Early embryonic death in mares

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Introduction

Early embryonic death (EED) is defined as loss of the conceptus before organogenesis is complete, generally regarded as before 40 days of gestation in the mare. Using ultrasonography, estimates of EED range from 5-23% between 12 to 50 days of gestation.[1-4] Whether critical periods exist during which the incidence of EED is highest has not been firmly established.[1] Overall, pregnancy losses are greatest early in gestation, before 35 days or even earlier before the embryo enters the uterus.[1,4,5] Estimates of the incidence of EED rely on the method used to diagnose pregnancy and the ability to detect EED.

Keywords: Early embryonic death, mare, infertility

Diagnosis

Although continued low progesterone is not compatible with pregnancy maintenance, a single low progesterone value does not necessarily indicate impending EED. Low progesterone concentration at more than one sampling is required before a diagnosis of EED or non-pregnant can be made.[6-8] Estrone sulfate is a product of a viable conceptus and, therefore, can be used as an indicator of pregnancy.[9] However, estrone sulfate is not a reliable indicator of fetal viability until at least 44 days of gestation,[10] and may not be useful as a valid indicator until after 80 to 90 days of gestation. Early pregnancy factor, based on the rosette inhibition test, has been proposed in a number of species as a means to diagnose pregnancy and therefore detect EED at a very early stage. Although shown to be unreliable for detection of pregnancy and non-pregnancy in cattle[11,12] and horses,[12] it has been suggested to be useful for monitoring the viability of equine embryos.[13]

When EED occurred between days 11-15 after ovulation, no ultrasonographic indication of impending EED was noted.[14] Later in gestation, however, indications of impending EED include an abnormal appearance of the conceptus, continued mobility of the vesicle, fluid surrounding the vesicle, absence of a heartbeat, decreased volume of conceptual fluids, disruption of conceptual membranes or edema of the endometrial folds.[14-16] (Figures 1 and 2)

Between days 20 and 45 of gestation, EED was first characterized by a speckled or granular appearance of the conceptus.[17] The conceptus then decreased in size and lost clear definition before disappearing completely within four to seven days of the onset of the granular appearance.[17] Doppler ultrasonography has been suggested as a tool to assess embryo viability or impending death.[16,18] In a normal pregnancy, blood flow is greater in the uterine arteries of the gravid horn. A relative decrease in flow in the arteries of the gravid horn compared to the nongravid horn was observed after the administration of exogenous prostaglandin F2a (PGF).[18]

Embryos that are smaller than normal may be lost at an increased rate compared to normal sized embryos, while oversized vesicles are not at risk.[19] Abnormal embryo development has been associated with the formation of trophoblastic vesicles that can be observed after day 22 of gestation that lack an embryo proper.[20] Potentially, these structures are formed after embryonic insult and death of the inner cell mass, the cells within the embryo that will form the embryo proper. (Figure 3) The trophoblast layer of the embryo continues to grow, resulting in a vesicle without development of the embryo proper. The vesicle may be small compared to a normal vesicle. Although the characteristic bulge associated with pregnancy may not be palpable, uterine and cervical tone are usually typical of a pregnant mare.

Diagnosis of EED by palpation and ultrasound is facilitated when the vesicle collapses and the contents spread out into the uterus. Although pregnancy loss can usually be detected by a loss of uterine tone and the palpable bulge, the bulge in the uterus may remain palpable for up to a week after conceptus death.[9] Cervical patency at the time of embryonic loss indicates that embryos are probably lost by passage through the cervix rather than by resorption.[21] If the mare is on supplemental progesterone, the progression of embryo loss may be changed, as the cervix will remain closed.

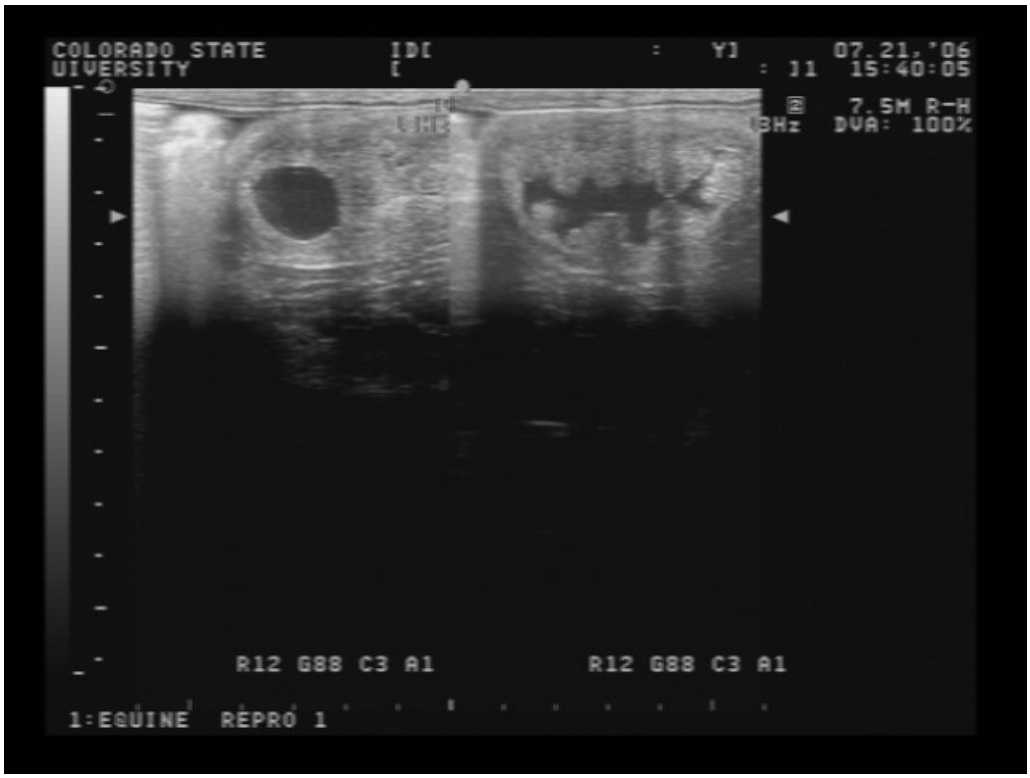

Figure 1. Embryonic vesicle in the left uterine horn (left image), with fluid within the lumen of the right uterine horn (right image). The fluid within the uterine lumen moved to surround the embryonic vesicle. In most cases, embryonic death will occur.

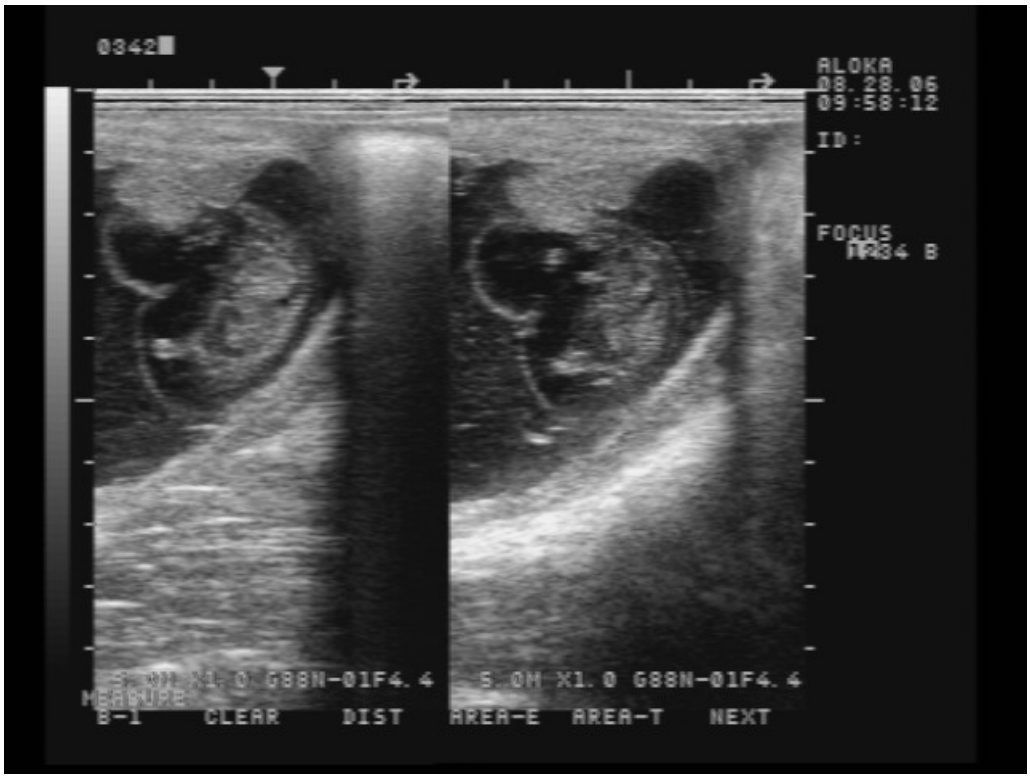

Figure 2. Aborting fetuses with disruption of membranes and granular debris. Heartbeats were not imaged in the thorax, and fetal features are less defined. After being lavaged from the uterus of a mare, the fetus and membranes were intact, but degenerative changes were occurring.

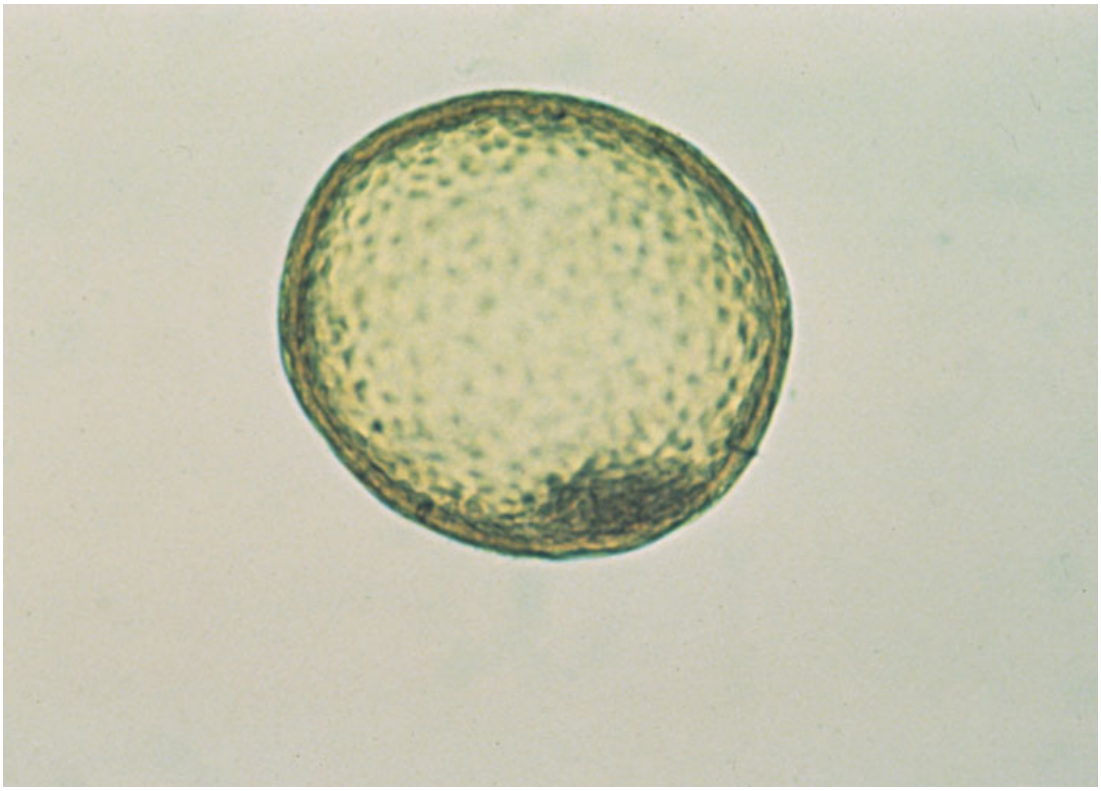

Figure 3. A 7-day embryo collected from the uterus of a mare. The embryo has a layer of trophoblast cells that will form the placental membranes. The thicker area of cells represents the inner cell mass, the cells responsible for formation of the embryo proper

Causes

Fertilization rates are apparently very high in horses,[22,23] although differences may exist between normal and subfertile mares.[22] One problem in the assessment of fertilization and EED is that the equine embryo remains in the oviduct for approximately 5.5 days before entry into the uterus,[24] and ovulatory or oviductal problems may affect early pregnancy rates. When recently ovulated oocytes or early embryos were recovered from the oviducts of mares, significantly fewer recently ovulated oocytes or embryos were recovered from old mares (≥ 20 years) than young mares (two to ten years), suggesting failure of ovulation or oviductal pickup of the oocyte. Therefore, fewer oocytes had the potential for fertilization and embryo development in the older mares, although fertilization rates were not different.[25]

Genetic abnormalities play a significant role in pregnancy loss in other species,[26] and similar findings can be expected in horses. Chromosomally abnormal cells have been identified in morphologically normal equine embryos[27] and an increased incidence of EED has been associated with certain family lines[28] or stallions.[29] The majority of chromosomal abnormalities leading to EED may not be inherited but arise during formation and ageing of gametes. These abnormalities may result in an inability of the resulting zygote to develop into a viable embryo.[30] Early embryonic death due to aged gametes may be increased when mating is not closely synchronized with ovulation.[30-32] Although insemination after ovulation can result in pregnancy, the incidence of EED is higher than when insemination occurs before ovulation[33] and increases as the interval from ovulation to insemination lengthens.[32]

Observable or unobservable defects in the embryo soon after its formation may alter or affect its metabolic function. Differential transport of unfertilized ova and embryos by the oviduct is apparently a function of the embryo rather than the oviduct.[23,34] Prostaglandin E2 (PGE), secreted by the equine embryo, is apparently responsible for instigating embryo transport.[35,36] Administration of exogenous PGE has reportedly hastened embryo transport.[34] Some degree of synchrony between the embryo and the uterus is critical for successful establishment of pregnancy. Therefore, pregnancy might not be established if an embryo was unable to produce sufficient quantities of PGE to induce

oviductal transport at the required time. Similarly, conditions resulting in increased concentrations of PGE may hasten transport resulting in early arrival of the embryo.

Substances produced by the embryo may be important for its continued survival after arrival in the uterus. Equine embryos produce estrogen which may be important in maternal recognition and maintenance of pregnancy.[37] Estrogen plays a role in the increased tone seen in the early pregnant mare which is influential in fixation of the embryo[38] and has a synergistic effect with progesterone to stimulate uterine specific proteins[39] which contribute to histotrophe.

In a retrospective study, embryo morphology was graded before transfer as excellent, good (minor morphological imperfections) or fair/poor (moderate or severe changes in morphology).[40] After transfer into recipient mares, pregnancy rates at 16 days were significantly higher for excellent and good embryos than for fair/poor embryos. However, although pregnancies were established at 16 days, the embryo loss rate from days 16 to 50 was higher for good than excellent embryos, and the embryo loss rate was similar for good and fair/poor embryos.[40] Results of the study demonstrate that relatively minor morphological defects in embryos may indicate reduced potential for normal embryo and fetal development.

Unrestricted conceptus mobility is essential for pregnancy maintenance and prevention of luteolysis.[41] If uterine contractions, the main propulsive force stimulating embryo movement,[14] are not sufficient to move the embryo throughout the uterus and inhibit luteolysis, EED may result. Uterine contractility is reduced in old versus young mares.[42] Removal of the endogenous progesterone source resulted in a reduction in uterine contractions and conceptus mobility and a failure of embryonic fixation.[21] The embryo was subsequently found more often in the uterine body,[21] a condition associated with increased EED.[43] It appears that prostaglandins also play a key role in embryonic mobility.[44] Inhibition of prostaglandins with flunixin meglumine results in a marked decrease in embryonic migration.[44] Experimental restriction of conceptus mobility has resulted in luteolysis followed by conceptus loss; however, administration of a progestogen was able to rescue the pregnancy.[41] Pathology of the uterus such as transluminal adhesions or endometrial cysts could restrict conceptus mobility sufficiently to mimic the experimental model resulting in EED.

A critical period in the establishment of pregnancy occurs at 14 to 16 days of gestation when the presence of the conceptus is needed to inhibit luteolysis.[41] Factors of conceptus origin inhibit the ability of the endometrium to release PGF between days 12 to 16 after ovulation and are essential for maintenance of luteal function.[45] If function of the corpus luteum is not maintained because the conceptus fails to block PGF release, either from inadequate migration or from retarded development of the conceptus, pregnancy loss will result.[41] (Figure 4)

A twin pregnancy rarely results in the birth of two healthy viable foals. If twins survive until late gestation, abortion, stillbirth or dystocia are likely outcomes. Fortunately, a natural reduction mechanism exists, provided both conceptuses are fixed in the same uterine horn, whereby one conceptus of a pair undergoes EED. The mechanism for embryonic reduction is hypothesized to be the result of deprivation of nutrients due to one conceptus preventing contact of the trilaminar omphalopleure of the other conceptus with the endometrium.[46]

Reproductive status of the mare may reflect maternal-embryonic interactions. Not surprisingly, pregnancy rates are lower and the incidence of EED higher for infertile mares than for normal mares.[47,48] Embryos were recovered at a reduced rate from subfertile mares,[49-52] although in one study, a majority of subfertile mares contributed a transferable embryo on at least one occasion after repeated embryo collection attempts.[53] Similar pregnancy rates have been found between subfertile and normal mares at two days after ovulation; however, the pregnancy rate at day 14 was reported to be greatly reduced in subfertile mares.[54] In general, subfertile mares tend to be older than fertile mares; therefore, some aspects of subfertility may be directly associated with aging.

In the past, fertility was considered to be primarily dependent on uterine environment. Pregnancy rates were similar for embryos from normal and subfertile mares when transferred into normal mares.[49] Moreover, initial pregnancy rates were similar after transfer of embryos to normal and subfertile recipient mares; however, subsequent EED was greater for subfertile recipients.[51] Recent evidence indicates other factors are involved. Transfer of embryos from normal mares to normal and subfertile recipients resulted in similar pregnancy rates at day 12 or 28, indicating that the uterus of a subfertile mare is capable of supporting an embryo from a normal mare.[55] Embryos collected from the oviducts of normal and subfertile donors four days after ovulation and transferred

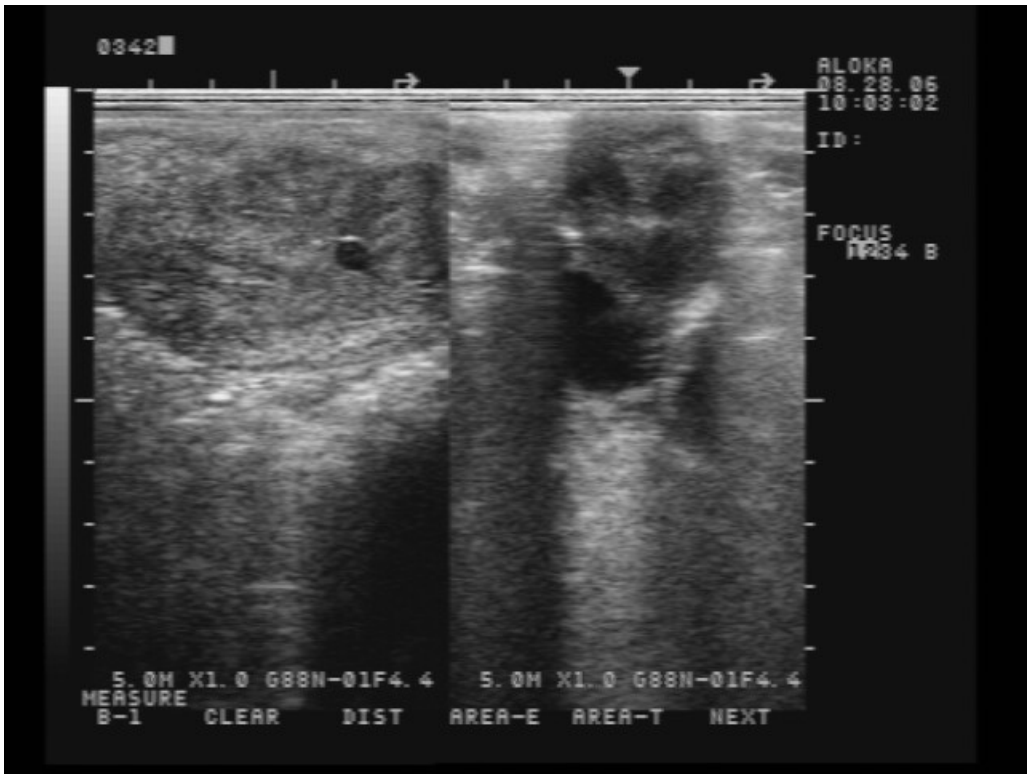

Figure 4. An embryonic vesicle at 11 days (left image) with a regressing CL on the right ovary. Unless exogenous progesterone is administered, the mare will return to estrus, and EED will occur.

to normal recipients resulted in lower pregnancy rates at day 14 for mares receiving embryos from subfertile donors than for mares receiving embryos from normal donors.[22] Other researchers have also found a lower pregnancy rate and an increased incidence of EED after transfer of embryos from subfertile mares when compared to those obtained from maiden mares.[52] In addition, abnormal embryos have been collected more often from subfertile mares than from maiden mares.[56]

Maternal aging has been shown to affect fertility and embryo loss in the mare. Extremes of age, either young or old, negatively affect fertility.[57,58] The high rate of EED in yearling mares has been attributed to immaturity, inadequate nutrition or physical stress.[57] Older mares were found to have a lower pregnancy rate and a higher loss rate compared with younger mares,[22,48,58] and a decrease in live foaling rate.[2,58-60] In a study of nearly 1400 mares in Newmarket, mares three to eight years of age experienced a 5% pregnancy loss between 15 and 35 days of gestation, while mares nine to 13 years of age had a 14% loss and mares 14 years of age or older showed a marked increase to approximately 22% EED.[3] In a controlled experiment, using semen from the same stallion and similar breeding management, the embryo loss rate between 12 and 39 days was 11% for young mares (five to seven years) and 62.5% for older mares (≥ 15 years),[42] suggesting differences in the ability of mares of different ages to establish and maintain a pregnancy. The effect of advanced age, however, is difficult to separate from acquired subfertility[61] and is confounded by the effect of parity since the two are generally correlated.[52] In one study, the incidence of embryonic resorption increased with parity.[2] The effect of age on fertility, at least in part, originates in the oocyte. When embryos were collected from the oviducts of young mares (two to nine years) and old mares (≥ 20 years) significantly fewer cells and poorer morphology were noted for embryos from old mares.[25] In a subsequent study, the researchers used oocyte transfer to compare fertility of oocytes collected from the follicles of young mares (six to ten years) or old mares (20 to 26 years).[62] Mature oocytes were transferred into the oviducts of young, inseminated recipients, and embryo development rates were determined. More oocytes from young than old mares (11/12, 92% and 8/26, 31%) resulted in embryonic vesicles. Results of the study indicate that intrinsic defects occur in the oocytes of old mares, and these defects may affect fertility and early embryo loss.

The repeatability of EED may depend on the inciting cause. Some authors indicate that mares which experience EED are more likely to again experience loss,[63] while others have failed to find

such an association.[48] Oviductal pathology may result in EED or fertilization failure. During post mortem examinations, salpingitis, which might affect embryo transport, oviductal secretions, and embryo support, was not uncommon.[64] Almost all oviducts were patent, although adhesions involving infundibula were common.[64] Uterine pathology may affect histotrophe production, which the early embryo relies on, and result in retarded embryonic development. Higher pregnancy rates have been reported when recipient mares had normal endometrium compared with mares that had moderate uterine pathology.[50] Endometrial cysts are common in older mares. Most mares over 17 years of age have some cysts, and mares over 11 years of age are four times as likely to have cysts as younger mares. The effect of cysts on fertility is unclear, because studies are often confounded by other factors such as age and parity. A study of nearly 300 Thoroughbred mares found that cysts did not affect the ability to establish or maintain pregnancy; however, the time of initial pregnancy examination was not controlled and embryonic losses occurring before pregnancy examinations were not detected.[65] The odds ratio of establishing pregnancy was suggestive of a negative effect.[65] Other studies have corroborated the quantitative effect of cysts on fertility. If endometrial cysts are present in sufficient number or size, a decrease in fertility results; however, the effect on fertility is less than that caused by delayed uterine clearance. Although endometrial cysts may interfere with maternal recognition of pregnancy, once pregnancy is established they do not increase the likelihood of pregnancy loss.[65]

Progesterone, normally supplied by the corpus luteum, is essential for the maintenance of pregnancy.[63,66,67] Evidence pointing to primary luteal insufficiency as a major cause of EED is lacking.[5,68] Secondary luteal insufficiency due to premature luteal demise as a result of uterine pathology is a more likely cause of EED.[8] Pregnancy loss after ovariectomy or PGF administration can be prevented by progestogen supplementation.[6,7,63,66] Progesterone dependent endometrial proteins have been demonstrated in the mare, and the lack of these proteins following luteolysis may be associated with a decrease in the quality of histotrophe, contributing to EED.[69] Supplemental progesterone, however, has not improved pregnancy rates in normal mares receiving embryos.[70]

Although PGF release from the uterus is normally responsible for lysis of the corpus luteum, other potential sources of PGF exist which can cause either complete luteolysis or decreased luteal function resulting in EED.[71] Endotoxemia or other febrile conditions can stimulate PGF release sufficient to cause decreased luteal activity precipitating EED.[71] Mares in early gestation, before endometrial cup formation, are at more risk of pregnancy loss. Although it is commonly believed that the developing corpus luteum is unaffected by PGF until five days after ovulation, studies have shown that some mares will respond as early as three days after ovulation.[72] Furthermore, Irvine, et al.[73] have shown that repeated low doses of PGF are very effective in inducing luteolysis. These studies would seem to indicate that inflammatory conditions, even soon after ovulation, warrant attention.

Prostaglandin inhibitors such as flunixin meglumine are effective in blocking PGF release, but must be administered soon after the onset of endotoxemia.[71,74] Delaying flunixin until two hours after endotoxin administration failed to prevent pregnancy loss.[74] Exogenous progestogens, however, can maintain pregnancy even after luteal activity is compromised,[71,74] but should be continued until placental progestins are capable of maintaining pregnancy.[75] Human chorionic gonadotropin, although generally regarded as luteotrophic, has been shown to induce luteolysis, resulting in EED when given between 24 and 39 days of gestation.[76]

Endometritis is an important cause of EED in mares[48] and usually causes loss before maternal recognition of pregnancy. Once pregnancy is established, endometritis is a less frequent cause of EED,[4] provided anatomical defects are corrected. Embryonic death may result either indirectly as a result of inflammatory mediated prostaglandin release affecting luteal function or embryo migration or from a direct embryotoxic effect of the inciting agent or inflammatory process. For example, altered intrauterine concentrations of PGE, which play an important part in oviductal transport of embryos,[34] has been associated with endometritis in mares.[77] Direct embryotoxic effects of pathogenic organisms have been reported,[56] as well as luteolysis secondary to induced endometritis.[15] An inflammatory reaction to sperm cells in the uterus is part of the normal physiologic response to mating. In normal mares, fluid and debris are easily evacuated from the uterus and the inflammatory response subsides relatively quickly. A delay in uterine clearance results in persistent mating-induced endometritis (PMIE) which will result in EED, either directly or through luteolysis.[78] Treatment of PMIE often includes administration of drugs such as oxytocin or cloprostenol, a PGF analog, to

augment uterine contractility and improve clearance. Oxytocin is generally regarded as safe when administered during the periovulatory period. Cloprostenol, on the other hand, has been shown to depress progesterone concentrations during mid-diestrus when given in the immediate post-ovulatory period.[79-82] Although the literature on the effect of post-ovulatory cloprostenol on pregnancy rates is equivocal,[79,81,82] it would seem advisable to err on the side of caution and restrict cloprostenol use to the pre-ovulatory period and use only oxytocin after ovulation.

Contagious equine metritis, a transmissible venereal disease caused by *Taylorella equigenitalis*, commonly results in EED after initial infection.[83] Clinical signs are confined to the mare and vary from acute endometritis to an inapparent carrier state.[83] The stallion can also maintain the organism as a carrier.[83] Control has been achieved by identifying carriers, improved hygiene during breeding, use of artificial insemination and surgical ablation of the clitoral sinuses.[83]

Mare reproductive loss syndrome is associated with both EED and abortion. Although the exact etiology is still unclear, reproductive losses have been linked to ingestion of eastern tent caterpillars, specifically the setae of the caterpillars.[84] Administration of West Nile virus vaccine to pregnant mares has been shown to be safe and is not associated with EED.[85]

Early embryonic death has been associated with conception occurring too soon after foaling.[59,86] Other studies have not substantiated these claims and have found that although pregnancy rates may be lower, no greater losses occur after foal heat breeding than after later breedings.[48] Examining factors associated with an increase in EED, McKinnon et al. reported that pregnancy rates are lower in mares bred on foal heat when intrauterine fluid was observed ultrasonographically.[87] A similar relationship was found between the presence of intrauterine fluid at foal heat and a greater risk of EED.[88] In a study examining recovery rate and embryo quality in mares bred on foal heat compared with mares bred at a later estrus, the authors concluded that EED was not the reason for lower pregnancy rates in mares bred on foal heat, and hypothesized that sperm transport or oviductal conditions were more likely to blame.[89] The effect of lactation on the incidence EED is likewise unclear. Lactating mares have been reported to have a lower,[59] a higher,[86] or the same[60] incidence of EED as nonlactating mares.

An increased incidence of pregnancy loss has been associated with nutritional stress.[90,91] Mares suffering from malnutrition experienced an increase in EED, which could be averted by supplemental feeding.[91] In a later study, the authors reported an association of poor quality protein in the ration with increased pregnancy losses, which they attributed to inadequate production of progesterone by the corpus luteum.[92] Restriction of dietary intake was reportedly successful in reducing twin pregnancies to singletons in 60% of treated mares.[93] Although reportedly beneficial in cows, nutritional supplementation with chelated or inorganic minerals failed to show an effect on conception, pregnancy rate, or EED in mares.[94] No adverse effects on reproduction have been observed in obese mares.[90,95]

When mares at 16 to 22 or 32 to 38 days of gestation were hauled for nine hours without food or water, progesterone and adrenocorticotropic hormone increased, indicating acute stress; however, pregnancy losses were found to be no different than in non-transported mares.[96,97] Stress due to pain, infectious disease or emotional disturbances such as weaning have been associated with decreased progesterone.[98] Administration of corticosteroid similarly has resulted in a drastic yet transient decrease in progestogen which is apparently not a result of luteolysis mediated by PGF.[98]

Repeated palpation or ultrasound examinations have not been associated with an increase in EED.[99,100] Long-term anabolic steroid treatment has untoward effects on subsequent reproductive performance. A trend towards higher EED was observed in mares treated during the previous year with anabolic steroids.[47]

Occasionally, there may be a need to induce EED. Choice of the best method depends on the stage of gestation.[101] Within five days of ovulation, no consistently effective method exists because the developing corpus hemorrhagicum is relatively unresponsive to the effects of PGF and because the embryo, still within the oviduct, is protected from the uterine environment. From five days after ovulation until the formation of the endometrial cups at approximately 35 to 38 days, administration of PGF is the preferred method to terminate pregnancy. Other methods such as manual crushing of the vesicle and uterine infusions, though effective, carry the possibility of untoward side effects.[101] After the formation of the endometrial cups, repeated injections of PGF are usually needed to induce EED. In the case of twins, manual crushing of one vesicle is the technique usually employed to

reduce the pregnancy to a singleton. Most other reported methods do not consistently achieve the same success.[101]

Prevention or treatment

Effective methods to reduce contamination at breeding and treat endometritis should be employed to allow establishment of pregnancy. Procedures such as Caslick's surgery are necessary if anatomic defects such as poor perineal conformation are present. Maintenance of good general health and nutrition will likely improve pregnancy rates and reduce EED. Failure to maintain pregnancy due to premature release of PGF attributable to inflammatory processes has been successfully treated with flunixin meglumine.[102] In the same report, repeated EED attributed to a failure of the normal process of maternal recognition of pregnancy to maintain luteal function was successfully prevented with altrenogest despite regression of the primary corpus luteum and low endogenous progesterone.[102]

Although nonsteroidal anti-inflammatory drugs can inhibit PGF release resulting from endotoxin infusion, administration must occur within a very short time of endotoxin release, limiting their usefulness under natural conditions.[71,74] Conceptus death may not occur until as long as 48 hours after luteolysis,[74] and embryos can apparently continue to grow for some time after loss of endogenous progesterone.[21] Therefore, sufficient time exists to institute progestogen therapy to rescue the pregnancy.[21,71,74]

Pregnancy has been maintained in mares lacking sufficient endogenous progesterone with the daily administration of progesterone in oil or altrenogest, but was subsequently lost if therapy was stopped before day 50 of gestation.[66] Results of ovariectomy after day 50 are variable with some mares able to maintain pregnancy without exogenous progestogen. To ensure pregnancy maintenance, exogenous progestogen therapy should be continued through the first four months of gestation, until the placenta can provide adequate progestogens for pregnancy support.

Some workers suggest that if the uterine environment is normal, progesterone therapy is not needed.[8] The difficulty lies in determining if the uterine environment is normal from the viewpoint of the conceptus. Administration of altrenogest on days six to 20 to supplement endogenous progesterone or buserelin on day ten to bolster endogenous progesterone production did not significantly affect progesterone concentrations or conceptus size.[103] If exogenous progestogen therapy is to be instituted, sufficient dosages should be used. Minimum dosages to maintain adequate circulating concentrations in plasma include daily administration of 200 to 300 mg progesterone in oil or 22 mg altrenogest.[7,104] Lower doses or less frequent administration of these products were insufficient to maintain pregnancy.[6,7] Aqueous forms of progesterone are cleared too rapidly to be of use for pregnancy maintenance.[7] A long-acting injectable formulation of progesterone administered on a weekly basis has been shown to be effective in maintaining pregnancy.[105] Pregnancy was not maintained in ovariectomized mares using a number of synthetic progestogens, including medroxyprogesterone, norgestomet and megestrol acetate; however, altrenogest at 0.044 mg/kg was able to maintain pregnancy.[106] Although 22 mg altrenogest is reportedly sufficient for maintaining pregnancy,[67] other workers suggest that higher doses are needed to provide optimal uterine and cervical tone and are preferred for pregnancy maintenance.[67,107] McKinnon, et al.[67] propose that if the progesterone concentration at 24 hours after exogenous progestin therapy is 2.5 ng/ml or more, exogenous therapy may be unnecessary.

Viability of a pregnancy should be reaffirmed when long-term progestogen therapy is practiced. Retention of a conceptus after fetal death may result from continued treatment.[108] Intensive management alone may accomplish the objective of getting a mare to carry a foal to term. In a report of six mares which had lost their pregnancies two to five times in the preceding six years, five had normal term pregnancies without any special treatment other than good management and being mated to a highly fertile stallion.[109]

Acknowledgement. Figures courtesy of Dr. Elaine Carnevale.

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