EARLY EMBRYONIC DEATH IN MARES

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(Original Print-1999)

Losing a pregnancy creates a significant economic loss each year to those involved in the breeding industry. Because of this, much attention has been spent on trying to understand why pregnancy loss occurs. The focus of this article will be on the loss of pregnancy within the first 50 days of gestation, otherwise known as early embryonic death or EED.

Many researchers have reported fertilization rates to be over 90% per cycle in reproductively sound mares and 81 to 92% in subfertile mares. The similarities change however shortly after fertilization. According to Ball and coworkers (1986, 1989), embryonic loss between fertilization and day 14 occurs approximately 9% of the time in young, reproductively sound mares and over 60% in aged, subfertile mares. For the first 40 to 50 days, rate of EED is approximately 20% in fertile mares and over 70% for mares considered to be subfertile. During the 1999 season at ERC, we had 8 cases where EED was diagnosed. In each case the mare was diagnosed pregnant by ultrasound exam at day 14. Mares were then followed with multiple ultrasound exams and embryonic death was seen to occur anywhere from day 16 to day 41.

In 1992, a comprehensive review by Ball outlined many factors that contribute to early embryonic loss. These factors were described as maternal, embryonic or external in origin. Maternal factors pertain to changes that occur within the mare that affect the pregnancy. Alterations in progesterone levels, the uterine and oviductal environment, maternal age and postpartum breeding have all been implicated with causing EED, either directly or indirectly.

Progesterone is a hormone produced by differentiated tissue within the ovary called the corpus luteum or CL. The presence of progesterone following ovulation is critical for maintaining a pregnancy. Although this hormone has many functions, its primary purpose is to help sustain a uterine environment that will promote embryonic development. Inadequate progesterone levels will induce abortion allowing the mare to come back into heat. Progesterone levels can be measured and monitored throughout a pregnancy to identify a problem. This will however only tell the practitioner that there is insufficient endogenous progesterone being produced, not that it's the actual cause for EED.

Systemic levels of progesterone can only be maintained if maternal recognition of pregnancy occurs. The embryo needs to signal to the mare that it is present within the uterus. If there is not adequate communication then prostaglandin-$F_{2\alpha}$, a hormone produced within the uterus, will be released resulting in regression of the CL and a decrease in progesterone production. The embryo helps to communicate by moving throughout the uterus continuously from day 6 to 16 of gestation. Although not fully understood, it is thought that the embryo and/or the uterus is releasing certain proteins that aid in identifying one another. In 1998, Crossett and associates found that the progesterone-dependent protein, lipocalin, does travel from the uterine lining to the yolk sac cavity of the developing embryo as early as day 16 or 17 of gestation. Estrogen produced from an early staged embryo has also been postulated to be involved with maternal recognition of pregnancy. According to Sharp and associates (1989), there appears to be a "critical deadline" between days 9 and 14 after ovulation in which recognition of the conceptus occurs. Any restriction or delay in movement during this time may not prevent the release of prostaglandins, thus creating a uterine environment that initiates early embryonic death.

Disruption in the uterine environment can bring death upon the embryo. Endometritis or an

inflammation to the uterine lining induced by infection almost always results in premature prostaglandin release, CL regression and embryonic loss. Many researchers, including Ball and associates in 1988, have induced endometritis and subsequent embryonic death by inoculating pregnant mare with infective agents such as pathogenic bacteria or yeast. Fibrous tissue formed around the uterine glands can also create inadequate conditions for sustaining a pregnancy. These periglandular fibrotic lesions limit the ability of the glands to function properly and aid in embryonic development. An early study by Kenney (1978) found that fibrosis is a common cause of embryonic death between 40 and 90 days of gestation. The presence of uterine lesions increases with the age of the mare.

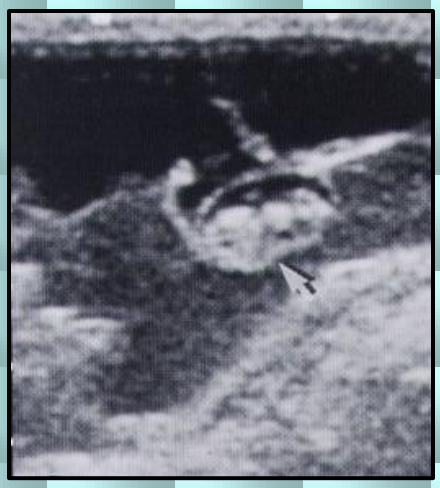
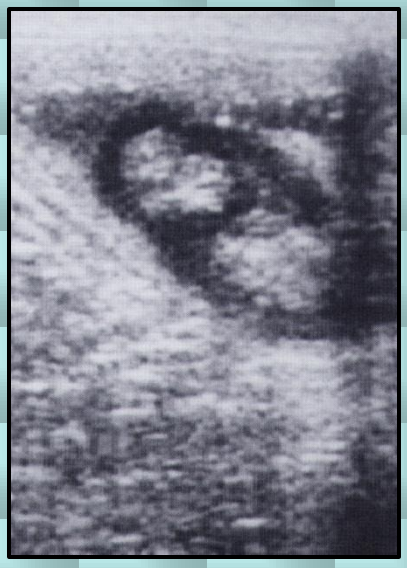


Figure 1 (Above). EED: Influx of uterine exudate.

Figure 2 (Right). EED: Resorption of fetal fluids.



Oviductal environment and embryo transport through the oviduct were implicated as being involved in EED. The time it takes for and equine embryo to travel through the oviduct is approximately 6 days. This period of time is thought to be necessary to assure that the uterine environment is favorable enough post breeding for the embryo to survive. Any alteration in oviductal transport could certainly affect embryonic development. In 1989, Ball did find that the uterus is capable of supporting embryonic development when embryos were transferred into a recipient as early as 4 days after ovulation. Although not examined directly, oviductal lesions may be a significant part of the environment that initiates early embryonic death. It is known that more oviductal lesions are found in aged mares. This coincides with an increased incidence of EED in older mares.

Increased EED rates have also been associated with postpartum or foal heat breedings. Under some circumstances the uterus becomes compromised in its ability to prepare for a new pregnancy. There may be a delay in uterine involution or a case of endometritis post-foaling. Delayed involution has also been suggested as a reason why a higher incidence of EED occurs in mares where the conceptus becomes fixed in the same uterine horn the previous pregnancy developed in. It may take longer for the tissue in the gravid horn to prepare for the next pregnancy.

Early embryonic death can be caused by one of many abnormalities from within the embryo as well. Retarded development of the embryo may result in missing the proverbial deadline for maternal recognition. In 1986 and 1987, Woods and coworkers found that embryos tended to be smaller and had a greater percentage of morphologic defects if they were recovered from subfertile mares rather than reproductively sound mares. The recoveries were performed before the embryo reached the uterus so uterine influence was not a factor. In addition, two other research groups discovered in 1989 that embryo survival rates following embryo transfer procedures were lower for embryos from subfertile mares. Aside from visible flaws, embryos destined for death may have insufficiencies in producing and/or releasing the estrogen and/or protein hormones thought to be involved with maternal recognition. Estrogen is produced by the conceptus as early as day 6-12. Decreased estrogen levels in the blood have been reported in mares that underwent early fetal loss. A recent study by researchers in Kentucky attempted to identify differences between day-12

and day-15 equine conceptuses with regard to gene expression of proteins produced during those stages of development. This is just one of many studies in the past few years that focused on identifying what embryos produce during early gestation that may or may not help to sustain their presence within the uterus.

One cannot disregard chromosomal and genetic abnormalities as playing a role in EED. Unfortunately, very little information is known specifically about the relationship between genetic defects in the equine embryo and EED. Many conclusions in this area have been drawn from studies of different species. Higher incidence of genetic abnormalities in cattle embryos have been reported when morphologic defects are present. Also, it is well documented that 50 to 60% of human abortions during the first trimester are a result of genetic flaws. Caution must be used when making judgments based on other species because there is a high degree of variation amongst species.

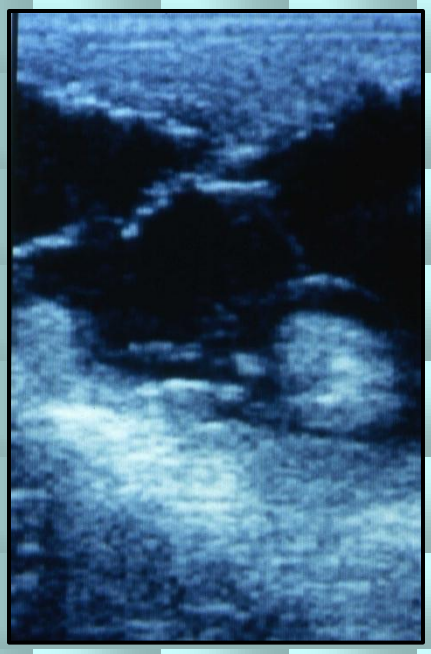


Figure 3. EED: Dissociation of fetal membranes.

Stress, season and environmental temperatures, and stallion influence have all been evaluated by researchers to determine what effect these external factors have on embryo development. Stress can be physical, nutritional or disease in nature. Clearly, physical stress such as transporting a mare during early pregnancy causes cortisol to be released which results in a decrease of progesterone blood levels. Less definitive is whether or not the changes in serum cortisol and progesterone concentrations are enough to increase embryo death rates. A few early studies did find that stressful situations, such as trailering, had a significant impact on early embryonic loss. In contrast, Baucus and associates (1990) showed no difference in loss of pregnancy when comparing mares transported 9 hours versus those not transported. Although many mares leave ERC during the early stages of pregnancy, to our knowledge there has been no pregnancy loss following transport. There is probably very little to worry about if the mare travels well, showing no overt signs of stress. Eventhough the data is conflicting on this matter, we at ERC will always recommend a conservative approach. If possible, wait until the mare is at least 50 days pregnant before transporting.

Nutritional status has long been thought of as having an influence on embryonic development. As early as 1965, studies reported increased EED rates in mares fed a poor diet. In more recent years, researchers have found a high correlation between diminished body condition and increased incidences of pregnancy loss. Henneke and associates (1984) found an increased loss of pregnancy between day 30 and 90 if the mare lost body condition 90 day before to 90 days after foaling.

Some evidence suggests that there are seasonal increases in EED in mares. Two studies reported a slightly higher incidence of EED in early spring compared to the summer months. Another researcher postulated that either early spring conditions or increased richness in pasture quality were responsible for dilating the cervix. As a result of cervical dilation, bacterial placentitis would occur ending in pregnancy loss. Exposure to elevated environmental temperatures has long been a concern but little is actually documented about the effects of prolonged exposure to the early pregnant mare. One cattle study has shown an increased number of degenerative 7-day embryos present if heifers were exposed to heat 30 hours after estrus.

As mentioned earlier, embryonic death can be a result of uterine infections. Complications from several diseases can use different pathways to have the same detrimental effect. Endotoxemia is a common byproduct of disease. A compromise to the intestinal wall allows for a release of

endotoxins into the bloodstream. The body responds by releasing prostaglandins, causing luteolysis and subsequent death to the embryo. Administration of flunixin meglumine (banamine) within 48 hours after the onset of endotoxemia can block prostaglandin release and prevent loss of the embryo. Any stallion may be the cause of early embryonic loss if he passed on a venereal disease. In addition, researchers have found in other species that the incidence of pregnancy loss rises if females are mated to subfertile males. To date, very little data has been published examining the role subfertile stallions have on EED.

Most cases of EED are noticeable by ultrasound examination. Detectable changes are: 1.) irregular shape to embryonic vesicle; 2.) embryo smaller than normal; 3.) loss of the embryonic heartbeat; 4.) visible presence of endometrial folds; 5.) continued mobility of the vesicle; 6.) fluid accumulation within the uterus; and 7.) vesicle dislodgment and fluid loss. Unfortunately there is no single method of prevention or treatment for EED since there are several causes for EED. Progesterone supplementation is probably the most common method of treatment since so many causes usually affect endogenous progesterone levels. Administration of Regumate, a progesterone analog, has been proven to maintain pregnancy in ovariectomized mares. For mares that habitually abort, progesterone therapy may also be beneficial. Administration can be started as early as 5 days post-ovulation and continued past 150 days. At approximately day 50 -70, the placenta also becomes a significant source of progesterone. Natural reduction of progesterone levels begins by days 120-150. Because of this, supplementation with Regumate can be slowly and safely weaned off at this time.

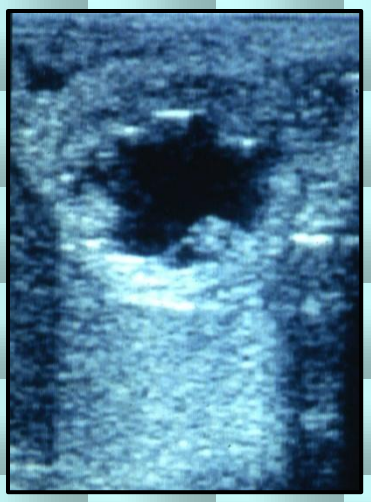


Figure 4. EED: Irregular border to the embryonic vesicle.

Another possibility for treatment is nonsteroidal anti-inflammatory drugs, such as flunixin meglumine (banamine). As mentioned earlier, banamine has been used in those cases where endotoxemia is suspected. The safety of using banamine long term in pregnant mares is unknown. Therefore, its use for this purpose has been limited in practice. Early embryonic death can be minimized by maintaining adequate nutritional care as well as preventing infectious disease and other environmental stresses.

When evaluating reproductive efficiency of the mare and financial loss to the breeding industry, EED must be examined further. A better understanding of this natural reduction mechanism may lead to a means of minimizing its effect on the industry.