The early fetal life of the equine conceptus

D.C. Sharp *

Department of Animal Science, University of Florida, Gainesville, FL 32601, USA

Abstract

This paper will discuss development of the equine conceptus, especially from the perspective of the maternal environment in which it develops and to which it has considerable influence. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Horse; Reproduction; Embryo; Placenta; Embryo–maternal interaction

1. Introduction

There is much interest and concern about the early fetal life of the equine conceptus for many reasons. For one thing, it is a time of highly unique specialization and reproductive strategy. For another thing, it is a time of high pregnancy failure, and therefore deserving of a better understanding. Cossar Ewart (1897) stated that he had been asked by Lord Arthur Cecil if he could account for “…so many mares breaking service from the sixth to the ninth week (of pregnancy).” He further stated that he felt it was “…in many cases due to the detachment and escape of an embryo.” These quotes from one of the first comprehensive study of equine embryology emphasized the occurrence of pregnancy losses during the early weeks of pregnancy, but neither Ewart or the breeders of that time could appreciate the relatively high incidence of embryonic loss in the first three or four weeks of pregnancy. That would have to wait for the development of diagnostic ultrasound almost 100 years later. Ewart’s approach to the problem was entirely appropriate, however, in that he studied the formation of placental membranes on the theory that the losses could be explained by some disruption or aberration in the supply of nourishment to the developing embryo. Perhaps he was not too far off.

* Corresponding author.

0378-4320/00/$ - see front matter © 2000 Published by Elsevier Science B.V. All rights reserved.

PII: S0378-4320(00)00138-X
2. Extraembryonic membranes and their formation

Discussion of the formation of extraembryonic membranes is also covered elsewhere in this symposium, and only a relatively basic description will be provided here.

2.1. Yolk-sac stage

One of the key features of the equine conceptus is the persistent yolk sac. Although most domestic animals develop a yolk sac, it quickly becomes nonfunctional and apparently does not contribute greatly to embryonic nutrition. In Equids, however, the yolk sac is a predominant structure for the first three to four weeks of pregnancy and is thought to play an important, if not critical, role in early embryonic nutritional supply. The yolk sac is formed by endodermal tissue which emanates from the inner cell mass and expands spherically, lying just under the outer trophoderm layer. The resultant bilaminar omphalopleure, or two-layered structure can be first observed by day 10 or 11, and persists, in ever diminishing size throughout pregnancy. Beginning about day 14, mesoderm from the embryonic disc begins developing interposed between the endodermal and ectodermal layers. The three layered structure so formed, or trilaminar omphalopleure, develops spherically from the region of the inner cell mass towards the abembryonic pole. As it does so, it is accompanied by vascular development as primitive blood islets form and a primitive circulatory system begins to take shape. The distal border of the developing trilaminar omphalopleure is demarcated by a prominent collecting vein and becomes known as the sinus terminalis. The sinus terminalis moves toward the abembryonic pole as mesoderm continues to develop between the endoderm and ectoderm, setting the stage for apparently spatially critical events later in pregnancy, including the site of umbilical attachment. More on this later. The yolk sac can still be recognized at term, but the question of when it ceases to contribute to embryonic or fetal well-being is unanswered. When placed into tissue culture and incubated in the presence of tritium-labelled leucine as amino acid precursor, it is clear that yolk sac tissue obtained from day 100 of pregnancy is still capable of synthesizing and secreting proteins of similar size class as yolk sac from day 16 (McDowell et al., 1990). The role these proteins play in early or late pregnancy is not known, but it seems clear that the yolk sac does not become totally inactive despite its greatly reduced volume. The receding yolk sac gives way to the advancing allantois, first recognized around day 21 or so, and it is likely that yolk sac function, whatever its role, begins to recede at that time as well. During the first three to four weeks of pregnancy, then, equine placentation can be described as choriovitelline. As the allantois forms and enlarges by the fourth or fifth week of pregnancy, the placenta becomes chorioallantoic because of the predominant allantoic sac which consists of endoderm, mesoderm and ectoderm, constituting a chorio-allantois (see Fig. 1).

Of special interest, the equine conceptus remains essentially spherical throughout the first six to 8 weeks of pregnancy in contradistinction to most domestic species in which the chorioallantois expands and elongates tremendously within the first two weeks of pregnancy. This architectural anomaly may be associated with further interesting and novel events of early pregnancy, such as the extreme intrauterine mobility of equine
conceptuses prior to day 16 or 17 (Ginther, 1993; McDowell et al., 1998). Perhaps it is the spherical nature of the equine conceptus that necessitates the development of a tough outer shell or acellular layer called the capsule. Again, the function is not well understood, but the capsule which surrounds the equine conceptus is a mucin-like network of glycoprotein which appears to originate largely from the underlying trophoderm (Betteridge, 1989). Readers are referred to Betteridge (2000) for more details on capsule formation and function. The argument could be made that the capsule provides a rigid structure for the equine conceptus, which enables it to shuttle back and forth in the uterine lumen in response to uterine contractions. Changes in the complex carbohydrate makeup of the capsule with stage of pregnancy could also reflect tissue remodeling processes which permit fixation or other maternal/conceptus interactions (Oriol et al., 1993). Likewise, presence of the capsule has been cited as a biological shield against bacterial and/or viral attack. Whatever the function(s) of this enigmatic structure, it is a familiar landmark to those who study this fascinating portion of equine pregnancy.

2.2. Conversion from choriovitelline to chorioallantoic placenta

As the allantois develops and the yolk sac recedes reciprocally, the embryo proper is lifted dorsally from a 6 o’clock position toward a 12 o’clock position. Similarly, the allantois, which arises from the hindgut of the embryo and forms under the embryo expands toward the abembryonic pole. As it does so, an exocoelom develops at the area of the advancing allantois and the retreating yolk sac. By about day 30 or so, the area of this exocoelom, which encircles the conceptus, takes on a characteristic appearance. This area is very avascular and varies in width from one to several millimeters. It is known as the chorionic girdle. The cells of the chorionic girdle are highly proliferative and begin
to invade the maternal endometrium between days 36 and 38 where they phagocytize maternal epithelial cells. Then, after the phagocytic stage, the chorionic girdle cells continue to migrate, penetrating the basement membrane of the maternal epithelial cells to the stroma, where they locate between the uterine glands. Here they differentiate and hypertrophy to become mature endometrial cups, large raised structures that protrude above the uterine endometrial surface. They often form a concave or depressed surface that resulted in their description as “cups”.

The mechanisms involved in formation of the chorionic girdle is a fascinating, as well as mysterious, aspect of equine early pregnancy. The proliferation of trophoblast cells over the exocoelom between the advancing allantois and the receding yolk sac suggests the action of a locally produced mitogenic stimulus. Stewart et al. (1995) reported that hepatocyte growth factor–scatter factor (HGF–SF) was localized by in situ hybridization in the allantoic mesoderm, and its receptor, the proto-oncogene c-met was localized in invasive trophoblast of the chorionic girdle. These authors interpreted their findings to indicate that mesoderm-derived HGF–SF could play an important role in proliferation of the chorionic girdle cells. Because of the fact that the chorionic girdle is close to, but not fused with, the underlying allantoic mesoderm, the effect of a mitogenic stimulus would be to stimulate cell proliferation in an area that becomes somewhat restricted, and the cells appear to “pile up” rather than expand linearly (Stewart et al., 1995).

One of the primary functions of the endometrial cups is the production of equine chorionic gonadotropin (eCG), although the primary purpose for this unique, highly glycosylated gonadotropin remains controversial. It is relatively clear that the secretion of eCG coincides temporally with the formation of multiple accessory corpora lutea, but is perhaps not so clear whether this is an essential event for maintenance of equine pregnancy. Likewise, it has been suggested that the secretion of eCG is timed so as to play a role in blunting or thwarting a maternal immune response to the interdigitation of chorionic villi with formation of the more advanced chorioallantoic placenta around day 60 or so (Samuel et al., 1974). Both of these proposed functions have some supportive evidence, but neither has been proven beyond doubt.

2.3. Formation of microcotyledonary placenta

Between days 50 and 60, chorioallantoic macrovilli (rudimentary irregularities or undulations) begin to elongate and transform into the microcotyledons that constitute the major nutrient exchange mechanisms of the placenta (Ginther, 1993). Some maternal gaseous exchange apparently begins to take place. Vascularity of both maternal and fetal tissues increases, and the epithelium of maternal crypts becomes reduced. By day 100 or so, the structures begin to have a distinct cotyledon-like appearance, albeit in miniature.

2.4. Summary

Ewart (1897) saw the changes in equine placentation as analogous to some of the structures of the chick embryo and a marsupial species (opossum). In fact, at one point he speculated that pregnancy loss in mares around the 6th to the 9th week of pregnancy
was an atavistic remnant of the marsupial reproductive strategy in which migration out of the uterus was necessary to achieve the next level of nutrition for the embryo. As quaint as that sounds, it may provide focus on the dilemma of the developing equine embryo to maintain a nutritive supply. While we may not understand the evolutionary forces that created the peculiarities of the early equine placenta, it seems reasonable that the resulting structures, yolk sac placenta, capsule, relatively late forming chorioallantoic placenta, chorionic girdle, and the very late forming microcotyledonary placenta, reflect changes in the embryo’s efforts to obtain sufficient nutrition for growth and development.

3. Chorionic girdle as a luteotropin

The temporal association between eCG and formation of the accessory corpora lutea has been known for a considerable time (Cole and Hart, 1930; Cole et al., 1931). Furthermore, it is now widely accepted (Ginther, 1993; Murphy and Martinuk, 1991) that eCG is indeed luteotropic; it has been demonstrated to stimulate progesterone production from luteal tissue in vitro (Squires et al., 1974), including tissue from the primary CL and the accessory CL that form later in pregnancy. Furthermore, Daels et al. (1998) have demonstrated that eCG significantly increased not only progestin secretion, but estrogen secretion as well. Albrecht and Daels (1997) reported that this increase in estrogen secretion is associated with changes in expression of key steroidogenic enzymes as measured by immunolocalization. Albrecht and Daels (1997) stated that the intensity of immunolocalized staining for P450 17α-hydroxylase (17α-OH) increased after the onset of eCG, whereas the intensity of immunolocalization of P450 aromatase (AROM) increased during pregnancy before the onset of eCG secretion and diminished after the onset of eCG secretion. Thus the above certainly provide compelling evidence for a luteotropic function of eCG, but do not imply that such a luteotropic event is necessary for maintenance of pregnancy. On the other hand, the report of Hinrichs et al. (1985) that ovariectomized mares could serve as embryo transfer recipients if provided a continuous source of progesterone argues against the need for a further boost in circulating progesterone concentrations at the time of accessory CL formation. Similarly, Squires et al. (1983a,b) and Squires and McKinnon (1987) demonstrated that administration of a continuous, relatively low dose of progesterone or similar progestin could maintain pregnancy in ovariectomized, embryo transfer recipient mares. These latter authors stated that the circulating concentrations of progesterone remained “consistently above 4 ng/ml in mares that maintained pregnancy.” They further stated their opinion that that was an important threshold level for pregnancy maintenance. Importantly, the mares did not receive an additional “boost” or surge of progesterone such as would result from formation of multiple accessory CL. These latter studies seem to provide strong evidence that the additional increase in progesterone is not a requirement for maintenance of pregnancy, but they do not indicate any deleterious effects either. Still it seems reasonable that the rise in circulating progesterone reflects the physiological consequence of gonadotropin secretion more than it reflects the physiological requirement.
4. Chorionic girdle as immunomodulator

The dilemma faced by female mammals during early pregnancy is that an antigenically foreign fetus will form intimate connections with the maternal endometrium necessitating long periods of modulation of the immune system so as not to reject the fetus and placenta, but maintain some degree of immune defense. In mares, the relatively prolonged time before such intimate attachments are formed (about 60 days) is unusual, and the temporal coincidence of placental attachment with chorionic girdle cell invasion and eCG secretion may not be accidental. The chorionic girdle cells, in addition to secreting eCG, appear to be the major source of fetal major histocompatibility complex (MHC) antigens (Antczak and Allen, 1989). This expression of MHC antigen by trophoblast tissue is unusual but is further complicated by the observation that mature endometrial cups do not appear to express paternal MHC antigen in appreciable quantity (Antczak and Allen, 1989). Furthermore, the endometrial cups are ultimately lost due to maternal leucocytic invasion.

5. Embryo—maternal interactions

The early life of the equine conceptus is taken up with maternal interactions for several reasons. However, the many unusual features of equine pregnancy make these interactions novel and interesting and certainly worthy of considerable study. The conceptus plays a role in controlling its own intrauterine environment, both by signaling its presence and assuring maternal recognition of pregnancy, and by contributing directly to the steroid environment of the intrauterine lumen. The equine conceptus migrates throughout the uterus prior to pregnancy recognition, likely serving to signal its presence uniformly, and to garner uterine secretions from throughout the entire uterus. The architecture of the equine conceptus likely also plays an important role in its orientation long before proper conceptus—maternal interdigitation occurs. Invasion of the endometrium by cells of the chorionic girdle certainly provides a unique conceptus—maternal interaction that is, as stated above, poorly understood.

6. Transport through the oviduct

The equine conceptus is unusual in that transport of ova through the oviduct occurs only if it is fertilized. This concept was reported by van Nie Kirk and Gerneke (1966), and further expanded by Betteridge and Mitchell (1972). The latter authors flushed oviducts of mares and recovered unfertilized ova. They reported that the number of unfertilized ova recovered was well correlated with the number of ovulations intervening. Furthermore, Betteridge et al. (1979) demonstrated that ova treated with TEPA, which prevents cleavage past the 16-cell stage were not transported through the oviduct, whereas fertilized ova in more advanced stages of cleavage were transported. Thus, these data suggest that some conceptus factor is capable of influencing transport through the oviduct when the conceptus is capable of cleavage past at least the 16-cell stage.
More recently, it has been shown that conceptuses at the morula or early blastocyst stage secrete prostaglandin E2 (PGE), a uterotonic substance, which could contribute to tubal transport though induction of oviductal contractions locally, or through relaxation of the isthmic musculature Weber et al. (1995). Furthermore, local administration of PGE to the oviduct appears to hasten tubal transport, resulting in early arrival of fertilized ova to the uterus (Robinson et al., 1998). Some authors refer to this discriminatory transport of fertilized ova through the oviduct as a form of maternal pregnancy recognition. Indeed, it is, but the term “maternal recognition of pregnancy,” as applied by Short (1967) has been classically used to describe the conceptus signal which prevents loss of the maternal corpus luteum at about 14 days after ovulation. Therefore, this author will retain the expression “maternal recognition of pregnancy” as a definition of luteal maintenance, not tubal transport.

7. Intrauterine environment

The early equine conceptus relies on its choriovitelline placenta to supply nourishment from uterine secretions for perhaps the first three to four weeks of pregnancy, if not beyond. That suggests that the uterine environment is extremely important to the developing equine conceptus, and merits discussion. It is clear that progesterone is essential to provide the appropriate intrauterine environment for conceptus development. It is also clear that the mare starts to prepare for a potential pregnancy with each and every luteal phase, regardless of whether or not she has been bred. Apparently it is a more acceptable trade-off, from the evolutionary standpoint, to prepare for a pregnancy that may not occur than to fail to prepare for one that does. The reproductive strategy of the mare is to make that decision at the time of maternal recognition of pregnancy.

Comparison of total intrauterine protein, as well as specific intrauterine proteins indicates the parallel production of such proteins up to the final day for maternal pregnancy recognition. Fig. 2 demonstrates the increase in a specific intrauterine protein, uteroferrin, in nonpregnant and pregnant mares. The increases in uterine luminal uteroferrin are not different in nonpregnant or pregnant mares until after day 14, the final deadline day for pregnancy recognition. By day 16, however, the differences between the two statuses are significant (Zavy et al., 1982). It seems reasonable to speculate that the differences in this one representative protein, uteroferrin, may reflect life or death differences as far as the conceptus is concerned.

The requirement for progesterone to drive uterine secretions has been shown in a variety of ways. For one, ovariectomized mares can be made suitable for pregnancy by simply administering progesterone, as demonstrated by Hinrichs et al. (1985). For another, removal of progesterone from pregnant mares by surgical ablation of the corpus luteum, or by administration of prostaglandin, leads to pregnancy loss. However, reintroduction of progesterone can salvage the pregnancy. Interestingly, reintroduction of progesterone has a deadline as well. In experiments in which corpora lutea were regressed by administration of PGF on day 14, and progesterone administration (150 mg/day) begun on day 15, 16 or 17 (n = 5 mares per group) embryo survival was 100%. However, when progesterone was administered beginning on day 18, none of the
embryos survived (Sharp, 1995). A similar observation was reported by Ginther (1985). One wonders if the few days of grace during which a lack of progesterone can be tolerated reflect a role for yolk sac nutrient stores.

8. Embryo mobility

It is now well accepted that the trans-uterine mobility of the equine conceptus is an important feature of the pregnancy recognition process. McDowell et al. (1998) demonstrated that restricting the trans-uterine mobility of equine conceptuses resulted in failure of the maternal recognition of pregnancy. In retrospect, the trans-uterine migration of equine conceptuses makes sense in light of the observations that uterine endometrium is capable of secreting PGF in vitro (Vernon et al., 1981), yet coincubation of conceptus membranes with endometrium from pregnant mares results in reduction in PGF secretion in vitro (Berglund et al., 1982; Sharp and McDowell, 1985). This suggests that the PGF-inhibitory effect of the equine conceptus may be somewhat transient, and the trans-uterine mobility of the conceptus permits frequent, or intermittent interaction to reduce PGF secretion.

9. Uncoupling the oxytocin/PGF cascade

The luteolytic cascade involves occupation of the oxytocin receptors and subsequent synthesis and release of PGF. In the presence of a conceptus, however, the synthesis and secretion of PGF are abrogated. The conceptus signal which accomplishes this is not known; however, presence of a conceptus uncouples the oxytocin-induced release of
Fig. 3. Endometrial prostaglandin synthesis inhibitory activity (expressed as arbitrary inhibitory units) in microsomal and cytosolic fractions of equine endometrium from day 16 pregnant and nonpregnant mares.

Fig. 4. Schematic representation of potential mechanisms of maternal recognition of pregnancy in mares. Oxytocin (OT) binds to its receptors (OT$_R$) initiating the arachidonic acid (AA) cascade and production of prostaglandin F2α (PGF). In pregnant mares, the oxytocin receptor numbers are reduced as is apparent affinity (represented by smaller font), leading to potentially reduced signal to arachidonic acid cascade. In addition, an endometrial produced prostaglandin synthesis inhibitor (EPSI) blocks conversion of AA to PGF powerfully, leading to luteal maintenance via luteostasis.
PGF. This has been demonstrated by administration of oxytocin to nonpregnant and pregnant mares, with the resultant blockade of PGF release in pregnant mares (Goff et al., 1987). Likewise, stimulation of the cervix of nonpregnant and pregnant mares results in oxytocin release in mares of both statuses, but PGF release, measured as PGF metabolite (PGFM) occurred only in nonpregnant mares (Sharp et al., 1997; Betteridge et al., 1985). Furthermore, the latter workers demonstrated a reduction in oxytocin receptors in pregnant mares. Thatcher (1995) reported presence of an endometrial prostaglandin synthesis inhibitor (EPSI) isolated from bovine endometrium. There is also evidence that mares may express an EPSI as well. Watson and Sertich (1989) first demonstrated the presence of a conceptus-induced, endometrium-produced inhibitor of PGF. We have also observed such activity in the microsomal fraction of pregnant mares, although the chemical structure of the equine EPSI is yet to be determined (see Fig. 3).

Interestingly, the discovery of an equine endometrial prostaglandin inhibitor begs the question of what it is about the presence of a conceptus that permits or stimulates its expression. It would seem that further work is required to understand the maternal/conceptus interaction that permits continuation of pregnancy in mares. Fig. 4 indicates a current theory of maternal recognition of pregnancy in which occupancy of the endometrial oxytocin receptors activates the arachidonic acid cascade and prostaglandin F2α (PGF) production. In pregnancy, reduced OT receptor number and affinity (Sharp et al., 1997) may reduce activation of the arachidonic acid cascade. Additionally, the pregnant mare endometrium contains a prostaglandin synthesis inhibitor (EPSI) which produces a potent blockade to the conversion of arachidonic acid to PGF. Likely, these two mechanisms work together to assure luteal maintenance.

**References**


Betteridge, K.J., Eaglesome, M.D., Mitchell, D., 1979. Embryo transport through the Me’s oviduct depends upon cleavage and is independent of the ipsilateral corpus luteum. J. Reprod. Fertil. (Suppl. 27), 387–394.


