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Review Article

The Role of Conceptus–maternal Signalling in the Acquisition of Uterine Receptivity to Implantation in Mammals

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Contents
Implantation is a pivotal step in the establishment of mammalian pregnancy. Although implantation strategies vary between species, many aspects of conceptus–maternal signalling necessary to induce uterine receptivity to implantation are conserved. The temporal 'window' for the initiation of implantation is short and precisely controlled by endocrine, paracrine and autocrine factors. An invariable prerequisite for the development of uterine receptivity to implantation is continuous exposure of the endometrium to progesterone which, after a species-specific interval, downregulates progesterone receptor (PGR) expression in the epithelium and stimulates the production of progestamedins. Uterine receptivity involves temporal changes in the expression of genes, leading to modifications in surface, extracellular matrix and secretory characteristics that support growth, proliferation, migration and attachment of the conceptus. Moreover, a complex interplay between endometrial progestamedins and estramedins and conceptus-derived oestrogens, cytokines and interferons (INFs), prostaglandins (PGs) and cortisol is crucial to the preparation for implantation. Understanding the individual roles and combined actions of conceptus and endometrial autocrine and paracrine factors in the development of uterine receptivity to implantation is essential for translational research into strategies to reduce pregnancy loss in man and animals.

Introduction

Implantation and the establishment of a stable placental attachment are critical aspects of successful pregnancy in eutherian mammals. Implantation begins with the apposition and adhesion of the conceptus to the uterine epithelium, which triggers a complex and coordinated chain of reciprocal secretory and physical interactions between the trophectoderm and the endometrium (Carson et al. 2000; Dey et al. 2004). To ensure successful implantation, this initial conceptus–maternal interaction has to take place during a limited period of time known as the 'window of receptivity to implantation' (Carson et al. 2000; Dey et al. 2004). This window of receptivity is regulated by the actions of progesterone and, in some species, oestrogens which control the expression of locally produced paracrine and autocrine factors (progestamedins and estramedins) that support conceptus development and implantation (Fazleabas et al. 2004; Slayden and Keator 2007; Bazer et al. 2008).

The time of onset, duration and the strategies that characterize the process of implantation vary tremendously among mammals. In some species, including higher primates and rodents, implantation is 'invasive' and, after a brief initial attachment, the trophectoderm invades through the endometrial epithelium into the stroma (Wimsatt 1975). Following initial invasion, there are interspecific differences in how and where the conceptus establishes itself, for example the rodent blastocyst comes to lie between the epithelial folds ('eccentric' implantation), while the human blastocyst burrows into the endometrial stroma underneath the epithelium ('interstitial' implantation) (Wimsatt 1975). Most large domestic animals show a 'non-invasive' or 'central' type of implantation, in which the trophectoderm forms a stable attachment with the surface of the luminal or glandular epithelium by means of interdigitation and without any invasion or erosion of maternal tissues (Wimsatt 1975).

Preparation for Implantation

The pre-implantation phase in species with an 'invasive' implantation is very short, ranging between 4 days in rodents and 6 days in women (Sharkey and Smith 2003). By contrast, species with a 'non-invasive' implantation often show a protracted pre-implantation period, with the most familiar examples including 16 days in pigs, 19 days in cattle and approximately 40 days in horses (Samuel et al. 1974; Guillomot et al. 1981; Bazer and Johnson 2014). During the pre-implantation period, the conceptus migrates through the uterine lumen to the eventual site of implantation. In ruminants and pigs, after hatching from the zona pellucida the initially spherical conceptus elongates to a tubular and then a filamentous form that maximizes its area of contact with the endometrium to ensure adequate transmission of the pregnancy recognition (antiluteolytic) factor and facilitate uptake of nutrients. The equine conceptus instead remains spherical at least in part because, after zona hatching, it is still completely enveloped by a tough glycoprotein capsule that prevents both elongation and direct physical contact between the trophectoderm and the endometrium until at least day 22 of gestation (Quinn et al. 2007). As in ruminants and pigs, however, the equine conceptus still needs to maximize its area of contact with the endometrium to adequately suppress uterine PGF2α secretion and signal its presence to its dam, this is achieved by continued migration throughout the uterine lumen between days 7 and 17 of pregnancy (McDowell et al.

1988). Unlike the other large domestic species, the equine trophoblast also as an 'invasive' component in the form of the chorionic girdle, the cells of which detach from the rest of the conceptus between days 35 and 40 of pregnancy and invade through the endometrial glandular epithelium, reaching the stroma where they become binucleate and establish the endocrinologically active endometrial cups (Allen and Stewart 2001).

Apposition and adhesion

In most mammals, the initial attachment of the conceptus to the endometrium requires alteration in expression of anti-adhesive mucins, in particular mucin-1 (MUC1), on the apical surface of the luminal epithelium of the endometrium (Carson et al. 1998). During the 'window of receptivity to implantation', MUC1 expression is reduced over the entire endometrium in sheep, mice and pigs or locally at the site of blastocyst attachment in rabbits. The loss of MUC1 is achieved either by steroid hormone (progesterone and oestrogens) regulation or by the local action of cell surfaces proteases, and it is believed that the down-regulation of MUC1 unmasks adhesion molecules on the luminal and glandular epithelium that permit initial apposition and then stable adhesion between the trophectoderm and maternal extracellular matrix. Exceptions to this mechanism are women and mares, which do not show any loss of MUC1 during both the initial conceptus–endometrium attachment or during the invasive phase of implantation (accomplished by the blastocyst in women or by invasive trophoblast cells during endometrial cup formation in mares) (Carson et al. 2006; Wilsher et al. 2013). In this respect, it may be significant that studies of human endometrium indicate that MUC1 carries selectin ligands throughout the secretory phase of the menstrual cycle, including the mid-secretory (receptive) phase, and in contrast to what was previously believed, MUC1 is now thought to have pro-adhesive capacities, besides its more established anti-adhesive properties, which may contribute to initial conceptus–uterine attachment (Carson et al. 2006).

Adhesion molecules with the ability to bind specifically to carbohydrates, such as selectins, galectins, heparin sulphate proteoglycans, cadherins, heparin-binding EGF-like growth factors and CD44, are responsible for the early stages of conceptus–endometrial attachment (Carson et al. 2000). After the initial low-affinity attachment, stable adhesion is achieved by the binding of integrins, present on both the trophectoderm and luminal epithelium, to extracellular matrix components (ECM) such as fibronectin, oncofetal fibronectin, vitronectin, laminin, osteopontin, insulin-like growth factor binding protein 1 and the latency-associated peptide linked to transforming growth factor beta (Fazleabas et al. 2004; Burghardt et al. 2009). Whereas the majority of integrins are constitutively expressed throughout the cycle, others such as the αV, α4, α5, β1 and β3 subunits, are upregulated during the 'window of implantation' in women and domestic species (Burghardt et al. 2002). Besides their adhesive functions, integrins play an important role in trophectoderm cytoskeleton organization, migration and invasion (Burghardt et al. 2002). In ruminants and pigs, the rapid elongation of the conceptus during the pre-implantation period is characterized by a complex cytoskeletal reorganization mediated by the action of cadherins, integrins and ECM, as indicated by the absence of elongation in conceptuses cultured in vitro (Burghardt et al. 2009). Similarly, integrins and ECM seem to play an important role in the cytoskeletal changes required for trophoblast outgrowth and invasion in species with invasive implantation (Bazer et al. 2009b). In response to ligand binding, integrins stimulate the rapid formation of macromolecular complexes known as 'focal adhesion complexes' that alter the shape of mechano-sensory molecules and elicit intracellular biochemical signals (mechano-transduction) which in turn regulate cellular metabolism and gene expression (Bazer et al. 2009b; Bazer and Johnson 2014). These integrin-mediated signals have extensive crosstalk with growth factors, cytokines, G-protein-coupled receptors and nutrient signalling pathways (Bazer et al. 2009b). Studies on human and ovine trophoblast have shown that the mTOR signalling pathway plays a central role in this mechano-transduction signalling. Focal adhesion assembly brought about by integrins binding to ECM components, and in particular osteopontin, induces the activation of PI3K-AKT1-mTOR-P70S6K-RPS6 or Erk1/2 or p38 cell signalling or Myosin II motor activity, which act in concert to stimulate adhesion, migration and cytoskeletal remodelling of trophectoderm cells (Al-Shami et al. 2005; Kim et al. 2010).

Invasion, fusion or interdigitation

Differences among mammals exist in the degree of interaction between the trophoblast and the maternal endometrium. In species with invasive implantation, such as primates and rodents, the trophectoderm is initially highly proliferative and undergoes syncytial formation to form a syncytiotrophoblast which, after developing a stable adhesion to the uterine luminal epithelium, penetrates it to reach the underlying stroma (Bazer et al. 2009b). The endometrium then loses the vascular endothelial cells resulting in the formation of the maternal blood sinusoids of the haemochorial placenta. The invasion of the endometrium by the blastocyst induces decidualization of the stromal cells, during which the latter undergo hyperplasia and hypertrophy, and start to produce prolactin and ECM proteins (osteopontin, laminin and fibronectin), contemporaneous to stromal invasion by numerous immune cells (Bazer et al. 2009a). In species with a non-invasive implantation, the degree of interaction between trophectoderm and endometrium is more restricted. In ruminants, binucleate trophectoderm cells migrate and fuse with the luminal epithelium to form multinucleated syncytia (synepitheliochorial placenta),

while in pigs and horses the trophectoderm progresses no further than interdigitating with the intact luminal epithelium (epitheliochorial placenta), with the notable exception of the chorionic girdle cells of the horse (Allen and Stewart 2001; Bazer et al. 2009b).

Maternal Recognition of Pregnancy

Maintenance of pregnancy requires prolongation of the secretory function of the primary corpus luteum (CL) beyond its cyclical lifespan in order to guarantee adequate progesterone production to support the endometrial secretory functions which in turn sustain conceptus growth, development, implantation and placentation (Short 1969). Among mammals, various signalling strategies are adopted by the conceptus in order to guarantee maternal recognition of pregnancy. Signals from the conceptus may be luteotrophic (directly acting on the CL to promote luteal function), antiluteolytic [preventing the luteolytic prostaglandin (PG) F2 alpha reaching the CL] or luteostatic (protecting the CL against the luteolytic action of PG F2 alpha).

In primates, the Chorionic Gonadotropin (CG) secreted by the syncytio-trophoblast cells acts on luteal cells via the LH/CG receptor to prevent demise and prolong CL function (Fazleabas et al. 2004). CG production decreases after approximately 60 days of pregnancy, when the placenta takes over the production of the progesterone required to support pregnancy. In rodents, prolactin (PRL) is released from the anterior pituitary at mating and acts as a luteotrophic signal up to day 12 of gestation, after which time luteal function is prolonged and maintained by lactogenic hormones produced by the decidua and conceptus (Soares 2004). In ruminants, conceptus-derived interferon-tau (INFT) downregulates the transcription of oestrogen receptor alpha (ESR1) in the uterus, which in turn suppresses the upregulation of oxytocin receptors (OXTR) thus disabling the OXT-PGF2 alpha feedback loop responsible for generating luteolytic pulses of PGF2alpha (Bazer et al. 2008). In pigs, the oestrogens secreted by the conceptus exert their antiluteolytic action in concert with PRL, redirecting the PGF2alpha produced by the endometrium away from the uterine vasculature (endocrine) and towards the uterine lumen (exocrine) where they are effectively sequestered from the CL and metabolized (Bazer and Thatcher 1977). In addition, the increased expression of PGE2 synthase during early porcine pregnancy in both conceptus and endometrium changes the PGE2/PGF2α ratio in favour of the luteotrophic/luteostatic PGE2 (Ziecik et al. 2008). Although the exact pregnancy recognition-signalling pathway in the horse is unknown, it is now clear that as in ruminats the presence of the conceptus in the uterus uncouples the OXT-PGF2alpha feedback loop responsible for generating luteolytic pulses of PGF2alpha. However, in the mare this is achieved by blocking the cyclical upregulation of OXTR (Starbuck et al. 1998) and inhibiting the expression of PGHS 2 (Boerboom et al.) during the first 15–16 days of pregnancy, and thereafter by reducing the expression of the PGF2alpha receptor (de Ruijter-Villani et al. 2014). It is only after endometrial cup formation, at approximately day 36 of pregnancy, that the endometrial cup cells of trophoblast origin start secreting the luteotropic CG which not only acts on the primary corpus luteum to further enhance progesterone production, but stimulate the formation of multiple accessory corpora lutea (Allen and Stewart 2001). Similarly to primates, equine CG production also decreases, in this case after approximately 120 days of pregnancy, when the placenta has taken over the function as primary producer of the progestagens required to support pregnancy (Allen and Stewart 2001).

Endometrial Receptivity

Across all eutherian mammals, the temporal window for the successful initiation of implantation is very restricted and precisely controlled by endocrine, paracrine and autocrine factors. This short interval, also known as the 'window of receptivity to implantation', is characterized by highly synchronized reciprocal interactions ('communication') between the trophectoderm of the conceptus and the endometrium. Successful implantation requires precise coordination between the development of the newly formed conceptus and the cyclical 'maturation' of the endometrium (Bazer et al. 2009a,b; Banerjee and Fazleabas 2010).

In most mammals, the maximal degree of conceptus–uterine asynchrony tolerated by the embryo is $\pm$ 2 days (Pope 1988; Barnes 2000; Tavaniotou et al. 2002); the major exception is the equine conceptus which is able to tolerate up to 5 days of negative asynchrony (uterus less advanced than the conceptus) showing only a mild retardation in development but without any apparent reduction in ability to produce a viable pregnancy (Wilsher et al. 2010; Jacob et al. 2012). The unique ability of equine embryos to adjust to a wide level of uterine asynchrony could be due to the fact that mares show a rapid post-ovulatory rise in progesterone concentrations compared to other domestic species (Wathes and Lamming 1995; Wilsher and Allen 2009), combined with the exceptionally long pre-implantation period in this species (Samuel et al. 1974) and the presence of a glycoprotein capsule which delays direct apposition between the conceptus trophectoderm and the endometrium until at least day 22 of pregnancy (Oriol et al. 1993); all of which may make it easier for the conceptus to catch up, slow down or communicate with the uterus to better attune their developmental states. Several studies on domestic species have demonstrated that embryo-uterine asynchrony alters growth and development of the conceptus, which accelerates in a more advanced uterus and slows down in a negatively asynchronous uterus (Pope 1988; Wilsher et al. 2010). The timing and length of exposure of the uterus to progesterone appears to play a causative central role in accelerating or decelerating

conceptus development as shown in experiments on ewes, where early progesterone supplementation (from 36 h post-mating) increased the rate of growth and development of the conceptuses (Satterfield et al. 2006). The exact mechanism by which the duration of uterine exposure to progesterone controls conceptus development is still unclear; however, it has been hypothesized that increasing duration of exposure to progesterone leads to changes in the release of growth factors, secretory proteins, amino acids, sugars and ions from the endometrium (Satterfield et al. 2006) that presumably coordinate conceptus development. It is interesting to note that, with the exception of the horse, the induction of severe embryo-uterine asynchrony in domestic species is usually associated with early embryonic mortality and implantation failure regardless of whether conceptus growth is accelerated or retarded; this is probably due to exposure to 'out of phase' histotroph which in turn induces irreversible and fatal alterations in conceptus development (Pope 1988; Barnes 2000).

Reproductive steroid hormones and the development of uterine receptivity

Uterine receptivity to implantation is initiated and maintained by the action of steroid hormones. While progesterone is essential for implantation and pregnancy maintenance in all mammals, the requirement for oestrogens appears to be species specific (Dey et al. 2004). Nevertheless, a coordinated spatiotemporal regulation of the expression of progesterone (PGR) and oestrogen receptors in the endometrium appears to be a prerequisite for uterine receptivity (Spencer et al. 2007). In all eutherian mammals, continuous exposure of the endometrium to progesterone during diestrus or early pregnancy negatively regulates PGR expression in the luminal and glandular epithelium, whereas its expression in stromal cells does not change (Carson et al. 2000; Slayden and Keator 2007; Spencer et al. 2007; de Ruijter-Villani et al. 2014). It has been speculated that, during the pre-implantation period, the selective action of progesterone on stromal cells stimulates the production of stroma-derived progestamedins, which in turn exert paracrine action on the endometrial epithelium and conceptus trophectoderm regulating the production of endometrial secretions and the development of the conceptus (Slayden and Keator 2007; Bazer et al. 2009b; de Ruijter-Villani et al. 2014). At present, only a small number of progestamedins have been identified, but these include Hepatocyte growth factor (HGF) and Fibroblast growth factors 7 (FGF7) and 10 (FGF10). The receptors for those progestamedins (MET proto-oncogene for HGF; FGF2IIIb for FGF7 and FGF10) have been identified on both uterine epithelial and trophectoderm cells (Spencer and Bazer 2002; Slayden and Keator 2007). HGF binding to the Met tyrosine kinase receptor leads to the activation of both the MAPK/ERK and Akt/PKB signalling pathways (Stewart 1996; Maroun and Rowlands 2014), and studies in primates and rodents have shown that HGF thereby stimulates trophoblast migration and proliferation (Stewart 1996). Similarly, FGF7 promotes proliferation and differentiation of trophoblast cells by phosphorylation of its receptor, FGF2IIIb and subsequent activation of the MAPK/ERK pathway (Ka et al. 2007). In addition, FGF7 has been shown to stimulate CG production by human trophoblast cells (Taniguchi et al. 2000). Contrary to the dogma that FGF7 and 10 are uniquely expressed by the endometrial stroma, FGF7 production in pigs seems to be primarily a function of epithelial cells (Bazer and Johnson 2014) and, although progesterone stimulation alone is enough to initiate the expression of FGF7 in the porcine epithelium (Bailey et al. 2010), oestrogens produced by the early conceptus are also able to directly upregulate its production (Ka et al. 2007).

The importance of histotroph

In all species, the early pre-implantation conceptus is entirely dependent on uterine secretions, also known as histotroph, for its survival and development. Studies on uterine gland-knockout sheep and mice have proved that the absence of adequate histotroph results in conceptus growth-retardation, failure of implantation and decidualization and finally conceptus death (Filant and Spencer 2014). The exact composition of histotroph is unknown and changes over time; however, it has been shown to be composed of ions, amino acids, carbohydrates, proteins, lipids, growth factors, proteases and their inhibitors, cytokines and other substances that are synthesized or transported and secreted by the uterine epithelium (Bazer et al. 2009a; Filant and Spencer 2014). Both the transport and the de novo synthesis of those substances necessitates a fine tuning of transcription and translation of genes in the luminal and glandular endometrial epithelium (Filant and Spencer 2014). Studies in sheep have shown that, during the window of implantation, the endometrial epithelium expresses, among others, genes that encode secreted factors (CTGF, GRP, WNT11), amino acid transporters (SLC1A1, SLC1A4, SLC1A5, SLC7A1, SLC7A2 SLC7A5, SLC7A8, SLC43A2), glucose transporters (SLC2A1, SLC2A5, SLC2A12, SLC5A1, SLC5A11), secreted migration and adhesion factors (LGALS15, osteopontin), a regulator of calcium/phosphate homoeostasis (stanniocalcin 1), secreted peptidases (CTSH, CTSL, CTSS, CTSZ), secreted protease inhibitors (CST3, CST6) and an immunomodulatory factor (SERPINA14) (Spencer et al. 2007; Filant and Spencer 2014). The gene products are able to alter the histotroph by enhancing the secretion of glucose, selected amino acids, cytokines and growth factors required to support growth, differentiation and implantation of the conceptus (Filant and Spencer 2014). Similarly, knock-out studies in mice have proven that successful implantation and decidualization is dependent on the expression, in luminal and glandular epithelium, of genes encoding for cytokines and their receptors (LIF, IL6ST), a secreted adhesion factor (Cdh1), growth factors (Areg, Ihh), regulators of

calcium homoeostasis (Calb1, CALCA), enzymes (Hdc, Hegf1, Ptgs1, Ptgs2), an immunomodulatory factor (Irg1) and transcription regulators (Klf5, Msx1, Msx2) (Filant and Spencer 2014). The expression of these genes is initiated and regulated by the binding of progesterone to both endometrial epithelial and stromal PGRs, as demonstrated by tissue recombinant studies in mice where the ablation of PGR either in the epithelium or the stroma of the uterus prevented progesterone-dependent Indian hedgehog (Ihh) upregulation (Simon et al. 2009). Progesterone may also regulate endometrial function through non-classical progestin receptors, as demonstrated by the transient progesterone-dependent upregulation of Ihh in the endometrial epithelium of PGR-null mice (Matsumoto et al. 2002). This indicates that the progesterone-dependent genomic responses in the uterus are not exclusively coordinated by PGRs. Moreover, although the expression of these pro-implantation genes in the uterine epithelium is progesterone dependent, available evidence suggests that progesterone and progestamedin stimulation alone is not enough to trigger adequate endometrial expression of most, and that conceptus-derived factors (such as oestrogens, cytokines, interferons and prostaglandins) are equally important in regulating their expression (Spencer et al. 2007; Bazer et al. 2009b).

Conceptus factors that regulate uterine receptivity

Conceptus oestrogens

Early porcine and equine conceptuses secrete large amounts of oestrogens from at least day 10 of pregnancy which, combined with the fact the uterine glandular epithelium in those species maintains the expression of oestrogen receptor alpha (ESR1) during early pregnancy, suggests a direct action of conceptus oestrogens on the uterine epithelium (Allen and Stewart 2001; Bazer and Johnson 2014; de Ruijter-Villani et al. 2014). Beside their antiluteolytic action, conceptus oestrogens in the pig are able to increase the expression of genes within the uterine epithelium (also called estramedins), which support implantation and conceptus development (Bazer and Johnson 2014). Genes identified as estramedins in porcine endometrium include AKRIBI, B2M, CD24, FGF7, IRF2, MXI, NMB, SLA21-8, osteopontin, STC1 and EDG7 (Johnson et al. 2009).

The role of intra-uterine cytokines

Many cytokines are expressed by the uterus and conceptus during early pregnancy; however, only a few have been proven to support conceptus growth and implantation. Among these, cytokines of the Interleukin (IL)-6 family, such as Leukaemia inhibitory factor (LIF), IL-6 and IL-11, play important roles in conceptus implantation and decidualization in various species (Singh et al. 2011). These cytokines act primarily through the janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway stimulating cell proliferation, differentiation and survival. Leukaemia inhibitory factor (LIF) produced by the endometrial glands during early pregnancy has been implicated in the establishment of uterine receptivity to implantation in primates, rodents, pigs and ruminants. LIF has also been shown to enhance adhesion of both endometrial epithelial cells and trophoblast cells to extracellular matrix via phosphorylation of STAT3 (Auernhammer and Melmed 2000; Kimber 2005). Moreover, in mice maternal LIF is necessary for blastocyst development and implantation, as demonstrated by the failure of wild-type embryos to survive in the uterus of LIF-knockout dams unless the latter receive LIF supplementation (Stewart et al. 1992). Similarly, knockout studies on IL-6 and IL-11 in mice showed that absence of these interleukins in the dam results in defective decidualization and compromised implantation sites (Singh et al. 2011). Cytokines of the IL-1 family also play a central role in the implantation process, as evidenced by implantation failure in mice subjected to repeated injections of an IL-1beta (IL1B) antagonist during the pre-implantation period (Bazer and Johnson 2014). IL1B produced by the early conceptus in primates, rodents and pigs is able to stimulate CG (in primates) and HSD11B1-derived cortisol expression in trophoblast cells, and to enhance both LIF and prostaglandin production by the endometrium (Imakawa et al. 2004)(Bazer et al. 2009b). Moreover, it has been suggested that conceptus-derived IL1B regulates trophoblast elongation and oestrogen synthesis in pigs (Bazer and Johnson 2014). Cytokines of the transforming growth factor beta (TGFB) family have also been implicated in the implantation process. TGFBs are synthesized by both conceptus and endometrium as precursor molecules that bind to the latency associated peptide (LAP). This dimer can bind to a TGFB-binding protein and be secreted into the extracellular matrix, where it remains inactive until activation by integrins expressed by the conceptus or the endometrial luminal epithelium (Bazer and Johnson 2014). TGFBs stimulates proliferation and differentiation of endometrial and trophoblast cells. Moreover, TGFBs promote conceptus–endometrium adhesion by increasing fibronectin synthesis, cell adhesion to fibronectin and the formation of focal adhesions (Singh et al. 2011; Bazer and Johnson 2014). To activate TGFB, integrins have to bind to the Arg-Gly-Asp (RGD) sequence within the LAP. Recent evidence suggests that TGFB and integrins regulate conceptus elongation in pigs, indeed intra-uterine infusion of LAP containing a requisite RGD sequence (LAP-RGD) in early pregnant gilts prevents conceptus elongation, whereas infusion of a recombinant mutant of LAP that does not have the sequence (LAP-RGE) does not affect conceptus elongation (Massuto et al. 2010). In species with invasive implantation, TGFB inhibits trophoblast proliferation and enhances the invasive properties of the trophoblast cells by activating the SMAD and MAPK pathways and up-regulating metalloproteinase secretion (Singh et al. 2011).

Conceptus interferons

A common feature of domestic animal, rodents and primates conceptuses is the production of type I and/or type II Interferons (IFNs) (Bazer et al. 2009a); type I IFNs include IFNA, IFNB, IFND, IFNT and IFNW1, while type II IFNs include only IFNG. During the peri-implantation period, human conceptuses express IFNA, IFNB and IFNG, rodents express IFNA and IFNB, ruminants IFNT, horses IFND and pigs INFD and IFNG (Cencic et al. 2003; Bazer et al. 2009a). Receptors for both type I and type II IFNs are expressed in all endometrial cell types and by trophectoderm; it is therefore likely that IFNs exert both paracrine and autocrine effects (Bazer et al. 2009a; Dorniak et al. 2013a; Bazer and Johnson 2014). Although only IFNT is best known for exerting a direct antiluteolytic effect during pregnancy recognition in ruminants, the IFNs as a whole appear to affect uterine receptivity, decidualization and placental development through the induction of IFN-stimulated genes in the endometrium (Johnson et al. 2009). Studies on human, bovine and ovine cells *in vitro* have shown that IFNs are able to act on target cells to activate the transcription of an interferon regulatory factor (JAK-STAT-IRF pathway), which in turn induces classical IFN-stimulated genes such as B2M, GBP2, IFI27, IFIT1, ISG15, IRF9, MIC, OAS, RSAD2, STAT1 and STAT2 (Dorniak et al. 2013a). Although all endometrial cell types express IFN receptors, *in vivo* studies on ruminants and pigs have indicated that classical IFN-stimulated genes are induced only in endometrial stroma and deep glandular epithelium (Dorniak et al. 2013a; Bazer and Johnson 2014). The reason for this selective response is that luminal and superficial glandular epithelia express IRF2, a potent transcriptional repressor for genes with promoters containing IFN-stimulated response elements. Recent studies in pigs have demonstrated that IRF2 expression in the luminal epithelium is induced and controlled by the oestrogens produced by the early conceptus (Dorniak et al. 2013a). IFNs also appear to be able to stimulate non-classical IFN-stimulated genes such as CST3, CST6, CTSL, GRP, HIF2A, hydrosteroid dehydrogenase (HSD)11B1, IGFBP1, LGALS15, SLC2A1, SLC2A5, SLC5A11, SLC7A2, WNT7A, specifically in endometrial luminal and glandular epithelium. Interestingly, all these non-classical IFN-stimulated genes support growth, proliferation, migration and attachment of the conceptus trophectoderm (Bazer et al. 2009a,b; Dorniak et al. 2013a). The expression of these non-classical IFN-stimulated genes is probably MAPK and PI3K-dependent and requires induction by progesterone and loss of PGR in the epithelia (Dorniak et al. 2013a). Other non-canonical mechanisms of action of IFNs on the endometrium include the production of prostaglandin and HSD11B1-derived cortisol (Dorniak et al. 2011, 2013a; Bazer and Johnson 2014).

Conceptus prostaglandins

Domestic animal, primate and rodent conceptuses are able to produce prostaglandins (mainly PGE2 and PGF2alpha in horses and pigs, PGE2 and PGI2 in ruminants, and PGE2 in primates and rodents) from very early stages of development (Holmes et al. 1990; Tan et al. 2005; Stout and Allen 2002; Waclawik 2011; Spencer et al. 2013). Moreover, studies in ruminants and rodents have demonstrated that prostaglandins (PGs) are critical regulators of conceptus elongation, implantation and decidualization, as the administration of PG synthase inhibitors prevents conceptus elongation in sheep, reduces the number of implantation sites and diminishes the amount of decidual tissue in rodents (Dey et al. 2004; Spencer et al. 2013). Since receptors for PGs are present in all endometrial and conceptus cell types, PGs of conceptus origin have probably both paracrine and autocrine actions on endometrial receptivity and conceptus development (Dorniak et al. 2013a; Spencer et al. 2013). Prostaglandins are also able to enhance endometrial cortisol production by increasing the expression and activity of HSD11B1, an enzyme capable of catalysing the conversion of inert cortisone to active cortisol, and decreasing the activity of HSD11B2, an enzyme that oxidizes cortisol to cortisone. Moreover, PGs are able to directly trigger the expression of genes capable of stimulating trophectoderm proliferation and implantation, such as GRP, IGFBP1, SLC2A5 and LGALS15, in the uterine epithelium of non-pregnant sheep (Spencer et al. 2013). Recent evidence supports the hypothesis that PGs, together with other conceptus-derived signals, stimulate the endometrium to further enhance progesterone- and IFN-induced uterine receptivity (Waclawik 2011; Spencer et al. 2013).

Intra-uterine cortisol

Both the endometrium and the conceptus are able to produce cortisol during early pregnancy. Progesterone, INFs and PGs have all been shown to stimulate HSD11B1 activity and thereby promote cortisol generation by the uterine luminal and glandular epithelium (Dorniak et al. 2013a). Cortisol in turn increases PG synthesis, by enhancing the expression and activity of phospholipase A2 and prostaglandin-endoperoxide synthase 2, thereby establishing a PG-cortisol positive feedback loop (Dorniak et al. 2013a). Given that glucocorticoid receptors are present in all endometrial cells and on trophectoderm during early pregnancy, it has been proposed that cortisol may have autocrine and paracrine functions during the peri-implantation period (Dorniak et al. 2013b). In women cortisol is known to have positive effects during early pregnancy such as stimulation of conceptus CG secretion, stimulation of trophoblast growth and invasive potential and stimulation of placental transport of amino acids, glucose and lactate (Dorniak et al. 2013a). Similarly, intra-uterine administration of cortisol in cyclic sheep upregulates the expression of

several genes able to stimulate conceptus elongation and implantation (such as HSD11B1, HIF1A, CTSL, CST6, CXCL10, GRP, LGALS15, SPP1, SLC2A1, SLC2A5, SLC2A12 and SLC1A5), whereas the inhibition of HSD11B1 in early pregnant sheep prevents conceptus elongation. Moreover, of all the genes stimulated by cortisol administration in the endometrium, only HSD11B1 is affected by infusion of the PTGS2 inhibitor, meloxicam, suggesting that the effects of cortisol are direct and not mediated by PGs (Dorniak et al. 2013b).

Conclusion

Although implantation strategies vary between species, eutherian mammals show many similarities in the conceptus–maternal signalling processes required to induce uterine receptivity to implantation. It seems clear that one universal prerequisite for the establishment of uterine receptivity is a period of continuous exposure of the endometrium to progesterone which downregulates the expression of PGR in the uterine epithelia and stimulates the production of progestamedins. There is also compelling evidence that uterine receptivity to implantation involves temporal expression in the endometrial epithelium of genes able to support growth, proliferation, migration and attachment of the conceptus. It is, however, not entirely clear what species-common pathways triggers the expression of these genes. Although the central role of endometrium-derived estramedins and progestamedins and of conceptus-derived oestrogens, cytokines and INFs, PGs and cortisol in the acquisition of uterine receptivity for implantation is increasingly clear, one key challenge is to understand the independent and combined actions of these conceptus- and endometrium-derived autocrine and paracrine factors. Comparative studies elucidating mechanism of receptivity to implantation in different species is essential for translational research into strategies to reduce pregnancy loss in man and animals.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

M. de Ruijter-Villani wrote the review paper, and T. Stout revised the paper critically.

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