MATERNAL RECOGNITION OF PREGNANCY IN THE MARE – A MINI REVIEW

RECONHECIMENTO MATERNO DA GESTAÇÃO NA ÉGUA - MINI REVISÃO

Maria M FGaivão[1] and Tom A E Stout[2]

1) ULHT – Universidade Lusófona de Humanidades e Tecnologias; maria.gaivao@gmail.com

2) Utrecht University, Department of Equine Sciences, Section of Reproduction, The Netherlands.

Abstract: *A number of features of early embryonic development in equids are unusual or unique; these appear to include the critical but poorly understood mechanism(s) responsible for the 'maternal recognition of pregnancy'. Maternal recognition of pregnancy is the physiological process by which a developing conceptus signals it presence to the maternal organism to prolong the lifespan of the primary corpus luteum (CL) and thereby ensure the continued supply of progesterone that is essential for embryonic survival and development. However, it is not yet clear what the primary conceptus signal to ensure CL prolongation in the horse is, and while a number of potential contributors to maternal recognition and the establishment of pregnancy have been proposed, none have been able to satisfactorily fulfill the criteria required of an intrauterine luteostatic or antiluteolytic factor. On the other hand, it is generally accepted that maternal recognition of pregnancy is of critical importance and that failure to either send or receive the signal appropriately is likely to lead to early embryonic death. Indeed, pregnancy loss at or soon after the expected time of maternal pregnancy recognition (days 10-16 of gestation) is a common, but unpredictable (and therefore difficult to prevent), occurrence in clinical practice and a considerable source of financial loss to the breeding industry*

Resumo: *Nos equinos, um grande número de eventos das fases iniciais do desenvolvimento embrionário são únicos e distintos daqueles que se verificam em outras espécies, incluindo os críticos mas pouco esclarecidos mecanismos de reconhecimento materno da gestação. Este processo fisiológico, através do qual o concepto sinaliza a sua presença ao organismo materno assegurando a manutenção do corpo lúteo (CL) primário e, consequentemente, dos níveis de progesterona necessários à sobrevivência e desenvolvimento do embrião, está pouco esclarecido. De facto, ainda não é claro qual o sinal embrionário primário que assegura a manutenção do CL nos equinos e, apesar do número de potenciais factores que contribuem para o reconhecimento e manutenção da gestação não parar de crescer, nenhum é capaz por si só de satisfazer todos os critérios que caracterizam um factor anti-luteolítico ou luteostático. Por outro lado, é geralmente aceite o conceito de que o reconhecimento materno da gestação é um fenómeno fisiológico de extrema importância e que qualquer falha, quer no envio quer na recepção do sinal apropriado, pode levar a morte embrionária. De facto, a perda de gestações durante ou à volta do espaço de tempo em que ocorre o reconhecimento materno da gestação (i.e dias 10-16 após a ovulação) é comum mas imprevisível (e, portanto, difícil de controlar e prevenir) na prática clínica, sendo uma causa de grandes perdas económicas.*

INTRODUCTION

Current State of Art

'Maternal recognition of pregnancy' (MRP) is the physiological process by which the developing conceptus signals its presence to the maternal organism to prolong the lifespan of the primary corpus luteum (CL) and thereby ensure a continuing supply of the progesterone on which embryonic survival and development are critically dependent (18, 1, 26). As such, MRP is an essential step in the establishment of pregnancy in many eutherian mammals. However, the nature of this process in the horse differs markedly to that in other large domestic animal species. In particular, the equine embryo remains discrete, spherical and enveloped by an unusual acellular glycoprotein capsule throughout the period of MRP, rather than undergoing the dramatic elongation seen in both the pig and ruminants and which, in these latter species, serves to ensure that the developing conceptus comes into contact with as much of the endometrium as possible. Instead, the goal of interacting with as much endometrium as possible during the period of pregnancy recognition is achieved in the equids by the conceptus migrating continuously throughout the entire uterine lumen propelled by myometrial contractions stimulated by conceptus-directed prostanoid production (12, 28). As well as being essential for successful pregnancy recognition and maintenance in the mare (20, 23), conceptus migration may also aid development by allowing more effective harvesting of the uterine secretions that form the only nutrient source in the period before the formation of the definitive (chorioallantoic) placenta; a process which does not begin in earnest until as late as day 45 of gestation in the horse (26).

An unusual and early additional form of ‘pregnancy recognition’ also occurs in the horse, in that only developing embryos are able to initiate transport into the uterus; unfertilized ova are retained at the ampullary-isthmic junction of the oviduct where they slowly degenerate (31, 7). The ability of the oviduct to differentiate between unfertilized oocytes and developing embryos is based on the fact that only the latter are able to secrete prostaglandin E2 (PGE2). Indeed, when an embryo reaches the compact morula stage of development on Day 5 (Day 0 = day of ovulation) it begins to secrete appreciable quantities of this hormone (34), which acts locally to relax the circular smooth muscle fibers in the oviduct wall causing the ampullary-isthmic sphincter to open, and thereby allowing the embryo to pass through and enter the uterus. In fact, the equine embryo spends nearly all of its 6-day period of oviductal development close to the ampullary-isthmic junction, whereas passage through the isthmus is rapid (hours: 35). This extraordinarily prolonged period during which the equine embryo develops in the oviduct is in marked contrast to the 48 h at which the 4-cell pig embryo (11) or the 72 h at which the 8-cell ruminant embryo (for review see 17) enter the uterine lumen; moreover, it is disadvantageous with respect to the development of certain assisted reproductive technologies, such as embryo cryopreservation (for review see 30).

Embryos typically enter the uterine lumen between 144 and 168 hours after ovulation (4, 5). At around the time of uterine entry, the equine embryo undergoes blastulation and its first morphologically obvious cell differentiation, into trophectoderm and inner cell mass (ICM). Indeed, while incipient blastocyst cavity formation has been recorded as early as 5.5 days after ovulation, Day 6.5 and early Day 7 blastocysts tend to show incompletely segregated ICMs, and it is not until late on Day 7 that equine blastocysts typically have a well defined ICM and the zona pellucida begins to dehiscence to reveal the newly-formed, underlying blastocyst capsule (8).

In the absence of a conceptus, the mare will return to oestrus as a result of the luteolysin, prostaglandin F2α (PGF2α; 10), which is released in intermittent pulses by the endometrium from around days 12-14 after ovulation. Whereas in the ruminant species endometrial PGF2α is transferred directly from uterine vein to ovarian artery by a counter-current exchange system created by the intimate intertwining of these two vessels, no such anatomical relationship exists in the mare (15) and the PGF2α is instead transported to the ovary via the systemic circulation. The intrauterine migration of the conceptus appears to be necessary to ensure frequent repeated interaction with a large proportion of the endometrium so as to adequately suppress (32, 6) PGF2α secretion. In recent years, it has become clear that cyclical luteolysis in the mare depends on oxytocin secreted either by the hypothalamus or the endometrium itself (33) acting on endometrial receptors to trigger the synthesis and release of the luteolytic pulses of PGF2α.

In the pregnant mare, the presence of the conceptus suppresses the normal cyclical increase in OT receptor numbers and affinity (24, 27), and thereby inhibits the synthesis and secretion of PGF2α (22, 16). It has also been suggested that pregnant mare endometrium produces a potent prostaglandin synthesis inhibitor (EPSI) that blocks the conversion of arachidonic acid to PGF2α (25). Although these two mechanisms may work in concert to prevent luteolysis, the biochemical signal(s) with which the equine conceptus “informs” the mare of its presence and initiates these processes has yet to be identified. Unlike in ruminants, the equine conceptus does not produce significant quantities of interferon-like molecules with anti-luteolytic properties (3). And while the equine conceptus, like the pig, does secrete appreciable amounts of estrogens from as early as day 6 after ovulation (21) and throughout the period of MRP (9), there is no convincing evidence that conceptus oestrogens are anti-luteolytic in the mare. The completion of classical maternal recognition of pregnancy (i.e., successful prolongation of the lifespan of the primary CL) is temporally coupled with a cessation in conceptus migration on Days 16-17 (fixation) after ovulation (14, 19, 20, 28), where fixation appears to be a result of the continued increase in conceptus diameter combined with a marked increase in uterine tone, such that the conceptus becomes lodged at the base of one of the uterine horns (13,14) (Figure 1). Beyond the period of conceptus fixation and before the development of the endometrial cups at around day 35 of gestation, the uterus appears to belatedly develop the ability to secrete PGF2α in response to oxytocin challenge, suggesting that continued prevention of luteolysis in the day 18-35 period must depend on an alternative mechanism (29).

CONCLUSION

In conclusion, our knowledge of the unique features of early equine embryonic development has improved greatly during the last few years, and the number of postulated contributors to MRP and the establishment of pregnancy is beginning to grow. Because of the relatively high incidence of pregnancy loss at around the time of MRP, there is a clear need to identify conceptus-derived signals that are involved in effecting pregnancy recognition, and it is to be expected that such signals will be secreted by viable pregnancies in the period between arrival of the embryo in the uterus and fixation at the base of one of the uterine horns (i.e. days 7-17). However, it is not yet clear whether the embryonic signaling molecule will be detectable in, or able to exert its effects if introduced via, the systemic circulation. Nor is it clear exactly how the conceptus engineers the temporary suppression in the ability to release PGF2α in response to oxytoxin challenge. This highlights how little we currently understand of the complex signal transduction pathways that

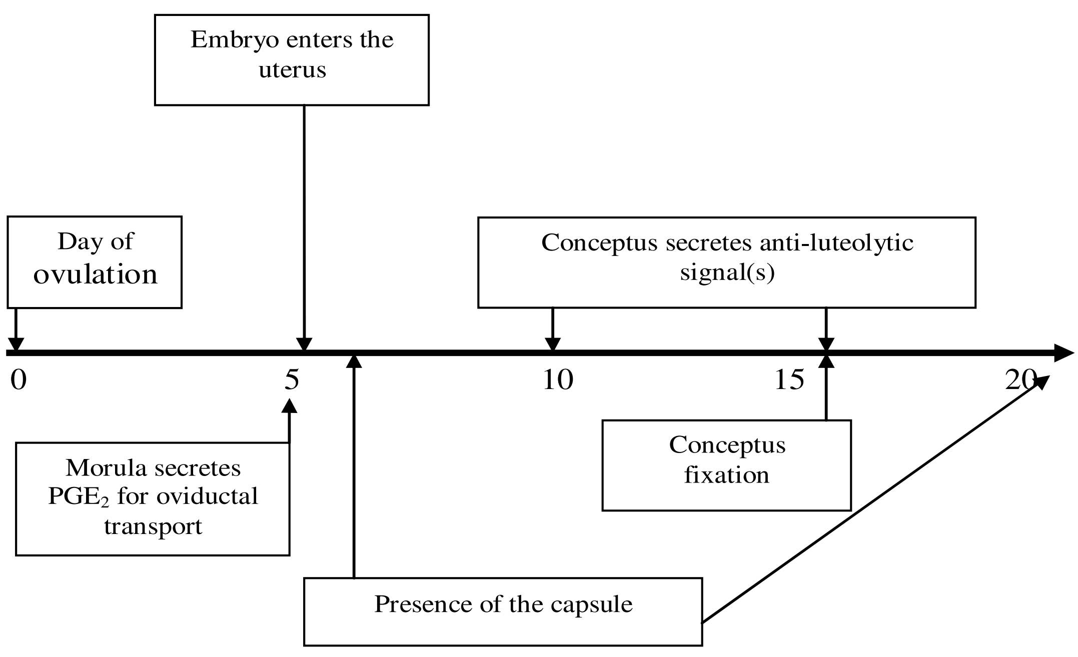

Figure 1 - Significant events involved in the establishment of pregnancy in the mare. The embryo enters the uterus at around Day 5-6 as a result of PGE_2 secretion. Subsequently, and due largely to the presence of the blastocyst capsule, the conceptus remains spherical and mobile within the uterine lumen until Day 16 (fixation), when maternal recognition of pregnancy has been completed (adapted from 2**).**

are likely to interact during this critical developmental process. In addition, it is becoming increasingly clear in many species that MRP is a sufficiently important process that even where a well characterized primary signaling pathway or factor exists, there may be additional less well described back-up pathways to ensure corpus luteum prolongation in the event of marginal production of the primary MRP factor. Similarly, it is likely that successful establishment and maintenance of pregnancy in the horse involves a combination of overlapping signaling events as part of an ongoing maternal-fetal "dialogue".

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