progestins from P5.

Abnormal changes in the dam’s progestin profile, such as an abrupt drop or a gradual premature rise, may be diagnostic for fetal stresses such as hypoxia or mild ischemic events resulting from placentitis, medical, or anesthetic compromise. Maternal plasma progestin concentrations do not change in conditions that directly affect the fetus, such as in herpes virus infection or in acute severe ischemic insults that may occur during colic surgery in late gestation. Cortisol is not produced by the fetal adrenals until the last few days of gestation, because the fetal adrenal lacks 17 α-hydroxylase, the enzyme needed for the conversion of progesterone to cortisol. Part of the rise in maternal plasma progestins in late gestation is likely a result of stimulation of the fetal adrenals by fetal ACTH. The fetal adrenals produce pregnenolone, and the fetoplacental unit metabolizes the pregnenolone to progestins (Fig. 5). The stimulus for production of 17 α-hydroxy-
pregnancy is a consistent developmental change across species. Increase in fetal HPA function results in increases in estrogen and prostaglandin production in humans and animal species such as sheep and cattle. In the fetus, prostaglandin E$_{2}$ (PGE$_{2}$) maintains the patency of the ductus arteriosus, regulates fetal breathing movements, and stimulates cortisol production by the fetal adrenal. In most animals, there is a marked decrease in the placental (or ovarian) output of progesterone before birth. At term, the influence of progesterone on the myometrium declines and uterine growth is reduced. The increase in wall tension caused by continued fetal growth becomes translated into increased expression of contraction-associated proteins (CAP) genes and myometrial activation. In the mare, mechanical stretch may contribute to the great incidence of pre-term birth of twins.

The relationship between the maturation of the equine fetal HPA, the maternal levels of estrogen, progesterol, and progesterone, and the delivery is not clear. Final maturation of the equine fetal HPA axis occurs extremely late in gestation compared with other species. The equine fetal adrenal glands produce cortisol only in the last 5 to 7 days of gestation. Estrogen in mares does not increase in maternal plasma in late gestation like other species. Progestins rise in the last 3 wk of gestation in mares, only to fall dramatically 48 h before birth. Prostaglandin F$_{2a}$ (PGF$_{2a}$) has a slow and gradual rise to 20-fold increase in concentrations at birth. Although the mechanism for activation of myometrial contractility is not known in the mare, it is likely that the process is influenced by the fetal HPA.

Preparation of the myometrium for parturition may evolve slowly over weeks, as reported in the primate, or rapidly and within 24 h, as seen in the ruminant. In the primate, there is a switch from low-amplitude, long-lasting mecanoelectrical activity, called contractions, to prominent, short-lasting contractions around the onset of darkness several nights before delivery. The switch from contractions to contractions is reversible: it occurs over a number of nights before delivery with myoelectrical activity returning to low-amplitude contractions during the day. The switch is also progressive, because nightly contractions increase in frequency and amplitude until the neonate is born. Work from our laboratory suggests that uterine contractility during late gestation in the mare is similar to that of the primate and human. The mare also seems to experience contractions and contractions, although definitive conclusion cannot be made without measurements of myometrial pressure. The mare exhibits low-amplitude clusters of myometrial activity that last for ≥1 min (large spike
bursts punitive contractions) and high-amplitude epochs of activity that are <30 s in duration (small spike bursts punitive contractions). The duration and number of large spike clusters vary little as gestation progresses (punitive contractures), whereas the number of small spike bursts (punitive contractions) begins to increase at night in the last 6 days of gestation and continues to increase at night until parturition (Figs. 6 and 7). It is not uncommon for owners or foaling attendants to complain of sweating, rolling, and straining in mares during the night in the last 10 days of gestation. These “warming-up” and “cooling-down” episodes are likely associated with an increase in the nightly contractions that occur in the last week of gestation in the mare.

Fig. 6. Number of spike-burst clusters per hour in control mares and in mares with experimentally induced ascending placentitis during the last 10 days of gestation. Results are least square means ± SEM. (A) Total clusters. (B) Small clusters. (C) Large clusters. Total clusters per hour began to increase 6 days before parturition in control mares as a result of an increase in the small clusters (p < 0.0001). Total, small, or large spike-burst clusters did not change in mares with placentitis. Taken with permission from McGlothlin et al.36
Regulation of the patterns of myoelectrical activity in the primate and ruminant is associated with increases in maternal plasma estrogen and oxytocin. Rising estrogen during late pregnancy plays a supportive role in establishing nocturnal uterine activity that is mediated by maternal oxytocin. Estrogen stimulates an increase in oxytocin production, an increase in oxytocin receptor availability and uterine sensitivity, and an increase in prostaglandin synthesis.\textsuperscript{26} In the primate, the nightly increase in uterine activity seems to be initiated by a surge in maternal estradiol concentration in the early evening hours during the last 10–12 days of pregnancy. There is a corresponding nightly increase in contractions that eventually results in labor.\textsuperscript{27,28} The estrogen pattern in the mare in late gestation is quite different than that seen in other species. There is a slight rise in daily plasma estradiol 17β concentrations in the dam in the last weeks of gestation, although total plasma estrogen concentrations are decreasing.\textsuperscript{9} We have found that plasma estradiol 17β seems to be released in pulses in late gestation. These pulses are most prominent at night with the greatest difference between day and night hours during the 6 days preceding parturition (Figs. 8 and 9).\textsuperscript{29} We propose that the nightly pulses in estradiol 17β are associated with the nightly progressive rise in myoelectrical activity seen in the last 6 days of gestation in the mare. The pattern of release of oxytocin in the last week of gestation in the mare is not known.

4. Effects of Uterine Infection

Uterine Infection and Premature Labor

In women, intrauterine infection is highly associated with idiopathic pre-term labor.\textsuperscript{30–32} Bacteria ascend from the maternal vagina, infect maternal and fetal gestational tissues near the cervix, and establish an inflammatory focus. The host responds with an inflammatory process that results in prostaglandin production and subsequent uterine activity. Pro-inflammatory cytokines increase prostaglandin output by the amnion and the cho-
rion. The human fetal membranes and decidua are also capable of synthesizing cytokines. These inflammatory changes are associated with accelerated maturation of the fetal HPA axis in the primate. Accelerated maturation of the fetal HPA axis is thought to occur directly or indirectly from the effects of the pro-inflammatory cytokines or from prostaglandins on the fetal brain.

In the mare, inoculation of the cervical canal in late gestation (days 285–293 of gestation; n = 8) with *S. equi* was associated with an increase in the mRNA expression of the pro-inflammatory cytokines IL-6 and IL-8 in the placenta. Allantoic concentrations of PGE2 and PGF2α were elevated in the last 48 h of gestation in the inoculated mares. All inoculated mares delivered prematurely. In the aforementioned experiment, one of eight mares with experimentally induced placitis delivered a viable foal prematurely at day 309 of gestation (20 days after bacterial inoculation). The foal lived with minimal nursing care. These findings indicate that experimental ascending placitis caused by *S. equi* is associated with a classic pro-inflammatory cytokine response and premature delivery and that it may induce accelerated maturation in the foal. The mechanisms by which labor and delivery are regulated in spontaneous parturition and in infection may be entirely different. Unlike the control mares, mares with experimentally induced placitis did not exhibit a rise in small-burst clusters (putative contractions) in the last week of gestation (Fig. 6). They exhibited an increase in the duration and intensity of the large spike bursts (putative contractions) in the 4 days preceding parturition. The increase in large spike bursts (putative contractions) may be associated with a rise in intrauterine pressure, which could result in cervical relaxation, dilation, or delivery. An increase in small spike bursts (putative contractions) were only observed in infected mares that were in active labor. These findings are clinically important, because many equine clinicians empirically treat mares with clenbuterol and other β agonists in an attempt to “stop” premature contractions that may not occur in clinical cases of placitis. In addition, mares with placitis are frequently placed on progesterone or Regumate in an attempt to block uterine contractions. The latter two drugs may be beneficial, because they may block the formation of prostaglandins in late gestation. Daels et al. has shown that altrnogest (Regumate) and progesterone in oil-block endogenous prostaglandin production in mares in mid-gestation when mares were given cloprostrenol to induce abortion.

Endocrine Changes Associated With Uterine Infection

Our studies and those of others show that maternal plasma progesterins may rise prematurely or may fall precipitously during chronic infection or after a surgical or medical insult in a manner similar to that seen in normal mares during the last 15–20 days of gestation. A study by Rossdale et al. evaluated progesterin and mammary secretions in 25 mares that exhibited clinical signs of premature delivery (mammary development and/or vaginal discharge). By infusing air through an endoscope, this study made placental separation in the region of the cervix to determine its effect on the length of gestation and progesterin concentrations. This experiment was performed in seven experimental mares between 228 and 267 days of gestation. Sixteen of the 25 clinically affected mares and 5 of the 7 experimental mares exhibited a premature rise in progesterins. The five experimental mares mentioned above delivered 28–75 days after insult. The remaining two experimental mares did not exhibit a rise in progesterone, and they aborted 9 and 16 days after placental separation. Santschi et al. evaluated progesterins in pregnant mares referred for medical and/or surgical colic. Progesterin concentrations did not change in 16 of 22 mares (range of gestation, 17–336 days) during their hospital stay. Twelve of 22 mares foaled normally. Three of 22 mares died, and 1 of 22 mares aborted 10 days after discharge. Progesterin concentrations declined in the remaining six mares (205–215 days of gestation). Five of six mares aborted, and one of six mares delivered a severely compromised foal at term. These data indicate that a premature rise in maternal plasma progesterins may be an indication of accelerated fetal maturation or fetal stress. A premature fall in progesterins often results in abortion.

5. Diagnostic Methodologies

Our ability to accurately evaluate fetal viability in a mare carrying a high-risk pregnancy is fair at best. If and when we do recognize that a fetus is in trouble, our only option for intervention is to treat the mare with drugs, because inducing parturition to deliver a premature foal has disastrous consequences. Unfortunately, not all mares with placental infection show signs of infection like vaginal discharge and udder development. If the mare does show signs of impending delivery >2 wk before the due date (using 335 days as the normal gestation time), a veterinarian can assist the mare’s owner by performing a number of procedures to determine the extent of the problem. The veterinary examination is helpful in determining how the mare should be managed (i.e., should she be shipped to a referral hospital, can she be treated at home, what drugs should be used for treatment). The goal of the treatment is to prolong the pregnancy, because chronic infection of the placenta is sometimes associated with accelerated maturation of the fetus. Therefore, a fetus can be born as early as 305 or 310 days of gestation if it has been subjected to prolonged in utero stress; however, it can survive with neonatal care.

Procedures that a veterinarian may suggest for evaluating fetal viability include rectal and vaginal examination, transabdominal and transrectal ultra-
sonography, and hormonal analysis. Abnormal vaginal fluids should be cultured to determine antibiotic or antifungal sensitivity. Transrectal ultrasonography of the placenta is more helpful in identifying mares with ascending placental infection in late gestation than is transabdominal ultrasonography, because over 90% of placental infections are caused by bacteria ascending through the vagina. The veterinarian performs transrectal ultrasonography to determine if the placenta has thickened or detached from the uterus. Not all mares that are at risk of premature labor will exhibit changes on transrectal ultrasonography. In a project that we conducted at the University of Florida, 15 of 19 mares (60%) that were infected experimentally exhibited placental thickening and/or placental separation before they aborted or delivered a foal prematurely.

Ultrasoundographic Evaluation of the Caudal Placenta
Measuring the combined thickness of the uterus and placenta by transrectal ultrasonography is relatively simple. After the rectum is cleared of feces, the transducer, preferably a 5.0- or 7.5-MHz probe, is positioned at the cervical-placental junction. The uterus can be identified as a fluid-filled structure. When the cervix and uterus are visualized, the probe is positioned 2.5-5 cm cranial of the cervical-placental junction and moved laterally until the middle branch of the uterine artery is visualized along the ventral aspect of the uterine body. The image is frozen, and the combined thickness of the uterus and placenta (CTUP) is measured at the caudal, ventral edge of the uterus between the middle branch of the uterine artery and the allantoic fluid (Fig. 10). The CTUP should not be measured along the dorsal border of the uterus. The placenta is normally thicker and edematous in that area, because it is not stretched by the weight of the fetus. A study by Renoudin et al. evaluated placental thickness throughout pregnancy in light horses and established normal values for various stages of gestation. The guidelines are CTUP of <8 mm from 271 days of gestation (dGa) to 360 dGa (month 10), <10 mm 301-330 dGa (month 11), and <12 mm 331-360 dGa (month 12). These measurements were obtained in Quarter horses. The CTUP may be slightly higher in Warmbloods and lower in ponies. The majority of mares will have a CTUP of ≤12 mm at foaling. A CTUP >15 mm in horse mares and >12 mm in pony mares after 310 dGa is associated with placental dysfunction.

In ascending placentitis, the placenta can separate from the endometrium in the area of the cervical scar. The detached placenta will appear as a ribbon of tissue floating in fluid (Fig. 11). The fluid between the endometrium and the detached placenta may contain hydropoic, swarming particles (Fig. 12). Normally, the allantoic fluid has some gray floating particles (slightly hydropoic). The amniotic fluid is generally more echogenic than the allantoic fluid. If the echogenicity of the fluid compartments is gray, it is likely that there is an on-going placental or fetal abnormality (Fig. 13). Abnormalities associated with an increase in the echogenicity of the fetal fluids include meconium in the amniotic, inflammatory debris, and hemorrhage. In mares at risk of placental compromise, we recommend that they be examined at least twice, 2–3 days apart. This examination will help determine if placental separation or the CTUP is increasing or if the treatment has possibly slowed the inflammatory process.

Maternal Plasma Progestins
In the presence of fetal stress caused by placental damage or maternal illness, progrenolone production by the fetal adrenals seems to be precociously enhanced in the last trimester. This results in a premature rise in maternal plasma progestins. Plasma progestosterone concentrations in a pregnant mare after 180 dGa are negligible, and concentrations measured by commercial RIAs or ELISAs represent placental progestins. We can detect changes in maternal plasma progestins in clinical cases, because the antibodies used to detect progesterone in RIAs and ELISAs react with many of the progestins produced by the fetoplacental unit. The degree of cross-reactivity of fetoplacental progestins with RIA and ELISA assays differ between assays, and therefore, laboratories. However, blood can be collected from mares in late gestation to qualitatively determine if a change from baseline is occurring. In normal pregnant mares, plasma progestin concentr-
Fig. 12. Ultrasoundographic image of the caudal reproductive tract of the same mare as in Fig. 11. This image was taken 6 days after cervical inoculation with bacteria. Endo, endometrium; CA, chorionicallantois; Alm, allantoic fluid. The placenta is separated from the endometrium with pus in the space between the two tissues. CTUP is thickened below the separation (CTUP = 1.28 cm).

Fig. 13. Ultrasoundographic image taken from a mare with hydrops allantois and secondary placentitis. Note the hyperechoic nature of the allantoic and amniotic fluids. The amnion is thickened and cystic.
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...tion and every 48–72 h after inoculation. A CTUP >1.2 cm was considered abnormal. Progestins were measured every three days in control mares from 265 dGn until parturition and every other day in experimental mares. Plasma progestins were compared before and after inoculation in experimental mares and between groups. Assessment of the plasma progestin data revealed that a minimum of three plasma samples taken 48–72 h apart after either surgical instrumentation or bacterial inoculation were needed to identify mares that exhibited a change in progestin concentration from a baseline sample taken the day before experimentation (Fig. 14). In the experimental model, a plasma progestin profile was considered to be abnormal (rise or fall) if the three progestin samples taken after the first sample (baseline) increased or decreased by >50% of the first (or baseline) value. In clinical practice, one may examine a pregnant mare after plasma progesterin have begun an abnormal rise or fall. In the latter situation, if the value obtained for the progestin sample lies outside the normal reference range for the laboratory and the second and third samples are also out of the range, it is likely that the fetus is stressed.

We found that transrectal ultrasonography parameters and plasma progesterin concentrations in the control mares in the experimental model were similar to those reported in the literature.40 Control mares delivered healthy foals at term. Four of the 15 mares with placentalitis could not be identified clinically, because they did not exhibit a vaginal discharge or precocious mammary development. Fourteen of 15 mares (93%) exhibited changes in their plasma progesterin profiles. Plasma progesterin decreased sharply in the seven mares that aborted within 7 days of inoculation and increased in seven of eight mares that carried their fetus for >15 days after inoculation. All inoculated mares exhibited histological changes in the cervical star region of the placenta consistent with ascending placentitis. Nine of 15 (60%) inoculated mares had a CTUP >1.2 cm before delivery. Four of the seven mares that aborted <7 days after inoculation and two mares that carried for >15 days had CTUP <1.2 cm. Two foals from inoculated mares were born on 318 and 314 dGn; both were viable and precociously mature. Fourteen of 15 mares were identified when both transrectal ultrasonography and plasma progesterin profiles were performed. However, four of the 15 inoculated mares (26.6%) did not exhibit clinical signs. Therefore, ultrasonography and plasma progesterin profiles are useful diagnostically only if the mare exhibits either vaginal discharge or premature udder development.

6. Treatment Modalities

There is still much that needs to be learned about the pathophysiology of placentitis in the mare before appropriate treatment protocols can be developed. Current data indicate that a number of approaches should be investigated. Our working hypothesis is that in the presence of an ascending bacterial infection, organisms enter the chorionic nutrient and induce an increase in the expression of pro-inflammatory cytokines in the placental tissue. This results in the release of PGF2α and PGE2 into allantoic fluid, which mediates the events that lead to premature delivery of the foal. If future studies support this hypothesis, then treatment protocols need to be directed at (1) inhibiting bacterial growth and their invasion of the placenta, (2) blocking the expression and release of pro-inflammatory cytokines and prostaglandins, and (3) maintaining uterine quiescence during treatment. The following section reviews past and current literature on drug treatment in the mare in late gestation. This in-depth review will help the veterinary clinician decide what, if any,
treatments to use when faced with a mare that is impending to foal prematurely.

**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 placental/abortion include *S. equi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and nocardioform species. Fungal and viral organisms can also infect the placentas 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. Common approaches to antimicrobial treatment include oral administration of trimethoprim sulfadiazine or parenteral administration of penicillin and gentamicin or ceftiofur.

Information on placental penetration of antibiotics in horses is scant. Serittich and Vaala administered commercially used antibiotics to 11 normal-foaling mares to evaluate the efficacy of the drugs in penetrating fetal membranes. Beginning 7 days before the expected foaling date, allantoic and amniotic fluid samples were taken using an ultrasound-guided needle. Mares were randomly placed into one of four groups: (1) potassium penicillin G (22,000 IU/kg, q 6 h, IV) and gentamicin sulfate (2.2 mg/kg, q 6 h, IV), (2) trimethoprim sulfadiazine (5 mg/kg, q 12 h, PO), (3) gentamicin sulfate (2.2 mg/kg, q 6 h, IV), (4) potassium penicillin G, (22,000 IU/kg, q 6 h, IV). Antibiotics were administered to mares after initial ultrasound examination until delivery. Allantoic fluid samples were obtained four days after initiation of treatment and at foaling before the amniotic membrane ruptured. Serum samples were obtained daily during the course of treatment. Penicillin was detected in mare serum at normal concentrations and in allantoic fluid of one mare, but it was not found in amniotic fluid or foal serum. Gentamicin concentrations in mare serum were within normal limits, but it was less than the detectable assay range of 0.5 μg/ml in fetal fluids and foal serum. Trimethoprim sulfadiazine was recovered from both amniotic (n = 2) and allantoic (n = 4) fluid, and it was detected in 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 detect the drugs. They felt that concentrations of penicillin and gentamicin in the fetal compartment were not high enough to combat fetal infection but that gentamicin would not pose a risk to the developing fetus if given to a pregnant mare. Concentration of trimethoprim sulfadiazine in fetal fluids was high enough to combat most bacteria sensitive to the drug.

Santschi and Papich monitored the pharmacokinetics of gentamicin in late pregnancy and early lactation. They also evaluated placent al transfer of gentamicin given to three mares 60 min before they were induced to foal with oxytocin. Serum was collected from foals at 10, 20, 40, 60, and 120 min after delivery. Gentamicin concentrations were measured with a fluorescence polarization immunoassay. Gentamicin was detected in all serum samples at expected concentrations in mares. However, it was not detected (minimum detection limit of 0.27 μg/ml) in foal serum or in the one amniotic fluid sample collected. The authors concluded that gentamicin did not readily pass through the equine placenta, but the serum and fetal fluids may have been collected too soon after gentamicin was given for it to distribute into fetal fluids.

Workers at University of Florida recently studied drug transfer across the equine placenta using microdialysis, a technique that provides continuous measurement of drugs. Five mares between 260-271 dGa were treated with potassium penicillin G (22,000 IU/kg, q 6 h, IV), gentamicin (6.6 mg/kg, q 24 h, IV), and flunixin meglumine (mg/kg, q 12 h, IV). A microdialysis probe was placed in the allantoic cavity of each mare using ultrasound guidance, and a second probe was placed in the jugular vein to simultaneously monitor drug levels in the mare's systemic circulation and in the fetal compartment. Serum and allantoic fluid samples were collected over 24 h. Analysis of microdialysate samples showed that both antibiotics were present in allantoic fluid, albeit at lower concentrations than were present in serum (Figs. 15 and 16). Elimination rates for penicillin G and gentamicin in allantoic fluid were slower than that of serum; therefore, drugs were detected for a longer period of time in allantoic samples. The authors concluded that the pharmacokinetic pattern might represent compartmentalization of drugs in the pregnant mare. Some drugs may persist longer in allantoic fluid than in serum, because fetal fluids may be isolated from the mechanisms responsible for systemic drug elimination. Penicillin concentration in allantoic fluid reached the minimum inhibitory concentration (MIC) against *S. equi*. Gentamicin concentrations in allantoic fluid seemed adequate to be effective against *Escherichia coli* or *Klebsiella pneumoniae*. Two of the pregnant mares then received an intracervical inoculum of *S. equi* to determine drug penetration across diseased fetal membranes. Penicillin and gentamicin were detected in allantoic fluid of the two infected mares; however, because sample size was small, accurate pharmacokinetic data could not be generated. Flunixin meglumine was not detected in allantoic fluid of control and infected mares, because it was protein-bound. Therefore, it was too large to penetrate the pores of the microdialysis membrane.

In a second study, workers at the University of Florida used the same methodology to study the pharmacokinetics of trimethoprim sulfadiazine and pentoxyfylline in allantoic fluid of pregnant mares.
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Allantoic Concentrations

Plasma Concentrations

Fig. 15. Allantoic and plasma concentrations of potassium penicillin G in a pregnant pony mare.

Advantages of using trimethoprim sulfa in mares with placentalis include oral bioavailability and good uterine penetration. Pentoxifylline was evaluated, because it may inhibit production of pro-inflammatory cytokines.\textsuperscript{44,45} Five mares received an intracisternal inoculation of \textit{S. equi} 5 days before the microdialysis study, and five mares served as controls. All mares were treated with oral trimethoprim sulfa (30 mg/kg, q 12 h) and oral pentoxifylline (8.5 mg/kg, q 12 h) for 14 days beginning on the day of the microdialysis study. Preliminary data indicate that trimethoprim sulfa efficiently penetrates the placental membranes in normal mares. The pharmacokinetics of trimethoprim and sulfadiazine were analyzed independently in microdialysate samples. Initial peak plasma concentrations of both drugs were similar; however, serum concentrations decreased more quickly than allantoic concentrations (Fig. 17). Pentoxifylline was also detected in allantoic fluid, but concentrations declined more rapidly than trimethoprim sulfa. Four of five infected mares aborted. Three mares aborted after drug therapy was stopped (10, 17, and 19 days after the last day of treatment), one mare aborted after 13 days of treatment, 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, either alone or in combination, can delay preterm delivery in mares with placentalis.

In a large clinical study regarding treatment of mares diagnosed with placentalis in Kentucky,\textsuperscript{46} investigators examined records of 477 mares over 6 yr. Fifteen mares were diagnosed with placentalis. Criteria for treatment included udder development and increased thickness of the uteroplacental unit identified by transrectal ultrasonography, placental separation, and/or vulvar discharge. Mean gestational age at diagnosis was 8.6 mo. Mares were treated with a combination of systemic antibiotics
(trimethoprim sulfa, ceftiofur, or penicillin and gentamicin), pentoxyfylline, altrenogest, and non-steroidal anti-inflammatory agents (NSAIDs). Mares were treated until abortion or delivery of a foal. Twelve of 15 (84%) treated mares carried their foals to term, and 11 of 15 (73%) treated mares 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 antibiotic and anti-inflammatory treatment may positively impact pregnancy outcome in mares with placentitis. Further studies are needed to examine the effect of individual drugs and/or length of treatment on neonatal outcome.

Anti-Inflammatory Therapy

Inflammation has recently been identified as a perpetrator of pre-term 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 pre-term delivery. Bacteria or bacterial products in fetal membranes stimulate cell-mediated immune mechanisms with subsequent release of pro-inflammatory cytokines from macrophages and decidua. In turn, pro-inflammatory cytokines stimulate release of PGE₂ and PGF₂α from the endometrium. Then, prostaglandins initiate uterine contractions. Work on mares indicates that pro-inflammatory cy-
tokines, PGE₂, and PGF₂α, are increased in fetal fluids and placental tissue of mares with experimentally induced placitis.  

Work on humans has been directed at identifying factors that interfere with the release of pro-inflammatory cytokines and the synthesis of prostaglandins. Sadowsky et al.  

induced uterine contractions in chronically catheterized pregnant monkeys by infusing IL-1β, a pro-inflammatory cytokine, into the amniotic cavity. Monkeys were treated with indomethacin, a potent cyclooxygenase inhibitor, in an effort to inhibit prostaglandin synthesis. Uterine activity increased seven-fold from baseline in control monkeys but did not increase in animals that received both IL-1β infusion and indomethacin treatment concurrently. Concentrations of white blood cells and cytokines increased in amniotic fluid after IL-1β infusion in both indomethacin-treated and untreated animals. However, amniotic fluid PGE₂ and PGF₂α only increased in control monkeys. Results from this study showed that indomethacin is effective in blocking prostaglandin-induced uterine contractions after intra-amniotic cytokine infusion, but it does not inhibit production of pro-inflammatory cytokines. 

The same group also examined the efficacy of immunomodulators, dexamethasone or interleukin-10, in preventing IL-1β-induced uterine contractions. Using a similar study design as the indomethacin experiment, IL-1β was infused into the amniotic space in 13 chronically instrumented Rhesus monkeys. Monkeys then received one of three treatments: (1) IV dexamethasone beginning 1 day before IL-1β infusion and continuing until 2 days after infusion (n = 4), (2) IV and intra-amniotic injection of interleukin-10 before IL-1β infusion and continuing for 3 days after infusion (n = 5), or (3) IL-1β infusion only (control, n = 5). Infusion of IL-1β, in the absence of dexamethasone or IL-10, initiated increased uterine activity and increased concentrations of intra-amniotic pro-inflammatory cytokines, prostaglandins, and leukocytes. Monkeys that were not treated with immunomodulators delivered fetuses prematurely. Administration of dexamethasone prevented pre-term delivery of fetuses. Dexamethasone and IL-10 treatment inhibited amniotic prostaglandin synthesis, but it did not provoke a marked effect on pro-inflammatory cytokine synthesis. Results revealed that immunomodulators play an important role in tempering the effects of pro-inflammatory cytokines and prostaglandins in inflammatory-mediated pre-term labor. The group then studied the effects of antibiotics alone (ampicillin) or antibiotic therapy plus dexamethasone and indomethacin in delaying pre-term labor in monkeys infected with an intra-amniotic inoculation of group B. streptococci. Results showed that ampicillin alone was effective in eradicating bacteria from the amniotic fluid of infected animals; however, it did not block elevations in amniotic fluid cytokines, prostaglandins, or uterine contractions. Concentrations of amniotic fluid cytokines and prostaglandins were suppressed in animals treated with ampicillin, dexamethasone, and indomethacin. It seems that combined therapy is needed to stem bacterial infection and to suppress the subsequent inflammatory response. 

The effectiveness of anti-inflammatory therapies in equine pregnancy is not well documented. Le Blanc et al. identified elevated concentrations of PGE₂ and PGF₂α in allantoic fluid samples collected within 48 h of abortion or delivery in mares with experimentally induced placitis. Allantoic concentrations of cytokines (IL-1, IL-6, TNF α) did not differ between infected and control mares. However, mRNA expression of IL-6 and IL-8 was elevated in placenta of infected mares. Murchie et al. attempted to determine if the potent anti-prostaglandin agent, flunixin meglumine, penetrated the placenta in both normal and experimentally infected mares. However, flunixin meglumine was not detected, because it was protein bound and too large to pass through the microdialysis pores. The Florida group showed that pentoxifylline, a xanthine derivative with anti-inflammatory cytokine effects, crossed the equine placenta of both normal and experimentally infected pregnant pony mares. 

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 pre-term labor. A variety of agents have been used including magnesium sulfate, β-sympathomimetic agents (ritodrine and terbutaline), prostaglandin synthesis inhibitors (indomethacin, sulindac, ibuprofen, and aspirin), calcium channel blockers (nifedipine), and oxytocin antagonists (atobasaban). 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 this times, glucocorticoids can be administered to the mother in an effort to expedite fetal maturation. Side effects from tocolytic agents can be significant and may include cardiac arrhythmia, pulmonary edema, and/or myocardial ischemia (β-sympathomimetics), hypotension (nifedipine), and gastrointestinal disturbance and oligohydramnios (indomethacin). 

Clenbuterol, a β-sympathomimetic agent, is used in the clinical equine practice. The effects of clenbuterol administration on uterine tone, maternal heart rate, and fetal heart rate were examined by Card and Wood. Clenbuterol was administered intravenously (300 μg) to four pregnant mares at 30, 40, 50, and 60 dGA and then one time per month until parturition. The final dose was administered when the mare was thought to be close to parturition, which was determined by measuring the con-
centrations of calcium and magnesium (120 ppm) in the mare’s milk with water hardness test strips. Fetal heart rate, maternal heart rate, and uterine tone (measured by palpation) were recorded. Mares and fetuses experienced transient tachycardia after drug administration. Resting uterine tone changed significantly after clenbuterol administration to mares early in gestation. Uterine relaxation was less profound when clenbuterol was given in late gestation. Uterine tone decreased within 3 min of drug administration and persisted up to 120 min. The authors concluded that clenbuterol effectively induced uterine relaxation for up to 120 min throughout gestation. Additionally, side effects were minimal and transient.

A more recent study reported the effects of clenbuterol when administered to 29 pony mares late in gestation. Beginning on day 320 of gestation, changes in mammary secretion electrolyte were monitored using a calcium strip test. Treatment started when calcium levels reached 13 mM (4 squares reacted on the strip test). Fifteen mares were treated with one of three doses of clenbuterol, IV: 0.6 mg (n = 6); 1 mg (n = 5); 1.5 mg (n = 4). Fifteen mares were treated with saline. Mares were treated one time per day at 10:00 p.m. until parturition. There were no differences between groups for length of gestation, number of treatments, time to foaling, or fetal outcome. Mares in the low-dose treatment groups (0.6 mg and 1 mg) showed no side effects, whereas 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 may have promoted cervical relaxation and subsequent parturition. Based on the side effects detected when clenbuterol is administered to pregnant mares and the lack of effect for delaying normal parturition, the authors suggest that this agent has limited usefulness in horses.

Treatment with progestins has long been advocated to promote uterine quiescence in pregnant mares with uterine pathology. The actual rationale for progestin use in late pregnancy is not clear. Presumably, the anti-prostaglandin effect of progestins contribute to reduced myometrial activity by interfering with up-regulation of prostaglandin and oxytocin receptors. Without receptor formation, gap junction formation is inhibited and uterine contractility prevented. Daels et al. tested the effects of progesterone and altronegest, a synthetic progesterin, on pregnancy maintenance in mares treated with the prostaglandin analog, cloprostenol. Sixteen mares with pregnancies ranging from 93 to 153 dCa were evaluated. Cloprostenol (250 µg, IM) was administered to all mares for 5 consecutive days. Progesterone (300 mg, q 24 h, IM) was administered to eight mares beginning 18 h after cloprostenol treatment and discontinued 18 h after the last cloprostenol treatment. Altronegest (44 mg, q 24 h) was administered to eight mares, orally, beginning 12 h after cloprostenol and discontinuing 12 h after the last cloprostenol treatment. Five of eight mares in the progesterone-treated group maintained pregnancies after cloprostenol treatment, whereas three mares aborted during treatment. All eight mares treated with altronegest-maintained pregnancies. All control mares (six mares from 82 to 102 dCa) aborted after cloprostenol treatment. Administration of exogenous progestins to mares treated with cloprostenol was associated with a decrease in concentration of endogenous prostaglandin metabolites. Results showed that progesterin supplementation prevented prostaglandin-induced abortion in most cases. Findings support the use of progesterin supplementation in mares at risk for pre-term labor.

Progesterin supplementation is currently being implemented in humans to halt pre-term labor. A recent double-blind, placebo-controlled study showed a beneficial effect when women with a documented history of spontaneous pre-term delivery were treated with progesterone. The incidence of recurring spontaneous pre-term delivery was lower in women treated with 17α-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 that facilitate myometrial contractions or interferes with prostaglandin-induced myometrial contractions stimulated by pro-inflammatory cytokines is unknown.

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

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