How to Manage Early Embryonic Death

Tom A.E. Stout, VetMB, PhD

Author's address: Utrecht University, Department of Equine Sciences, Yalelaan 114, Utrecht, The Netherlands; e-mail: T.A.E.Stout@uu.nl. © 2012 AAEP.

1. Introduction

During the past 20 years, per-cycle and per-season pregnancy rates have improved markedly in well-managed horse farms. By contrast, early embryonic death (EED) has remained a significant cause of frustration and economic loss, with approximately 15% of pregnancies detected at day 15 after ovulation failing to survive to term.[1] Moreover, the majority (>60%) of these losses occur before day 42 after ovulation, a period when pregnancy maintenance is critically dependent on progesterone produced by the primary corpus luteum (CL), and when a number of essential developmental events take place, including maternal recognition of pregnancy, embryogenesis and initial organogenesis, disintegration of the blastocyst capsule, endometrial cup formation, and the onset of definitive (chorio-allantoic) placenta formation.[2] Despite its economic impact, surprisingly little is known about why EED occurs and whether, how and to what extent EED can be prevented. This is largely because EED is usually diagnosed retrospectively (ie, the conceptus has already disappeared or is obviously dying), when it is no longer possible to reliably establish the initiating cause. In addition, there has been a tendency to assume that the underlying problem is probably "progesterone insufficiency," more because this is a deficit that can be addressed pharmacologically than because there is any real evidence to suggest that inadequate maintenance of CL function is a common cause of EED. Recently, there have been attempts to determine factors other than progesterone insufficiency that cause or predispose to EED.

Causes of Early Embryonic Death

Factors contributing to EED can be broadly divided into embryonic abnormalities, inadequacy of the maternal environment and "external" factors. Indeed, it is increasingly clear that abnormalities of the embryo per se account for a significant proportion of EEDs. In some cases there may even be ultrasonographically detectable signs that conceptus development is not progressing normally, such as an abnormally small vesicle or failure to develop an embryo properly at the appropriate time.[3] In addition, it appears that a high percentage of equine embryos contain chromosomally abnormal cells, mostly in insignificant quantities but occasionally in proportions certain to compromise embryo viability.[4] Although little is known about the true impact of chromosomal abnormalities on fertility and EED in horses, it is likely that they contribute to the increased incidence of EED characteristic of older mares, even when oocyte or embryo transfer is used to negate uterine inadequacy,[5] and in mares inseminated "too long" after ovulation.[6] In addition, it has recently become apparent that repeated EED

NOTES

Orig. Op.	OPERATOR:	Session	PROOF:	PE's:	AA's:	COMMENTS	ARTNO:

can occur in mares that themselves carry a stable chromosomal abnormality (eg, a translocation) that has remained undiagnosed because it causes no other obvious clinical signs.[7] Similarly, it is likely that in some of the stallions associated with above-average rates of EED, the underlying problem is embryonic chromosome abnormalities arising either from karyotypic abnormalities of the stallion or from DNA damage or instability in his sperm.[8]

"Deficiencies of the maternal environment" is a broad concept encompassing diverse factors such as insufficient maternal progesterone; inadequate provision of nutrients from an aged, degenerate, or inadequately synchronized endometrium (eg, after embryo transfer); and infection/inflammation as a result of unresolved postbreeding endometritis, endometritis acquired subsequently (ie, ascending or by "reactivation of dormant micro-organisms") because, for example, of inadequate closure of the cervix, the vestibular-vaginal "sphincter," and/or the vulva. Uterine infection can also be induced during embryo transfer, either because the embryo was recovered from an infected mare or because of the accidental introduction of bacteria during transfer; the limited ability of the diestrous uterus to combat bacterial proliferation explains the relative ease with which contamination at this time results in an active infection. EED due to endometritis may present as the accumulation of uterine fluid or widespread and marked uterine edema, despite the presence of a conceptus and subsequent conceptus death will result either from direct infection of the embryo or via luteolysis due to prostaglandin F (PGF)$_{2\alpha}$ release from an inflamed endometrium.

Although it is tempting to assume that inadequate maternal progesterone is a major contributor to EED, there is little evidence to support this assumption.[2] Indeed, even when maternal recognition of pregnancy and maintenance of the CL do fail, the failure is often paired with abnormal conceptus development (eg, "small for dates" vesicle) such that it is impossible to determine cause and effect. Nevertheless, luteal failure certainly does occur and can, for example, be induced by PGF$_{2\alpha}$ release from organs other than the uterus, for example, in the case of systemic disease involving endotoxemia.[9] Moreover, it appears that the day 18 to day 35 pregnant mare is particularly vulnerable to luteolysis because the inhibition of endometrial PGF$_{2\alpha}$ release that underlies maternal recognition of pregnancy wanes after the cessation of conceptus mobility on day 17.[5] Indeed, various manipulations (eg, twin aspiration) and hormones (eg, oxytocin, estrogen, human chorionic gonadotropin [hCG]) have been shown to elicit endometrial PGF$_{2\alpha}$ secretion during the day 18 to day 35 period. Although this PGF$_{2\alpha}$ release rarely results in complete luteolysis, it is possible that there is a mare-specific minimum threshold for progesterone concentrations below which pregnancy may be endangered because, for example, the uterus is no longer able to provide the nutrients required for conceptus development. Age-related endometrial degeneration can also markedly affect the supply of nutrients to a conceptus, and although this classically leads to fetal compromise later in gestation, EED can result if endometrial degeneration is severe or if large lymphatic cysts impede conceptus migration or nutrient uptake. Finally, there are reports that stress in the form of pain, systemic disease, weaning, transport, changes in group structure, poor nutrition, or extremes of temperature can predispose to pregnancy loss.[10,11] Although the extent to which stress contributes to EED is not known, it is nevertheless prudent to minimize exposure to the potential stressors listed above during early pregnancy.

Treatment or Prevention of Embryonic Death

Many of the measures that can be taken to reduce the risk of EED are nonspecific and involve attention to good breeding management (eg, prevent postbreeding endometritis and correct anatomical defects predisposing to pneumovagina or urovagina), selection of mares (eg, retire mares with poor endometrium quality or a badly damaged cervix), minimizing stress, and minimizing the risk of introducing or spreading infectious disease among broodmares.

There are few pharmacological means of preventing EED, and not all of the underlying causes are amenable to treatment. Progestagen supplementation can avert EED when a mare shows clear signs of returning to estrus despite the presence of an apparently normal conceptus in her uterus, has a history of repeated EED associated with loss of the primary CL, is endotoxemic, or has undergone severe acute stress likely to compromise CL maintenance. In these cases, the logical approach is to supplement with a suitable progestagen (eg, altrenogest[a]) from as soon as CL failure is suspected or systemic disease is identified, or, in the case of repeated EED, from before day 6 after ovulation. When the incident takes place in early gestation, supplementation should continue until adequate maternal progesterone production is certain, that is, from some time after day 75 of gestation, by which time the placenta has assumed the role as major provider of the progestagens required to maintain pregnancy. Alternatively, progestagen supplementation can be discontinued from around day 45 if ultrasonographic examination demonstrates formation of equine chorionic gonadotropin (eCG)-induced accessory CLs or endogenous plasma progesterone concentrations exceed 4 ng/mL. Although progesterone supplementation clearly has a role in protecting pregnancies threatened by maternal progesterone deficiency or endotoxin- or inflammation-induced PGF$_{2\alpha}$ release, it is important to subsequently monitor the pregnancy for viability because many will fail despite the progestagen therapy (suggesting an alternative underlying cause),

and the progestagens will then prevent the mare returning to estrus.

An additional treatment that has been shown to improve pregnancy rates, presumably by preventing EED, is the administration of a single injection of 20 to 40 μg buserelin[b] between days 8 and 12 after ovulation. Although this treatment leads to a fairly consistent 5% to 10% increase in pregnancy rates,[12] it is not known how it exerts its effects, although it does not appear to be by boosting circulating progesterone concentrations or preventing luteolysis.[13] The utility of systemic antibiotics to counter suspected endometritis in early pregnant mares is less clear; the use of a 5-day course of broad-spectrum antibiotics to treat embryo transfer recipients that receive an embryo from an obviously infected donor (ie, very cloudy flush) seems to be a sensible precautionary measure. On the other hand, if an early pregnant mare shows ultrasonographic indications of endometritis (uterine free fluid or marked edema), antibiotics are generally ineffective at saving the conceptus and more likely to result in false hope and wasted time.

2. Conclusions

In summary, although EED is a significant cause of loss to the horse breeding industry, it is difficult to predict, may occur without any premonitory signs, and is, in many cases, not treatable or preventable. While some methods of reducing specific types of pregnancy loss are now commonplace (eg, early detection and resolution of twin pregnancy, maintaining a closed broodmare band, progesterone therapy in cases of suspected failure of pregnancy recognition/endotoxemia/severe stress), development of strategies to combat EED has been slowed by inadequate understanding of the underlying causes.

References and Footnotes

1. Allen WR, Brown L, Wright M, et al. Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in England. *Equine Vet J* 2007;39:438–445.
2. Allen WR. Luteal deficiency end embryo mortality in the mare. *Reprod Dom Anim* 2001;36:121–131.
3. Vanderwall D, Squires EL, Brinsko SP, et al. Diagnosis and management of abnormal embryonic development characterized by formation of an embryonic vesicle without an embryo in mares. *J Am Vet Med Assoc* 2000;217:58–63.
4. Rambags BPB, Krijtenburg PJ, Drie HF, et al. Numerical chromosomal abnormalities in equine embryos produced in vivo and in vitro. *Mol Reprod Dev* 2005;72:77–87.
5. Stout TAE. The early pregnancy. In: Samper JC, editor. *Equine Breeding Management and Artificial Insemination*. St Louis: WB Saunders; 2008:223–239.
6. Newcombe JR, Cuervo-Aranga J. The effect of time of insemination with fresh cooled transported semen and natural mating relative to ovulation on pregnancy and embryo loss rates in the mare. *Reprod Dom Anim* 2011;46:678–681.
7. Lear TL, Lundquist J, Zent WW, et al. Three autosomal chromosome translocations associated with repeated early embryonic loss (REEL) in the domestic horse (Equus caballus). *Cytogenet Genome Res* 2008;120:117–122.
8. Kenney RM, Kent MG, Garcia MC. The use of DNA index and karyotype analyses as adjuncts to the estimation of fertility in stallions. *J Reprod Fertil* 1991;(Suppl 44):69–75.
9. Daels PF, Stabenfeldt GH, Hughes JP, et al. Evaluation of progesterone deficiency as a cause of fetal death in mares with experimentally induced endotoxemia. *Am J Vet Res* 1991;52:282–228.
10. van Niekerk CH, Morgenthal JC. Fetal loss and the effect of stress on plasma progestagen levels in pregnant Thoroughbred mares. *J Reprod Fertil* 1982;(Suppl 32):453–457.
11. van Niekerk FE, van Niekerk CH. The effect of dietary protein on reproduction in the mare, VII: embryonic development, early embryonic death, foetal losses and their relationship with serum progestagen. *J S Afr Vet Assoc* 1998;69:150–155.
12. Newcombe JR, Martinez TA, Peters AR. The effect of the gonadotropin-releasing hormone analog, buserelin, on pregnancy rates in horse and pony mares. *Theriogenology* 2001;55:1619–1631.
13. Stout TAE, Tremoleda JL, Knaap J, et al. Mid-diestrus GnRH-analogue administration does not suppress the luteolytic mechanism in mares *Theriogenology* 2002;8:567–570.

Orig. Op.		OPERATOR:	Session	PROOF:	PE's:	AA's:	COMMENTS		ARTNO: