ASSESSMENT OF FETAL HEALTH IN THE PREGNANT MARE

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Introduction

Assessing the health of the fetus forms an essential component of the critical care of the high risk pregnant mare. Although fetal health assessments are not performed routinely in late gestation, this practice is becoming more prevalent in mares that have a history of recurrent abortion or those considered a high risk for fetal loss. The techniques available to assess fetal health have improved over recent years together with a greater understanding of mare and foal/fetal physiology which has lead to improved therapies and a reduction in premature delivery / abortion rates, particularly for mares with placentitis.

Incidence and causes of fetal abortion

The incidence of early embryonic losses (<Day 35) are more frequent than fetal losses in later pregnancy (7 % versus 2.5 %, Allen et al 2007). However early losses may be less significant because the option exists to re-mate the mare whereas in later pregnancy, after formation of the endometrial cups and secretion of equine chorionic gonadotrophin, rebreeding the mare is difficult and she usually will remain barren for that year. Abortion rates increase between 3 and 10 months of pregnancy while unexplained intrapartum death or stillbirth are significant (13.7%) causes of fetal abortions in late gestation (Ricketts et al 2001; Smith et al 2003). These losses are traumatic for all concerned and have a significant economic impact for the owner and horse breeding industry as a whole. Reasons for late gestation losses vary between different geographical regions. In the UK the major causes are umbilical cord compromise (torsions, vascular lesions, long cords) and placental compromise (placentitis, placental separation, placental abnormalities) whereas in the USA the major cause of late gestation abortions are feto-placental infections (Giles et al 1993; Smith et al 2003). Infectious diseases such as equine herpes virus always present a high risk situation in intensively managed horse populations while emerging diseases over the last decade, for example, mare reproductive loss syndrome (MRLS), equine amnionitis and fetal losses (EAFL), and fescue grass toxicosis, are all associated with environmental factors and have had devastating consequences on the horse industry in the affected areas (Sebastian et al 2008).

Diagnosis of fetal compromise

The first step towards preventing fetal abortion is identification and diagnosis of fetal compromise in the late pregnant mare. This in itself presents a significant veterinary and technical challenge. In many cases of fetal compromise, the mare does not display

clinical signs and often the first indication of a problem is when the mare aborts. This is particularly true when there are umbilical cord lesions. In mares with placental abnormalities, due to the chronic nature of this disease, clinical signs may develop and typically include premature lactation and vaginal discharge. Indirect monitoring of fetal health primarily relies upon measurement of maternal hormone concentrations (oestrone sulphate, progestagens) or mammary secretion electrolyte concentrations. Before the 1990's, direct monitoring of the fetus was limited to fetal electrocardiograms performed over short (5-10 min) periods using hand held electrodes which provided a crude measure of fetal viability. With the advent of ultrasound scanning and technical improvements to transducers to allow greater (>20 cm) penetration of ultrasound waves, it is now possible to perform transabdominal scans on mares during late pregnancy and formulate biophysical profiles which correlate with fetal health. In the last 5 years or so, Doppler ultrasound technology has been applied to equine reproduction and provides an additional, essential tool for fetal health exams. A brief review of these diagnostic techniques will be presented, together with a more detailed description of Doppler technology including recent research results.

Maternal indicators of fetal health

1. Hormones

The feto-placental unit produces high concentrations of hormones during the second and third trimesters of pregnancy. These are critical for maintenance of the pregnancy and, therefore, provide a measure of feto-placental health. The concept of the feto-placental unit is based on the fact that the fetus synthesizes precursors for the production of oestrogens and progestagens from the fetal gonads and adrenal glands respectively; these substrates are then transported to the placenta and uterus where they are synthesized into the final hormone products (Fowden et al 2008). These are transported across the placenta into the mare's circulation for further metabolism. Local production of progestagens and oestrogens is important because they control many physiological processes within the utero-placental tissues. Progesterone maintains quiescence of the uterus during pregnancy by inhibiting the action of prostaglandins which cause muscular contractions. Oestrogens are important for uterine blood flow during pregnancy and promote the action of contractile associated proteins at parturition.

One of the most abundant oestrogens is oestrone sulphate (E1S) which is found in the fetal fluids, maternal and fetal plasma, maternal urine and milk. Measurement of E1S in mares' plasma or urine provides a simple test to confirm pregnancy from 80 days onwards. High E1S levels (> 100 ng/ml) indicate the presence of a viable fetus while low levels (< 10 ng/ml) indicate pregnancy loss or a barren mare. Attempts to predict fetal demise using plasma E1S tests have largely proved unsuccessful. Frequent blood sampling of mares induced to abort with prostaglandin between 90 and 150 days of pregnancy indicated that E1S levels only declined within 5 h of abortion (Daels et al 1995).

Progestagens, which is the collective term for progesterone and several related compounds, have proved more useful in predicting fetal health. This is because they are produced by the adrenal glands and, therefore, also provide a maker of fetal stress.

Cortisol, the main stress hormone, normally is not produced by the equine fetus until close to parturition (Fowden & Silver 1995). In chronic diseases particularly those involving the placenta, for example placentitis, maternal progestagen concentrations rise precociously associated with disease (Rossdale et al 1991). In general rising levels indicate fetal stress whilst rapidly declining or low levels indicate fetal death or imminent abortion (Ousey 2006; Morris et al 2007).

Relaxin is also produced by the equine placenta in high concentrations during pregnancy. Declining relaxin concentrations have been observed in pregnant mares with a range of diseases, and it may help to indicate fetal demise (Ryan et al 2009). Unfortunately specific assays for equine relaxin are not widely available and further work is needed to establish its role in the pregnant mare.

2. Mammary secretion (MS) electrolyte concentrations

All mammalian species studied show a similar pattern of MS electrotype changes before the onset of full lactation. MS concentrations of sodium and chloride decrease while potassium and calcium increase (Peaker et al 1979). This pattern has been observed in mares before parturition and has been used extensively to predict the timing of delivery (Ousey et al 1984). Primiparous (maiden) mares may be the exception because it is their first lactation and MS electrolyte changes are not always consistent. Mares that develop premature udder development before 300 days of gestation usually in response to fetal stress and/or placental abnormalities have aberrant electrolyte patterns (Rossdale et al 1991). These abnormal patterns indicate that a problem may exist but they cannot predict the timing of abortion because the MS electrolyte changes are inherently abnormal in these cases.

3. Fetal electrocardiogram (ECG) monitoring

Measurement of fetal ECG can be performed either via electrodes attached to the mare's abdomen with a receiver unit to detect the fetal (and maternal) cardiac electrical signals, or during a transabdominal ultrasound scan using a transducer with M-mode which measures fetal heart rate (FHR). With the advent of telemetric systems, stronger and smaller receiver units and advanced computer filtering systems, fetal ECG may be detected easily over long periods (days) in the unrestrained mare (Nagel et al 2010). However, the ultrasound method has the advantage that changes in fetal heart rate can be correlated with fetal behaviours. FHR normally declines gradually with increasing gestational age but accelerations (20-40 beats per minute increments) occur with fetal activity (Colles et al 1978; Bucca et al 2005). In healthy pregnancies, fetal activity is a frequent occurrence (every hour) but dormant periods increase towards term due to restriction of uterine space. Abnormal fetal heart rate patterns are observed during fetal compromise (Reef et al 1996). For example, sustained high FHR often indicate fetal distress while sustained low FHR indicate fetal hypoxia; also fetal arrhythmias are associated with poor fetal outcome. In human obstetrics, analysis of the fetal electrocardiographic waveform (the QRS and T complex) by computerized programmes, form a useful adjunct to conventional ECG analysis and also can identify fetal distress in the infant (Rosen et al 2004).

Direct monitoring of feto-placental health

1. Fetal and placental ultrasound assessment

Fetal ultrasonography reliably assesses fetal growth, presentation, activity and mobility throughout gestation. It also provides a qualitative assessment of fetal fluids, placental and cervical integrity and a quantitative measure of the combined thickness of the utero-placental unit (CTUP) at the cervix. Measurements of the fetal aortic diameter and eye orbit diameters provide a reliable measure of fetal growth (Renaudin et al 2000). Biparietal (head) diameter may provide a more crucial measure of intra-uterine growth retardation by identifying asymmetric fetal growth ie reduced organ / body growth with normal brain growth, termed the brain sparing effect. Ultrasonography may be used to measure increased (hydramniois and hydroallantois), or reduced (oligohyramnios), fetal fluid volumes, conditions which are relatively rare in the mare but are invariably associated with fetal demise. CTUP is generally increased in mares with placentitis or placental oedema and is used as a critical diagnostic indicator but some studies have reported no such effect in mares with placentitis (Troedsson 2001; Lof et al 2010). Areas of placental separation or thickening may be observed but only on the ventral abdominal surface. Other areas of the placenta may not be visualized reliably because they are too deep within the abdomen. Physical abnormalities of the cord may be identified although urachal and amniotic pouches are often present in apparently healthy pregnancies. The whole length of the umbilical cord cannot be observed after the first trimester and therefore some cord problems may be missed using linear ultrasonography alone.

2. Doppler assessment

Use of Doppler technology enables blood flow volume and direction to be assessed through a vessel and, therefore, provides a critical measure of blood supply to the uterus, placenta and vital organs of the fetus. Colour Doppler imaging provides information about the direction of blood flow whereas power Doppler reflects the strength of the Doppler signal. Doppler velocity signals displayed against time create a spectral waveform from which quantitative measures can be derived about the vascular perfusion of structures downstream from the measurement site (Dickey 1997). Thus Doppler recordings of the pregnant mare's uterine and umbilical arteries provide data about blood flow and resistance within the uterus and placenta, respectively. In human obstetrics, Doppler analysis provides a vital part of the health assessment of the fetus and scans are routinely performed at the end of the first trimester and later in pregnancy if indicated. Meta-analysis has demonstrated that increased resistance (to blood flow) in both the uterine and umbilical arteries during the second trimester in pregnant women correlates with fetal growth retardation, premature delivery and increased infant mortality (Papageorghiou et al 2004; Viero et al 2004). Blood flow volume and resistance have been measured by Doppler ultrasonography in the uterine arteries and umbilical arteries of healthy mares during pregnancy (Bollwein et al 2004; Ousey et al 2012). Blood flow increases particularly during the last trimester and correlates closely with growth of the fetus. There is an enormous increase in total uterine blood flow during pregnancy from approximately 150 ml/min at 14 days up to nearly 20,000 ml/min at term in Thoroughbred mares (Ousey et al 2012). The resistance index in the uterine arteries decreases during pregnancy, notably between 40 and 150 days, which coincides with formation and expansion of the placenta. Assessment of blood flow in the umbilical

arteries is more challenging due to the movement and coiled nature of these vessels and currently no data exists for blood flow volume; however, vascular resistance decreases between mid and late pregnancy (Bollwein et al 2004).

Because the technology is relatively new, there is little published data on blood flow parameters in mares with compromised pregnancies. My research investigated blood flow in older mares (> 15 years) with age related degenerative changes of their uterus (endometrosis). Previously Bracher et al (1997) showed that these older mares have poor placental development; Doppler examinations revealed they also had significantly lower uterine artery blood flow volume and proportionally lighter foals at birth compared with young, healthy mares (Ousey et al 2012). These data suggest that uterine perfusion may be compromised in older mares with poor placental development and this has adverse consequences on fetal growth. One clinical case of intra-uterine growth retardation in a Thoroughbred mare with prolonged pregnancy (foaled 386 days) demonstrated substantially reduced uterine blood flow measured with Doppler ultrasonography compared with healthy mares at term (340 days) (Ousey & McGladdery - unpublished observations). Therefore Doppler ultrasonography provides a useful measure of blood flow and resistance within the uterus and placenta and may help to identify growth retarded fetuses as a consequence of poor placental blood supply.

Conclusions and future research

The diagnostic tools described enable the veterinarian to form a better understanding of the degree of fetal compromise in high risk pregnancies. In acute situations, for example uterine torsion or colic, the endocrine parameters, ECG and linear ultrasound provide an immediate indication of fetal status and viability. For chronic diseases, for example placental or umbilical cord problems, linear and Doppler ultrasound provide important measures to predict long term outcomes. As a new technology, Doppler ultrasound can assess blood flow and vascular perfusion not only to the uterus and placenta but, in skilled hands, to vital fetal organs including the brain, liver and heart. Further research is needed to measure blood flow and other Doppler indices in mares with compromised pregnancies and correlate these parameters with the degree of fetal compromise, to predict fetal outcomes and to measure the direct effects of therapeutic interventions. A multi treatment approach to placental conditions, particularly placentitis, has already yielded some success with a reduction in mortality rates of affected fetuses (Bailey et al 2010). A number of novel treatment options need to be investigated. For example pentoxifylline (Trental) which promotes blood flow and blocks cytokines (Ousey et al 2010), immunomodulators (anti-inflammatory agents, glucocorticoids) which block cytokines and prostaglandin release and calcium channel blockers (Nifedipine) which inhibit uterine contractions.

References

Allen WR, Brown L, Wright M, Wilsher S. (2007) Reproductive efficiency of Thoroughbred Flatrace and National Hunt mares and stallions in England. *Equine vet. J*, 29, 338-345.

Bailey CS, MacPherson M, Pozor ML, Troedsson MHT, Benson S, Giguere S, Sanchez LC, LeBlanc MM, Vickroy TW. (2010) Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in mares with experimentally induced equine placentitis. *Theriogenol*ogy 74, 402-412.

Bollwein H, Weber F, Woschee I, Stolla R. (2004) Transrectal Doppler sonography of uterine and umbilical blood flow during pregnancy in mares. *Theriogenol*ogy 61, 499-509.

Bracher V, Mathias S, Allen WR. (1996) Influence of chronic endometritis (endometrosis) on placental development in the mare. *Equine vet. J*., 28, 180-188.

Bucca S, Fogarty U, Collins A, Small V. (2005) Assessment of feto-placental well-being in the mare from mid-gestation to term: transrectal and transabdominal ultrsonographic features. *Theriogenology* 64, 542-557.

Colles CM, Parkes RD, May CJ. (1978) Foetal electrocardiography in the mare. *Equine Vet J*., 10, 32-37.

Daels PF, Hussni M, Montavon SME, Stabenfeldt GH, Hughes JP, Odensvik K, Kindahl, H. (1995) Endogenous prostaglandin secretion during cloprostenol-induced abortion in mares. *Anim. Reprod. Sci.,* 40, 305-321.

Dickey RP. (1997) Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. *Human Reprod. Update*, 3, 467-503.

Fowden AL, Forhead AJ, Ousey JC. (2008) The endocrinology of equine parturition. *Exp. Clin. Endocrinol. Diabetes*, 116, 393-403.

Fowden AL, Silver M. (1995) Comparative development of the pituitary-adrenal axis in the fetal foal and lamb. *Reprod Dom Anim.* 30, 170-177.

Giles RC, Donahue JM, Hong CB, Turtle PA, Petrites-Murphy MB, Poonacha KB, Roberts AW, Tramontin RR, Smith B. Swerczek TW. (1993) Causes of abortion, stillbirth and perinatal death in horses: 3,527 cases (1986-1991). *JAVMA*, 203, 1170-1175.

Lof HK, Pires Neves A, Gregory JW, Winter GHZ, Garbade P, Wolf CA, Richter G, Jobim MIM, Gregory RM, Mattos, RC. (2010) Evaluation of the combined utero-placental thickness (CUPT) and of vulvar conformation as indicators of placentitis in pregnant mares. *Anim. Reprod. Sci*., 121S, S329-S330. *Abstract*.

Morris S, Kellemann AA, Stawicki RJ, Hansen PJ, Sheerin PC, Sheerin BR, Paccamonti DL, LeBlanc MM. (2007) Transrectal ultrasonography and plasma progestin profiles identities feto-placental compromise in mares with experimentally induced placentitis. *Theriogenol*ogy 67, 681-691.

Nagel C, Aurich J, Aurich C. (2010) Determination of heart rate and heart rate variability in the equine fetus by fetomaternal electrocardiography. *Theriogenology* 73, 973-983.

Ousey JC (2006) Hormone profiles and treatments in late pregnant mares. *Vet. Clin. N. Am. Equine Pract.*, 22, 727-747.

Ousey JC, Dudan, FE, Rossdale, PD. (1984) Preliminary studies of mammary secretions in the mare to assess foetal readiness for birth. *Equine vet. J*., 16, 259-263.

Ousey JC, Kolling M, Wright M, Willis D, Allen WR (2010) Effects of pentoxifylline on uterine blood flow, and placental and fetal development in young and aged mares with endometrosis. *Anim, Reprod. Sci*., 121S, S343-S344. *Abstract*.

Ousey JC, Kolling M, Newton R, Wright M, Allen WR (2012) Uterine haemodynamics in young and aged pregnant mares measured using Doppler ultrasonography. *Equine vet. J. Perinatol. Suppl*. In press.

Papageorghiou AT, Yu CK, Nicolaides KH. (2004) The role of uterine artery Doppler in predicting adverse pregnancy outcome. *Best Pract. Res. Clin. Obstet. Gynaecol.* 18, 383-396.

Peaker M, Rossdale, PD, Forsyth IA, Falk M. (1979) Changes in mammary development and the composition of secretion during late pregnancy in the mare. *J. Reprod. Fert. Suppl.* 27, 555-561.

Reef VB, Vaala WE, Worth LT, Sertich PL, Spencer PA. (1996) Ultrasonographic assessment of fetal well-being during late gestation: development of an equine biophysical profile. *Equine vet. J*., 28, 200-208.

Renaudin CD, Gillis CL, Tarantal AF, Coleman DA. (2000) Evaluation of equine fetal growth from day 100 of gestation to parturition by ultrasonography. *J. Reprod. Fert. Suppl*., 56, 651-660.

Ricketts SW, Barrelet A, Whitwell K. (2001) A review of the causes of abortion in UK mares and means of diagnosis used in an equine studfarm practice in Newmarket. *Pferdeheilkunde* 17, 589-592.

Rosen KG, Amer-Wahlin I, Luzietti R, Noren H. (2004) Fetal ECG waveform analysis. *Best Pract. Res. Clin. Obstet. Gynecol.*, 18, 485-514.

Rossdale, PD, Ousey, JC, Cottrill, CM, Chavatte, P, Allen, WR, McGladdery, AJ. (1991) Effects of placental pathology on maternal plasma progestagen and mammary secretion calcium concentrations and on neonatal adrenocortical function in the horse. *J. Reprod. Fert. Suppl.* 44, 579-590.

Ryan, PL, Christiansen, DL, Hopper, RM, Bagnell, CA, Vaala, WE, LeBlanc, MM. (2009) Evaluation of systemic relaxin blood profiles in horses as a means of assessing placental function in high-risk pregnancies and responsiveness to therapeutic strategies. *Ann. NY Acad. Sci*, 1160, 169-178.

Sebastian MM, Bernard WV, Riddle TW, Latimer CR, Fitzgerald TD, Harrison LR. (2008) Mare reproductive loss syndrome. *Vet Pathol*., 45, 710-722.

Smith, KC, Blunden, AS, Whitwell, KE, Dunn, KA, Wales, AD. (2003) A survey of equine abortion, stillbirth and neonatal death in the UK from 1988- 1997. *Equine vet. J*., 35, 496-501.

Troedsson MHT. (2001) Ultrasonographic evaluation of the equine placenta. *Pferdeheilkunde* 17, 583-588.

Viero, S, Chaddha, V, Alcazaleh, F, Simchen, MJ, Malik, A, Kelley, E, Windrim, R, Kingdom, JCP. (2004) Prognostic value of placental ultrasound in pregnancies complicated by absent end-diastolic flow velocity in the umbilical arteries. *Placenta* 25, 735-741.