ASCENDING PLACENTITIS IN THE MARE

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Introduction

Although incidence of placentitis is low, between 3-7%, in pregnant mares, it may lead to abortion, stillbirth or the birth of weak foals (Barr 2005). Besides the costs of neonatal care that is often necessary in case a viable foal is born, stillbirth and abortion lead to loss of income and a subfertile mare in the subsequent season. Of all abortions, premature deliveries and perinatal deaths in equines, about 10 to 30% of cases can be attributed to placentitis. As such placentitis is one of the most common reason of perinatal mortality (Gilles et al. 1993, Hong et al. 1993, Smith et al. 2003, Troedsson 2003, Laugier et al. 2011). Ascending placentitis, caused by bacteria ascending through the vagina and affecting the placenta, is the most common form of placentitis (Platt 1975, Whitwell 1988, Hong et al. 1993).

Most commonly opportunistic bacteria are involved with Streptococcus equi subsp. zooepidemicus isolated in the greatest number of cases, besides Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa (Platt 1975, Merkt 1985, Whitwell 1988, Acland 1993). The majority of cases involved bacterial infection, in about 15% of cases a fungal or mixed infection was seen (Smith et al. 2003). No breed predisposition is noticed however Thoroughbreds might be overrepresented (Macpherson and Bailey 2008).

As a result of the infection, foetal membranes will become oedematous, thickened, and can separate from its attachment to the uterus (Platt 1975). Due to its origin, placental pathology is mostly localized in the area of the cervix (Whitwell 1988, Mays et al. 2002). The infection, or the inflammation of the placental membranes alone, causes a lesser functionality of the membranes, the release of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6 and IL-8 from placental and foetal tissues in response to infection (Dudley 1997, LeBlanc et al. 2002, Mays et al. 2002) and may result in increasing prostaglandine synthesis (LeBlanc et al. 2002,

Evidence based medicine to support any therapeutic approaches are scarce, since not only experimental infection trials are expensive and difficult to maintain, but also most mares will abort without any symptoms, since placentitis in clinics externalizes itself as an insidious, hidden disease where diagnosis comes in late (Macpherson and Bailey 2008). Moreover endocrine regulation in the late term mare differs from other species with only subtle serum changes occurring very late in gestation just before expulsion.

**Diagnosis**

Early diagnosis enables a rapid intervention and will improve prognosis and pregnancy outcome although in most cases the diagnosis based on symptoms of the disease can be difficult because mares are rarely ill, nor do they have abnormal blood counts or changes in clinical parameters. The earliest sign, vaginal discharge, is not seen in every case (Macpherson and Bailey 2008) and the subsequent premature development of the udder is not a constant (Zent et al. 1999, LeBlanc et al. 2002, Bailey et al. 2010, LeBlanc 2010). Although in some cases no symptoms at all can be seen externally, in cases where a premature udder development is seen without vaginal discharge, the mare must be checked for presence of twins or an Nocardioform placentitis (LeBlanc 2010).

In the majority of cases, affected mares are pluriparous and might have anatomic defects of the genital tracts such as as pneumo- or urovagina and/or cervical incompetence (Platt 1975, Macpherson and Bailey 2008, LeBlanc 2010, Löf et al. 2014).

Besides the symptoms, a transrectal and transabdominal ultrasound examination of the placenta, foetal fluids and foetus, and serum profiles of hormones can be used to evaluate the foetal well-being and extent of the inflammation (LeBlanc 2010). A vaginal exploration, whether manually or through a speculum, is contraindicated. The vestibule of the mare harbours in fact a variety of germs that may be pushed to the cranial part of the vagina during such an examination (Hinrichs et al. 1988, Bucca and Fogarthy 2011). Question remains what extra information can be obtained by a vaginal examination. In woman the length and tone of
the cervix gives a good prognostic view on the course of a compromised gestation (Bucca and Fogarthy 2011). The tone and size of the cervix, as last physical barrier to the uterus, can be assessed by transrectal palpation and ultrasonography and cervical relaxation is a consistent feature in the last two months of gestation in the mare (Bucca and Fogarthy 2011) and can as such be of importance in order to estimate the prognosis of the affected gestation.

Post-partum, or after an abortion or stillbirth, a thorough examination of the placenta is essential and might reveal in case of ascending placentitis a thickened, discoloured, oedematous or even ulcerated chorioallantois at the level of the cervical star (Platt 1975, Hong et al. 1993, Mays et al. 2002). Although gross lesions on macroscopic inspection are not specific as they can be caused by other diseases as well (Löf et al. 2014). Furthermore, the ultrasonographic feature of a thickened placenta is not correlated with the total weight of the placenta after expulsion (Löf et al. 2014) although a more heavy placenta might be caused by inflammation and should be sampled for histology to exclude placentitis.

Ultrasonography

When evaluating the combined thickness of uterus and placenta (so called CTUP) one measures the ventral portion of the placenta just cranial of the cervix. It is more easy to perform when using the vasculature of the uterine branch of the vaginal artery, with its course ventral to the uterine border a little bit to the lateral side (on both sides), as a landmark (Renaudin et al. 1997, Bucca and Fogarthy 2011). As such one measures always at the same spot, and the hypoechoic area of the blood vessel makes it more easy to visualise the contours of the uteroplacental delineation. In a normal gestation, the placenta cannot be distinguished from the uterine wall (Macpherson 2006).

The CTUP thickens physiologically with increasing gestational age (Renaudin et al. 1997; Kelleman et al. 2002, Morris et al. 2006). A thickened CTUP can be seen in an inflamed placenta, and separation can become visible, sometimes with fluid and pus in between chorion and uterus, in the more advanced cases of placentitis (LeBlanc et al. 2004). In short, a CTUP of more than 1.2 cm at 9 months of gestation, or more than 1.5 cm at 11 months of gestation can be associated with placentitis (Renaudin et al. 1997, Troedsson et al. 1997, Troedsson 2001, Bucca et al. 2005, 2006). The thickening occurs quite fast most probably depending on the degree of contamination and inflammation, and in infectious trials it has been observed within 48h post inoculation (Bailey et al. 2012).
It has to be said that in other studies (Morris et al. 2007, Bailey et al. 2010, Löf et al. 2014) no different CTUP measures between mares with- and without placentitis were seen, or present in placentitis mares which lead the authors to the conclusion that CTUP is not a good indicator of ascending placentitis during the final month of gestation in Thoroughbred mares (Löf et al. 2014). However, if an increase in CTUP is seen, a degree of placental insufficiency might be present (Cummins et al. 2008) but other reasons of thickened placenta might have to be considered (Govaere et al. 2009, Souza et al. 2010). Moreover, Souza et al. (2010) concluded that “CTUP measurement in mares should not be the only parameter to estimate placental failure and impending abortion”.

Bailey and co-workers (2012) evaluated the use of Doppler measurements to diagnose placentitis in a clinical setting, however since the onset of the disease occurs when the uterine blood flow is already very high no differences could have been detected between affected or healthy cases.

Not only the aspect and size of the CTUP has to be checked, also the appearance of foetal fluids is evaluated and cloudy foetal fluids in the last 3 months of gestation indicate infection (LeBlanc 2010). Whilst checking the foetal fluids, foetal viability parameters (movement and heart rate) are assessed as well (LeBlanc 2010). Repeated ultrasound measurements and check-ups are necessary to minimize the margin for errors and since abrupt foetal movements can also cause the whirling up of cellular material causing a high density of vernix without clinical implications (Macpherson and Bailey 2008).

**Endocrine profiles associated with placentitis**

In the last trimester of pregnancy, the foeto-placental unit has an important role in the endocrine regulation of pregnancy and parturition. Endocrine profiles in the late term pregnant- and peri-parturient mare, are sequel of a delicate interplay of changing hormone levels and interactions that must coincide all in a timely and concerted manner to ensure a timely smooth birth of a viable foal. Any condition that affects the functionality of the foetus or the placenta will also alter the endocrine production, thus possibly disrupting this interaction. As such the foeto-placental functionality can be monitored by measuring progestins and/or oestrogens (Morris et al. 2007). However, while the disease process can alter the endocrine pathways and stimulate inflammatory and immune responses, only post
factum will these alterations be usable for diagnosis (Rossdale et al. 1991; Santschi et al. 1991; Ousey et al. 2005; LeBlanc 2010).

When using the measurements of progestins, it is advised to evaluate at least 3 consecutive samples obtained at 2 to 3 days interval (Morris et al. 2007). Different abnormalities in progestin patterns have been described (LeBlanc 2010). A premature rapid decline is observed in acute conditions with imminent foetal expulsion or in case of a dead foetus (Ousey 2006). An early rise in progestin serum concentrations is associated in placental pathology or foetal stress (Houghton et al. 1991; Rossdale et al. 1992; Ousey et al. 2005; Morris et al. 2007). Should these elevated progestin concentrations be maintained two to three weeks prior to 310 days of gestation, a certain degree of foetal hypothalamo-pituitary adrenal activation can be assumed and resulting foals are more likely to survive compared to those who didn’t have this activation (LeBlanc 2010). A last abnormality in progestin pattern is the failure to exhibit the normal prepartum rise in progestin as seen almost exclusively in mares after ergopeptine alkaloid exposure (fescue toxicosis) (Brendemuehl et al. 1995).

In the mare, maternal progestin concentrations are low until 3 weeks before parturition when they start to rise with a subsequent abrupt decrease 24h before parturition (Ousey et al. 2003). This rise is associated with the onset mammary electrolyte secretion and the fall in concentration is simultaneous with the rise in foetal cortisol levels (LeBlanc 2010). The alterations in electrolyte concentrations in mammary secretions can be used to predict foetal readiness in the normal, a term, mare, in preterm mares it can be an indicator of impending abortion (Ousey et al. 1984, Rossdale et al. 1991).

In most commercial progestin assays (RID and ELIZA) progestin cross reacts with progesterone and as such can be used to assess progestin concentration in the mare. Normal values between 2 and 12 ng/ml until the last 3 weeks are measured (Ousey et al 2005, Morris et al. 2007).

In a clinical setting mares could be monitored by sampling 3 times a week, or 4 samples with 48 h interval in the acute phase and biweekly to assess the effectiveness of therapy thereafter (Morris et al. 2007, Macpherson and Bailey 2008).
Oestradiol is sometimes used as a marker for foeto-placental compromise (LeBlanc 1997), and concentrations above 1000ng/ml between 150 days and 280 days are assumed to be normal while concentrations less than 500ng/ml have been associated with severely compromised or dead foals (Douglas et al. In LeBlanc 2010).

Relaxin serum levels are high at end of gestation and increase during labour, however relaxin concentrations vary and are different in between breeds and are of limited value as a placentitis marker in the mare (Klonisch et al. 1997; Ryan et al. 2009).

Prostaglandins are released locally and are rapidly metabolized and can’t be used for diagnosis (LeBlanc 2010).

Other (serum) markers to diagnoses placentitis are far from specific. Acute phase proteins (Haptoglobulin and Serum Amyloid A) will rise as a response to the infection, with SAA knowing a marked increase. Fibrinogen and white blood cell count did not alter post inoculation (Canisso et al. 2014). The finding that SAA increases in response to placentitis will give us new insights in the disease process since this increased concentration of SAA can only be explained by a contribution of the endometrium to this rise or, although always regarded as a local process, a systemic involvement in the pathogenesis of placentitis (Canisso et al. 2014).

Treatment

The underlying idea of treating the condition should be reducing the spread of the germs, eliminate the inflammation and thus prevent uterine contractions and preterm delivery of the foetus (LeBlanc 2010). The consideration to induce the parturition in order to obtain better chances for the foal’s survival is not an option (Jeffcott and Rossdale 1977; Leadon et al. 1982, 1986; Rossdale and Silver 1982). The foal and its chances of survival will benefit of any delay of preterm parturition. When a premature expulsion can be avoided, the maturation of the foal in a chronic stress situation is accelerated, which increases its chances of survival in a significant degree (Rossdale et al. 1991; LeBlanc et al. 2004; Bailey et al. 2007; Christiansen et al. 2009). The artificial acceleration of foal maturation with the aid of cortisone (at a dose rate of 100mg q 24h for 3 consecutive days) is sometimes considered although is not without risks (Rossdale et al 1992; Ousey et al 2006).
Most common used antimicrobial to treat placentitis is Trimetoprim sulfamethoxazole (TPS), with a good oral bioavailability in horses (Zent 1999) although activity might be reduced in presence of pus and some strains of Streptococci are resistant (Peyrou et al. 2003). Therapies with a combination of TPS en pentoxifylline (Pf) prolonged gestation but did not prevent stillbirth (Graczyk 2006).

Also penicillin and gentamycin have been used and concentrations in allantoic fluid reached about 80% of sero-concentrations, where TPS and Pf reach serum concentrations in the allantoic fluids (Murchie et al. 2006; Rebello et al. 2006). In affected mares gentamycin concentration are reduced below MIC of Gramm negative bacteriae whilst the clearance of penicillin G from the allantoic cavity is delayed resulting in possibly toxic concentrations to the foetus (Murchie et al. 2006). Specific posology and doses of therapeutic in case of placentitis are reviewed in LeBlanc et al 2010 and summarised in the handouts.

The administration of pentoxifylline (Pf) is based on its anti-inflammatory properties by reducing the amount of cytokines (TNFα and IL-1) (Lauterbach et al. 1996, Baskett et al. 1997). Although presumed previously, even a double dose of Pf ( a ratio of 17mg/kg BID) did not alter the artery blood flow (Bailey et al. 2012). In earlier trials, when given for a prolonged period of time, Pf did increase uterine blood flow and as such ameliorated oxygen transport (Bacher et al. 1997, Bacher et al. 2005) and, by alteration of flow characteristics of equine erythrocytes in vitro (Weis et al. 1994) might help in bacterial clearance and to impede colonisation as shown in rabbits (Heller et al. 1999). Normal therapies will use a dose of 8-10 mg/kg q 12h and maximal concentrations will be reached about 1 hour post administration (Liska et al. 2006).

Besides antimicrobials and anti-inflammatory drugs, several drugs with an activity to impede any preterm contraction of the uterus (β-sympathomimetic, prostaglandin synthesis inhibitors, calcium channel blockers, oxytocin antagonists) have been tested in woman (Lamont 2005). Although neither of these were able to prolong the pregnancy or improve the prognosis when used alone. In mares, the anti-prostaglandin effect of progesterone (analogues) could prevent prostaglandin induced abortion in most cases (Daels et al. 1996). Apparently, the upregulation of oxytocine and prostaglandin receptors is inhibited by the progesterone administration and without these receptors and the gap formation, uterine contraction is prevented (Garfield 1980). Commonly a dose of altrenogest of 0.088mg/kg bwt q 24h per os, is used.
When using β-sympathomimetica as clenbuterol in the mare, an uterine relaxation will be seen within minutes and for up to 2 hours (Card and Wood 1995) however, as clinical therapy no significant difference in gestation length was seen (Palmer et al. 2002). Moreover treated mares tended to foal earlier than controls as clenbuterol might have stimulated cervical relaxation and subsequent parturition and has as such limited use in placentitis in mares (Palmer et al. 2002).

The length of placentitis therapy (antibiotics, NSAID’s, altrenogest) is still under debate. Cures of 10-14 days of antibiotics with 7 days NSAID’s have been advised while others do treat until delivery of the foal (Zent et al. 1999, LeBlanc 2010). Prolonged antibiotic and NSAID therapy may result in gastro intestinal side effects and antibiotic resistance (LeBlanc 2009), and a protracted progestin therapy up to time of delivery might lead to a prolonged stage 2 and subsequential neonatal complications (Neuhauser et al. 2008). That is why it is advisable to stop progesterone therapy at 320 days of gestation (LeBlanc 2010). To add some more duality in this discussion, in most placentitis mares, even after a prolonged therapy, uterine swabs post foaling will still show bacterial growth (Bailey et al. 2010). Moreover long-lasting therapies can result in retention of dead foetuses, metritis and laminitis.

So, an early and aggressive therapy is necessary until clinical signs disappear (Rebello et al. 2006, Christiansen et al. 2009, LeBlanc 2009) whilst regularly checking the effect of the therapy and the viability of the foal is essential (Macpherson and Bailey 2008, LeBlanc 2010). Post-partum, mares that suffered placentitis should be checked for endometrial infection as soon as possible to select an appropriate therapy. When delayed, samples will always reveal a mixed infection (Macpherson and Bailey 2008, Bailey et al. 2010). In most cases; 3 consecutive days of uterine flushes in combination of NSAID’s and a week of antimicrobial therapy will clear the infected post-partum uterus.

Logically, after stage 3 a thorough examination of the placenta is essential. Bacterial sampling will not be of any use but a visual check-up for gross lesions (especially at the caudal chorionic part and cervical star region) and histological examination of the region of interest can help to diagnose placentitis (Mays et al. 2002).
Prognosis

As described above the mare’s health status is not affected unless severe complications occur. The prognosis for the foetus is acceptable when an early, aggressive therapy could be initiated although in a first line approach this is mostly difficult due to its insidious course and discrete signs (Bailey et al. 2010). Anyway, longer intervals from parturition to subsequent pregnancy (with on average 1 month extra) have been noted in affected mares compared to normal Thoroughbred mares (Hughes et al. 2014). Foals born from affected mares should be checked meticulously for signs of pre-or dysmaturity and septicaemia. The description of the whole scale of controls and therapeutic considerations of the neonate are beyond the scope of this paper. In anyway a foal serum cortisol level on top of the blood analysis with specific attention to the neutrophil/lymphocyte ratio will be part of the first screening. Until certainty and in the presence of suspected signs (clinical signs during pregnancy and or placental gross lesions) foals should be supervised closely, besides a strict control of colostrum intake and of essential viability parameters, and an antibiotic therapy should be started until deemed unnecessary.

Surviving foals might need expensive intensive care, will possibly never reach expected athletic performances and this is a consideration that should be communicated with the owner when initiating a therapy even during foetal life (LeBlanc 2010). In anyway, pre- and postnatal (epigenetic) conditions will determine the possible athletic capacities of the offspring in its later life (Axon et al. 1999, Hughes et al. 2014). In the US, costs for clinical care of foals born out of such compromised gestations have been estimated between 2 and over 10 000$ (Barr 2005). When Hughes et al. (2014) couldn’t see any differences in their retrospective study when looking at performances of yearlings and two-years born out of a compromised pregnancies versus controls born after an uneventful pregnancy, one should know that pre-test criteria included especially subclinical cases of placentitis and only surviving foals were taken into account.

Finally, besides mares that show clinical signs of ascending placentitis, also mares that suffered from placentitis in a previous pregnancy should be monitored from the 7th month of gestation on. Since the pathogenesis mostly involves an aberrant functionality of the physical barriers guarding the sterility in utero, when unsolved (or -solvable) they can resurge the infection-inflammation in any of the subsequent pregnancies.

(list of referred literature can be obtained upon simple request)