

Maternal Recognition of Pregnancy in the Mare

Dirk K. Vanderwall, DVM, PhD, Dipl. ACT

Northwest Equine Reproduction Laboratory
Department of Animal and Veterinary Science
Center for Reproductive Biology
University of Idaho
Moscow, Idaho, USA 83844-2201
dirkv@uidaho.edu

Introduction

In domestic animals, early pregnancy is characterized by dynamic interactions between the developing conceptus (embryo and associated extra-embryonic membranes) and the dam's reproductive tract. These interactions provide signals to the dam that pregnancy has been established and that changes must be made in the maternal system (in the reproductive tract and elsewhere in the body) to accommodate the developing conceptus. Historically, the term maternal recognition of pregnancy has been used to describe the specific process during early pregnancy in which the conceptus blocks luteolysis, the event in nonpregnant females that leads to the cessation of function of the corpus luteum (CL) and the return to estrus (heat). During luteolysis, the uterus secretes prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in a pulsatile manner, and the $PGF_{2\alpha}$ travels to the ovary where it disrupts function of the CL causing progesterone levels to decrease, which brings the female back into heat allowing another opportunity for mating and the establishment of pregnancy. In most domestic animals, the conceptus is responsible for blocking luteolysis by secreting a substance(s) that inhibits $PGF_{2\alpha}$ secretion from the uterus, ensuring continued secretion of progesterone from the CL, which is necessary for maintenance of the pregnancy.

Although the equine conceptus disrupts the luteolytic mechanism by blocking $PGF_{2\alpha}$ secretion from the uterus, the overall process of maternal recognition of pregnancy in the horse involves more than just the anti-luteolytic effect of the conceptus. Specifically, the mare "recognizes" the difference between an unfertilized oocyte and a fertilized oocyte (i.e., embryo) during the first week of gestation during a process of selective oviductal transport of the embryo. Because of the active role the conceptus plays in the process of maternal recognition of pregnancy, a review of early embryonic development is appropriate.

Early Embryonic Development

When a mature ovarian follicle ovulates, the unfertilized oocyte (egg) is surrounded by the cumulus oophorus, a sticky cellular gelatinous matrix. Once ovulated, the oocyte is picked up by the infundibulum of the oviduct (fallopian tube), and is presumed to be rapidly (~ minutes to hours) transported to the ampullary-isthmic junction of the oviduct where fertilization takes place. When fertilization occurs, the first cell division (cleavage) typically occurs within 24 hours, with subsequent divisions occurring at 12 to 24 hour intervals. After the first cleavage division, individual cells are termed blastomeres. Cleavage-stage embryos are commonly referred to by the number of cells present, such as one-cell, two-cell, etc., up to the 16-cell stage. Between the 16- and 32-cell stage, as individual blastomeres can no longer be readily identified,

the embryo is referred to as a pre-compact morula. Continued cell division and tight junction formation (membrane attachments between cells) lead to the formation of a compact morula, which is a compact mass of ≥ 32 blastomeres. The compact morula is the latest developmental stage typically found in the oviduct.

After entering the uterus on Day 5 to 6 postovulation, the morula develops into a blastula, which is characterized by the formation of a fluid-filled cavity (blastocoel) within the cluster of morula cells. During the initial stages of blastocoel development, the embryo is referred to as an early blastocyst. When the blastocoel has fully formed, the cells lining the blastocoel consist of a single layer of cells called the trophoblast. Most of the trophoblast will eventually contribute to the formation of the placenta. In addition to trophoblast cells, a population of cells at one pole project into the blastocoel forming the inner cell mass, which is the forerunner of the embryo-proper (the developing foal). Once the blastocoel is fully formed the size of the embryo starts to increase dramatically, and the embryo is referred to as an expanded blastocyst. Embryos collected for transfer are generally recovered nonsurgically from the uterine lumen on Day 7 or 8 postovulation at the blastocyst stage of development.

The embryo is referred to as a blastocyst through Day 10 postovulation. Between Days 9 and 12 postovulation, a single layer of cells originating from the inner cell mass migrates along the inner surface of the trophoblast surrounding the blastocoel. Once this bilayer of cells completely encircles the blastocoel, the fluid-filled cavity is referred to as the yolk-sac, and the embryo itself is referred to as a yolk-sac embryo. The yolk-sac lumen is directly continuous with the primitive gut lumen of the developing embryo-proper and, therefore, whatever the yolk-sac absorbs from the uterus becomes available to the embryo-proper. The yolk-sac does not contain stored food material as in birds, but with the development of associated blood vessels, it becomes an efficient organ for purveying nutritive material from the uterus to the rapidly growing embryo; the yolk sac functions in this capacity until true placental development and function occurs after Day 30.

During its early development, the equine embryo is surrounded by a series of changing extracellular coverings. At the time of ovulation, the oocyte is surrounded by the prominent zona pellucida. After the embryo enters the uterus, an acellular capsule is deposited as a thin layer on the inner surface of the zona pellucida. Formation of the capsule typically occurs coincidentally with formation of the blastocoel. Within a day or so of the first formation of the blastocoel and capsule, the zona pellucida is shed from the outside of the capsule. As the blastocyst expands, the capsule thickens at least until Day 11 and then disappears after Day 22 postovulation.

Selective Oviductal Transport

The mare is unique among domestic animals in that unfertilized oocytes are retained in the oviduct where they degenerate over a period of several months, while embryos are selectively transported through the oviduct into the uterus between Days 5 and 6 postovulation. In contrast, in other domestic animals both unfertilized oocytes and embryos are transported through the oviduct into the uterus. Our laboratory identified that the embryo initiates its own transport by secreting the hormone prostaglandin E_2 (PGE_2). We initially showed that the embryo starts secreting PGE_2 just before the time of oviductal transport, and that the amount of

PGE₂ secretion increases throughout the transport period. We also showed that oviductal cells have receptors for PGE₂, indicating they had the capacity to respond to the embryonic PGE₂ signal (leading to selective transport). However, those two pieces of evidence did not conclusively prove that embryonic PGE₂ secretion initiated selective oviductal transport. The conclusive evidence came when we administered exogenous PGE₂ into the oviduct of pregnant mares prior to the expected time of oviductal transport, and caused embryos to move through the oviduct into the uterine lumen two days earlier than they would have without the PGE₂ treatment. Subsequent work in other laboratories has confirmed the role embryonic PGE₂ secretion in initiating selective oviductal transport. Selective oviductal transport represents the first embryo-initiated event in the overall process of maternal recognition of pregnancy in the mare.

Anti-luteolytic Effect of the Conceptus

In nonpregnant mares, regression of the CL (luteolysis) occurs on Days 14 to 15 post-ovulation. During luteolysis, the uterus secretes PGF_{2α} in a pulsatile manner, which disrupts CL function causing progesterone levels to decrease, allowing mares to return to estrus. Currently, there are three lines of evidence the pulsatile pattern of PGF_{2α} secretion during luteolysis is initiated by pulsatile secretion of oxytocin from the posterior pituitary gland: 1) pulsatile secretion of oxytocin and PGF_{2α} are temporally related during luteolysis, 2) administration of exogenous oxytocin stimulates PGF_{2α} secretion in nonpregnant mares, and 3) continuous administration of exogenous oxytocin blocks luteolysis by interfering with uterine responsiveness to oxytocin. By causing CL regression, oxytocin-induced secretion of PGF_{2α} from the uterus initiates each successive estrous cycle, which ensures mares have multiple opportunities to become pregnant. However, once pregnancy is established, PGF_{2α} secretion must be prevented for maintenance of pregnancy to occur.

Equine conceptuses exert their antiluteolytic effect between Days 11 and 14 post-ovulation by blocking secretion of PGF_{2α} from the uterus. In vitro incubation of uterine tissue demonstrated the content and production of PGF_{2α} at the expected time of luteolysis was not different for non-pregnant and pregnant mares; therefore, the uterus of pregnant mares maintains the capacity to synthesize and secrete PGF_{2α}. This suggests that a conceptus-derived factor(s) is responsible for blocking secretion of uterine PGF_{2α}, but the effect is short-lived or transient. The identity of the conceptus secretory product(s) responsible for blocking PGF_{2α} secretion has not been determined.

During luteolysis, PGF_{2α} is secreted from the uterus into the systemic (whole body) circulation where it is delivered to both ovaries. In other words, secretion of PGF_{2α} from any part of the uterus can cause regression of the CL on either ovary; therefore, the conceptus must block PGF_{2α} secretion from the entire uterus in order to prevent luteolysis. The continuous transport of the conceptus throughout the uterine lumen appears to be the mechanism for delivering the conceptus-derived PGF_{2α} inhibitory factor(s) to the entire uterus. Equine conceptuses are transported throughout the uterine lumen from the time they are first detectable with transrectal ultrasonography (Day 9 or 10) until Day 16 when they become fixed at the base of one of the uterine horns. Uterine contractions are the propulsive force causing uterine transport/mobility of the conceptus. The critical role of intrauterine transport/mobility of the conceptus in blocking luteolysis was demonstrated in an elegant study by McDowell et al. (see

suggested reading list) in which they restricted the intrauterine movement of the conceptus; severe restriction of movement prevented the conceptus from blocking luteolysis leading to failure of the pregnancy when progesterone levels decreased due to secretion of $\text{PGF}_{2\alpha}$ from that area of the uterus the conceptus could not interact with.

Using an in vitro culture system, Berglund et al. first demonstrated that co-incubation of equine conceptus tissue with uterine tissue significantly decreased uterine secretion of $\text{PGF}_{2\alpha}$. In an effort to further characterize the conceptus factor(s) responsible for blocking $\text{PGF}_{2\alpha}$ secretion, Weithenauer et al. performed an elaborate study using the same in vitro co-incubation system; to estimate the molecular weight of the conceptus factor(s) that inhibited $\text{PGF}_{2\alpha}$ secretion, conceptus tissues were placed inside dialysis tubing “bags” with different molecular weight exclusion limits, which were then co-incubated with uterine tissue. Prostaglandin $\text{F}_{2\alpha}$ secretion was significantly reduced when conceptus tissues were enclosed within dialysis bags with exclusion limits of 12,000, 6,000 and 3,500, but not 1,000 M_r , suggesting the conceptus $\text{PGF}_{2\alpha}$ -inhibitory factor(s) had a molecular weight between 1,000 and 3,500.

Unfortunately, the in vitro studies described above were performed before it was known that oxytocin regulates pulsatile secretion of $\text{PGF}_{2\alpha}$, and that the equine conceptus must block oxytocin-induced $\text{PGF}_{2\alpha}$ secretion in order to initiate maternal recognition of pregnancy. In the studies by Berglund et al. and Weithenauer et al., the ability of conceptus tissue to block oxytocin-induced $\text{PGF}_{2\alpha}$ secretion was not tested; they demonstrated only that basal (i.e., continuous) uterine secretion of $\text{PGF}_{2\alpha}$ was decreased by co-incubation with conceptus tissue. Another plausible explanation for the results obtained by those authors, is the conceptus tissue secreted a substance(s) that had a non-specific anti-inflammatory effect on the uterine tissue, which decreased basal $\text{PGF}_{2\alpha}$ secretion. In order to conclusively demonstrate the co-incubation system is a valid in vitro model for studying the anti-luteolytic mechanism of the conceptus in mares, it must be demonstrated that co-incubation of conceptus tissue with uterine tissue significantly decreases oxytocin-induced $\text{PGF}_{2\alpha}$ secretion. We are currently performing a series of experiments to examine the effect of co-incubation of equine conceptus and uterine tissues on oxytocin-induced $\text{PGF}_{2\alpha}$ secretion. Once we have developed a valid system in which we can detect the ability of the conceptus to block oxytocin-induced $\text{PGF}_{2\alpha}$ secretion, we can perform subsequent experiments to identify the specific conceptus secretory product(s) responsible for blocking $\text{PGF}_{2\alpha}$ secretion.

Summary

The equine conceptus is an active participant in two critical periods of communication between the conceptus and the mare's reproductive tract during early pregnancy. The first event is selective transport of the conceptus through the oviduct into the uterus between Days 5 and 6 postovulation, which is initiated by the conceptus' secretion of the hormone PGE₂. The second event is the anti-luteolytic effect of the conceptus to maintain function of the CL, so that continued secretion of progesterone can support the pregnancy.

It has been postulated that failure of the conceptus to block PGF_{2α} secretion is one cause of early pregnancy loss in mares. Characterizing the mechanism by which equine conceptuses block uterine secretion of PGF_{2α} may lead to methods of preventing failure of pregnancy, which could increase the reproductive efficiency of mares. In addition, once the anti-luteolytic signal is known, detection of that chemical could potentially be used as the basis for an early pregnancy diagnostic test.

Suggested Reading

1. McDowell KJ, Sharp DC, Grubaugh W et al: Restricted conceptus mobility results in failure of pregnancy maintenance in mares. *Biol Reprod* 39:340-348, 1988.
2. Berglund LA, Sharp DC, Vernon MW et al: Effect of pregnancy and collection technique on prostaglandin F in the uterine lumen of Pony mares. *J Reprod Fertil Suppl* 32:335-341, 1982.
3. Weithenauer J, Sharp DC, McDowell KJ et al: Characterization of the equine conceptus prostaglandin-inhibitory product. *Proc Equine Nutr Physiol Symp* 10: 215-220, 1987.