REPRODUCTION
REVIEW

Comparative aspects of implantation

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Abstract

Uterine receptivity to implantation of blastocysts in mammals includes hatching from zona pellucida, precontact with uterine luminal (LE) and superficial glandular (sGE) epithelia and orientation of blastocyst, apposition between trophectoderm and uterine LE and sGE, adhesion of trophectoderm to uterine LE/sGE, and, in some species, limited or extensive invasion into the endometrial stroma and induction of decidualization of stromal cells. These peri-implantation events are prerequisites for pregnancy recognition signaling, implantation, and placentation required for fetal–placental growth and development through the remainder of pregnancy. Although there is a range of strategies for implantation in mammals, a common feature is the requirement for progesterone (P_4) to downregulate expression of its receptors in uterine epithelia and P_4 prior to implantation events. P_4 then mediates its effects via growth factors expressed by stromal cells in most species; however, uterine luminal epithelium may express a growth factor in response to P_4 and/or estrogens in species with a true epitheliochorial placenta. There is also compelling evidence that uterine receptivity to implantation involves temporal and cell-specific expression of interferon (IFN)-stimulated genes that may be induced directly by an IFN or induced by P_4 and stimulated by an IFN. These genes have many roles including nutrient transport, cellular remodeling, angiogenesis and relaxation of vascular tissues, cell proliferation and migration, establishment of an antiviral state, and protection of conceptus tissues from challenges by the maternal immune cells.

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Introduction

Uterine receptivity to implantation of blastocysts includes: 1) hatching from zona pellucida; 2) precontact with uterine luminal (LE) and, in some species, superficial glandular (sGE) epithelia and orientation of blastocyst/conceptus (embryo and extra-embryonic membranes); 3) apposition between trophectoderm and uterine LE/sGE; 4) adhesion of trophectoderm to uterine LE/sGE; and 5) depending on species, limited or extensive, endometrial invasion (Guillomot 1995). These peri-implantation events are prerequisites for placentation required for fetal–placental growth and development through the remainder of pregnancy. Secretory products (histotroph) from maternal uterine epithelia (histotroph), hematotrophic transfer of essential gases and nutrients, and coordinate signaling between conceptus trophectoderm and uterine epithelia are critical to conceptus growth and development, pregnancy recognition signaling, implantation, and placentation.

Following fertilization and intitiation of embryonic development in the oviduct, embryos enter the uterus and develop to the blastocyst stage prior to the initiation of implantation, regardless of species. In mares, unfertilized ova remain in the oviduct and never enter the uterus, perhaps due to failure to secrete prostaglandin E_2 (PGE_2; Weber *et al*. 1991). In all subprimate species, however, embryonic development fails prior to or at the early blastocyst stage when restricted to the oviductal environment (see Murray *et al*. 1971). This may be due to the lack of stimulation by a uterine factor(s) or the presence of a factor(s) that prevents embryonic development. Only in primates can pregnancy be established and maintained in the oviduct until the conceptus dies or ruptures through the wall of the oviduct to establish an abdominal pregnancy.

Implantation may be non-invasive (central) or invasive (interstitial or eccentric) depending on whether the trophectoderm invades through uterine LE/sGE into the stroma. Implantation in domestic animals is protracted and superficial with conceptus trophectoderm only attaching to uterine LE/sGE. The protracted pre-implantation period allows large spherical conceptuses to migrate (horse) and hatched blastocysts to migrate and then transition morphologically from spherical to tubular and filamentous conceptuses (swine and ruminant species) before apposition and attachment phases of implantation. The spherical equine conceptus is contained within a capsule that allows it to migrate

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between uterine horns 12–15 times per day to interact with uterine LE prior to apposition and attachment on about day 18 of pregnancy (see Hayes *et al*. 2008). During the preattachment period, conceptuses undergo differentiation of trophectoderm for the secretion of an antiluteolytic or luteotrophic pregnancy recognition signal for maintenance of functional corpus luteum (CL).

Changes in organization of the cytoskeleton of blastocysts/conceptuses of domestic animals are responsible for the morphological transition from spherical to tubular and filamentous forms in pigs (Geisert *et al*. 1982, Albertini *et al*. 1987, Mattson *et al*. 1990) as indicated in Fig. 1. Trophectoderm cells in the elongation zone are columnar compared with cuboidal in areas peripheral to the elongation zone. This modification is associated with changes in length and orientation of microfilaments along the lateral cell borders and redistribution of cytoplasm to the apical surface of trophectoderm cells. Specifically, orientation of microfilaments changes from horizontal to parallel relative to the lateral cell borders. Elongation of trophectoderm is initially through migration or condensation of trophectoderm cells into the region and plane of the embryonic disc to form the elongation zone. At about 10 mm diameter, alterations in microfilaments and junctional complexes of trophectoderm cells of conceptuses allow movement and redistribution of cells toward the ends of tubular and then filamentous conceptuses as they increase in length and decrease in diameter. Studies of pig blastocysts revealed that: 1) early cleavage stage embryos have filamentous actin concentrated at sites of contact between blastomeres; 2) compacting morulae accumulate actin at the margins of blastomeres, which is associated with interdigitating cell processes; and 3) trophectoderm cells of expanding blastocysts exhibit pericellular distribution of actin that later forms continuous actin-rich lateral borders and stress fibers along their basal surface. As expanded pig blastocysts elongate, abundant microvilli on the apical surface of trophectoderm cells are lost and changes occur in basolateral aspects of membranes with contiguous pericellular F-actin being essential for the expansion and elongation of conceptus trophectoderm. Thus, actin-based modifications in trophectoderm cells appear to be responsible for shaping them for progressive axial elongation and circumferential narrowing. The actin cytoskeleton is considered essential for conceptus elongation because constricted regions along the length of filamentous conceptuses contain polarized trophectoderm cells with a distinct F-actin array, and regions are more elongated and polarized in filamentous conceptuses due to actin-based contractile forces that change morphology.

The organization and bundling of actin filaments in cells are response to mechanical forces (i.e. mechanotransduction) transmitted through plasma membrane adhesion molecules that link adjacent cells and/or extracellular matrix (ECM) molecules. Mechanotransduction requires anchorage of actin filaments to the plasma membrane via interactions with the cytoplasmic tails of cadherins and integrins. Cadherins are single-pass transmembrane proteins that mediate homophilic adhesion. In epithelial cells, formation of cadherin-mediated cell junctions is accompanied by remodeling of the cytoskeleton. Cadherin–catenin complexes can actively induce actin polymerization as well as serve as signaling nodes by activating tyrosine kinases and phosphatases and interacting with adapter proteins

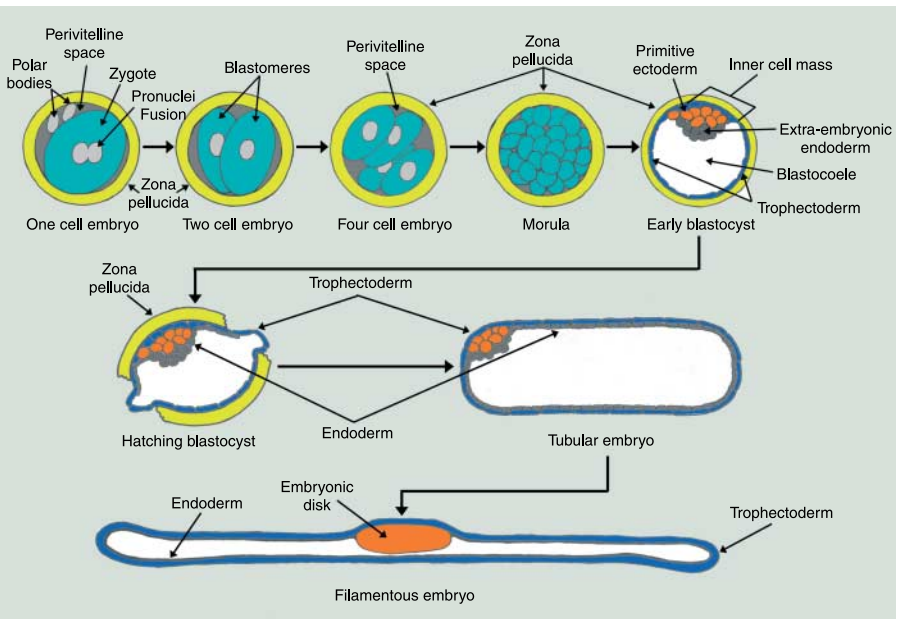

Figure 1 Early pregnancy events in domestic animals. Fertilization occurs in the oviduct, and morula-stage embryos enter the uterus where they develop into spherical blastocyst and hatch from the zona pellucida by actions of proteases. Thereafter, spherical blastocysts migrate, assume a tubular and then a filamentous form due to rapid elongation of trophectoderm before initiation of implantation. Implantation involves apposition and transient attachment followed by firm adhesion of trophectoderm to uterine lumenal and superficial glandular epithelia.

(Goodwin & Yap 2004). The initial events of conceptus elongation appear to involve actin reorganization involving homophilic adhesion and actin reorganization involving cadherin–catenin. However, initial adhesive interactions between conceptus trophectodern and uterine LE ultimately appear to involve other force-bearing adhesion molecules linked to counter receptors and ECM proteins. This may account for the fact that conceptuses of pigs and ruminants do not elongate *in vitro*.

Initial attachment of conceptus trophectoderm to uterine LE appears to require loss of anti-adhesive components, mainly mucins, from the glycocalyx of LE/sGE that sterically inhibit attachment (see Burghardt *et al*. 2009). For example, MUC1 is an intrinsic transmembrane mucin localized to the apical surface of uterine LE/sGE that is considered a barrier to implantation. The abundance of MUC1 on uterine LE/sGE is reduced during the peri-implantation period (mouse, pig, and sheep) or at sites of blastocyst attachment (human and rabbit) due to activation of cell surface proteases and loss of expression of progesterone receptors (PGR) in uterine LE/sGE. Loss of MUC1 unmasks adhesion and attachment molecules on uterine LE/sGE to permit initial apposition and stable adhesive interactions between maternal ECM and stromal cells in species in which implantation involves invasion beyond LE/sGE. Initial attachment is mediated by low-affinity carbohydrate ligand-binding molecules including selectins and galectins and, perhaps heparan sulfate proteoglycan, heparin-binding EGF-like growth factors, cadherins, and CD44. But stable adhesions with integrins expressed on trophectoderm and uterine LE and their ECM ligands are required for implantation through their roles in adhesion, migration, invasion, cytoskeletal organization, and bidirectional signaling (see Johnson *et al*. 2003, Brayman *et al*. 2004).

In humans, expression of $\alpha_v\beta_3$ and $\alpha_4\beta_1$ integrins increase in LE during the window of implantation (Kao *et al*. 2002). These and other integrins at both maternal and conceptus interfaces along with integrin-binding matrix proteins such as fibronectin, oncofetal fibronectin, vitronectin, secreted phosphoprotein 1 (SPP1 or osteopontin), laminin, insulin-like growth factor binding protein 1 (IGFBP1), and the latency-associated peptide linked to one or more isoforms of transforming growth factor-β (TGFB) are critical for both non-invasive and invasive implantation (Fazleabas *et al*. 2004, Kashiwagi *et al*. 2007). These and other ECM molecules are likely bridging ligands for stable adhesion between apically expressed maternal and fetal integrins.

There is an increasing experimental evidence that mechanotransduction involving integins and ECM proteins plays an important role in adhesion and remodeling of the conceptus during elongation and adherence of trophectoderm to uterine LE during the peri-implantation period. For example, integrins expressed at the apical surfaces of cultured ovine and porcine uterine LE and/or trophectoderm cells are rapidly activated by several ECM proteins attached to microcarrier beads resulting in rapid formation of macromolecular complexes known as focal adhesions (Johnson *et al*. 2003). Focal adhesions function to transmit force at cell adhesion sites by organizing and bundling the actin cytoskeleton and serve as signaling centers from which numerous intracellular pathways can regulate cell growth, proliferation, survival, gene expression, development, tissue repair, migration, and invasion. Development of focal adhesions *in vivo* at the maternal–conceptus interface during initial stages of implantation and placentation has not been reported, no doubt due in part to the complexity of isolating the initial attachment sites. However, treatments that block integrin attachment reduce the number of implantation sites in mice and rabbits (Illera *et al*. 2000, 2003). Large focal adhesions along with abundant ECM have been detected later in pregnancy at the apical surfaces of LE and conceptus trophectoderm in interplacentomal attachment sites in sheep. These expand in area as pregnancy progresses reflecting adaptation of the placenta to tensile, compression, and shear loads imposed by increasing fetal and placental growth (Burghardt *et al*. 2009).

Global gene profiling, using high-density microarray technology, comparing endometrial tissues from late proliferative and secretory phases of the menstrual cycle indicated that about 20% of the changes were attributed to genes encoding cell surface receptors, adhesion and ECM proteins, and growth factors, including markers of uterine receptivity in humans such as glycodelin and SPP1, stromal cell-specific IGFBP1 and 2, PGE_2 receptors, interleukin 15 (IL15), and TGFB type II receptor (see Carson *et al*. 2002, Hess *et al*. 2007). Notably, SPP1 expression by uterine GE increased 12-fold during the receptive phase in women and up to 60-fold during pregnancy in rats suggesting a direct role in embryo–uterine interactions (see Kao *et al*. 2002). Similar microarray studies are addressing uterine gene expression in cattle during early pregnancy (see Spencer *et al*. 2008).

Despite differences in duration of the pre-implantation period and type of implantation (non-invasive versus invasive), initial stages of apposition and attachment are common across species (see Spencer *et al*. 2007, Bazer *et al*. 2008, 2009). Functional changes in uterine LE/sGE include a decrease in the apical glycocalyx, cytoskeletal remodeling of LE, and loss of polarity (see Burghardt *et al*. 2009). The initial stages of implantation are depicted in Fig. 2 that also depicts differences in interactions between trophectoderm and uterine epithelia during the peri-implantation period in domestic animals (non-invasive implantation) with those of rodents, carnivores, and primates (invasive implantation). For example, intimate contact between

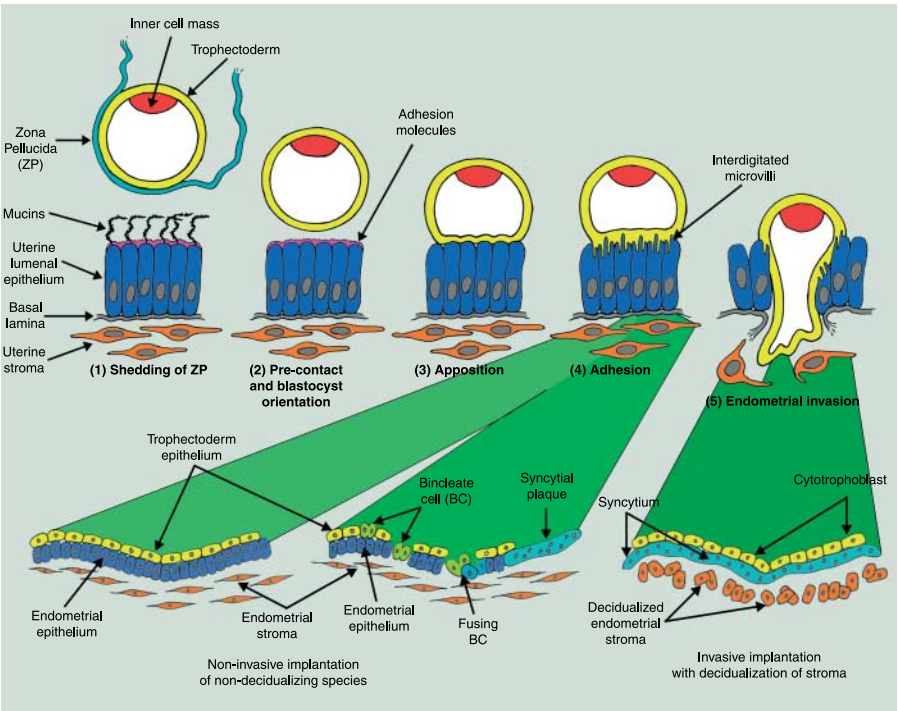

Figure 2 The phases of blastocyst implantation. Shedding of the zona pellucida is phase 1 and allows for expansion of the spherical blastocyst after that time it may migrate and transition from a spherical to tubular and filamentous form (domestic animals) or remain spherical prior to implantation. Phase 2 is a pre-contact period during which blastocysts may migrate and undergo orientation without definitive cellular contact between the conceptus trophectoderm and endometrial epithelia, and initiate pregnancy recognition signaling. Phase 3 is the apposition phase during which the conceptus trophectoderm associates closely with endometrial LE for unstable adhesion and, in ruminants, develop finger-like villi or papillae that extend into the superficial ducts of the uterine glands for stable adhesion and to absorb histotroph. Phase 4 is the adhesion phase characterized by the trophectoderm becoming firmly adhered to endometrial LE and, in some species, superficial glandular epithelium. In ruminants, this is the period of interdigitation of trophectoderm and endometrial LE in both caruncular and intercaruncular areas of the endometrium in preparation for development of cotyledons on the chorion and caruncles on the uterine endometrium to form placentomes. Also during phase 4, the mononuclear trophectoderm cells differentiate into trophoblast giant binucleate cells. Phase 5 is unique to species in which there is invasive implantation of the blastocyst through the uterine luminal epithelium and into the uterine stroma that becomes decidualized.

trophectoderm/chorion and uterine LE is maintained in pigs and horses throughout pregnancy as these species have diffuse epitheliochorial placentae. Ruminant conceptuses form binucleate trophectoderm cells that invade and fuse with uterine LE to form multinucleated cells or syncytia and a synepitheliochorial placenta. The binucleate cells (BNC) produce placental lactogen (CSH1). Attachment of the chorioallantoic membranes of ruminant placentae with uterine caruncles devoid of uterine glands induces development of placental cotyledons. The resultant structure consisting of maternal caruncles and placental cotyledons is the placentome, the major site for transfer of nutrients and gases.

Blastocysts of carnivores, rodents, and primates exhibit invasive implantation in that they invade and implant in the endometrial stoma. During initial contact, the trophectoderm is highly proliferative and undergoes syncytial formation to form a syncytiotrophoblast cell layer, which develops stable adhesion with uterine LE followed by penetration of syncyiotrophoblasts into the uterine wall and into uterine blood arterial vessels. Loss of maternal vascular endothelial cells results in the formation of maternal blood sinusoids in the hemochorial placentae of higher primates and rodents (see Fig. 2), whereas hemoendothelial placentae of carnivores retain the endothelial layer.

Mononuclear cytotrophoblasts underlie syncytiotrophoblasts and these cells migrate out of the trophoblast layer. In contrast to expansion (horse) or elongation (ruminants and pigs) of conceptuses in domestic animals to establish a large surface area for nutrient and gas exchange, species with an invasive type of implantation accomplish this by achieving close contact between trophectoderm and maternal blood. However, secretions from uterine glands appear important in humans throughout the first 3 months of gestation, whereas histotroph from uterine glands is important throughout gestation in species with epitheliochorial and synepitheliochorial placentae (Burton *et al*. 2007).

Compaction of the morula and formation of blastocysts are similar for baboon, macaque, and human, including differentiation of blastocysts prior to

implantation. The earliest indications of implantation in primates include attachment to and penetration of uterine LE by trophoblast followed by its invasion into adjacent uterine glands; however, most of the trophoblast remains superficial to the basal lamina of uterine LE. Then, ectoplasmic processes of syncytial trophoblast penetrate the basal lamina, invade into subjacent superficial maternal blood vessels, and form junctional complexes with endothelial cells. Consequently, maternal blood vessels are dilated and the stratum compactum stroma is edematous at implantation sites and some uterine glands become surrounded by trophoblast cells that do not penetrate the basal lamina. Vascular lacunae then form and fill with maternal blood in areas with syncytial clefts formed by a single layer of syncytiotrophoblast over cytotrophoblast with numerous microvilli protruding into the cleft in baboons, macaque, and humans. During early stages of lacunar formation, cytotrophoblast cells are present within superficial maternal capillaries and enter maternal arterioles as early as day 12 and are common by day 14 of gestation. The blood-filled lacunae enlarge and lift the developing placental disk above the endometrial surface and stromal edema increases at the implantation site. In addition, endovascular cytotrophoblast cells migrate into venules and uterine glands, but not into the endometrial stroma. During the lacunar stage, epithelial plaques form around the necks of uterine glands so that the developing placenta is subsequently superficial to the endometrial surface. Then, decidualization of the endometrial stroma occurs in response to implantation and this is followed by formation of the placenta.

Invasive implantation induces decidualization in primates, which involves hyperplasia and hypertrophy of stromal cells and their secretion of prolactin (PRL), ECM proteins, SPP1, laminin, and fibronectin, invasion by numerous immune cells, and formation of cell–cell contacts. Decidualized stroma produces many endocrine and paracrine factors that control trophoblast invasion by generating a local cytokine environment that promotes trophoblast attachment. Varying degrees of decidualization occur in all species with extensive stromal transformation occurring in species with invasive implantation (rodents and primates), moderate transformation occurring in species with synepitheliochorial placentae (ruminants), and minor changes occurring in species with epitheliochorial placentae (pig and horse).

Endocrine regulation of implantation involves a highly synchronized series of reciprocal interactions between conceptus trophectoderm and uterine endometrium during the 'window of receptivity' to implantation (see Fazleabas *et al*. 2004, Slayden & Keater 2007, Spencer *et al*. 2007, Bazer *et al*. 2008, 2009). Uterine receptivity to implantation requires actions of progesterone (P_4) and/or estrogen on the uterus to regulate locally produced cytokines, growth factors, homeobox transcription factors, and cyclooxygenase-derived prostaglandins through autocrine and paracrine pathways. Endometrial receptivity also requires silencing expression of PGR and/or estrogen receptor-α (ESR1) in uterine LE/sGE and GE, but continued expression of PGR in uterine stromal cells and myometrium. The effects of P_4 on PGR-negative uterine epithelia are likely then mediated by P_4 acting on PGR-positive stromal cells to stimulate expression of growth factors called 'progestamedins' that include fibroblast growth factors-7 (FGF7) and -10 (FGF10) and hepatocyte growth factor (HGF). Histotroph is required to support conceptus development; therefore, the conceptus produces hormones and cytokines to stimulate or silence expression of genes by uterine LE/sGE and GE as necessary for pregnancy recognition signaling for maintenance of a functional CL to produce P_4 required for establishment and maintenance of pregnancy. Early studies of endocrine regulation of expression of proteins secreted by uteri of pigs (uteroferrin, ACP5; Knight *et al*. 1973) and sheep (serine protease inhibitors or uterine milk proteins; Moffat *et al*. 1987) revealed a requirement for long-term treatment with P_4, indicating that effects of P_4 were not mediated by a 'classical' steroid receptor-type mechanism of action. Based on current evidence, long-term treatment with P_4 is required in order to down-regulate PGR as a prerequisite to expression of proteins such as uteroferrin and uterine milk proteins in response to one or more progestamedins (Spencer *et al*. 1999, Spencer & Bazer 2002).

P_4, the hormone of pregnancy, is required for establishment and maintenance of pregnancy in all mammals (see Bazer *et al*. 2009). The paradox is that endometrial epithelia cease expressing PGR prior to implantation in all mammals studied. For example, loss of PGR in ovine endometrial LE/sGE and GE occurs by days 11 and 13 of the oestrous cycle and pregnancy respectively; but stromal cells and myometrial cells express PGR throughout gestation. The loss of PGR in uterine epithelia appears to be a prerequisite for implantation, as well as epithelial cell proliferation and differentiated functions as directed by specific factors produced by PGR-positive stromal cells in mammals (see Fig. 3).

The cell-specific expression of progestamedins is also of interest with respect to conceptus development. Pig blastocysts are not invasive in the uterus likely because of abundant secretion of protease inhibitors by uterine epithelia (Fazleabas *et al*. 1982), but pig blastocysts are invasive when transferred to an ectopic site such as kidney capsule (Samuel & Perry 1972). Therefore, contrary to dogma, uterine LE is in direct contact with non-invasive conceptus trophectoderm cells that express FGFR2(IIIb) respond directly to FGF7 with respect to proliferation and differentiated functions of trophectoderm cells in pigs (see Ka *et al*. 2007, Bazer *et al*. 2008). In ruminants, uterine LE and conceptus trophectoderm interact to form BNC that produce PRL3D1 and form

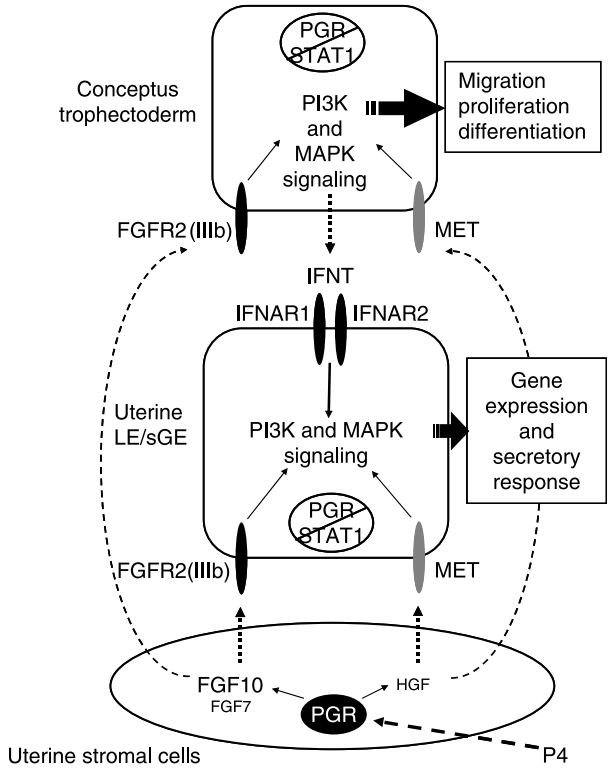

Figure 3 Hypothesis on the roles of progesterone, progestamedins (FGF7, FGF10, and HGF), and interferon τ (IFNT) on gene expression and secretory functions of ovine uterine luminal (LE) and superficial glandular (sGE) epithelia that lack both progesterone receptor (PGR) and signal transducer and activator of transcription 1 (STAT1) and glandular epithelium (GE) that lack PGR. Ovine uterine LE/sGE lack detectable PGR and STAT1, indicating that P_4 and IFNT use non-classical-signaling pathways to regulate expression of P_4-induced and IFNT-stimulated genes and GE lack PGR. Importantly, stromal cells remain PGR positive throughout pregnancy. Progesterone increases production of stromal-derived progestamedins in the ovine uterus, particularly FGF10. Therefore, our hypothesis is that progestamedins act on uterine LE, sGE, and GE, as well as conceptus trophectoderm cells that express FGFR2(IIIb) and MET receptors for FGF7/FGF10 and HGF respectively to activate MAPK and PI3K cell signaling. Progestamedins and type I IFNs activate the PI3K- and AKT1-signaling pathways in other cell types (Bazer *et al.* 2009).

syncytia, and, with loss of uterine LE, trophectoderm cells are in direct contact with uterine stromal cells that express FGF10 and HGF and perhaps FGF7 expressed by tunica intima of blood vessels that can interact with their respective receptors on trophectoderm cells, i.e. FGFR2(IIIb) and MET (see Bazer *et al.* 2008, 2009). Expression of novel genes by uterine LE and sGE is discussed in more detail in the sections on implantation in ruminants and in pigs. In species with invasive placentae, it is also true that trophectoderm cells achieve direct contact with stromal cells that produce progestamedins and/or estramedins that can directly stimulate proliferation and differentiated functions of trophoblast cells (Slayden & Keater 2007).

Maternal recognition of pregnancy

Maintenance of pregnancy in mammals requires functional CL to produce P_4 to support secretory functions of the endometrium that sustain early embryonic development, implantation, and placentation. Maternal recognition of pregnancy signaling from conceptus to the maternal system may be either luteotrophic, i.e. a hormone acts directly on CL to maintain luteal function, or antiluteolytic, i.e. the hormone prevents uterine release of luteolytic prostaglandin $F_{2\alpha}$ (PGF). The result is the maintenance of functional CL for production of P_4 that is permissive to actions of interferons (IFNs), growth factors, and cytokines responsible for uterine receptivity to implantation in most mammals (Fazleabas *et al.* 2004, Soares 2004, Spencer *et al.* 2007). In primates, CGB acts directly via LHCGR on luteal cells for the maintenance of structural and functional integrity of CL (Fazleabas *et al.* 2004). In rodents, mating induces release of PRL from the anterior pituitary, which acts as the initial luteotrophic hormone for CL formation and production of P_4 to about day 12 when lactogenic hormones from conceptuses and uterine decidua take over to maintain luteal function (Soares 2004). In ruminants (sheep, cow, and goat) and pigs, antiluteolytic hormones for pregnancy recognition and CL maintenance are interferon τ (IFNT) and estradiol (E_2) respectively. IFNT silences expression of ESR1 (estrogen receptor α) that precludes estrogen stimulation of expression of oxytocin receptors (OXTR) and, therefore, OXT-induced release of luteolytic pulses of PGF to prevent CL regression (see Bazer *et al.* 2008). In pigs, E_2, acting in concert with PRL, exerts antiluteolytic effect on uterine epithelia to prevent endocrine release of luteolytic PGF by directing secretion of PGF into the uterine lumen (exocrine release) where it is metabolized (Bazer & Thatcher 1977). Pig conceptus trophectoderm expresses both a type I (IFN δ, IFND) and type II (IFN γ, IFNG) IFN during the period of conceptus elongation between days 14 and 20 of pregnancy, but neither of these IFNs has been shown to be antiluteolytic (Cencič *et al.* 2003).

A common feature of the peri-implantation period of pregnancy in domestic animals, rodents, and primates is the production of type I and/or type II IFNs by trophectoderm, which induce and/or stimulate expression of IFN-stimulated genes (ISGs) in the uterus in a temporal and cell-specific manner. Although IFNT is the only known IFN to act as the pregnancy recognition signal, IFNs appear to affect uterine receptivity, decidualization, and placental growth and development in primates, ruminants, pigs, and rodents (see Bazer *et al.* 2008, 2009). The IFN family includes one type II IFN (IFNG), as well as multiple type I IFNs that include IFNA, IFNB, IFND, IFNT, and IFNW1. IFNT is unique to ruminants and IFND is unique to pigs (Cencič *et al.* 2003) and horses (Tayade *et al.* 2009). All type I IFNs

bind a common receptor composed of two subunits, IFNAR1 and IFNAR2, to induce cell signaling via the JAKs and tyrosine kinase 2 (TYK2) pathways respectively (Darnell *et al*. 1994). Signaling by type II IFNG involves activation of JAK1 and JAK2 constitutively associated with IFNGR1 and IFNGR2 subunits of type II IFNR respectively. IFNG stimulates autophosphorylation and subsequent tyrosine phosphorylation and homodimerization of STAT1 allowing STAT1 homodimers to translocate to the nucleus and bind GAS elements in promoter regions of IFNG-regulated genes (Leanza *et al*. 2007). There is evidence that IFNs are expressed by human placentae (IFNA, IFNB, and IFNG), decidua (IFNA, IFNB, and IFNG) and fetal membranes (IFNA and IFNG), as well as conceptus trophectoderm of sheep (IFNT) and pig (IFND and IFNG), and rodent uteri and/or conceptuses (IFNA and IFNB; see Bazer *et al*. 2008, 2009). These IFNs have classical antiviral, antiproliferative, and immunosuppressive effects, as well as unique biological activities.

Uterine receptivity to implantation requires P_4 that is also permissive to actions of IFNs, CG (chorionic gonadotropin), and lactogenic hormones (Fazleabas 2007, Joyce *et al*. 2007*a*, 2007*b*, Slayden & Keater 2007). The paradox is that cessation of expression of PGR and ESR1 by uterine epithelia is a prerequisite for uterine receptivity to implantation, expression of genes for secreted proteins, and selective transport of molecules into the uterine lumen that support conceptus development. Downregulation of PGR and loss of expression of some proteins by uterine LE, such as MUC1, appear to be a prerequisite for uterine receptivity to implantation. Silencing expression of *PGR* in uterine epithelia requires that P_4 acts via PGR-positive uterine stromal cells to induce expression of progestamedins, e.g. FGF7, FGF10, and/or HGF to exert paracrine effects on uterine epithelia and conceptus trophectoderm that express receptors for FGF7 and FGF10 (*FGFR2(IIIb))* and HGF (MET; proto-oncogene *MET*; Slayden & Keater 2007, Bazer *et al*. 2008, 2009). Many ISGs are P_4-induced and IFN-stimulated; however, the mechanism whereby actions of P_4 and IFNs on uterine epithelia lacking PGR and the classical JAK/TYK2 cell-signaling mechanisms is not known. But it is likely that their effects are mediated via progestamedins and IFN receptors via non-classical cell-signaling pathways such as MAPK and phosphoinositide-3 kinase (PI3K) to affect gene expression and uterine receptivity to implantation (Platanias 2005). Type I IFNs use the same receptor, but activate unique signaling pathways to differentially affect gene expression in uterine LE/sGE, GE, and stromal cells. Cell-specific gene expression in the ovine uterus is due, at least in part, to restriction of expression of interferon regulatory factor 2 (IRF2), a potent transcriptional repressor of ISGs, to uterine LE/sGE (Choi *et al*. 2001).

In spite of expression of IRF2 in ovine uterine LE/sGE, there is a growing list of genes discovered to be expressed by those cells that are P_4-induced and IFNT-stimulated during the period of uterine receptivity to implantation and conceptus development (see Spencer *et al*. 2007, Bazer *et al*. 2008). For example, the energy substrate for mammalian conceptuses switches from pyruvate to glucose at the blastocyst stage, which is coordinate with increases in expression of glucose transporter genes by conceptus trophectoderm and uterine epithelia, some of which (SLC2A1 and SLC5A11) are P_4-induced and IFNT-stimulaed (Gao *et al*. 2009*a*). Similarly, significant increases in amino acids in the ovine uterine lumen occur coincidentally with increases in expression of cationic amino acid transporters SLC7A1 and SLC7A2 in uterine LE/sGE in response to P_4 and IFNT (Gao *et al*. 2009*b*, 2009*c*). Conceptus trophectoderm also expresses specific transporters for uptake of glucose and amino acids from the uterine lumen. Glucose, leucine, and arginine are nutrients that stimulate proliferation of trophectoderm cells by activating the glutamine:fructose-6-phosphate amidotransferase (GFPT1)-mediated FK506 binding protein 12-rapamycin associated protein 1 (FRAP1, formerly mTOR) signaling pathway (Wen *et al*. 2005). Arginine is also essential for fetal–placental growth and development through effects on synthesis of nitric oxide (NO) and polyamines that stimulate vascular functions and DNA and protein synthesis for proliferation and differentiation of cells respectively (Wu & Morris 1998, Wu *et al*. 2004).

Other genes induced by P_4 and further stimulated by *IFNT* in ovine uterine LE/sGE during the peri-implantation period include galectin 15 (*LGALS15*), cathepsin L (*CSTL*), cystatin C (*CST3*), *WNT7A*, hypoxia inducible factors (*HIF*) *1A* and *2A*, gastrin-releasing peptide (*GRP*), and *IGF2*. In contrast, major histocompatibility complex class I molecules and β2-microglobulin that regulate immune rejection responses are silenced in LE/sGE, perhaps to protect the conceptus allograft. Similar results have been reported for pigs (Joyce *et al*. 2008). Details on the regulation of expression and potential functions of LGALS15, cathepsin L, cystatin C, WNT7A, HIF1A, HIF2A, GRP, IGF2, MHC class I molecules, and β2-microglobulin have been reported recently (see Spencer *et al*. 2007, Bazer *et al*. 2008, 2009).

SPP1 is a secreted ECM protein that is upregulated in the uterus during early pregnancy in humans, mice, rabbits, goats, sheep, and pigs (see Johnson *et al*. 2003). SPP1 contains an Arg–Gly–Asp (RGD) sequence that mediates binding to cell surface integrin receptors, including $\alpha_v\beta_3$, $\alpha_5\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_5$, $\alpha_v\beta_6$,and $\alpha_8\beta_1$, as well as alternative binding sequences for interactions with $\alpha_4\beta_1$, $\alpha_9\beta_1$, and $\alpha_4\beta_7$. Binding of SPP1 to these various receptors elicits diverse effects including cell-to-cell and cell-to-ECM adhesion, leukocyte, smooth muscle cell and endothelial cell chemotaxis, endothelial and epithelial cell survival, and fibroblast, macrophage, and tumor cell migration. SPP1 is secreted by ovine

uterine GE from day 13 of pregnancy in response to P_4, and the 45 kDa form is the most abundant and has greater binding affinity for $\alpha_v\beta_3$ integrin than the 70 kDa form. In pregnant pigs, estrogens secreted by the elongating day 12 conceptuses induce synthesis and secretion of SPP1 specifically in the maternal uterine LE in direct apposition to the implanting conceptus. SPP1 protein is abundant on both the apical surface of uterine LE and conceptus trophectoderm from the beginning of the attachment phase of implantation in many species. Binding of SPP1 to integrin receptors on conceptus trophectoderm and uterine LE stimulates changes in proliferation, hypertrophy, migration, survival, and adhesion of cells, as well as remodeling of conceptuses during elongation and adherence of trophectoderm to LE during the peri-implantation period (see Johnson *et al.* 2003) as depicted in Fig. 4.

The nutrient sensing pathway is via FRAP1 (FK506 binding protein 12-rapamycin associated protein 1, formerly known as mTOR, RAFT, and RAPT), a highly conserved serine–threonine protein kinase that responds to changes in concentrations of amino acids, glucose, hormones, and mitogens (Schmelzle & Hall 2000). As indicated in Fig. 4, FRAP1 is critical for growth and proliferation of cells involved in conceptus development through effects on translation of proteins required for trophoblast differentiation in mice (Martin & Sutherland 2001). Disruption of the *Frap1* gene leads to post-implantation lethality due to impaired cell proliferation and hypertrophy in both the embryonic disc and trophoblast (Gangloff *et al.* 2004, Murakami *et al.* 2004). Other components of FRAP1 complexes also play important roles as knockout of *Raptor*, *Mlst8* (Guertin *et al.* 2006), *Rictor* (Shiota *et al.* 2006, Guertin *et al.* 2007), and *Mapkap1* (Jacinto *et al.* 2006) genes results in disfunction of mTORC1 and mTORC2 respectively with fetal lethality occuring at different stages of development. mTORC1 (FRAP1, mLST8, and RAPTOR) is associated with cell proliferation, protein synthesis, and gene expression, whereas mTORC2 (FRAP1, mLST8, RICTOR, and MAPKAP1) is associated with cell migration and changes in organization of the cytoskeleton (see Fig. 4). The mRNAs for *FRAP1*, *LST8*, *MAPKAP1*, *RAPTOR*, *RICTOR*, *TSC1*, *TSC2*, *RHEB*, and *EIF4EBP1* are localized to uterine LE/sGE, GE, and stromal cells of ovine uteri, as well as trophectoderm

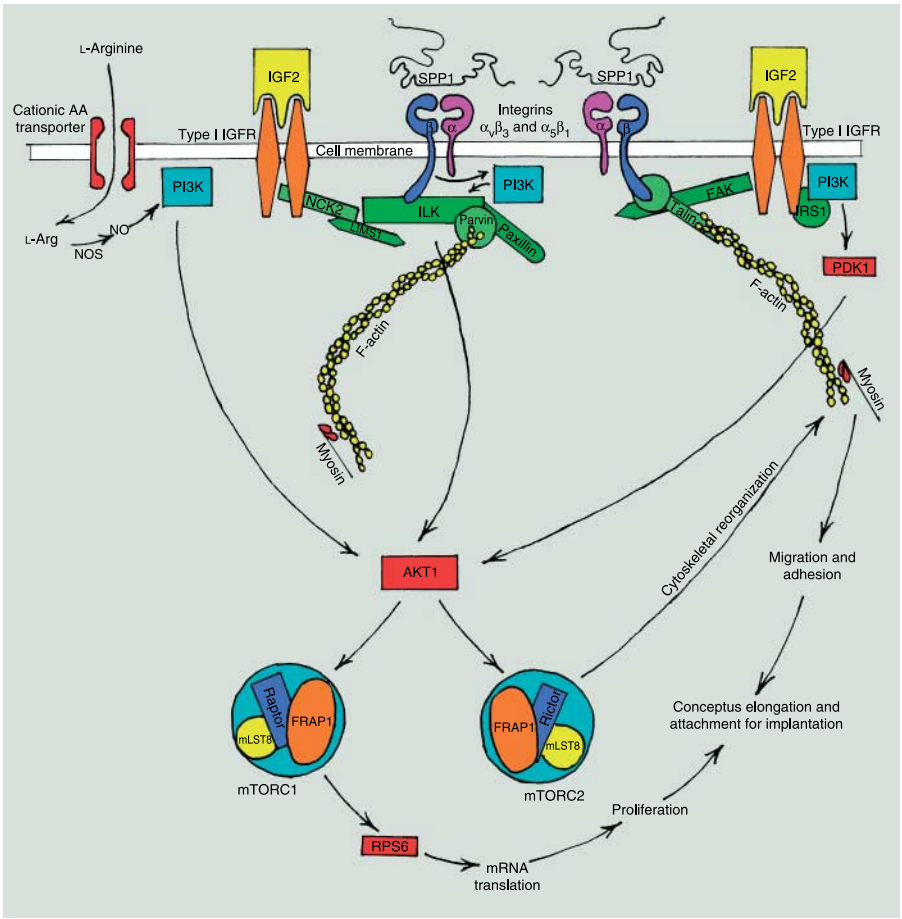

Figure 4 Model for induction of cell signaling for proliferation, migration, adhesion, and cytoskeletal remodeling of conceptuses. Our working model summarizes pathways whereby IGF2, Arg and SPP1 may activate FRAP1 cell-signaling pathways via pathways that converge on AKT1 and FRAP1/mTOR cell signaling via mTORC1 (cell proliferation and gene expression) and/or mTORC2 (cell migration, cell survival, and cytoskeletal proteins) to affect oTr cells in conceptuses for transition from spherical to tubular and filamentous forms that can signal pregnancy recognition, as well as undergo implantation and placentation. AKT1, proto-oncogenic protein kinase 1; L-Arg, arginine; FAK, focal adhesion kinase; FRAP1, FK506 binding protein 12-rapamycin associated protein 1, also known as mTOR, mammalian target of rapamycin; IGF2, insulin-like growth factor 2; type I IGFR, type I insulin-like growth factor receptor; ILK, integrin-linked kinase; IRS1, insulin receptor substrate 1; LST8, G protein β-subunit-like protein; NCK2, non-catalytic region of tyrosine kinase, beta; NO, nitric oxide; NOS, nitric oxide synthase; PI3K, phosphatidylinositol 3-kinase; LIMS1, LIM and senescent cell antigen-like domains 1; RPS6, ribosomal protein S6; SPP1, secreted phosphoprotein 1.

and endoderm of ovine conceptuses (Gao *et al*. 2009*e*). The abundance levels of *LST8*, *MAPKAP1*, *RHEB*, and *EIF4EBP1* mRNAs increase in ovine endometria during early pregnancy and in response to P_4 and IFNT.

The proteins induced via mTORC1 and mTORC2 in trophectoderm and endoderm of ovine conceptuses include NOS, ODC1, and GTP cyclohydrolase (GCH1, the key enzyme in *de novo* synthesis of tetrahydrobiopterin, an essential cofactor for NO production; Gao *et al*. 2009*f*). In mice, for example, leucine, arginine, and glutamine are transported by system $B^{0,+}$ (solute carrier family 6 (neurotransmitter transporter), member 14 (SLC6A14)) in mouse blastocysts (Lewis & Kaye 1992, Jamshidi & Kaye 1995, Bode 2005) in response to estrogen (Van Winkle & Campione 1987, Sloan & Mager 1999), and other hormones also regulate trophectoderm differentiation through the FRAP1-dependent pathway (Hara *et al*. 1998, Wang *et al*. 1998). Glucose may regulate the activity of FRAP1 via insulin and insulin-initiated signaling or via an insulin-independent pathway to regulate proliferation of trophectoderm cells (Patel *et al*. 2001, Ruvinsky *et al*. 2005, Wen *et al*. 2005).

In the human placenta, amino acid transporters belong to different families and systems (Regnault & Hay 2006, Grillo *et al*. 2008), but little is known about their expression in uterine endometria and conceptuses. In contrast, various glucose transporters identified in preimplantation embryos provide a mechanism for glucose uptake and utilization. The solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1) may be important for transporting glucose into the uterus and/or into the conceptus (Pantaleon *et al*. 1997), because of its ubiquitous nature and high abundance in peri-implantation blastocysts (Scheepers *et al*. 2004, Shiota *et al*. 2006), while SLC2A3 may be involved in the uptake of glucose by cells (Pantaleon *et al*. 1997) and expression of SLC2A4 in syncytiotrophoblast can be regulated by maternal insulin and concentrations of glucose in maternal blood (Ericsson *et al*. 2005).

Translation of genes critical to trophoblast development, differentiation, and motility including *IGF2*, *ODC1*, *NOS3*, *NOS2A*, and *NOS1* is stimulated via the FRAP1– RPS6KB1 pathway (Nielsen *et al*. 1995, Kimball *et al*. 1999). IGF2 is associated with placental and fetal growth (Ohlsson *et al*. 1989), RPS6KB1 is under control of FRAP1 (Nielsen *et al*. 1995) to induce NO production (Kaliman *et al*. 1999). Interestingly, a positive autocrine feedback loop may exist between FRAP1 and IGF2 to promote cell growth (Nielsen *et al*. 1995). ODC1 can regulate conceptus development and differentiation by catalyzing the synthesis of polyamines that associate with DNA and nuclear proteins to produce normal chromatin required for proliferation of trophectoderm and formation of multinucleated trophectoderm cells (see Bachrach *et al*. 2001). ODC1 and polyamines are important for trophectoderm motility and receptivity of uterine epithelial cells to adhesion by trophectoderm of mouse blastocysts (Martin *et al*. 2003), rapid growth of the placenta and fetus, as well as increases in placental blood flow in pigs and sheep (see Wu *et al*. 2005). NO promotes blastocyst attachment and trophectoderm motility, possibly through modifications of the ECM, and stimulation of vasodilation of maternal capillaries, through PI3K/AKT/FRAP1 induced by HGF, and/or by stimulating expression of SPP1 (see Guo *et al*. 2005). Both NOS1 and NOS3 are present in ovine trophectoderm during the peri-implantation period and NOS2A increases during early- and mid-gestation (Kwon *et al*. 2004), and NOS1 is expressed by uterine endometria and conceptuses of rats (Mara *et al*. 1995).

Transporters of neutral and acidic amino acid transporters are also expressed in uteri of pregnant ewes and in conceptus trophectoderm and endoderm with some being induced by P_4 and further stimulated by IFNT (see Gao *et al*. 2009*d*). The marked increases in the utero-placental transport of amino acids are necessary to support rapid placental and fetal growth during pregnancy. Thus, studies with sheep and pigs have shown that intrauterine growth retardation is associated with impaired placental transport of amino acids.

Expression of chemokine, cc motif, ligand 1 (CCL1) and CCL2 increases between days 13 and 19 of pregnancy in eosinophils of ovine uteri treated with P_4 and intrauterine IFNT (Asselin *et al*. 2001). Trophoblast giant BNC first appear on day 14 in sheep and are transformed into syncytial plaques as trophoblast BNC fuse with uterine LE to produce trinucleate fetomaternal hybrid cells (Wooding 1992). Eosinophils may participate in this process by inducing cell death in uterine LE via perforin, granzyme B, and/or FAS ligand (Costain *et al*. 2001) to allow for intimate contact between trophectoderm and endometrial stroma to enhance conceptus development by direct exposure to stromal-derived growth factors.

Endometrial expression of ESR1, PGR, and OXTR is not affected by either ovine PRL3D1 or ovine GH; however, expression of serine protease inhibitor, kunitz-type, 1 (SPINT1 or uterine milk proteins) by GE increases in response to both PRL3D1 and GH only if ewes are treated first with intrauterine IFNT between days 11 and 21, and then either PRL3D1 or GH and P_4 daily from days 16 to 29 after onset of estrus (see Spencer *et al*. 2004). However, the mechanism whereby IFNT permits GE to be responsive to PRL3D1 and GH during gestation is not known.

Many ISGs induced by IFNT in ruminants (see Spencer *et al*. 2007) are among the most upregulated genes in human endometrial stromal cells co-cultured with human trophoblast (Popovici *et al*. 2006) or treated with human trophoblast-conditioned medium (Hess *et al*. 2007) and in endometria of baboons, domestic animals, and laboratory animals (see Bazer *et al*. 2008, 2009). The mechanisms whereby IFNT exerts temporal

and cell-specific effects on expression of ISGs in ovine endometrial cells are novel in that: 1) P_4-induced gene expression by uterine epithelia is likely mediated by a progestamedin(s) that is also permissive to stimulatory effects of IFNT; 2) IFNT stimulation of ISGs can be via the classical STAT1-dependent cell-signaling pathway (GE and SC) or via a non-classical cell-signaling pathway (LE/sGE); 3) non-classical cell-signaling mechanisms unique to LE/sGE proximal to conceptus trophectoderm result in expression of P_4-induced and IFNT-stimulated genes that affect conceptus remodeling, attachment, nutrient transport, and implantation; and 4) classical cell signaling in GE and stromal cells results in expression of classical ISGs, e.g. antiviral genes.

IFNs and uterine receptivity in primates

There is abundant information on uterine receptivity to implantation in baboons, but the focus here is on humans (see Fazleabas 2007). Syncytiotrophoblast cells of human conceptuses secrete CG from days 8 to 10 for pregnancy recognition, and implantation begins on days 7–9 post-ovulation. During pregnancy, PGR expression is limited to endometrial decidual tissue to insure a P_4-responsive endometrium permissive to establishment and maintenance of pregnancy. The window of uterine receptivity to implantation in women is between days 6 and 10 post-ovulation and invasion of the conceptus into the endometrium begins on about day 11. The CG acts via LHCGR expressed by uterine epithelia and stromal cells to induce decidualization of stromal cells that: 1) secrete PRL and IGFBP1; 2) increase edema; 3) express α-smooth muscle actin and prostaglandin-endoperoxide synthase 2 (*PTGS2*) genes; 4) increase angiogenesis and blood flow; and 5) express leukemia inhibitory factor (LIF). IL1β (IL1B) from trophoblast also increases expression of IGFBP1 and decidualization of endometrial stromal cells.

During the window of implantation in humans, P_4 stimulates morphological development of uterine glands and secretory activity by GE and a decrease in ESR1 marks the onset of uterine receptivity. Thereafter, ESR1 and PGR are restricted to the basalis zone of the endometrium prior to implantation and PGR remain abundant in uterine stromal cells. The window of implantation in humans is characterized by expression of integrin heterodimers $\alpha_1\beta_1$, $\alpha_4\beta_1$, and $\alpha_v\beta_3$, which can bind fibronectin, vitronectin, thrombospondin, von Willebrand factor, bone sialoprotein 1, L-selectin, and SPP1. L-selectin binding to $\alpha_4\beta_1$ is associated with establishment of connections between invading conceptus trophectoderm and maternal vasculature that extends to placentation, while $\alpha_v\beta_3$ and SPP1 localization to pinopods of endometrial LE is a marker of implantation. Glycodelin may be required for implantation, whereas LIF and calcitonin are considered to be essential for implantation.

Secretions of uterine GE increase in response to P_4 and contain uteroglobin, histone A2, spermidine/spermine acetyltransferase 2, secretory leukocyte protease inhibitor, and metallothionein. Stromal cells of humans and macacque also secrete proprotein convertase 6 (PCSK5) at the implantation site. However, P_4 also suppresses expression of proteins including TGFB, MMP11, proenkephalins, cysteine/glycine-rich protein 2, collagen type $VII_{\alpha 1}$, and frizzled-related protein 4, while FGF7 has anti-apoptotic effects on uterine GE.

Type I and type II IFNs produced by human placenta and decidual cells (see Aboagye-Mathiesen *et al*. 1996) may: 1) regulate proliferation of trophoblast or other cells in the uterus; 2) exert immunosuppressive effects by suppressing mitogen-induced proliferation of T- and B-cells; 3) protect the conceptus from viral infections; 4) regulate cellular differentiation and expression of cell surface antigens; 5) stimulate expression of ε-globin, a component of embryonic hemoglobin; and 6) suppress expression of proto-oncogenes such as *EGFR*, *c-erB-2*, and *CSF1R* to affect trophoblast growth and differentiation. As noted earlier, ISGs are among the most upregulated genes in human endometrial stromal cells treated with human trophoblast-conditioned medium (see Hess *et al*. 2007). Kumar *et al*. (2001) found guanylate-binding protein 1 (GBP1) to be induced by both IFNA and IFNG and it is considered a marker of uterine receptivity to implantation, although its function is not known. The Mx proteins, also GTPases, are induced by type I IFNs and may protect against viral infection. Li *et al*. (2001) reported expression of p27 (cyclin-dependent kinase inhibitor 1b; CDKN1B), which has high homology to interferon-regulated gene 1 (IRG1), to increase in Ishikawa cells in response to IFNA, and that E_2 and IFNA exert synergistic effects on abundance of CDKN1B that preceded cell proliferation. The *CDKN1B* gene is also expressed during the window of implantation in humans and is considered essential for control of normal endometrial proliferation (Erkanli *et al*. 2006). The shift in endometrial production from PGF to PGE is associated with implantation and, in humans, IFNA suppresses P_4-regulated production of basal PGF, but not PGE_2 (Mitchell & Smith 1992). Of particular interest is the report that IFNA may stimulate transcription of the *CGB* gene without effects on cell proliferation (Iles & Chard 1989); however, these results are from studies using a bladder tumor cell line, which have not been confirmed using trophectoderm cells.

Studies of cytokines regulating development of human conceptuses may also have undesirable effects. For example, the combined effects of tumor necrosis factor-α (TNF), IFNG, and IL1B may lead to pregnancy failure due to loss of blood supply and conceptus death (Peyman & Hammond 1992).

IFNs, estrogens, and uterine receptivity to implantation in pigs

Pregnancy recognition in the pig results from secretion of estrogens by conceptus trophectoderm between days 11 and 15 of pregnancy, which induces a mechanism to redirect PGF secretion from the uterine vasculature to the uterine lumen (see Bazer & Thatcher 1977). These estrogens also modulate expression of uterine genes considered essential for implantation, and inappropriate exposure of the pregnant uterus to estrogen on days 9 and 10 results in degeneration of pig conceptuses by day 15 (Ross *et al*. 2007). Both PGE_2 and lysophosphatidic acid (LPA) have proposed roles in pregnancy recognition signaling. Expression of PGE_2 synthase by trophoblast and endometrium decreases production of PGF in favor of PGE_2 to support CL maintenance (Ziecik *et al*. 2008). In addition, LPA increases in uterine luminal fluids and endometrium of pigs in response to estrogens, binds to its receptor, EDG7, in pig conceptuses (So *et al*. 2008), and may be critical for migration and spacing of blastocysts prior to implantation in pigs as has been reported for mice (Ye *et al*. 2005).

Pig conceptus trophectoderm secretes both IFND and IFNG during the peri-implantation period (see Cencič *et al*. 2003, Joyce *et al*. 2007*a*, 2007*b*), which, on day 15 of pregnancy, co-localize to peri-nuclear membranes typically occupied by endoplasmic reticulum and golgi apparatus, as well as cytoplasmic vesicles within clusters of trophectoderm cells along the endometrial LE. This expression is accompanied by *de novo* appearance of zona occludens one (ZO1), a marker of epithelial tight junctions, on their basal aspect, suggesting changes in endometrial polarity. Pig conceptus IFNs do not have antiluteolytic effects, but stimulate secretion of PGE_2 by pig endometrium (see Bazer *et al*. 2008). Further expression of several IFN-responsive genes in pig endometrium has been described (see Hicks *et al*. 2003, Joyce *et al*. 2007*a*, 2007*b*, 2008).

Estrogens and IFNs regulate endometrial genes that affect conceptuses during pregnancy in pigs. Estrogens secreted by the conceptus induce SPP1 expression in uterine LE, whereas stromal induction of STAT1 correlates with IFNG and IFND secretion by the conceptus. Indeed, administration of exogenous E_2 to ovariectomized pigs induces *SPP1* mRNA in endometrial LE (White *et al*. 2005), while intrauterine infusion of conceptus secretory proteins containing IFND and IFNG into cyclic pigs treated with exogenous estrogen increases expression of STAT1 (Joyce *et al*. 2007*a*). Upregulation of SPP1 within uterine LE and STAT1 within stroma and GE is only in close proximity to a conceptus, which implies paracrine regulation of these genes by conceptus estrogens and IFNs. It is likely that effects of estrogen on the endometrium are restricted to regions near the conceptus due to the fact that the pig endometrium converts E_2 to estrone and then to biologically inactive estrone sulfate (Flood 1974). Pig trophectoderm has sulfatase enzyme activity to convert estrone sulfate back to biologically active estrone to upregulate genes such as *SPP1* in LE. In contrast, initial increases in expression of STAT1 in stromal cells are restricted to sites of contact between the conceptus and uterus, although IFNG synthesis and secretion by pig conceptuses appear to be similar in magnitude to IFNT production by sheep conceptuses (Joyce *et al*. 2007*a*). Indeed, STAT1 expression increases universally in the stroma and GE of pregnant sheep independently of conceptus location within the lumen, presumably due to the high levels of secretion of IFNT by conceptuses (Spencer *et al*. 2007). Perhaps the spatial pattern of STAT1 expression in the pig uterus requires that IFND and IFNG act synergistically to upregulate expression of ISGs. Interactions between type I and type II IFNs have been demonstrated (Decker *et al*. 1989). High levels of IFNG may act on uterine stroma and GE to increase intracellular interferon-stimulated gene factor 3 (ISGF3) that permits lower levels of IFND to maximally upregulate STAT1 expression in close proximity to the implanting pig conceptus. To date, a limited number of estrogen- and IFN-stimulated genes have been localized in pig endometrium (see Hicks *et al*. 2003, White *et al*. 2005, Joyce *et al*. 2007*a*, 2007*b*, 2008, Ka *et al*. 2007, Ross *et al*. 2007, So *et al*. 2008, Song *et al*. 2009). Type I and type II IFNs each induce expression of largely non-overlapping sets of genes and they may also have synergistic interactions to affect physiological responses (see Levy *et al*. 1990). Although IFNG may enhance uterine receptivity to implantation in pigs, highly localized and abundant expression of IFNG, TNF, IL1B, and IL1R may be associated with arrested conceptus development between days 15 and 23 of pregnancy (Wessels *et al*. 2007).

IFNs and uterine receptivity to implantation in rodents

Type I IFNs are present in high concentrations in placenta, but not maternal or fetal tissues in mice and viral infections as early as day 7 of pregnancy induce IFNA, IFNB, and IFNG (Platt & Hunt 1997). Reese *et al*. (2001) reported increased expression of IFNB and several ISGs in uterine implantation sites of mice following treatment with E_2 to terminate delayed implantation. Li *et al*. (2001) found expression of IFNA and IRG1 in uteri of pregnant rats to increase between days 1 and 4 and then decrease following implantation. *Irf1* mRNA was most abundant in uteri of rats treated with both E_2 and IFNA. In support of findings of Li *et al*. (2001), Austin *et al*. (2004) and Bany & Cross (2006) reported that mouse trophoblast giant cells express IFNA that, in turn, induced expression of ISG15 during the peri-implantation period.

Microarray analyses revealed significantly altered expression of genes at implantation sites in mice, including upregulation of interferon-activated gene 202 and downregulation of genes for histocompatibility 2, T-region locus 23, *IRF6*, and MHC class I and class II (Reese *et al*. 2001). IFNG is expressed by uterine LE and GE, trophoblast cells, and degenerating metrial gland cells of mice (Monk *et al*. 2005); however, most IFNG in decidua of mice at mid-gestation may be from uterine natural killer cells (Ashkar *et al*. 2000). Major changes in uterine spiral arteries between days 9 and 10 of gestation in mice do not occur in IFNG null or type II IFNR null mice or in alymphoid mice (Ashkar *et al*. 2000, Monk *et al*. 2005). Thus, endothelial cells, vascular smooth muscle cells, and stromal cells of the uterus may be targets of action of IFNG. Furthermore, IFNG is targeted to heparan sulfate that is essential for implantation in mice (Kirn-Safran *et al*. 2008), and this may protect IFNG from inactivation and increase its stability (Lortart-Jacob & Grimaud 1991) to allow protracted effects of IFNG on vascular development in decidual tissue. Type I and type II IFNs modify gene expression at implantation sites in rodents to affect the conceptus directly or to exert indirect effects through actions on blood vessels and decidual cells required for successful implantation and pregnancy (Lash *et al*. 2006).

Summary

In reviewing the wide range of strategies for implantation in mammals, it seems clear that P_4 must downregulate expression of PGR in uterine epithelia and that progestamedins must regulate gene expression by uterine epithelia, perhaps in a more precise way than P_4. Further results from studies of pigs counter the dogma that progestamedins are unique to stromal cells. Rather, pigs and other species with true epitheliochorial placentae require key interactions between uterine epithelia and trophectoderm, including expression of FGF7 by porcine uterine LE, during the peri-implantation period. In ruminants, there is shedding of uterine LE, and in primates, there is invasion of the blastocyst into the uterine stroma to assure direct contact between trophectoderm and stromal cells and their secreted progestamedins and/or estramedins that affect conceptus development. There is also compelling evidence that uterine receptivity to implantation involves temporal and cell-specific expression of ISGs that may be induced directly by an IFN or induced by P_4 and stimulated by an IFN. The affected genes have many roles including nutrient transport, cellular remodeling, angiogenesis, and relaxation of vascular tissues, cell proliferation and migration, establishment of an antiviral state, and protection of conceptus tissues from challenges by the maternal immune cells. One key challenge is to understand independent and combined actions of progestamedins and IFNs on uterine epithelia that lack both PGR and STAT1, as well as the range of effects mediated by lactogenic hormones and CGB on uterine endometria of rodents and primates during implantation and placentation. The comparative study of mechanisms of uterine receptivity to implantation should reveal improved strategies to enhance reproductive health and fertility in humans and animals.

Declaration of interest

The authors declare that there is no conflict of interest that prejudices the impartiality of this scientific work.

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