INTRODUCTION

To have a successful equine breeding program, producers must successfully manage animals both pre- and post-breeding to maximize the health of the mare and ensure the delivery of a healthy foal. The following information is designed to provide a basic understanding of how to identify pregnant mares, outline major events in pregnancy development, and identify some primary issues that can cause complications in pregnant mares.
PREGNANCY DIAGNOSIS

For most horse breeders, the timeliness and accuracy of pregnancy diagnosis is essential. Without additional equipment and skills, pregnancy diagnosis in the mare can be difficult. The most effective means of diagnosis are rectal palpation or transrectal ultrasonography. Other options for diagnosis include analysis of hormone concentrations and a return to estrus behavior. Relying on return to estrus, estrogen, progesterone, or equine chorionic gonadotropin (eCG) is not as effective or as accurate as ultrasound or palpation, as those indicators may be influenced by endocrine disruptions or show false positives in animals that have lost the pregnancy.

For rectal palpation, the examiner may be able to feel the cervix, which will be rigid and firm as if the mare was in diestrus (or under strong progesterone influence). Early in pregnancy (around 18 to 22 days) the uterus will have some tone and rigidity, but the conceptus pocket generally cannot be felt. A palpatator will identify uterine tone that is consistent with pregnancy but generally cannot palpate the vesicle. “Vesicle” is a term often used to describe the early developing embryo. The palpation of the ovaries is of little use in the mare as the corpus luteum (CL), the structure responsible for pregnancy maintenance in early gestation, is difficult to palpate with accuracy. The CL’s ability to maintain early pregnancy is reliant on its production of the hormone progesterone. Each time the reproductive tract is manipulated, which is done to a greater extent with palpation than with ultrasound, the pregnancy is placed at risk. This manipulation can stimulate an inflammatory response and the production of uterine prostaglandin. Prostaglandin can then act directly on the CL to cause its destruction, which would stop progesterone production and ultimately terminate the pregnancy.

In most cases, the use of ultrasound is the gold standard for pregnancy diagnosis in the mare. In addition to being more reliable than palpation, ultrasound can be performed with accuracy earlier post-ovulation, and it may provide additional insight into the pregnancy. The earliest that most technicians are willing to ultrasound with great accuracy is approximately 14 days after assumed ovulation or actual insemination. This allows some flexibility if ovulation occurred some time after insemination.

When ultrasounding for pregnancy, it is essential to scan the entire uterine body, uterine horns, and ovaries. Scanning the uterine body and horns will both allow identification of an embryonic vesicle (if one is present) and reveal any potential abnormalities such as cysts (Figures 1 and 2).

Figure 1. Ultrasound image of a uterine cyst (large, black cavity) identified during initiation of the breeding season. Note the symmetry of the cyst and placement within the uterine horn. There is also apparent edema in the uterine endometrium. It’s important to note that early pregnancies (around days 14 to 16) can have a similar appearance to the spherical cyst represented above.

Figure 2. An ultrasound scan that shows multiple cysts located within the uterine horn.
These abnormalities may closely resemble embryonic vesicles and subsequent ultrasound examinations may be necessary to track growth and/or changes in location. Ovaries should be scanned for presence of large CL if a vesicle is located. Identification of a second CL may warrant another look for twins, as this is too early for the presence of an accessory CL. The vesicle at this age may only be 13 to 18 millimeters in size. Size measurements of the vesicle may provide a more accurate conception date.

Never walk away from a mare on a day-14 positive pregnancy diagnosis without rechecking. If ultrasounds are readily available, weekly checks will help keep track of embryonic life and growth. By day 21 or 22, the embryonic mass should be visible and approximately 4 to 5 millimeters in length. The heartbeat should be evident by day 24 as a small flicker in the center of the embryonic mass (Figure 3). These early pregnancy checks allow for a more rapid return to the breeding program in a non-pregnant mare.

Gestation is the period of time that begins immediately following fertilization and ends when the foal is delivered. In general for the mare, this period of time ranges 320-370 days but averages approximately 338. Length of gestation may differ as a result of breed, season, sex of the foal, and body condition of the mare. Table 1 outlines critical steps in early conceptus development. Early conceptus development is often a focal point as this is the time when most pregnancies are lost. Understanding the events occurring in utero for that age embryo or fetus, may assist a producer in determining cause of the pregnancy loss. Figure 4 outlines the key hormones that are at work during gestation.

Early pregnancy diagnosis should be confirmed at 40 to 60 days, recognizing that the majority of losses will occur prior to 40 days in gestation. The 35-to-40-day window is also important because this is the timeframe in which the embryo becomes attached to the endometrial wall of the mare and endometrial cup formation occurs. After approximately 35 days in gestation, eCG can be found in the blood, and eCG is then responsible for continuing to maintain the existing CL. The presence of eCG is also the point at which accessory CLs may be formed. A positive pregnancy diagnosis at day 40 to 45 is generally a good final positive pregnancy diagnosis unless anything abnormal happens with the mare or if any abnormal behavior is noticed from the mare. A 60-day pregnancy check via ultrasound will allow the identification of distinguishable features such as the chest, stomach, head, and backbone. This is also the timeframe that allows for the determination of fetal sex. After approximately 70 days, the fetus is too large for easy imaging.

Figure 3. A 26-day-old “embryonic vesicle,” or embryo. A line separates the allantoic cavity (embryonic and extraembryonic tissues) from the yolk sac [source of early nutrition], which is now regressing. At this time, the heartbeat could also be visualized.

Figure 4. Key hormones responsible for pregnancy maintenance in the horse. Early in gestation, multiple developmental changes are necessary for the hormonal changes to maintain pregnancy. Sufficient estrogen produced by the placenta (with precursors for hormone production sourced primarily from the fetal gonads) is the primary maintenance hormone after approximately 150 to 180 days. Prior to this point, ovarian progesterone is responsible for pregnancy maintenance (Adapted from W.R. Allen).
UNDERSTANDING THE RISK FACTORS THAT COMPROMISE PREGNANCY SUCCESS

Changes in the developing conceptus as well as the mare’s internal and external environment can have a marked influence of the success of the pregnancy. To more accurately identify causes of death, there are three major classifications that follow with conceptus development. There are many methodologies used to determine the timeframe referred to as “embryonic” development versus that which is “fetal” development.

Most often, the conceptus is referred to as an embryo when the yolk sac is used as its nutritional source and the development of organs and external anatomical features unique to the horse are incomplete. The fetus stage is identified when a horse can be recognized by external anatomical form with complete organogenesis and the umbilical cord is formed with the conceptus, depending on a maternal nutrition source. Typically, this change occurs around the time when the placental portion of the conceptus embeds within the uterus of the dam, approximately 40 to 50 days into development. The three major classifications of conceptus death are:

EARLY EMBRYONIC DEATH
• A death that occurs prior to recognition of the pregnancy, around days 12 to 14. In this case, the mare will have a normal return to estrus. Without an ultrasound, this loss is the most difficult to recognize.

LATE EMBRYONIC DEATH
• A death that occurs after maternal recognition but before formation of endometrial cups, around day 40 of gestation. In this case, the mare will have a delayed return to estrus, and therefore, this loss is more recognizable to most producers.

Critical in Early Conceptus Development

Table 1. Developmental milestones in early fetal development of the horse.

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<th>Conceptus age</th>
<th>Developmental milestones</th>
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| 1 to 6 days   | • The conceptus is located in the oviduct.  
• The conceptus develops two cell types: the inner cell mass, which continues to develop into the embryo, and the trophoblast, which forms the fetal portion of the placenta. |
| 6 to 16 days  | • The conceptus moves into the uterus around day 6.  
• Days 12 to 14 are critical for recognition of the conceptus by the mare.  
• For this recognition, the embryo is moving! This 10-day window is spent with the embryo moving about the uterus prior to becoming stationary around day 16, a period referred to as “fixation.” |
| Technical note | After fixation, the embryo loses its spherical shape. This is an important point for ultrasound technicians. |
| 22 to 25 days | • Heartbeat is present.  
• Embryo begins to attach to the uterine wall. |
| 35 to 40 days | • Embryo is attached around days 36 to 38.  
• After attachment begins, the endometrial cup forms.  
• These cups produce equine Chorionic Gonadotropin (eCG).  
• eCG helps produce a second CL that will help to maintain pregnancy |
| Technical note | If a pregnancy is lost following day 40, the mare is typically not considered a candidate for rebreeding that year. |
| 80 days       | • Conceptus now fills both uterine horns. |
| 100 to 140 days | • Endometrial cups disappear. |
| 150 to 180 days | • The placenta takes over (fetal gonads serve as primary source of hormonal precursors) maintaining pregnancy.  
• The CLs regress and there is a rise in estrogen. |
| 180 days      | • Peak in fetal estrogen production. |
FETAL DEATH
• A death that occurs after the endometrial cup forms and the conceptus embeds within the dam’s uterus. Most of these losses can be identified if mares are observed frequently. These mares will not be candidates for rebreeding in the same season.

As the classifications of death changes, generally so do the reasons for the death. In most animals, as conceptus age increases, the likelihood of conceptus death decreases. Therefore, the embryonic period is most volatile for pregnancy survival.

REASONS FOR EMBRYONIC DEATH
An estimated 5-25 percent of all pregnancies are lost during the embryonic period.

**Breeding on Foal Heat**
• Embryonic death is more likely to occur in mares bred on foal heat if the mare has not had sufficient uterine involution or is battling a uterine infection.

• Set standards for breeding on foal heat, which include clearing of all infections and only breeding mares that had an easy, clean foaling.

**Stress**
• Stress can include abnormal exercise, nutrition, climate, palpation, or some of these in combination.

• Events such as transportation or changing of herdmates may prove ultimately too stressful for certain mares. Avoidance of these activities may prove most useful in those animals that are difficult to breed or have lost a pregnancy previously.

• Lactational stress may increase energy demands on a mare and decrease her ability to sustain pregnancy. This loss is of greater prevalence in older mares.

**Mare Hormonal Issues**
• Progesterone is the pregnancy maintenance hormone of the early gestation mare. Without sufficient quantities, the CL will be lysed and the embryo sloughed.

• Uterine infection may lead to a rise in circulating prostaglandin, which will ultimately cause destruction of the CL and cease circulating progesterone for pregnancy maintenance.

  • In both of the above conditions, supplemental progesterone may be administered or a GnRH injection can be administered some 10 to 12 days following breeding for formation of a secondary CL.

  • A uterine infection has other deleterious effects on the ability of the uterus to sustain a pregnancy which will not be overcome with additional endogenous or exogenous progesterone.

  • Lactation may alter circulating hormone concentrations, in particular progesterone. Higher rates of loss are associated with nursing than non-nursing mares.

**Endometritis (Uterine Infection)**
• Inability of the uterus to sustain a pregnancy and/or the premature lysing of the CL by an increase in circulating prostaglandin.

• If a mare has a uterine infection, it should be cleared prior to initiation of a breeding program. See potential hormonal consequences in the “mare hormonal issues” item above.

  • In addition to hormone changes, uterine infections may result in damage to the uterine lining and glandular layers responsible for nourishment of the early conceptus.
• There has been some success with uterine lavage early in pregnancy (when the embryo is still in the oviduct) if the infection is mating induced.

• Maximize sterility when breeding mares, whether naturally or artificially. This will reduce the incidence of introduction of new infections.

**Embryonic abnormalities**

• Abnormalities may result from genetic issues associated with one parent or both parents.

• An older dam may lead to decreased oocyte quality as a result of increased chromosomal abnormalities.

• Abnormalities may result from decreased oocyte or sperm quality. To reduce the risk of losses associated with gamete quality, the timing of insemination is imperative.

• Insemination following ovulation, generally more than six hours after ovulation, has led to an increased rate of embryonic death. In this situation, the oocyte is still capable of being fertilized, but the delay in fertilization has resulted in an aged oocyte that leads to slowed embryonic development. This delay in development could ultimately leave the embryo incapable of being recognized by the dam to prevent luteolysis (lysing of the CL).

• Poor semen quality is a factor that affects both conception rates in the mare and embryonic development. Other than stallion factors, poor semen handling techniques and insemination too early (relative to ovulation) may result in a reduction in semen quality.

**REASONS FOR FETAL DEATH**

**Twins**

• Twins are associated with late-term fetal abortion. Generally, this abortion occurs as a result of increased fetal stress caused by a lack of free space within the uterus. Uterine crowding leads to an increase in fetal cortisol (stress hormone), which initiates a cascade of events that result in premature expulsion of (most often) non-viable fetuses.

• Early termination of one embryo is the preferred method of treating twins while still allowing for one viable offspring. This early manual crushing of a twin is best performed prior to day 16 when the embryos are still free-moving within the uterus. After this point, procedures become more complicated and costly.

**Placentitis**

• Placentitis is most often caused by an ascending infection that ultimately enters the mare’s uterus. The fetal membranes, in response to the invading bacteria, begin to thicken. As the thickening continues, they eventually begin to separate from the uterus at the site of infection. The lack of placental contact with the uterus can lead to decreased oxygen and nutrients to the developing foal. Uniquely, this stress initiates more rapid maturation in the foal and, most often, an early birth. In some cases, this early birth may lead to a viable foal. Unfortunately, in others, the organs did not mature at the same rate following stress induction, and the foal is incapable of living outside of the uterus or may require extensive care.

• Unfortunately, placentitis does not have many clinical symptoms, so the condition often goes unrecognized.

• Frequent hormone assays can help with identification, as stress-induced maturation is often marked by a rapid, premature increase in progesterone (prior to 310 days in gestation). Earlier than 10 months in gestation, insufficient estrogen may be used to determine a compromised fetus. After 10 months, the natural reduction in fetal gonad size also decreases circulating estrogen.
• Transrectal ultrasound may also be capable of identifying a thickened placenta or a prematurely separating placenta.

• Mares that have previously suffered from placentitis are at a higher risk rate and may need additional therapies including antibiotics, anti-inflammatories, or hormone supplementation.

**Uterine Torsion**

• Uterine torsion is seen in mares after 7 months of gestation. Uterine torsion has similar symptoms to colic and can be confirmed as torsion by rectal palpation.

• Prevention of uterine torsion is not well understood. Some factors that predispose a mare to uterine torsion are anatomical in nature, like large abdomen and long broad ligament. Additional factors such as an active or large fetus and the mare rolling or falling may increase the incidence rate.

• There are both non-surgical and surgical methods of treatment for uterine torsion. With early identification and immediate remediation, some pregnancies have continued to term. Complications with the torsion such as gastrointestinal tract involvement and/or uterine damage lead to increased mortality of foal and potentially mare.

**Mare Reproductive Loss Syndrome (MRLS)**

• MRLS is associated with ingestion of the eastern tent caterpillar.

• MRLS is prevented by removing the pest, their larvae, and foliage on which they may have accumulated.

**Premature Placental Separation**

• Premature placental separation most often occurs during parturition, however, it may occur during mid to late gestation.

• Stress throughout gestation is a common cause of this condition.

• There is a reduction in nutrient and oxygen between the mare and foal. This mimics what happens in mares suffering from placentitis.

• Premature placental separation may be induced by mares with MRLS or mares exposed to toxic tall fescue during late gestation.

**FINAL THOUGHT**

Even with best practices and best intentions, some unavoidable factors will compromise pregnancy success. However, it is possible to reduce risk factors that are readily apparent. A conscientious producer interested in maximizing pregnancy success rates should be aware of the critical steps in conceptus development, reasons for conceptus loss, methods to avoid, and management practices that are conducive to successful establishment and maintenance of pregnancy in the mare.
REFERENCES


