Veterinary and Animal Science 28 (2025) 100441

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

The early pregnancy in mares - What do we still not know?

Marta Siemieniuch-Tartanus

Department of Large Animals Diseases with the Clinic, Institute of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 100, 02-787 Warsaw, Poland

ARTICLE INFO

Keywords:
Embryo
Conceptus
Early pregnancy
Maternal recognition of pregnancy
Mare

ABSTRACT

Equine conceptus development is a critical study area due to its significant implications for the equine breeding industry. Following a high fertilization success rate of 71–96 % (Ball, 1988), it is concerning that 30–40 % of developing embryos may not survive beyond the crucial initial two weeks of gestation, coinciding with the onset of gastrulation (Ball, 1988).

The pregnancy in mares lasts 330–345 days. The underlying molecular mechanisms of the embryonic period are not fully explained in mares. During early pregnancy in mares, the following takes place: the descent of the blastocyst from the oviduct to the uterus around 5–6 days post-fertilization; migration of the spherical conceptus, surrounded by a glycoprotein capsule in the uterine lumen; fixation of the vesicle near the base of the uterine horn around 16–17 days post-fertilization; implantation; development of endometrial cups that produce chorionic gonadotropin, as well as an additional corpus luteum that produces progesterone. In mares, there is no clear determination of what constitutes the early signal of pregnancy recognition. The results of previous research indicate that mechanical stimuli, *i.e.* the movement of the conceptus in the uterine lumen, trigger a cascade of molecular events in the endometrium responsible for the luteostasis and the maintenance of early pregnancy in mares.

This study aims to provide a synthetic summary of the knowledge we have gained so far about early pregnancy and to attempt to answer the question of what molecular mechanisms underlie maternal recognition of pregnancy in mares.

1. Introduction

The development of equine conceptuses is a critical area of study due to its significant implications for the equine breeding industry. Despite high fertilization rates ranging from 71 % to 96 % (Ball, 1988), a substantial number of embryos-approximately - 30–40 % - do not survive past the early stages of gestation, particularly during the first two weeks when gastrulation occurs (Ball, 1988). This early embryonic loss represents a considerable economic burden, especially given the advancements in treatments for common sub-fertility issues and assisted reproductive technologies aimed at improving reproductive success.

The causes of early embryonic death are diverse and can include factors such as persistent mating-induced endometritis and subclinical endometritis (LeBlanc & Causey, 2009; Siemieniuch et al., 2016, 2019; Gajos et al., 2015), endometrial fibrosis (Szóstek et al., 2012, 2013), cervical incompetence, and corpus luteum (CL) failure, as well as chromosomal and genetic anomalies (LeBlanc, 2004). This problem is particularly pronounced in older mares with a history of reproductive challenges, where studies have reported embryonic loss rates between 62 % and 73 % in the critical days following ovulation. In these cases, the loss is often attributed to intrinsic embryonic defects rather than uterine issues (Ball et al., 1989).

Given these challenges, researchers and practitioners in the equine reproductive field must continue investigating the underlying mechanisms of embryonic development and loss. Understanding these factors can lead to improved strategies for monitoring early pregnancy and enhancing the chances of successful gestation, thereby mitigating the economic impacts and improving the overall efficiency of equine breeding programs.

Pregnancy in mares lasts about 330–345 days. The first 40 days of pregnancy constitute the embryonic period, followed by the fetal period. The embryonic period in horses lasts until the embryo reaches the peak of the embryonic vesicle, the yolk sac undergoes reduction, and the formation of the umbilical cord begins (Ginther, 1998). The embryonic period in mares can be divided into several stages: 1) the descent of the embryo from the oviduct to the uterus on the 5th-6th day after

---

E-mail address: marta_siemieniuch-tartanus@sggw.edu.pl.

https://doi.org/10.1016/j.vas.2025.100441

fertilization; 2) the intrauterine migration of the spherical conceptus, during which the yolk sac plays a dominant role in nourishing the embryo; 3) fixation of the conceptus on the 16th day after fertilization; 4) orientation of the embryonic vesicle – the positioning of the pole of the embryonic vesicle containing the embryo downward; 5) formation of endometrial cups and additional corpora lutea after five weeks from fertilization (Ginther, 1998). In mares, there is no clear determination of what constitutes the early signal of pregnancy recognition. This period in mares occurs around 10–13 days post-fertilization. Previous studies suggest the existence of a complex maternal mechanism for pregnancy recognition, which includes the migration of the embryonic vesicle in the uterine lumen, production of luteotropic prostaglandin E_2 (PGE_2), changes in the direction of luteolytic prostaglandin $F_2\alpha$ secretion ($PGF_2\alpha$), secretion of estradiol and estrone, a decrease in the expression of the oxytocin receptor in the endometrium, as well as the production of many other biologically active substances and the complicated regulation of the expression of many genes. This study aims to provide a synthetic summary of the knowledge we have gained so far about early pregnancy and to attempt to answer the question of what molecular mechanisms underlie maternal recognition of pregnancy in mares.

2. Methodology

Electronic databases of published scientific literature were the main source for this review. PubMed, Scopus and Google Scholar were searched for early pregnancy and maternal recognition of pregnancy in mares. Additional articles of interest were obtained through cross-referencing of published literature. The primary key terms used were “early pregnancy”, “gastrulation”, “embryo”, “maternal recognition of pregnancy”, and “mare” which resulted in 927 search hits. Papers not related to processes of equine embryo development and migration or the mechanisms of maternal recognition of pregnancy were excluded, leaving 86 papers that met the criteria for inclusion. Only English language papers were taken into consideration.

3. Physiology of early pregnancy in mares

Early pregnancy in mares is characterized by several unique features that distinguish horses from other mammalian species. These include: an extended period of the embryo presence in the oviduct (Freeman et al., 1991), the passage of only viable embryos from the oviduct to the uterus (Betteridge et al., 1974; J.A. Weber et al., 1991), the movement of the spherical conceptus, surrounded by a glycoprotein capsule (Oriol et al., 1993), within the uterine lumen (Ginther, 1983; Betteridge et al., 1982), and the formation of endometrial cups producing equine chorionic gonadotropin (eCG) (Wooding et al., 2001).

The process of early embryonic development begins with fertilization in the oviduct, leading to the formation of a zygote that contains all the genetic information necessary for development. Following fertilization, the zygote undergoes a series of rapid cell divisions known as cleavage, resulting in the formation of a morula, which is a compact ball of cells. As the morula develops, it consists of two distinct groups of cells: a smaller cluster of internal cells and a larger group of peripheral cells known as blastomeres. The peripheral cells are destined to become the trophoblast (or trophectoderm), which plays a critical role in implantation and the formation of the placenta. Most of these trophoblast cells will not contribute to the embryo itself but will instead give rise to the chorion, the outer layer of the fetal portion of the placenta. Initially, the morula lacks an internal cavity. However, during a process called cavitation, the trophoblast cells secrete fluid that forms small cavities (lacunae) within the mass of cells. These cavities eventually merge to create a single large cavity known as the blastocele. The formation of the blastocyst, characterized by the presence of the blastocele, marks a significant milestone in mammalian embryonic development and sets the stage for further differentiation and development of the embryo (Gilbert, 2000).

Following blastocyst formation, the cells within the blastocyst differentiate into two primary lineages: the trophoblast and the inner cell mass (ICM). The trophoblast is responsible for forming the placenta and facilitating implantation into the uterine wall, while the ICM will give rise to the embryo. After this initial allocation, both lineages undergo distinct biochemical and developmental changes. The trophoblast cells begin to express specific genes that promote their role in implantation and placentation, such as those involved in cell adhesion and invasion. In contrast, the ICM cells experience a series of changes that prepare them for subsequent differentiation into the three germ layers during gastrulation: ectoderm, mesoderm, and endoderm. This process involves the activation of specific transcription factors and signaling pathways that guide the cells toward their future fates (Imakawa et al., 2004).

As gastrulation approaches, the ICM cells undergo processes such as cell signaling, proliferation, and migration, leading to the establishment of the body plan of the developing embryo. These early developmental events are critical for ensuring the proper formation of tissues and organs in the later stages of embryogenesis (Gaivão & Stout, 2012).

The embryo descends into the uterus in the late morula or early blastocyst stage approximately 144 h (6–6.5 days) after ovulation. The movement of the embryo to the uterus is driven by PGE_2, synthesized by the living embryo from about the 4th-5th day after ovulation. PGE_2 exhibits a relaxing effect on the uterine smooth muscle, counteracting its contractility; it may also facilitate the transport of the embryo toward the uterus by relaxing the muscle in the area of the isthmus of the oviduct (J.A. Weber et al., 1991). Upon reaching the uterine lumen, the embryo rapidly grows, continuing to maintain its spherical shape. On Day 7 post-ovulation, the ICM is located on one side of the blastocyst, with a discontinuous layer of cells beneath it. Primitive endoderm cells, which are essential for early embryonic development, are initially scattered and found lining the mural trophoblast (the outer layer of the blastocyst). By Day 8, these isolated endoderm cells begin to coalesce, forming a continuous layer that constitutes the bilaminar blastocyst. This process of forming a primitive endoderm layer from scattered cells is noted to be unusual and significantly different from what is observed in other mammals (Enders et al., 1993). The formation of the bilaminar blastocyst is a critical step, as it sets the stage for further differentiation and development of the embryo, ultimately leading to the formation of the three germ layers that will give rise to all tissues and organs in the developing organism (Enders et al., 1993).

POU5F1, also known as Oct3 and Oct4, is a pivotal transcription factor that plays a crucial role in the regulation of early embryonic development and the maintenance of pluripotency in stem cells. Its significance was first highlighted in studies conducted on mice (Scholer et al., 1989b), where it was found to be expressed in pluripotent cells, particularly in embryonic stem cells and in the inner cell mass (ICM) of blastocysts. The expression of POU5F1 is essential for sustaining the pluripotent state of these cells, enabling them to differentiate into various cell types during development. Research has shown that any spontaneous or induced loss of pluripotency in stem cells correlates with decreased expression of POU5F1 (Scholer et al., 1989a). This underscores the transcription factor’s critical role not only in maintaining pluripotency but also in guiding the differentiation processes that follow during embryogenesis. The expression pattern of the POU5F1 gene, which encodes a key transcription factor involved in maintaining pluripotency in early embryonic development, exhibits significant variability among different species. In many species, maternal POU5F1 protein is present in oocytes and can be detected in early embryos. The transition to embryonic expression typically occurs around the 4- to 16-cell stage of development (Palmieri et al. 1994; Cauffman et al. 2005). The study by Choi et al. (2009) provides important insights into the role of the POU5F1 protein during the early development of equine embryos. POU5F1 protein is initially found in both the cytoplasm and nucleus of immature equine oocytes. Its levels decrease during the first five days of embryonic development, likely due to the depletion of maternal protein and mRNA stores. After day 5, POU5F1 levels increase again, indicating the activation of transcription from the embryonic genome. POU5F1 is expressed in the trophectoderm (TE) of equine blastocysts produced *in vitro*. However, this expression is suppressed in the TE of embryos recovered from the uterus, whether produced *in vivo* or transferred after *in vitro* production. This suggests that the uterine environment is crucial for the proper regulation of POU5F1 and subsequent differentiation of the TE. The abnormal expression of POU5F1 *in vitro* cultured equine embryos may contribute to developmental issues, such as the failure to form the embryonic capsule and improper allocation of cells to the inner cell mass (ICM) and endoderm. This supports findings from previous studies indicating that *in vitro* conditions do not adequately replicate the *in vivo* environment needed for normal equine embryo development (Buehr et al., 2003; Choi et al., 2009). The lack of normal POU5F1 signaling observed in equine embryos *in vitro* may explain the failure of the embryonic capsule to form in cultured equine embryos (Choi et al. 2004), and underlie the apparent failure of allocation of internal cells to ICM and endoderm in these embryos (Hinrichs et al. 2007). The development of equine embryos *in vitro* can be influenced by various transcriptional factors. While some transcriptional factors have been identified in the context of horse embryogenesis, it is indeed possible that additional factors remain to be discovered.

Around 9 days post-ovulation, the conceptus (the products of conception of all stages of development, embryo proper plus the extra-embryonic tissues) begins to migrate within the uterine cavity. Between days 9 and 15 post-fertilization, it moves within the uterine cavity 10–20 times a day. The intense motility of the conceptus is associated with the high contractile activity of the muscle layer of the uterus at that time. The conceptus moves at a speed of 3.4 mm/min (Ginther, 1998). The greatest activity of the equine conceptus occurs between days 10–14 post-ovulation, up to about day 16 when the embryo anchors at the base of one of the uterine horns (Ginther, 1983). It has been found that the migration of the conceptus throughout the entire uterus is essential for maintaining pregnancy, as closing one horn leads to luteolysis and pregnancy termination (McDowell et al., 1985; Stout & Allen, 2001). During the migration within the uterus, the conceptus signals its presence to the endometrium, triggering signaling cascades in the endometrium and ovaries that lead to further production of progesterone by the corpus luteum (CL) (D.C. Sharp et al., 1989).

4. The capsule

The zona pellucida is a crucial structure in the reproductive process of many large domestic animals, serving as the primary extracellular coating that surrounds the oocyte and developing conceptus before implantation. It plays an important role in fertilization, protection of the oocyte, and early embryonic development. Approximately 8 days post-ovulation, the zona pellucida, which surrounds the embryo, begins to break down. Instead, an acellular glycoprotein capsule primarily consisting of mucin proteins (Oriol et al., 1993) and uterocalin, produced under the influence of progesterone, which binds fatty acids and retinol, forms around the blastocyst (Oriol et al., 1993; Smits et al., 2012; Squire et al., 2001). This blastocyst capsule provides additional protection and support to the developing conceptus during the critical early stages of gestation, specifically during the second and third weeks post-fertilization. The presence of the blastocyst capsule in horses is significant because it helps maintain the integrity of the developing embryo as it moves through the uterine environment. This unique adaptation is thought to be important for the successful implantation and development of the equine embryo, contributing to the overall reproductive success of the species (Betteridge et al. 1982). The transition from the zona pellucida to the capsule is an important developmental process in equine embryos. Initially, the zona pellucida provides a protective environment for the embryo as it travels through the reproductive tract. However, as the embryo enters the uterus, the capsule becomes the primary protective layer. The capsule is composed of glycoproteins and plays a crucial role in supporting the embryo's development and facilitating interactions with the uterine environment. This change occurs around day 7 post-ovulation, which is critical for further embryonic development. The reduction in thickness of the zona pellucida and its eventual disappearance allow for the embryo to establish connections with maternal tissues, which are essential for nutrient transfer and hormonal signaling.

The presence of this capsule gives the embryo a spherical shape while moving inside the uterus. It is assumed that it also plays a role in protecting the embryo from potential infectious agents (Oriol, 1994). It has also been noted that during the migration of the conceptus, proteins produced by uterine glands adhere to the capsule, and it may play an important role in nourishing the embryo, especially since its growth during this period is very intense (Allen, 2001). Embryos that do not form a capsule are unable to develop properly. This is likely related to the fact that other biologically active factors are associated with the glycoproteins of the capsule, which play a role in the development of the embryo. Furthermore, the capsule possesses anti-adhesive properties, allowing the embryo to migrate within the uterine lumen, which is one of the most significant anti-luteolytic mechanisms (Stout et al., 2005; Arar et al., 2007). Around the 21st day of pregnancy, the glycoprotein capsule disappears (Allen, 2001).

5. Maternal recognition of pregnancy in mares

Maternal recognition of pregnancy in the mare is a unique process that differs significantly from the mechanisms seen in other large domestic animals. In mares, the conceptus, which is the developing embryo and associated tissues, plays a crucial role in signaling its presence to the mother to ensure the continued production of progesterone, a hormone vital for maintaining pregnancy (Kastelic et al., 1987; Allen, 2001; Spencer et al., 2004). In most large domestic species, such as cattle and sheep, the conceptus produces specific signals that directly act on the maternal uterus to prevent the regression of the corpus luteum (CL) after fertilization. In cows, for instance, interferon-tau is the key signal that inhibits prostaglandin production, which would otherwise lead to the lysis of the CL (Johnson & Bazer, 2024).

In the case of horses, it has not been clearly established what signals confirm early pregnancy (Bazer, 1992; Johnson & Bazer, 2024).

The unique characteristics of equine embryonic development, particularly the maintenance of a discrete and spherical shape, set it apart from other species such as pigs and ruminants. In those species, rapid elongation of the embryo serves to maximize contact with the endometrium, which is crucial for pregnancy recognition. In contrast, equine embryos achieve a similar goal through a different mechanism: they migrate continuously throughout the entire uterine lumen. This continuous migration is facilitated by myometrial contractions, which are likely stimulated by the production of prostanoids from the conceptus. This distinct approach allows the equine embryo to interact with the endometrial lining effectively, ensuring adequate signaling for the maintenance of pregnancy (Gastal et al., 1998; Stout & Allen, 2001).

The migrating conceptus is responsible for the production of a biochemical signal that prevents luteolysis and maintains progesterone production by the corpus luteum (CL), which provides the appropriate uterine environment for the growth and survival of the embryo (Short, 1966). In horses, it is most likely a combination of mechanical stimuli (the migration of the conceptus within the uterine lumen) along with the secretion of various biologically active substances, such as 17-α-hydroxyprogesterone (Goff et al., 1993), estrogens (Flood et al., 1979), prostaglandins (PGs) (T.A.E. Stout & Allen, 2002), and interferons (Cochet et al., 2009). The migration of the embryonic vesicle within the uterus counteracts the production and secretion of $PGF_2\alpha$ by the endometrium, which has a strong luteolytic effect, meaning it causes the destruction of the CL that supports the pregnancy and the resumption of cyclic ovarian activity. In mares, the so-called maternal recognition of pregnancy is largely the result of the movement of the conceptus within the uterine lumen (Martal et al., 1994) and plays a role until approximately day 15 post-fertilization (Martal et al., 1990). The presence of the embryo inhibits the pulsatile release of $PGF_2α$ in response to oxytocin (OT) released from the posterior pituitary (Vanderwall et al., 1998) or from the endometrium (Stout et al., 2000) between days 10 and 16 after ovulation (Goff et al., 1987). From day 12 of pregnancy, there is a decrease in the expression of genes for PG synthases (PTGS2) and OT receptors in the endometrium of pregnant mares (Boerboom et al., 2004). Moreover, equine embryos produce interferon D (IFND) (Lefèvre et al., 1998), PGE_2 (T.A. Stout & Allen, 2002), insulin-like growth factor 1 (IGF1) (D.C. Sharp et al., 1989), and estradiol (E2) (Zavy et al., 1979), all of which may participate in maternal recognition of pregnancy, embryo migration (Stout & Allen, 2001), angiogenesis and vasculogenesis/vascularization (Silva et al., 2005), and secretory activity of the uterus (Zavy et al., 1982). In cyclic mares, the increase in $PGF_2α$ concentration in both the uterine venous blood and uterine flushings is observed between days 14 and 16 of the cycle. This rise in $PGF_2α$ coincides with luteolysis, marked by a decline in plasma P4 levels, as $PGF_2α$ is a key mediator in the regression of the CL. Luteal cells have a high density of $PGF_2α$ receptors from Day 14 of the estrous cycle up until estrus, as well as on Day 18 of pregnancy. This suggests that the luteal cells remain responsive to $PGF_2α$ during this critical period (Bazer et al., 2009; de Ruijter-Villani & Stout 2015). However, in the context of pregnancy, the presence of the equine conceptus plays a significant role in altering the normal luteolytic process. The conceptus exhibits migratory behavior between the two uterine horns until it becomes fixated on Day 18 of pregnancy. During this time, it exerts an antiluteolytic effect, leading to a reduction in $PGF_2α$ concentrations in uterine fluids and venous plasma. Interestingly, in pregnant mares, there is no observed pulsatile pattern of $PGF_2α$ secretion. Furthermore, the conceptus inhibits the endometrial production of $PGF_2α$ in response to cervical stimulation (via the Ferguson Reflex) and exogenous oxytocin (OXT). This inhibition is associated with a decrease in the expression of endometrial oxytocin receptors (OXTR) in pregnant mares, which further supports the idea that the presence of the conceptus alters the normal endocrine responses that typically occur during the estrous cycle (Bazer et al., 2009; de Ruijter-Villani & Stout 2015).

In the case of pregnant mares, there is a decrease in the concentration of luteolytic $PGF_2α$, and additionally, there is an absence or a significantly reduced number of OXTR in the uterine smooth muscle, without which uterine contractility will not be possible. The lack of OXTR activity ensures relaxation of the uterus, which is essential during pregnancy. The mechanism that conditions the movement of the conceptus within the uterus during early pregnancy is not clearly explained. Between days 8 and 20 post-fertilization, high concentrations of estradiol and estrone secreted by the equine conceptus are observed. However, experiments involving the administration of these hormones to prolong the endocrine activity of the CL have not yielded conclusive results, nor has the administration of PGE_2 alone. Swegen et al. (2017) conducted a proteomic analysis of embryos collected from the uterus on the 8th day post-ovulation. The embryos were then cultured *in vitro*, and the secretory products released into the culture medium, as well as those contained in the blastocoel of the embryos and the acellular capsule surrounding the embryo, were subjected to proteomic analysis after the first and second days of culture. Blastocysts produced a pregnancy-specific proteinase (PAG) on day 10, and the secretion of prostaglandin receptor inhibiting protein (PTGFRN) and a progesterone potentiating factor (FKBP4) was also confirmed in the fluid present in the blastocoel (Swegen et al., 2017). Since the PGE_2 receptors were not detectable as expressed in the equine embryo, the results obtained suggest that PGE_2 produced by the embryo mainly acts on the endometrium, while $PGF_2α$ affects both the embryo and the endometrium (Vegas et al., 2022). It can further be presumed that the production of estrogens and/or proteins by the embryo in early pregnancy inhibits the synthesis and release of the luteolytic $PGF_2α$ by the endometrium and sustains the secretory activity of the CL, participating in the luteostasis and maintenance of early pregnancy.

The rapid development of advanced molecular methods in recent years has allowed for the use of modern techniques to assess the transcriptome and proteome of both the endometrium and the embryo during maternal recognition of pregnancy (Smiths et al., 2018, 2020; Vegas et al., 2021; Scaravaggi et al., 2019; C. Klein, 2015; C. Klein & Troedsson, 2011). The transcriptome of endometrial biopsies was analyzed using microarrays and RNA sequencing (RNA-seq) (Klohonatz et al., 2015). The transcriptome in the glandular, luminal epithelium and stromal cells of the endometrium was also evaluated using laser capture microdissection (Vegas et al., 2022; Scaravaggi et al., 2019). In the search for factors that may play a role during maternal recognition of pregnancy, the transcriptome of embryos (Smits et al., 2020; C. Klein, 2015; C. Klein & Troedsson, 2011) and the miRNA profile in uterine fluid (Smits et al., 2020) were analyzed. One of the recently published studies examined the transcriptome, miRNA, and proteome of horse embryonic vesicles between days 10 and 13 of pregnancy, which is the period during which maternal recognition of pregnancy occurs (Vegas et al., 2022). miRNAs are considered regulatory factors for gene expression during pregnancy, especially during embryonic development (Morales Prieto & Markert, 2011; Reza et al., 2019). The research by Vegas et al. (2022) found that the expression profile of mRNA, miRNA, and the proteome is dynamic and more characteristic of the size of the embryonic vesicle rather than its age. The expression profile changes with the growth of the embryonic vesicle; one profile is characteristic of smaller vesicles (2.5–5 mm), while the other is typical for larger vesicles (7–11 mm) (Vegas et al., 2022). An increased expression of mRNA for genes associated with steroidogenesis was observed, including HSD3B2 (hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2, involved in P4 synthesis), CYP11A1 (cytochrome P450 family 11 subfamily A member 1), and STAR (steroidogenic acute regulatory protein) in larger embryos, especially those from day 13 of pregnancy (Vegas et al., 2022). Genes for enzymes responsible for the production of estradiol E2 were also up-regulated at this stage of development, including cytochrome P450 family 17 subfamily A member 1 (CYP17A1), CYP19A1 (aromatase, cytochrome P450 family 19 subfamily A member 1), and HSD17B1 (estrogen activation, hydroxysteroid 17-beta dehydrogenase 1) (Vegas et al., 2022). Since the equine embryo synthesizes PGE_2 at a very early stage of development, this prostaglandin has naturally been considered as one of the factors in maternal pregnancy recognition. The expression of mRNA and protein for genes belonging to the phospholipase A2 (PLA2) group, responsible for the release of arachidonic acid from cell membrane phospholipids, which is a precursor for prostaglandin synthesis, has been confirmed. The expression of mRNA and protein of PTGS1 (prostaglandin-endoperoxide synthase 1, also known as COX-1) and PTGS2 (prostaglandin-endoperoxide synthase 2, also known as COX-2) has been confirmed in embryos on days 10–13 of pregnancy and was not differentiated based on the size of the embryonic vesicle. PTGS-1 and 2 are responsible for converting arachidonic acid to prostaglandin H (PGH_2), which is then further metabolized by specific prostaglandin synthases to PGE_2 and $PGF_2α$.

In contrast to ruminants, in which interferon tau is the main signal for maternal pregnancy recognition (Bazer, 1992; Johnson & Bazer, 2024), the role of interferons in pregnancy recognition in horses remains unclear and in this case the horse conceptus is much more similar to the pig conceptus in making IFNs that are not antiluteolytic (La Bonnardiere, 1993; La Bonnardierre et al., 1991). Considering the results of transcriptomic studies, essentially none of them are considered the sole signal for early pregnancy recognition (Budik et al., 2010; C. Klein & Troedsson, 2011; Lefèvre et al., 1998; Vegas et al., 2022).

6. Fixation and implantation of the embryo

The fixation of the embryo at the base of one of the two horns of the uterus occurs 15–17 days after fertilization. The embryo does not need to be located on the same side as the ovary from which the oocyte was released. Although in mares bred during the post-foaling estrus, implantation usually occurs in the previously non-pregnant horn, which is related to the faster involution of that uterine horn. Around the 16th day, the conceptus changes its shape from round to triangular and significantly increases in size. The cessation of the conceptus's migration and its fixation is associated with several phenomena. The reduction of progesterone receptor expression in the uterine epithelium and the loss of the glycoprotein capsule of the embryo allow for the attachment of the embryonic trophoblast to the endometrial epithelial cells, inducing the proliferation of trophoblast cells and modifying the epithelial glycocalyx in the uterus (Carson et al., 1998; Dy et al., 2004; Wilsher et al., 2013). Rather, processes related to the desialylation of the capsule allow for the expression of the embryo's adhesive capabilities (Arar et al., 2007). Progesterone produced by the CL and embryonic estradiol most likely stabilize the attachment of the trophectoderm to the epithelial cells of the endometrium by increasing the expression of integrin αVβ3, fibronectin, fibrinogen, and CD44 (C. Klein, 2015). During a rectal examination during this period, a change in the tension of the uterine wall can be felt, which becomes thicker and more elastic. The increase in uterine wall tension is most likely related to the increased amount of estradiol secreted by the embryo. Once the conceptus is established at the base of one of the horns, there is the orientation of the embryo, which involves positioning the embryo in a ventral location within the vesicle (Ginther, 1998; Silva & Ginther, 2006). Genes encoding cytokines, growth factors, hormones, and their receptors are strongly expressed in the trophectoderm, the endometrium, or both structures between the 3rd and 4th week of pregnancy in mares (C. Klein & Troedsson, 2011; C. Klein, 2015). These include, among others: genes encoding aromatase, leukemia inhibitory factor and its receptor, interleukin-6, proteins belonging to fibroblast growth factors, and proteins that bind insulin-like growth factors (C. Klein & Troedsson, 2011; de Ruijter-Villani et al., 2013; Herrler et al., 2000).

The yolk sac in mares, unlike in most domestic animals, plays a significant role in embryo development for a relatively long time, and for the first 3–4 weeks of pregnancy, it is an essential structure that nourishes the embryo (Sharp, 2000). The yolk sac absorbs nutrients from the uterine environment and provides them to the developing conceptus. On day 21 post-fertilization, an amniotic cavity can be seen on ultrasound imaging. As the pregnancy progresses, the yolk sac space decreases in favor of the allantoic cavity, and around day 30 of pregnancy, their spaces become comparable, with the embryo located between them. During this period, the embryo begins to move upward in the embryonic vesicle, while the yolk sac gradually diminishes, and the allantoic cavity enlarges. At the junction of the yolk sac and the amniotic cavity, around the 30th day of pregnancy, a chorionic ring forms, which is a structure ranging in width from one to several millimeters. The chorionic ring is formed by cells derived from the trophoblast, which migrate to the endometrium between the 36th and 38th days of pregnancy and ultimately settle in the connective tissue stroma of the uterine mucosa, between the uterine glands, creating structures characteristic of this species, raised above the surface of the endometrium, called endometrial cups. The endometrial cups produce the pregnancy hormone - equine chorionic gonadotropin (eCG) (choriogonadotropin from pregnant mares). The hormone eCG exhibits biological actions similar to follicle-stimulating hormone (FSH) and luteotropic hormone (LH), in a ratio of 1.4:1. It is believed that the role of eCG is to support the additional corpora lutea that form during this period of pregnancy, thus acting as a luteotropic hormone (Ousey, 2004). Approximately during the formation of the endometrial cups and after they begin their hormonal activity, *i.e.* around the 40th-42nd day of pregnancy, the embryonic period ends, and the fetal period begins. The endometrial cups reach their maximum size around the 60th-70th day of pregnancy and produce eCG until about the 100th-120th day of pregnancy. If the embryo or fetus dies after the 35th day of pregnancy, the cyclic sexual activity of the mare is inhibited until the endometrial cups regress. As a result, the mare will not conceive again in that breeding season. Additional corpora lutea form on the ovaries as a result of the ovulation of ovarian follicles, or there may be luteinization without ovulation of the oocyte. The role of these corpora lutea is to provide additional production of progesterone, which plays a crucial role in maintaining pregnancy during the early stages in mares.

7. Conclusion

Early pregnancy in mares involves a series of biological processes, without which proper embryo development, and subsequently implantation and placentation, would be impossible. The dialogue between the endometrium and the embryo during maternal recognition of pregnancy is very complex and, as seen from research, is not limited to the production of one or several factors by the embryo. The process of maternal recognition of pregnancy in mares can rather be compared to an orchestra performing a complicated piece; without the participation of even one instrument, the concert would be incomplete.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of this paper. No conflict of interest is present.

This manuscript is a review and does not need any ethical statement.

Ethical statement

This manuscript is a review and does not need any ethical statement.

CRediT authorship contribution statement

Marta Siemieniuch-Tartanus: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The embryo and *in vitro* laboratory photos come from the Department of Large Animal Diseases and the Clinic, Warsaw University of Life Science, for which I thank Dr. Monika Petrajtis-Gołobów. I thank Professor Kiyoshi Okuda, Obihiro University of Agriculture and Veterinary Medicine, Japan for critical evaluation of the manuscript.

References

Allen, W. R. (2001). Fetomaternal interactions and influences during equine pregnancy. *Reproduction, 121*, 513–527.

Arar, S., Chan, K. H., Quinn, B. A., Waelchli, R. O., Hayes, M. A., Betteridge, K. J., & Monteiro, M. A. (2007). Desialylation of core type 1 O-glycan in the equine embryonic capsule coincides with immobilization of the conceptus in the uterus. *Carbohydrate Research, 342*, 1110–1115. https://doi.org/10.1016/j.carres.2007.02.016

Ball, B. A. (1988). Embryonic loss in mares. Incidence, possible causes, and diagnostic considerations. *Veterinary Clinics of North America: Equine Practice, 4*, 263–290.

Ball, B. A., Little, T. V., Weber, J. A., & Woods, G. L. (1989). Survival of day-4 embryos from young, normal mares and aged, subfertile mares after transfer to normal recipient mares. *Journal of Reproduction and Fertility, 85*, 187–194.

Bazer, F. W. (1992). Mediators of maternal recognition of pregnancy in mammals. *Proceedings of the Society for Experimental Biology and Medicine, 199*, 373–384.

Bazer, F. W., & Johnson, G. A. (2024). Early Embryonic Development in Agriculturally Important Species. *Animals, 14*, 1882. https://doi.org/10.3390/ani14131882

Bazer, F. W., Spencer, T. E., Johnson, G. A., Burghardt, R. C., & Wu, G. (2009). Comparative aspects of implantation. *Reproduction, 138*, 195–209. https://doi.org/10.1530/REP-09-0158

Betteridge, K. J., & Mitchell, D. (1974). Direct evidence of retention of unfertilized ova in the oviduct of the mare. *Journal of Reproductive Fertility, 39*, 145–148. https://doi.org/10.1530/jrf.0.0390145

Betteridge, K. J., Eaglesome, M. D., Mitchell, D., Flood, P.. F., & Beriault, R. (1982). Development of horse embryos up to twenty-two days after ovulation: observations on fresh specimens. *Journal of Anatomy, 135*, 191–209.

Boerboom, D., Brown, K. A., Vaillancourt, D., Poitras, P., Goff, A. K., Watanabe, K., Dore, M., & Sirois, J. (2004). Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. *Biology of Reproduction, 70*, 391–399. https://doi.org/10.1095/biolreprod.103.020800

Budik, S., Lussy, H., & Aurich, C. (2010). Quantification of different type I interferon transcripts in equine embryos at days 10 to 16 of gestation. *Animal Reproduction Science, 121*, 307–308. https://doi.org/10.1016/j.anireprosci.2010.04.058

Buehr, M., Nichols, J., Stenhouse, F., Mountford, P., Greenhalgh, C. J., Kantachuvesiri, S., Brooker, G., Mullins, J., & Smith, A. G. (2003). Rapid loss of Oct-4 and pluripotency in cultured rodent blastocysts and derivative cell lines. *Biology of Reproduction, 68*, 222–229. https://doi.org/10.1095/biolreprod.102.006197

Carson, D. D., DeSouza, M. M., Kardon, R., Zhou, X., Lagow, E., & Julian, J. (1998). Mucin expression and function in the female reproductive tract. *Human Reproduction Update, 4*, 459–464. https://doi.org/10.1093/humupd/4.5.459

Cauffman, G., Van de Velde, H., Liebaers, I., & Van Steirteghem, A. (2005). Oct-4 mRNA and protein expression during human preimplantation development. *Molecular Human Reproduction, 11*, 173–181. https://doi.org/10.1093/molehr/gah155

Choi, Y. H., Harding, H. D., Hartman, D. L., Obermiller, A. D., Kurosaka, S., McLaughlin, K. J., & Hinrichs, K. (2009). The uterine environment modulates trophectodermal POU5F1 levels in equine blastocysts. *Reproduction, 138*, 589–599. https://doi.org/10.1530/REP-08-0394

Choi, Y. H., Roasa, L. M., Love, C. C., Varner, D. D., Brinsko, S. P., & Hinrichs, K. (2004). Blastocyst formation rates in vivo and in vitro of in vitro-matured equine oocytes fertilized by intracytoplasmic sperm injection. *Biology of Reproduction, 70*, 1231–1238. https://doi.org/10.1095/biolreprod.103.023903

Cochet, M., Vaiman, D., & Lefèvre, F. (2009). Novel interferon delta genes in mammals: cloning of one gene from the sheep, two genes expressed by the horse conceptus, and discovery of related sequences in several taxa by genomic database screening. *Gene, 433*, 88–99. https://doi.org/10.1016/j.gene.2008.11.026

de Ruijter-Villani, M., van Boxtel, P. R., & Stout, T. A. (2013). Fibroblast growth factor-2 expression in the preimplantation equine conceptus and endometrium of pregnant and cyclic mares. *Theriogenology, 80*, 979–989. https://doi.org/10.1016/j.theriogenology.2013.07.024

de Ruijter-Villani, M., & Stout, T. (2015). The role of conceptus-maternal signaling in the acquisition of uterine receptivity to implantation in mammals. *Reproduction in Domestic Animals, 50*, 7–14. https://doi.org/10.1111/rda.12527

Dy, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., & Wang, H. (2004). Molecular cues to implantation. *Endocrinology Reviews, 25*, 341–373. https://doi.org/10.1210/er.2003-0020

Enders, A. C., Schlafke, S., Lantz, K. C., & Liu, I. K. (1993). Endoderm cells of the equine yolk sac from day 7 until formation of the definitive yolk sac placenta. *Equine Veterinary Journal, 25*(S15), 3–9.

Flood, P. F., Betteridge, K. J., & Irvine, D. S. (1979). Oestrogens and androgens in blastocoelic fluid and cultures of cells from equine conceptuses of 10-22 days gestation. *Journal of Reproduction and Fertility. Supplement, 27*, 413–420.

Freeman, D. A., Weber, J. A., Geary, R. T., & Woods, G. L. (1991). Time of embryo transport through the mare oviduct. *Theriogenology, 36*, 823–830. https://doi.org/10.1016/0093-691X(91)90348-H

Gaivão, M., & Stout, T. (2012). Equine conceptus development – a mini review. *Revista Lusófona de Ciência e Medicina Veterinária, 5*, 64–72.

Gajos, K., Kozdrowski, R., Nowak, M., & Siemieniuch, M. J. (2015). Altered secretion of selected arachidonic acid metabolites during subclinical endometritis relative to estrous cycle stage and grade of fibrosis in mares. *Theriogenology, 84*, 457–466. https://doi.org/10.1016/j.theriogenology.2015.03.038

Gastal, M. O., Gastal, E. L., Torres, C. A., & Ginther, O. J. (1998). Effect of PGE2 on uterine contractility and tone in mares. *Theriogenology, 50*, 989–999. https://doi.org/10.1016/s0093-691x(98)00202-7

Gilbert, S. F. (2000). Early embryonic development. In S. F. Gilbert (Ed.), *Developmental Biology* (6^{a}, pp. 185–373). Sunderland: Sinauer Associates, Inc.

Ginther, O. J. (1983). Mobility of the early equine conceptus. *Theriogenology, 19*, 603–611.

Ginther, O. J. (1998). *Equine pregnancy: physical interactions between the uterus and conceptus, 44* pp. 73–104). American Association of Equine Practitioners Proceedings.

Goff, A. K., Leduc, S., Poitras, P., & Vaillancourt, D. (1993). Steroid synthesis by equine conceptuses between days 7 and 14 and endometrial steroid metabolism. *Domestic Animals Endocrinology, 10*, 229–236. https://doi.org/10.1016/0739-7240(93)90027-9

Goff, A. K., Pontbriand, D., & Sirois, J. (1987). Oxytocin stimulation of plasma 15-keto-13,14-dihydro prostaglandin F2a during the oestrous cycle and early pregnancy in the mare. *Journal of Reproduction and Fertility. Supplement, 35*, 253–260.

Herrler, A., Pell, J. M., Allen, W. R., Beier, H.. M., & Stewart, F. (2000). Horse conceptuses secrete insulin-like growth factor-binding protein 3. *Biology of Reproduction, 62*, 1804–1811. https://doi.org/10.1095/biolreprod62.6.1804

Hinrichs, K., Choi, Y. H., Walckenaer, B. E., Varner, D. D., & Hartman, D. L. (2007). In vitro-produced equine embryos: production of foals after transfer, assessment by differential staining and effect of medium calcium concentrations during culture. *Theriogenology, 68*, 521–529. https://doi.org/10.1016/j.theriogenology.2007.04.046

Imakawa, K., Chang, K. T., & Christenson, R. K. (2004). Pre-implantation conceptus and maternal uterine communications: molecular events leading to successful implantation- Review. *Journal of Reproduction and Development, 50*, 155–169. https://doi.org/10.1262/jrd.50.155

Kastelic, J. P., Adams, G. P., & Ginther, O. J. (1987). Role of progesterone in mobility, fixation, orientation, and survival of the equine embryonic vesicle. *Theriogenology, 27*, 655–663.

Klein, C. (2015). Novel equine conceptus/endometrial interactions on Day 16 of pregnancy based on RNA sequencing. *Reproduction, Fertility and Development, 28*, 1712–1720. https://doi.org/10.1071/RD14489

Klein, C. (2015). Novel equine conceptus? Endometrial interactions on day 16 of pregnancy based on RNA sequencing. *Reproduction, Fertility and Development, 28*, 1712. https://doi.org/10.1071/RD14489

Klein, C., & Troedsson, M. H. (2011). Maternal recognition of pregnancy in the horse: A mystery still to be solved. *Reproduction, Fertility and Development, 23*, 952–963. https://doi.org/10.1071/RD10294

Klein, C., & Troedsson, M. H. (2011). Transcriptional profiling of equine conceptuses reveals new aspects of embryo-maternal communication in the horse. *Biology of Reproduction, 84*, 872–885. https://doi.org/10.1095/biolreprod.110.088732

Klohonatz, K. M., Hess, A. M., Hansen, T. R., Squires, E. L., Bouma, G. J., & Bruemmer, J. E. (2015). Equine endometrial gene expression changes during and after maternal recognition of pregnancy. *Journal of Animal Science, 93*, 3364–3376. https://doi.org/10.2527/jas.2014-8826

La Bonnardiere, C. (1993). Nature and possible functions of interferons secreted by the preimplantation pig blastocyst. *Journal of Reproduction and Fertility Supplement, 48*, 157–170.

La Bonnardiere, C., Martinat-Botte, F., Terqui, M., Lefevre, F., Zouari, K., Martal, J., & Bazer, F. W (1991). Production of two species of interferons by Large White and Meishan pig conceptuses during the peri-attachment period. *Journal of Reproduction and Fertility, 91*, 469–478. https://doi.org/10.1530/jrf.0.0910469

LeBlanc, M. M. (2004). *Infertility: mares that conceive and lose their pregnancy*. Ithaca: International Veterinary Information Service.

LeBlanc, M. M., & Causey, R. C. (2009). Clinical and subclinical endometritis in the mare: both threats to fertility. *Reproduction in Domestic Animals, 44*, 10–22. https://doi.org/10.1111/j.1439-0531.2009.01485.x

Lefèvre, F., Guillomot, M., D'Andréa, S., Battegay, S., & La Bonnardière, C. (1998). Interferon-delta: The first member of a novel type I interferon family. *Biochimie, 80*, 779–788. https://doi.org/10.1016/S0300-9084(99)80030-3

Martal, J., Assal, N. E., Assal, A., Zouari, K., Huynh, L., Chene, N., Reinaud, P., Charpigny, G., Charlier, M., & Chaouat, G. (1994). Immunoendocrine functions of trophoblast interferons (IFN-ô or TP-1 or trophoblastin) in the maternal recognition of pregnancy. In S. R. Glasser, J. Mulholland, & A. Psychoyos (Eds.), *Endocrinology of Embryo-Endometrium Interactions (195-216)*. *Reproductive Biology*. Boston, MA: Springer. https://doi.org/10.1007/978-1-4615-1881-5_16.

Martal, J., Degryse, E., Charpigny, G., Assal, N., Reinaud, P., Charlier, M., Gaye, P., & Lecocq, J. P. (1990). Evidence for extended maintenance of the corpus luteum by uterine infusion of a recombinant trophoblast alpha-interferon (trophoblastin) in sheep. *Journal of Endocrinology, 127*, R5–R8. https://doi.org/10.1677/joe.0.127r005

McDowell, K. J., Sharp, D. C., Peck, L. S., & Cheves, L. L. (1985). Effect of restricted conceptus mobility on maternal recognition of pregnancy in mares. *Equine Veterinary Journal. Supplement, 3*, 23–24. https://doi.org/10.1111/j.2042-3306.1985.tb04586.x

Morales Prieto, D. M., & Markert, U. R. (2011). MicroRNAs in pregnancy. *Journal of Reproductive Immunology, 88*, 106–111. https://doi.org/10.1016/j.jri.2011.01.004

Oriol, J. G. (1994). The equine embryonic capsule: Practical implications of recent research. *Equine Veterinary Journal, 26*, 184–186.

Oriol, J. G., Betteridge, K. J., Clarke, A. J., & Sharom, F. J. (1993). Mucin-like glycoproteins in the equine embryonic capsule. *Molecular Reproduction and Development, 34*, 255–265. https://doi.org/10.1002/mrd.1080340305

Ousey, J. C. (2004). Peripartal endocrinology in the mare and foetus. *Reproduction in Domestic Animals, 39*, 222–231. https://doi.org/10.1111/j.1439-0531.2004.00507.x

Palmieri, S. L., Peter, W., Hess, H., & Scholer, H. R. (1994). Oct-4 transcription factor is differentially expressed in the mouse embryo during establishment of the first two extraembryonic cell lineages involved in implantation. *Developmental Biology, 166*, 259–267.

Reza, A. M. T., Choi, Y.-J., Han, S. G., Song, H., Park, C., Hong, K., & Kim, J-H. (2019). Roles of microRNAs in mammalian reproduction: from the commitment of germ cells to peri-implantation embryos. *Biological Reviews of the Cambridge Philosophical Society, 94*, 415–438. https://doi.org/10.1111/brv.12459

Scaravaggi, I., Borel, N., Romer, R., Imboden, I., Ulbrich, S. E., Zeng, S., Bollwein, H., & Bauersachs, S. (2019). Cell type-specific endometrial transcriptome changes during initial recognition of pregnancy in the mare. *Reproduction, Fertility and Development, 31*, 496–508. https://doi.org/10.1071/RD18144

Scholer, H. R., Balling, R., Hatzopoulos, A. K., Suzuki, N., & Gruss, P. (1989a). Octamer binding proteins confer transcriptional activity in early mouse embryogenesis. *EMBO Journal, 8*, 2551–2557.

Scholer, H. R., Hatzopoulos, A. K., Balling, R., Suzuki, N., & Gruss, P. (1989b). A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor. *EMBO Journal, 8*, 2543–2550.

Sharp, D. C. (2000). The early fetal life of the equine conceptus. *Animal Reproduction Science, 60-61*, 679–689. https://doi.org/10.1016/S0378-4320(00)00138-X

Sharp, D. C., McDowell, K. J., Weithenauer, J., & Thatcher, W. W. (1989). The continuum of events leading to maternal recognition of pregnancy in mares. *Journal of Reproduction and Fertility. Supplement, 37*, 101–107.

Sharp, D. C., McDowell, K. J., Weithenauer, J., & Thatcher, W. W. (1989). The continuum of events leading to maternal recognition of pregnancy in mares. *Journal of Reproduction and Fertility. Supplement, 37*, 101–107.

Short, R. V. (1966). Implantation and the maternal recognition of pregnancy. In G. E. W. Wolstenholme, & M. O'Connor (Eds.), *Ciba Foundation Symposium on Foetal Autonomy* (pp. 2–26). London, UK: J. and A. Churchill Ltd.

Siemieniuch, M. J., Szóstek, A. Z., Gajos, K., Kozdrowski, R., Nowak, M., & Okuda, K. (2016). Type of Inflammation differentially affects expression of interleukin 1β and 6, tumor necrosis factor-α and toll-like receptors in subclinical endometritis in mares. *PLoS One, 11*, Article e0154934. https://doi.org/10.1371/journal.pone.0154934

Siemieniuch, M. J., Kozdrowski, R., Szóstek-Mioduchowska, A. Z., Raś, A., & Nowak, M. (2019). Evidence for increased content of PGF2α, PGE2, and 6-keto-PGF1α in endometrial tissue cultures from heavy draft mares in anestrus with endometritis. *Journal of Equine Veterinary Science, 77*, 107–113. https://doi.org/10.1016/j.jevs.2019.02.014

Silva, L. A., & Ginther, O. J. (2006). An early endometrial vascular indicator of completed orientation of the embryo and the role of dorsal endometrial encroachment in mares. *Biology of Reproduction, 74*, 337–343. https://doi.org/10.1095/biolreprod.105.047621

Silva, L. A., Gastal, E. L., Beg, M. A., & Ginther, O. J. (2005). Changes in vascular perfusion of the endometrium in association with changes in location of the embryonic vesicle in mares. *Biology of Reproduction, 72*, 755–761. https://doi.org/10.1095/biolreprod.104.036384

Smits, K., Govaere, J., Peelman, L. J., Goossens, K., de Graaf, D. C., Vercauteren, D., … Van Soom, A. (2012). Influence of the uterine environment on the development of in vitro-produced equine embryos. *Reproduction, 143*, 173–181. https://doi.org/10.1530/REP-11-0217

Smits, K., Willems, S., Van Steendam, K., Van de Velde, M., De Lange, V., Ververs, C., Roles, K., Govaere, J., Van Nieuwerburgh, F., Peelman, L., Deforce, D., & Van Soom, A. (2018). Proteins involved in embryo-maternal interaction around the signalling of maternal recognition of pregnancy in the horse. *Scientific Reports, 8*(1), 5249. https://doi.org/10.1038/s41598-018-23537-6

Smits, K., Gansemans, Y., Tilleman, L., Van Nieuwerburgh, F., Van De Velde, M., Gerits, I., Ververs, C., Roels, K., Govaere, J., Peelman, L., Deforce, D., & Van Soom, A. (2020). Maternal recognition of pregnancy in the horse: are microRNAs the secret messengers? *International Journal of Molecular Sciences, 21*(2), 419. https://doi.org/10.3390/ijms21020419

Spencer, T. E., Burghart, R. C., Johnson, G. A., & Bazer, F. W. (2004). Conceptus signals for establishment and maintenance of pregnancy. *Animal Reproduction Science, 82-83*, 537–550.

Squire, S., Stewart, F., Beachamp, J., & Kennedy, M. W. (2001). Uterocalin, a lipocalin provisioning the preattachment equine conceptus: Fatty acid and retinol binding properties, and structural characterization. *Biochemical Journal, 356*, 369–376. https://doi.org/10.1042/0264-6021:3560369

Stout, T. A. E., & Allen, W. R. (2001). Role of prostaglandins in intrauterine migration of the equine conceptus. *Reproduction, 121*, 771–775. https://doi.org/10.1530/rep.0.1210771

Stout, T. A. E., & Allen, W. R. (2002). Prostaglandin E2 and F2a production by equine conceptuses and concentrations in conceptus fluids and uterine flushings recovered from early pregnant and dioestrous mares. *Reproduction, 123*, 261–268. https://doi.org/10.1530/rep.0.1230261

Stout, T. A. E., Lamming, G. E., & Allen, W. R. (2000). The uterus as a source of oxytocin in the cycling mare. *Journal of Reproduction and Fertility. Supplement, 56*, 281–287.

Stout, T. A. E., Meadows, S., & Allen, W. R. (2005). Stage-specific formation of the equine blastocyst capsule is instrumental to hatching and to embryonic survival in vivo. *Animal Reproduction Science, 87*, 269–281. https://doi.org/10.1016/j.anireprosci.2004.11.009

Stout, T. A., & Allen, W. R. (2002). Prostaglandin E(2) and F(2 alpha) production by equine conceptuses and concentrations in conceptus fluids and uterine flushings recovered from early pregnant and dioestrous mares. *Reproduction, 123*, 261–268.

Swegen, A., Grupen, C. G., Gibb, Z., Baker, M. A., de Ruijter-Villani, M., Smith, N. D., Stout, T. A. E., & Aitken, R. J. (2017). From Peptide Masses to Pregnancy Maintenance: A Comprehensive Proteomic Analysis of The Early Equine Embryo Secretome, Blastocoel Fluid, and Capsule. *Proteomics, 17*(17-18). https://doi.org/10.1002/pmic.201600433

Szóstek, A. Z., Lukasik, K., Galvão, A.. M., Ferreira-Dias, G. M., & Skarzynski, D. J. (2013). Impairment of the interleukin system in equine endometrium during the course of endometrosis. *Biology of Reproduction, 89*, 79. https://doi.org/10.1095/biolreprod.113.109447

Szóstek, A. Z., Siemieniuch, M. J., Lukasik, K., Galvão, A. M., Ferreira-Dias, G. M., & Skarzynski, D. J. (2012). mRNA transcription of prostaglandin synthases and their products in the equine endometrium in the course of fibrosis. *Theriogenology, 78*, 768–776. https://doi.org/10.1016/j.theriogenology.2012.03.024

Vanderwall, D. K., Silvia, W. J., & Fitzgerald, B. P. (1998). Concentrations of oxytocin in the cavernous sinus of mares during luteolysis: Temporal relationships with concentrations of 13,14-dihydro-15-ketoprostaglandin F2-alpha. *Journal of Reproduction and Fertility, 112*, 337–346. https://doi.org/10.1530/jrf.0.1120337

Vegas, A. R., Podico, G., Canisso, I. F., Bollwein, H., Alminana, C., & Bauersachs, S. (2021). Spatiotemporal endometrial transcriptome analysis revealed the luminal epithelium as key player during initial maternal recognition of pregnancy in the mare. *Scientific Reports, 11*, 22293. https://doi.org/10.1038/s41598-021-01785-3

Vegas, A.. R., Podico, G., Canisso, I. F., Bollwein, H., Fröhlich, T., Bauersachs, S., & Almiñana, C. (2022). Dynamic regulation of the transcriptome and proteome of the equine embryo during maternal recognition of pregnancy. *FASEB BioAdvances, 4*, 775–797. https://doi.org/10.1096/fba.2022-00063

Weber, J. A., Freeman, D. A., Vanderwall, D. K., & Woods, G. L. (1991). Prostaglandin E2 secretion by oviductal transport-stage equine embryos. *Biology of Reproduction, 45*, 540–543. https://doi.org/10.1095/biolreprod45.4.540

Weber, J. A., Freeman, D. A., Vanderwall, D. K., & Woods, G. L. (1991). Prostaglandin E2 hastens oviductal transport of equine embryos. *Biology of Reproduction, 45*, 544–546.

Wilsher, S., Gower, S., & Allen, W. R. (2013). Persistence of an immunoreactive MUC1 protein at the feto-maternal interface throughout pregnancy in the mare. *Reproduction, Fertility and Development, 25*, 753–761. https://doi.org/10.1071/RD12152

Wooding, F. B., Morgan, G., Fowden, A. L., & Allen, W. R. (2001). A structural and immunological study of chorionic gonadotrophin production by equine trophoblast girdle and cup cells. *Placenta, 22*, 749–767. https://doi.org/10.1053/plac.2001.0707

Zavy, M. T., Mayer, R., Vernon, M. W., Bazer, F. W., & Sharp, D. C. (1979). An investigation of the uterine luminal environment of non-pregnant and pregnant pony mares. *Journal of Reproduction and Fertility*, (Suppl. 27), 403–411.

Zavy, M. T., Sharp, D. C., Bazer, F. W., Fazleabas, A., Sessions, F., & Roberts, R. M. (1982). Identification of stage-specific hormonally induced polypeptides in the uterine protein secretions of the mare during the oestrous cycle and pregnancy. *Journal of Reproduction and Fertility, 64*, 199–207. https://doi.org/10.1530/jrf.0.0640199