Embryo-maternal communication during the establishment of equine pregnancy

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Abstract

Over the last 30 years, improvements in veterinary management have resulted in higher per cycle pregnancy rates in commercial horse breeding programmes. However, the incidence of early pregnancy loss has not diminished and ~ 10% of pregnancies detected on day 15 fail to survive beyond day 42 of pregnancy. To develop strategies to combat pregnancy loss, it is first necessary to understand why these pregnancies fail. In this respect, a sizeable proportion probably results from intrinsic embryonic abnormalities that cannot be remedied; another significant subset is, however, likely to result from an 'inadequately receptive' uterus or failure of the uterus to physiologically adapt to the developing conceptus' changing needs, as a result of inadequate embryo-maternal communication. Communication between embryo and dam starts during the oviductal period, as evidenced by selective transport of viable embryos into the uterus on day 6 postovulation, whereas unfertilized oocytes remain in the oviduct. Conversely, retarded development exhibited by embryos produced in vitro demonstrates the importance of the oviductal environment to the early embryo. Once in the uterus, the embryo must steer maternal physiology to ensure adequate nutrient provision and prepare the endometrium for implantation. This communication includes 'maternal recognition of pregnancy' signalling to extend the lifespan of the primary corpus luteum and ensure continued secretion of progesterone. Although the identity of the embryonic pregnancy recognition signal remains unknown, our understanding of how endometrial function is altered to avert luteolytic prostaglandin $F_{2\alpha}$ release and become receptive to implantation has improved.

Keywords: Equine embryo, endometrium, maternal recognition of pregnancy, implantation

Introduction

Over the last 30 years, a range of technological and pharmacological breakthroughs, allied to a better understanding of factors that compromise fertility, has led to a marked rise in the per cycle pregnancy rate in intensively managed horses.[1] During the same period, assisted reproductive technologies (e.g. embryo and oocyte transfer) and more recently ovum pick up and intracytoplasmic sperm injection (ICSI) for in vitro embryo production,[2] have been adopted commercially either to circumvent specific forms of subfertility or to aid genetic selection. Despite successful introduction of these techniques to overcome subfertility, and general improvement in reproductive efficiency, there has been little progress in combating one of the major sources of economic loss within horse breeding, namely early pregnancy loss.[3] After initial detection of pregnancy at around day 15 postovulation, ~ 5 - 15% of all pregnancies will fail to reach term in young healthy mares, often without any obvious explanation for why the pregnancy was lost. Pregnancy losses are even more common in older (> 18 years) mares,[4] or following the transfer of in vitro produced embryos,[5] where > 25% of day 15 pregnancies are reported to fail. Interestingly, more than half of all pregnancy losses occur in the first 6 weeks of pregnancy.[6] This strong bias to early loss is presumably in large part because conceptuses with serious intrinsic defects, such as gross abnormalities of chromosome number (aneuploidy), are most likely to fail during early development.[7] Moreover, the equine conceptus undergoes an incredible transformation during the first 7 weeks of life, from a microscopic ball of undifferentiated cells that imbibes nutrients from the surrounding oviductal fluids, to a fetus with recognisable body parts and precursors of all of the major organs required for extra uterine life, that is provided with nutrients and respiratory gasses by a dedicated placenta. To enable this remarkable growth and development, uterine physiology needs to evolve in parallel with conceptus development. One of the defining events in the conceptus directed adaptation of maternal physiology is the so called 'maternal recognition of pregnancy' (MRP), a process by which the day 10 - 14 equine conceptus suppresses cyclical luteolysis, and thereby extends the lifespan of the primary corpus luteum. This safeguards the supply of maternal progesterone required to stimulate endometrial production of histotroph on which the conceptus depends for nutrition before the development of a stable placental attachment from

around day 40.[8] Conceptus derived signals also combine with luteal progesterone to modify endometrial physiology in preparation for implantation. Clearly, therefore, the way in which the early embryo and its dam communicate to ensure that uterine physiology is coordinated with embryonic development is critical to the successful maintenance of pregnancy. This paper discusses some of the important elements of embryo-maternal communication during the first 6 weeks of equine pregnancy.

Embryo development in the oviduct and descent into the uterus

The equine embryo remains in the oviduct for an unusually long interval and finally descends into the uterus on day 6 postovulation.[9] During its sojourn in the oviduct, the embryo's genome is activated and it progresses to the late morula stage of development. Although little is known about embryo and oviduct interaction, the microenvironment of the oviductal ampulla clearly plays an active role in supporting early development; indeed, although in vitro produced (IVP) equine embryos reach the blastocyst stage at a similar time to in vivo embryos (7 - 8 days postfertilization), at this stage they contain many fewer cells,[10] a higher percentage of which are apoptotic[11,12] and, following transfer to a recipient mare, IVP embryos lag 2 - 3 days behind in development and are more prone to early embryonic death.[5] A positive influence of the oviduct on equine embryo development is also illustrated by the more rapid development and increased likelihood of reaching the blastocyst stage of 2 - 4 cell embryos transferred into the oviduct of a recipient mare soon after ICSI, compared to embryos maintained in vitro.[12] Although it also appears that the developing embryo induces changes in oviductal epithelial cell gene and protein expression,[13,14] the presence of an embryo in the oviduct does not appear to affect the mare's subsequent ability to support pregnancy, as demonstrated by the high initial and ongoing pregnancy rates in well synchronised embryo transfer recipient mares.[15] Conversely, the early equine embryo has been reported to influence maternal physiology beyond the confines of the oviduct, in the form of the 'early pregnancy factor' (EPF) reported to be detectable in the serum of pregnant mares from day 2 postovulation.[16] Although it is unclear whether EPF plays any further role in pregnancy recognition or maintenance, a reliable assay for EPF would be invaluable for investigating the scale and timing of embryonic loss and potentially for improving the efficiency of ET programmes, (e.g. by ensuring that only mares 'guaranteed' to deliver an embryo are flushed). Unfortunately, field trials of an 'early conception factor' assay were disappointing, with a high rate of false positives limiting the ability of the test to discriminate between pregnant and nonpregnant mares.[17]

The equine embryo's oviductal phase is brought to an end by an enigmatic form of embryonic signalling, referred to as selective oviductal transport. Only developing embryos are able to reliably descend into the uterus, whereas unfertilized oocytes remain trapped in the oviductal ampulla.[18] This selective transport is mediated by prostaglandins, in particular prostaglandin E_2 (PGE_2) that viable embryos begin to secrete from around day 4 postfertilization.[19] The early postfertilization embryo is retained in the ampulla of the oviduct by a tightly closed ampullary-isthmic junction; the PGE_2 secreted by the developing morula relaxes the circular smooth muscle of the isthmus, causing it to dilate and allow the embryo to pass through into the uterus.[20] Failure of viable embryos to initiate oviductal transport does not seem to occur, since tubal pregnancy has not been reported as a complication in mares.

Glycoprotein capsule

Another unusual feature of equine pregnancy that both depends on and, almost certainly plays a role in, early embryo-maternal communication, is the formation and subsequent disappearance of the acellular glycoprotein 'capsule'. The capsule forms between the trophectoderm and zona pellucida very soon after the embryo enters the uterus, roughly coincident with blastocyst formation.[21] The mucin-like glycoproteins that initially make up the capsule are secreted by the trophectoderm;[22,23] however, they are unable to cross-link to form a confluent structure in vitro.[10] It therefore appears that capsule coalescence requires input from the endometrium, a supposition strengthened by the report of increased capsular glycoprotein production by IVP embryos exposed to uterocalin,[24] a progesterone dependent endometrial protein that associates strongly with the capsule of early uterine stage conceptuses.[25] Nevertheless, it is not entirely clear whether the primary uterine contribution to capsule formation is in the form of structural elements, or by providing a microenvironment that facilitates cross-linking and assembly of the trophectoderm-derived glycoproteins into a confluent structure.

Soon after capsule formation is completed, the day 7 embryo 'hatches' from its zona pellucida; however, its newly formed 'tertiary embryo coat' remains as a physical barrier between the trophectoderm and the endometrium. The capsule subsequently increases in thickness until ~ day 17, developing a bilaminar appearance that may reflect an additional (presumably endometrial) source of structural components.[25,26] Soon after embryo fixation (cessation of migration), however, dissolution of the capsule begins, with a rapid decline in dry weight and loss of continuity somewhere between days 20[8] and 23;[27] by day 30 the capsule has disintegrated completely.[8] Although it is not known how the capsule is dissolved, the process is likely to involve both trophectodermal and endometrial enzymes. Moreover, there appears to be a central role for progesterone in stimulating enzymatic dissolution, since induction of luteolysis shortly before conceptus fixation (day 16) prevents capsule dissolution and the desialylation and degradation of capsule-associated proteins associated with normal degradation.[27,28]

Although the functions of the capsule are largely speculative, that it is interposed between the trophectoderm and endometrium means it almost certainly plays some role in mediating embryo-maternal interaction and communication. Moreover, the capsule is essential for conceptus survival in utero, since removing it from early day 7 embryos by micromanipulation prior to embryo transfer prevented the embryos from developing into ultrasonographically visible pregnancies.[29] Conversely, although IVP embryos have no confluent capsule when they reach the blastocyst stage, they have accumulated capsular glycoproteins within the perivitelline space. Subsequently, they develop an apparently normal capsule following transfer to the uterus of a suitable recipient mare. Physically, the capsule is both tough and elastic and it has been proposed to help the blastocyst maintain its spherical shape, and to provide mechanical protection during the 'mobile phase' when the relatively delicate conceptus vesicle is 'squeezed' around the uterine lumen by myometrial contractions.[30] In addition, the capsular glycoproteins are rich in negatively charged sialic acid residues that have been postulated to confer antiadhesive properties and thereby facilitate conceptus migration;[27] indeed, the end of the conceptus mobile phase is associated with extensive desialylation of the capsule.[31] In this way, the capsule plays at the very least an indirect role in embryonic maternal recognition of pregnancy signalling (i.e. by promoting migration). More intriguingly, the capsule has been proposed to play an active role in embryo-maternal dialogue by acting as a 'mail-box'[32] to store, modify or transfer endometrial proteins involved in nutrient transport (e.g. uterocalin) or in stimulating trophectoderm cell proliferation. Similarly, the capsule may act as a repository for trophectodermal proteins that modulate endometrial function, or act in a paracrine fashion to regulate trophectoderm cell migration or proliferation (e.g. IGFBP3[33]).

Maternal recognition of pregnancy signalling

The archetypal embryo-maternal signalling process during early intrauterine development is MRP, during which the developing embryo biochemically signal(s) its presence to its dam to ensure that she undergoes the physiological adjustments necessary to maintain a uterine environment conducive to embryonic survival.[34] Since the pivotal determinant of this 'supportive environment' is a continuing supply of luteal progesterone, MRP is usually taken to refer specifically to the conceptus initiated events that prolong the lifespan of the primary corpus luteum. We now know that MRP in the mare involves suppression of the endometrium's ability to release large pulses of the luteolytic hormone, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), in response to hypothalamic-pituitary[35] (and/or endometrial)[36] oxytocin, during days 10 - 16 postovulation.[37] To ensure sufficient delivery of the presumed conceptus antiluteolytic signal to the endometrium to adequately suppress $PGF_{2\alpha}$ release and thereby avert luteolysis, the spherical equine conceptus must migrate throughout the entire uterine lumen during the day 10 - 16 period when luteolysis would otherwise occur. Indeed, when equine conceptuses were surgically restricted to a single uterine horn, luteolysis was not blocked.[38] Somewhat paradoxically, conceptus vesicle migration appears itself to be driven by myometrial contractions induced by prostaglandins produced either directly by the conceptus or locally in the uterine wall in response to other conceptus derived mediators.[30]

One of the reasons why it has not been possible to definitively identify the conceptus factor responsible for initiating MRP in the mare may be that the process, as in other species, involves multiple signals and more than one phase. Even though the identity of the MRP signal(s) remains a mystery, it has been established that the initial suppression of endometrial $PGF_{2\alpha}$ secretion involves a

conceptus directed down regulation of endometrial prostaglandin endoperoxide synthase 2 (PTGS2) activity[39,40] accompanied by a reduction in endometrial oxytocin receptor (OXTR) expression.[40] However, following the cessation of conceptus mobility at around day 16, the endometrium does develop the ability to secrete $PGF_{2\alpha}$ in response to oxytocin,[41,42] a change that is reflected by contemporaneous upregulations in PTGS2 and OXTR expression.[40] This implies that prevention of luteolysis beyond the time of conceptus fixation depends either on the disappearance of the oxytocin 'trigger' (e.g. hypothalamic-pituitary OT release only occurs in a defined time window) and/or on disabling of another link in the luteolytic cascade. In this latter respect, expression of the endometrial $PGF_{2\alpha}$ receptor (PTGFR), that is thought to play an important role in establishing the local feedback ($PGF_{2\alpha}$ - OXT) and feedforward ($PGF_{2\alpha}$ - $PGF_{2\alpha}$) loops essential to generating the large pulses of $PGF_{2\alpha}$ release required to ensure luteal destruction, is down regulated until at least day 21 of pregnancy;[40] this may explain why inadvertent luteolysis is not very common in the early postfixation period, despite the presence of sufficient OXTRs and PTGS2.

Given that the oxytocin responsiveness central to cyclical luteolysis develops at around day 10 postovulation,[37,43] it is logical to assume that conceptus signalling required for MRP must also begin at or before day 10. Frustratingly, the conceptus signal(s) responsible for down regulating PTGS2 and OTXR expression, and thereby effecting the first phase of MRP, remains elusive.[44] It has been reported that the equine conceptus product that inhibits endometrial $PGF_{2\alpha}$ secretion must be small, with a molecular weight estimated at ~ 1 - 6 Kda[45] or 3 - 10 Kda;[46] however, these studies did not identify specific candidates. Similarly, while changes in the transcriptome of conceptuses during the MRP period revealed various conceptus derived molecules likely to play roles in embryo-maternal interaction,[47] it did not unearth a putative antiluteolytic signal. Nevertheless, the transcriptomic data adds to the list of hormones, cytokines and growth factors, such as PGE_2,[42] IGF-1,[48] and estrogens,[49] secreted by MRP stage equine conceptuses and likely to play important roles in driving other events critical to pregnancy maintenance, such as conceptus migration,[30] increased uterine vascularity,[50] and changes in uterine gland secretions that modify the composition of histotroph.[51] Indeed, transcriptomic studies have indicated a significant number of genes differentially regulated in the endometrium of pregnant and nonpregnant mares between days 8 -14 postovulation. These genes are regulated by estrogen or PGE_2.[52,53] Recently, we examined the effect of asynchronous embryo transfer on the transcriptome of both conceptus and endometrium; one of the most striking findings was that hundreds of genes relating to 'extracellular exosome' were differentially regulated in either the conceptus membranes or the endometrium as a result of asynchrony between the two, suggesting that extracellular vesicles play an important role in trafficking molecules important to conceptus endometrial communication.[54]

Preparing endometrium for implantation and placentation

Implantation involves the establishment of a stable attachment to facilitate more efficient, hemotrophic exchange between the conceptus and endometrium. A normally developing embryo and an appropriately 'primed' uterus are prerequisites for successful implantation; even then, carefully coordinated communication between the embryonic trophectoderm and maternal endometrial epithelium is required to further modify the endometrial surface and thereby 'permit' attachment and stimulate the trophectoderm cells to attach and subsequently either invade into (chorionic girdle cells), or interdigitate with (other chorionic cells), the endometrium.[55,56] Initial attachment takes place during the 'window of receptivity to implantation',[57] when the previously nonreceptive endometrial surface is modified to allow conceptus adhesion. An important aspect of endometrial preparation is a period of exposure to progesterone that results in a down regulation of progesterone receptors (PRs) in the luminal and glandular epithelial cells but, possibly crucially, not in the stromal cells.[55] These stromal cells are proposed to produce 'progestamedins' that allow progesterone to fine tune the preparations of the endometrial epithelium for its role in implantation, despite the 'switching off' of their own PRs.[55]

One of the first steps in implantation is intimate apposition of the trophectoderm to the endometrium, an event that can only occur once the capsule starts to disintegrate at around day 23 of pregnancy.[27] In most species, attachment of the trophectoderm to the endometrium requires removal of surface glycocalyx components that previously inhibited this process and, in particular, mucins that coat the endometrial luminal epithelium (e.g. MUC1[58]). In the mare, there does not appear

to be a reduction in endometrial MUC1 expression in preparation for attachment,[59] but it may be significant that the blastocyst capsule is itself rich in mucin-like glycoproteins. As discussed previously, these capsular glycoproteins are desialylated at the time of conceptus fixation[26] by a process that must be progesterone dependent, since it fails if luteolysis is induced prior to fixation.[27] In short, desialylation of the capsular glycoproteins may be an equine specific equivalent to the progesterone primed dismantling of endometrial surface mucins in the preparation for trophectoderm-endometrium attachment. Whether degradation of the capsule alone is sufficient to permit attachment is not known, and it seems more likely that luteal progesterone and conceptus derived signals combine to further promote and stabilize trophectoderm-endometrium attachment.

The need for coordinated interaction between the progesterone primed endometrium and the developing conceptus if pregnancy is to proceed normally is clearly illustrated by the delayed development of equine embryos transferred into the uterus of a recipient mare that ovulated 5 days after the donor.[60] Indeed, it is likely that a species specific minimum period of progesterone priming is required for the endometrium to be able to both provide adequate histotrophic nutrition and become receptive for implantation. Moreover, recent transcriptomic studies identified a range of cytokines, growth factors and corresponding receptors that are upregulated either in the trophectoderm, the endometrium or both, in response to luteal progesterone and/or local trophectodermal hormones including estrogens and PGE_2, in the third and fourth weeks of pregnancy.[52,61] There also appear to be roles for molecules known to be instrumental to implantation in other species, such as leukaemia inhibitory factor,[62] osteopontin, integrins and various members, receptors and binding factors from the insulin-like and fibroblast growth factor families.[47,61,63] However, it is not known how the endometrial surface is modified to play its contrasting roles in the two distinct implantation events seen during equine pregnancy, namely invasion of the highly proliferative chorionic girdle cells versus interdigitation of the remaining, noninvasive chorionic cells.[8] Nevertheless, deficiencies in endometrial receptivity and/or aberrations in the embryo-maternal signalling processes required for implantation almost certainly contribute to the high incidence of pregnancy loss between the end of the conceptus mobile phase (day 16) and the onset of definitive placental formation (day 40).[8]

Conclusion

During the first 6 weeks of pregnancy, the equine conceptus develops from a single cell ‘zygote’ to a fetus with a functional circulation and precursors of all of the organs required for postnatal life. To ensure the successful establishment and maintenance of pregnancy, embryo-maternal signalling plays critical roles in ensuring luteal maintenance and, together with luteal progesterone, in preparing the endometrium for its role in implantation and establishment of a more stable platform for nutrient and gaseous exchange.

Conflict of interest

None to declare.

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