Diagnosis and management of placentitis

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Introduction

Placentitis is the most common cause of placental abnormality and in Thoroughbred (TB) mares in the UK, accounts for 9.8% of diagnosed abortions (Smith *et al.* 2003). To put this data into perspective at a population level the incidence risk is estimated to be 0.34% (Roach *et al.* 2021). Almost all cases in the UK are caused by an ascending infection that enters the uterus via the cervix with the most common pathogens isolated being β-haemolytic Streptococci (*Streptococcus equi.* var *zooepidemicus*), *Escherichia coli* and *Aspergillus spp.* In ascending placentitis infectious agents (bacteria and/or fungi) gain entry via the cervix inoculating the chorioallantois. Infection may subsequently gain access to the foetus via the umbilical cord or via translocation through the foetal fluids resulting in foetal sepsis and ultimately premature delivery or abortion. Placentitis can be insidious in nature; clinical signs may not develop until well into the disease process making effective treatment challenging. Typically, mares suffering from placentitis are middle aged and multiparous and may have previous history or poor perineal conformation (Canisso *et al.* 2015).

Pathophysiology of placentitis

Bacterial invasion and colonisation of the placenta results in neutrophil infiltration of the placenta, destruction of the villi and ultimately separation of the chorioallantoic membrane from the endometrium with collection of a neutrophilic exudate between. The ensuing inflammatory process is associated with an increase in the production of allantoic prostaglandin E2 and F2a, and subsequent activation of myometrial smooth muscle activity and premature activation of the foetal hypothalamic-pituitaryadrenal (HPA) axis leading to premature delivery or abortion (Rossdale *et al.* 1991, LeBlanc *et al.* 2002, Lyle *et al.* 2009). Recent work from Kentucky and Florida (Fedorka *et al.* 2019a, Macpherson *et al.* 2021) has further characterised the foetal-maternal immune response to placental infection demonstrating that the maternal response is largely pro-inflammatory whilst the foetal cytokine response is both pro- and anti-inflammatory with levels of cytokines and prostaglandins increasing within the amniotic fluid.

Diagnosis of ascending placentitis

The most common presenting sign of placentitis prior to abortion or premature delivery, is premature mammary development and lactation. Premature mammary development is not pathognomonic for placentitis and may indicate impending abortion due to a variety of infectious and non-infectious causes. Vulvar discharge is a very inconsistent clinical finding in mares with placentitis; it is likely that a discharge is often present in affected mares, but it may be produced in such small quantities that it is easily missed.

Transrectal and transabdominal ultrasound are both useful tools to investigate the condition. Subclinical disease may result in subtle ultrasonographic changes that are not easily distinguished from normal findings, however, placentitis has been demonstrated to cause a marked increase in thicknesses of the chorioallatois at the cervical pole. Under normal circumstances, the uterus and the placenta in this region are indistinguishable from one another and the combined thickness of the uterus and placenta (CTUP) is measured. Transrectal ultrasonography in late-gestation mares allows evaluation of the chorioallantois at the cervical pole and measurement of CTUP; it also allows for evaluation of foetal activity, foetal fluid character, foetal orbit measures and subjective amniotic evaluation.

Measurement of the combined thickness of the utero-placental unit (CTUP)

A 5-10 MHz linear transducer is positioned just cranial to the cervix and then moved laterally until a major uterine vessel is visible at the ventral aspect of the uterine body. The area between the uterine vessel and the allantoic fluid represents the CTUP. Where possible, at least three measurements should be taken and averaged. Measurements for the CTUP are obtained from the ventral aspect of the uterine body because the dorsal aspect of the uteroplacental unit may be oedematous in the last month of gestation in normal mares. The examiner should ensure that the amnion is not lying in close apposition to the chorioallantois as this will result in an artificially increased CTUP.

Normal values for the CTUP have been established in Quarter horses (Renaudin *et al.* 1997), Thoroughbreds (Troedsson and Zent, 2004, Colón 2008), Standardbreds (Bucca *et al.* 2005), Ponies and Arabians (Barnes *et al.* 2005), and Dutch Warmbloods (Hendricks *et al.* 2009). More recently other breeds of horse, Mule pregnancies and Donkeys have been studied (Table 1). The mean CTUP plateaus at approximately 4mm between the 4th and the 9th month of pregnancy, after which time it increased significantly by 1.5-2mm each month until the end of gestation (Renaudin *et al.* 1997). Considerable variation may exist however, the month to month increase in CTUP is consistent (Bucca *et al.* 2005). The author uses data from Renaudin and co-workers (1997) as a guide (Table 2). Le Blanc and co-workers (2004) suggested that a CTUP >15mm in horse mares and >12mm in pony mares after 310 days gestation (10 months) is associated with placental malfunction.

Table 1. Sources of placental scanning (CTUP) data on different breeds of horse and donkey.

Sub-species	**Breed**	**Location**	**Reference**
Horse	Quarter Horse	USA	Renaudin *et al.* 1997
	Thoroughbred	USA	Troedsson and Zent 2004, Colón 2008
	Standardbreds	Italy	Bucca *et al.* 2005
	Ponies, Arabians	USA	Barnes *et al.* 2005
	Dutch Warmbloods	The Netherlands	Hendriks *et al.* 2009
	Criollo	Brazil	Souza *et al.* 2010
	Spanish Pure Bred	Spain	Requena *et al.* 2017
	Mangalarga Marchador	Brazil	Campos *et al.* 2017
	Heavy Draft Horses	Japan	Kimura *et al.* 2018
Mule pregnancies	Trotter mare x Amiatino jack	Italy	Paolucci *et al.* 2012
Donkey	Martina Franca	Italy	Carluccio *et al.* 2016
Donkey	Dezhou	China	Magalhaes and Canisso 2022

Table 2. Normal values for CTUP (Adapted from Renaudin *et al.* 1997)

Month of gestation	**Gestation day**	**CTUP (mm)**
9th	241-270	<7.0
10th	271-300	<8.0
11th	301-330	<10.0
12th	331-360	<12.0

While an elevated CTUP may suggest pathology mild increases may require confirmation by the presence of other clinical signs as some normal mares may show marked thickening and oedema without further clinical signs or post-partum evidence of placental pathology (Hendriks

et al. 2009). Conversely, not all mares suffering from an ascending placentitis show detectable placental thickening and it is suggested that mares which abort within seven days of infection may not have time to develop placental thickening (Morris *et al.* 2007). CTUP measurements become somewhat invalidated by the presence of separation of the

Chorioallantois from the endometrium and accumulation of purulent material/fluid between, as this highly suggestive of an advanced disease process. Subjective assessment of the character of foetal fluids may yield additional information; fluids that persistently show increased echogenicity may have increased cellularity in relation to infection or inflammation (Morris *et al.* 2007), however it is important to differentiate this from the changes which occur with foetal movement as cellular debris is 'stirred up'. Mares clinically affected with ascending placentitis may require two or more serial trans-rectal ultrasonographic examinations in order to reliably identify changes on ultrasound.

Vaginal speculum examination of mares with a purulent discharge may reveal an exudate originating from the cervix. The examination will then enable swabbing of the cervical canal to determine the bacterial (or fungal) agent(s) involved and their antibiotic sensitivity pattern. Despite this advantage some commentators feel that vaginal examination via a speculum is contraindicated due to the risks of advancing bacteria forward from the vestibule to the cervix (Govaere *et al.* 2018). The author agrees that manual examination of the cervix may be contraindicated with the additional risk of mechanical stimulation of the cervix, however, the risks of performing a vaginal speculum examination may be outweighed by the benefits of acquiring a sample for sensitivity testing. The author will cautiously perform a speculum examination if either there is an obvious vaginal discharge and/or there is a significant increase in CTUP and/or separation evident on ultrasound.

Serial hormone measurements have been suggested as a means of diagnosing placental pathology. Mares with acute placentitis often demonstrate a rapid drop in plasma progestagen concentrations, whereas mares that develop chronic placentitis often have increased plasma progestin concentrations (Ousey *et al.* 2005; Morris *et al.* 2007). Plasma oestrogens but not androgens decline precipitously after experimentally induced bacterial placentitis, suggesting that maternal oestrogen concentrations may be useful as an early marker of placental insult (Ball *et al.* 2013, Canisso *et al.* 2016). Concentrations of the acute phase proteins serum amyloid A (SAA) and haptoglobin (Hp) rapidly increase subsequent to experimental induction of placentitis and remained increased until abortion suggesting they may be useful markers to help rule in or out placental infection; fibrinogen nor white blood cell concentrations appear to be useful markers for placentitis (Canisso *et al.* 2014).

Recently researchers in Kentucky undertook a field study to determine if cytokine changes could be detected in maternal serum of clinical cases; additionally, they screened for maternal steroid hormones and alfa-fetoprotein. Significant increases in the concentrations of serum cytokines (interleukin (IL)-2, IL-5, IL-6, IL-10, IFNγ, and TNF) were detected along with a significant increase in progesterone and alpha-fetoprotein and decreases in oestradiol-17β and the ratio of oestradiol-17β to progesterone in cases of ascending placentitis (Fedorka *et al.* 2021a, 2021b). As such these may be considered potential biomarkers for the prediction of placental infection and in the future, with the development of reliable, cost-effective laboratory assays, placental infection may be detected by screening mares' serum, rather than by trans-rectal ultrasound.

Treatment of ascending placentitis

Targeted treatment may result in resolution of the condition however very few of the treatments that will be described are licensed for use in equines or do not have a labelled indication for use in pregnant mares. This means that the prescribing clinician frequently needs to utilise the prescribing cascade guidelines when it comes to the selection of therapies and they also need to be aware of the current published evidence to justify the off-license or off-label use of such medications.

On the basis of our knowledge of the pathophysiology of ascending placentitis, treatment strategies are directed at (i) stopping spread of bacterial invasion; (ii) maintaining uterine quiescence; and (iii) blocking production of pro-inflammatory cytokines (LeBlanc, 2010). In practice this is achieved through a combination of therapies:

Antibacterial agents

Antibiotic choice should ideally be determined by culture and sensitivity results however, if these are not available broad-spectrum antibiotics should be instigated. Trimethoprim-sulfamethoxazole or a combination of potassium penicillin G and gentamycin have been demonstrated to penetrate placental and foetal tissues (Murchie *et al.* 2006, Rebello *et al.* 2006) and are therefore the antibiotics of choice. Practically speaking we extrapolate from this data to utilise the licensed equine formulations of trimethoprin-sulfadiazine and procaine benzylpenicillin (Table 3). Despite ceftiofur sodium being found to penetrate endometrial tissues it has not been found to cross to the foetal membranes and thus it is not recommended for cases of placentitis (Macpherson *et al.* 2014).

Recently enrofloxacin has been shown to cross the placenta in mares. Ellerbrock and coworkers (2019), demonstrated that ciprofloxacin, the active metabolite of enrofloxacin, concentrated in allantoic fluid with a 10-fold increase relative to foetal trough plasma concentrations. Concentrations in foetal fluids exceeded the minimum inhibitory concentration (MIC) for bacteria such as *E. coli* but not for *Streptococcus zooepidemicus* (Trundell *et al.* 2017). Foetal articular cartilage was examined post-mortem and no cartilage lesions were noted on gross inspection of any foetal articular surface. Nor were there any detectable toxic effects on the cartilage, kidney or liver of the foetus. More research is required to determine if foals develop cartilage lesions once they become weight bearing. In a separate study, Canisso and coworkers (2019) demonstrated that doxycycline hyclate crossed the foetal membranes with significantly higher concentrations in the allantoic than the amniotic fluids; in addition, doxycycline was detectable in foal plasma at parturition. Mares and their foals did not demonstrate any apparent clinical abnormalities, however further studies are necessary to determine if the detected concentrations are adequate for the treatment of bacteria such as *S. zooepidemicus* with an MIC in the region of 250ng/ml (Chapuis *et al.* 2021, Table 3).

Progestagen therapy

Altrenogest has been demonstrated to prevent preventing prostaglandin induced myometrial activity and is effective in preventing prostaglandin induced abortion in mares at a dose of 0.088mg/kg po sid (Daels *et al.* 1996) and as such is advocated to promote uterine quiescence in mares with high-risk pregnancies. Many other therapies to promote myometrial quiescence have been suggested, many extrapolated from other species, and have been largely anecdotal. Tocolytics such as isoxsuprine and clenbuterol have been shown to cause short term uterine relaxation however don't appear to be of practical use in equine placentitis.

Recent research demonstrated that equine placentitis is associated with a localised myometrial

progestin withdrawal, as well as a down regulation of progesterone receptors concurrent with a localised inflammatory response (El-Sheikh Ali *et al.* 2019). The investigators suggested that tocolytic drugs working via a non-PR associated mechanism may be more beneficial for the treatment of equine placentitis. It has also been demonstrated that altrenogest alters the immune system of the mare systemically and locally within the endometrium which is potentially mediated through the progesterone receptor (Fedorka *et al.* 2019b). Further study will be required to see if these findings will influence our decision to use altrenogest in our multimodal treatment approach.

Non-steroidal anti-inflammatories (NSAIDs)

NSAIDs such as flunixin meglamine (1.1mg/kg IV, or po bid) or phenylbutazone (2.2mg/kg IV, or po bid) are obvious choices to reduce inflammation and prevent prostaglandin-mediated abortion. Phenylbutazone is known to cross the placenta however, flunixin meglumine has not yet been detected in placental fluids, however, this may be a function of the methodology for detection (Macpherson and Giguère 2018).

Frocoxib is a cyclooxygenase-2 (COX-2) selective inhibitor offering the advantage of not impairing mucosal healing which has recently become licensed in the UK. In pregnant mares, firocoxib has equivalent plasma concentrations to non-pregnant mares and crosses the placenta into placental fluids (Giguère *et al.* 2016, Macpherson and Giguère 2018). In an experimental model of placentitis, investigators demonstrated that firocoxib treatment had a significant anti-inflammatory effect, suppressing cytokine and prostaglandin production in foetal fluids (Macpherson *et al.* 2018, 2021).

Other therapies

Acetylsalicylic acid (aspirin; 10-20mg/kg po bid) is another NSAID but also has an inhibitory effect on platelet aggregation by blocking the formation of thromboxane A_2. As a result the side effect of aspirin is that it reduces the ability off the blood to clot and excessive bleeding may result. Pentoxifylline is a competitive non-selective phosphodiesterase inhibitor with anti-cytokine activity. Additionally, it improves red blood cell deformability, reduces blood viscosity and decreases the potential for platelet aggregation. Pentoxifylline (8.5mg/kg po bid) crosses the equine placenta into foetal fluids (Rebello *et al.* 2006) and has been advocated as an agent in a multimodal approach (Bailey *et al.* 2010). Ousey and co-workers (2010) demonstrated that pentoxifylline at 17mg/kg po sid (double dose only once a day) for 70 days from day 50 until 120 of gestation increased uterine artery blood flow velocity during the first 30 days. They also demonstrated that the gestational age decline in uterine artery resistance was diminished suggesting that long-term treatment increases placental vascular resistance which is of unknown significance.

Sielhorst and co-workers (2018) looked at the effects of aspirin on uterine blood flow, placental development and foal birth weight. Placental weight and foal birth weight were not altered by the treatment (10mg/kg po sid-bid), however, time averaged mean velocity (TAMV) of blood flow was significantly increased in mares treated with 10mg/kg aspirin orally twice daily from day 285 of gestation to parturition. In addition, they found no significant difference in coagulation parameters as determined by thromboelastography, suggesting aspirin may be a safe adjunct therapy perhaps as a replacement to a more traditional NSAID.

Combination therapy

Initial research with the experimental model of placentitis (inoculation of *Streptococcus zooepidemicus)* suggested that aggressive anitibiotic therapy with trimethoprim-sulfamethoxazole alone could substantially improve pregnancy outcome; combination therapies with NSAID or aspirin or pentoxyfylline, with or without altrenogest achieved improved

outcomes both experimentally and in the field (Christiansen *et al.* 2010, Bailey *et al.* 2010, Troedsson and Zent 2004, Table 4). Evidence from Curcio and co-workers (2017) indicated a benefit of oestrogen supplementation. Mares receiving trimethoprim-sulfamethoxazole, flunixin meglumine and estradiol cypionate had 100% survival at parturition and at seven days. However, the trimethoprim-sulfamethoxazole, flunixin meglumine and altrenogest group however had better outcomes than the trimethoprim-sulfamethoxazole and flunixin meglumine only group suggesting a positive benefit of altrenogest (Table 4). The most recent work from investigators from Florida suggested that the combination of trimethoprim-sulfamethoxazole, altrenogest and firocoxib (0.3mg/kg po sid for one day, followed by 0.1mg/kg po sid) was very favourable with respect to pregnancy outcome and foal survival (Table 4, Varner *et al.* 2019). In the authors practice this combination is now the first line therapy for bacterial placentitis cases unless bacterial sensitivity suggests a different antibiotic should be utilised. It is worth noting that the potent anti-prostaglandin properties of firocoxib could potentially cause a delay in parturition and it may be necessary to withdraw therapy prior to anticipated parturition.

Recently a new concept of antibiotic delivery in mares with placentitis was proposed. Beachler and coworkers (2021) hypothesised that trans-cervical intrauterine infusion of antibiotics may more effectively treat placentitis pathogens than standard systemic routes. A single trans-cervical infusion of 2.4 million IU of procaine penicillin and 200mg of gentamicin in a total volume of 10ml was performed between 280 and 295 days of gestation in normal mares. All mares foaled without complication 12-58 days after antibiotic infusion at a mean gestational age of 322.7 ±12.7 days. The investigators suggested that procaine penicillin and gentamicin can be administered as a trans-cervical infusion in late-pregnant mares without affecting neonatal viability however, additional work is needed to demonstrate the efficacy of such an approach and we should be cautious in the application of this research.

Duration of therapy

The duration of therapy necessary to cure placentitis is unclear and it is difficult to determine treatment success diagnostically. Resolution of inflammatory markers and normalisation of CTUP may be an indicator of successful therapy but may not indicate eradication of bacteria and persistence of bacteria at the level of the chorioallantois may serve as a continued nidus of infection (Beachler *et al.* 2021). It is notable that more than 60% of experimentally infected mares remain culture positive post-delivery despite antibiotic therapy (Bailey *et al.* 2010; Diaw *et al.* 2010; Curio *et al.* 2017). Given these findings a minimum period of at least three weeks should be considered when using trimethoprim-sulfadiazine. Resurgence of disease after cessation of therapy clearly indicates failure to resolve either bacterial infection or the inflammatory cascade involved

If the treatment goals are accomplished, the gestation length of mares affected with placentitis should be similar to the expected normal duration of pregnancy and result in a live, well-developed foal with minimal health issues. In practice however, this is not always possible; if the condition is detected early and aggressive treatment initiated with a combination of systemic antibiotics, altrenogest, and non-steroidal anti-inflammatories the prognosis may be reasonable, the emphasis however, should be on early detection and timely therapy.

Table 3: Systemic antibiotic therapies for bacterial placentitis

Antibiotic	Dose	Comments	Reference
Ceftiofur sodium	Not indicated	Does not cross the foetal membranes (not detected or very low levels in allantoic fluids)	Macpherson *et al.* 2014
Ceftiofur crystalline free acid	Not indicated	Does not cross the foetal membranes (not detected or very low levels in allantoic fluids)	Macpherson *et al.* 2013
Doxycycline hyclate	10mg/kg po bid	Detectable in allantoic (73.55 ng/ml) and amniotic (8.32ng/ml) fluids, tissues, plasma (35.52ng/ml), and joint capsules of foals from the treated mares	Canisso *et al.* 2019
Enrofloxacin	5.5mg/kg IV sid (7.5mg/kg po sid)	Ciprofloxacin detectable in allantoic fluid in concentrations up to 876ng/ml; amniotic fluid (>100ng/ml)	Ellerbrock *et al.* 2019
Gentamicin sulphate	6.6mg/kg IV sid	Mean peak allantoic concentrations of 8.5mcg/ml. Peak conc. approx. 80% less than mare serum	Murchie *et al.* 2006
Potassium penicillin G	22,000 IU/kg IV qid	Mean peak allantoic concentrations of 9.8mcg/ml. Peak conc. approx. 80% less than mare serum; clearance rate reduced compared with serum	Murchie *et al.* 2006
Procaine benzylpenicillin	25mg/kg IM bid	Extrapolated from above	N/A
Trimethoprim-sulfamethoxazole	30mg/kg po bid	Allantoic fluid at concentrations similar to serum with maximum allantoic concentrations of 0.6mcg/ml trimethoprim and 7.0mcg/ml sulfamethoxazole	Rebello *et al.* 2006
Trimethoprin-sulfadiazine	30mg/kg po bid	Extrapolated from above	N/A

Table 4: Combination therapies and foal survival outcomes in the treatment of bacterial ascending placentitis.

Therapy combination	n	Treatment period	Foal survival	Type of study	Reference
Trimethoprim-sulfamethoxazole (30mg/kg po bid), altrenogest (0.088mg/kg po sid) and firocoxib (0.3mg/kg po sid for one day, followed by 0.1mg/kg po sid)	7	Until foaling/ abortion	100%	experimental*	Varner *et al.* 2019
Trimethoprim-sulfamethoxazole (30mg/kg IV bid for 10d), oestradiol cipionate (10mg/mare IM, q 3d for 3 treatments*) and flunixin meglumine (1.1mg/kg IV sid for 10d)	6	10 days *(9d oestradiol cipionate)	100%	experimental*	Curcio *et al.* 2017
Trimethoprim-sulfamethoxazole (30mg/kg IV bid for 10 d), altrenogest (0.088mg/kg IM, q 7d for two treatments*) and flunixin meglumine (1.1mg/kg IV sid for 10d)	7	10 days *(14d altrenogest)	85.7%	experimental*	Curcio *et al.* 2017
Trimethoprim-sulfamethoxazole (30mg/kg po bid), altrenogest (0.088mg/kg po sid) and pentoxifylline (8.5mg/kg po bid)	12	Until foaling/ abortion	83.33%	experimental*	Bailey *et al.* 2010
Trimethoprim-sulfamethoxazole (30mg/kg	6	Until foaling/ abortion	83.33%	experimental*#	*Christiansen et al*. 2010

po bid) and aspirin (50mg/kg po bid)					
Systemic antibiotics (trimethoprim-sulfamethoxazole, ceftiofur or penicillin and gentimicin), altrenogest and NSAIDs	15	Until foaling/ abortion	73.33%	field	Troedsson and Zent 2004
Trimethoprim-sulfamethoxazole (30mg/kg po bid)	6	Until foaling/ abortion	66.66%	experimental*#	Christiansen *et al.* 2010

*Experimental studies involve the intra-cervical inoculation with 1 x 10^7 colony forming units (CFU) of *Streptococcus equi* var *zooepidemicus* unless otherwise stated; # 2–10 x 10^6 CFU *Strep zooepidemicus.*

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