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Novel pathways for implantation and establishment and maintenance of pregnancy in mammals

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ABSTRACT: Uterine receptivity to implantation varies among species, and involves changes in expression of genes that are coordinate with attachment of trophectoderm to uterine lumenal and superficial glandular epithelia, modification of phenotype of uterine stromal cells, silencing of receptors for progesterone and estrogen, suppression of genes for immune recognition, alterations in membrane permeability to enhance conceptus-maternal exchange of factors, angiogenesis and vasculogenesis, increased vascularity of the endometrium, activation of genes for transport of nutrients into the uterine lumen, and enhanced signaling for pregnancy recognition. Differential expression of genes by uterine epithelial and stromal cells in response to progesterone, glucocorticoids, prostaglandins and interferons may influence uterine receptivity to implantation in mammals. Uterine receptivity to implantation is progesterone-dependent; however, implantation is preceded by loss of expression of receptors for progesterone (PGR) so that progesterone most likely acts via PGR-positive stromal cells throughout pregnancy. Endogenous retroviruses expressed by the uterus and/or blastocyst also affect implantation and placentation in various species. Understanding the roles of the variety of hormones, growth factors and endogenous retroviral proteins in uterine receptivity for implantation is essential to enhancing reproductive health and fertility in humans and domestic animals.

Key words: implantation / placentation / pregnancy / uterus / conceptus development

Introduction

Strategies for implantation

Implantation involves attachment of conceptus trophectoderm (Tr) of the developing conceptus (the embryo and its associated extra-embryonic membranes) to uterine luminal epithelium (LE) in a highly synchronized series of events requiring reciprocal secretory and physical interactions during a restricted period known as the 'window of receptivity' (Carson *et al.*, 2000; Dey *et al.*, 2004). The 'window of receptivity to implantation' is established by actions of progesterone and, in some species, estrogen (E2) that regulate locally produced cytokines, growth factors, homeobox transcription factors and cyclooxygenase-derived prostaglandins through autocrine and paracrine pathways (Paria *et al.*, 2002). A paradox is the role of progesterone to sequentially down-regulate expression of progesterone receptors (PGR) in uterine LE, as well as superficial (sGE) and mid- to deep-glandular (GE) epithelia as a prerequisite for endometrial receptivity to implantation; however, PGR continue to be expressed in stromal and myometrial cells of the uterus. Subsequent effects of progesterone on PGR-negative uterine epithelia are likely mediated by stromal cell-derived growth factors known as 'progestamedins' (Spencer and Bazer, 2002; Cunha *et al.*, 2004).

Implantation may be non-invasive (central) or invasive (interstitial or eccentric) depending on whether or not conceptus Tr invades through uterine LE into the stroma. Implantation in domestic animals differs from that of rodents and primates where the conceptus enters a receptive uterus and almost immediately attaches to uterine LE. Domestic animals have a protracted preimplantation period (the pre-receptive phase) during which the developing conceptus migrates throughout the uterine lumen. Equine blastocysts remain spherical and contained within a capsule prior to attachment, whereas pig and ruminant conceptuses shed the zona pellucida (hatch) and transform morphologically from spherical to tubular and filamentous forms. Pre-attachment conceptus development is accompanied by growth

and differentiation of the Tr that secretes a pregnancy recognition signal.

In all mammals, initial conceptus attachment requires alteration in the expression of anti-adhesive components, mainly mucins, contained in the glycocalyx of LE that sterically inhibit attachment (Brayman *et al*., 2004). The mucin, MUC1, exists as both an intrinsic transmembrane mucin and an alternatively spliced, secreted variant. Both forms are localized to the apical uterine LE to provide a barrier to attachment, but are generally reduced during the receptive phase (mice, pig, sheep) or locally at the site of blastocyst attachment (human, rabbit) due to activation of cell surface proteases (Carson *et al*., 2006).

Unmasking adhesion molecules on the surface of uterine LE permits initial contacts with Tr that progressively develop into more stable adhesion through interactions between Tr and maternal extracellular matrix (ECM), as well as stromal cells encountered following intrusion beyond the uterine LE during invasive implantation (Burghardt *et al*., 2002). Initial adhesion or attachment is mediated by molecules that contribute specific carbohydrate ligand binding including selectins and galectins, as well as heparan sulfate proteoglycan, heparin binding epidermal growth factor (EGF)-like growth factors, cadherins and CD44 (Carson *et al*., 2000). Low-affinity interactions are followed by stable adhesion involving integrins expressed on Tr and uterine LE and their ECM bridging ligands that also have roles in adhesion, migration, invasion, cytoskeletal organization and bidirectional signaling (Burghardt *et al*., 2002). In humans, expression of αvβ3 and α4β1 integrins increase in uterine LE during the window of implantation (Lessey *et al*., 1996). 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, and the latency associated peptide linked to transforming growth factor-beta (TGFB) are critical in species having non-invasive and invasive implantation (Burghardt *et al*., 2002). These and other ECM constituents such as insulin-like growth factor binding protein-1 (IGFBP1) (Simmons *et al*., 2009a) and galectin-15 (LGALS15) (Farmer *et al*., 2008) may function as bridging ligands to promote blastocyst expansion initially followed by stable adhesion between apically expressed maternal and fetal integrins. Other genes reported to be markers of uterine receptivity to implantation include laminin β3, microfibril-associated protein 5, angiopoietin-like 1, endocrine gland-derived vascular endothelial growth factor (VEGF) and nuclear localized factor 2 that, in a general thematic context, are associated with angiogenesis and vascularization of tissues (Haouzi *et al*., 2009).

During the initial stages of implantation conceptus/maternal interactions differ between domestic animals (non-invasive implantation) and rodents, carnivores and primates (invasive implantation) (Bazer *et al*., 2009b). Differences among species exist in the extent of interactions between Tr (gives rise to chorion) and maternal uterus at the interface between maternal and fetal cells giving rise to placental structures. For example, intimate contact between chorion derived from Tr and an intact LE is maintained in pigs throughout pregnancy (epitheliochorial placenta). Ruminant conceptuses form binucleate Tr cells which migrate and fuse with uterine LE and each other to form plaques of multinucleated syncytia (synepitheliochorial placenta). Binucleate Tr cells and the syncytia derived from binucleate cell migration and fusion are the source of placental lactogen as well as other hormones such as progesterone (Wooding *et al*., 1992). In both epitheliochorial and synepitheliochorial placentation, the conceptus remains within the uterine lumen throughout gestation. In ruminants, contact between the chorioallantois and caruncles, discrete sites on the endometrial mucosa devoid of uterine glands, leads to development of opposing highly vascularized cotyledons of the chorioallantois that form placentomes. The placentomes are critical for exchange of nutrients and gases across the placenta (Reynolds *et al*., 2005).

Carnivores, rodents, and primates exhibit invasive implantation where the blastocyst invades and implants deeply into the endometrial stoma and then the uterine LE is restored over the site of implantation. During initial contact, the Tr is highly proliferative and undergoes syncytial formation to form a syncytiotrophoblast cell layer which develops stable adhesion with uterine LE followed by penetration of syncytiotrophoblasts into the uterine wall to establish extensive contacts with the maternal vasculature. Loss of maternal vascular endothelial cells results in the formation of maternal blood sinusoids in hemochorial placentae of higher primates and rodents, whereas the hemoendothelial placenta of carnivores retains the endothelial layer. Mononuclear cytotrophoblasts underlie syncytiotrophoblasts and migrate out of the trophoblast layer as well as fuse together to maintain the syncytium.

Global gene profiling comparing endometrial tissue between proliferative and secretory phases of human endometria identified many differentially expressed genes including cell surface proteins/receptors, ECM molecules, secretory proteins, immune modulators and cytokines, cytoskeletal proteins, transport proteins and transcription factors as well as proteins involved in cholesterol trafficking, prostaglandin biosynthesis, detoxification, cell cycle regulation, signal transduction, transport and metabolism of nutrients, coagulation cascades, chemotaxis, phagocyte recruitment, angiogenesis and other cellular functions (Carson *et al*., 2002; Horcajadas *et al*., 2007; Savaris *et al*., 2008; Tseng *et al*., 2009). 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 insulin growth factor binding proteins-1 and -2 (IGFBP1, IGFBP2), prostaglandin E_2 receptor (EP2), interleukin-15 (IL15) and TGFB type II receptor for which expression increased. Notably, expression of SPP1 by uterine GE increased 12-fold during the receptive phase in women (Carson *et al*., 2002) and up to 60-fold during pregnancy in rats (Girotti and Zingg, 2003), suggesting a direct role in conceptus–uterine interactions. SPP1 is an abundantly secreted ECM protein up-regulated in uteri during early pregnancy in humans, mice, rabbits, goats, sheep and pigs (Johnson *et al*., 2003a). SPP1 contains an Arg-Gly-Asp (RGD) sequence that mediates binding to cell surface integrin receptors, including αvβ3, α5β1, αvβ1, αvβ5, αvβ6 and α8β1, as well as alternative binding-sequences for interactions with α4β1, α9β1 and α4β7. Binding of SPP1 to these various receptors elicits diverse effects including cell-to-cell and cell-to-ECM adhesion, chemotaxis of leukocytes, smooth muscle cells and endothelial cells, endothelial and epithelial cell survival, and migration of fibroblasts, macrophages and tumor cells. Similar microarray studies have addressed changes in uterine gene expression during early pregnancy in ruminants (Spencer *et al*., 2008).

Cytoskeletal changes mediate blastocyst expansion and elongation of conceptus Tr in non-invasive implantation and trophoblast outgrowth and invasion in invasive implantation

The mechanisms responsible for elongation of pig conceptuses are likely common to conceptuses of other livestock species that undergo rapid elongation during the peri-implantation period of pregnancy, i.e. a reduction in diameter and a rapid increase in length of Tr. Pig conceptus Tr cells in the elongation zone are columnar compared with cuboidal in areas peripheral to the elongation zone and that this structural modification is associated with changes in length and orientation of microfilaments (Geisert *et al*., 1982). That is, orientation of microfilaments in pig Tr cells change from horizontal to parallel relative to the lateral cell borders suggesting that elongation is initially through migration or condensation of Tr cells into the region of the embryonic pole forming the elongation zone in 10 mm diameter blastocysts. Within the elongation zone, alterations in microfilaments and junctional complexes of Tr cells and extension of filopodia from extra-embryonic endodermal cells allow movement and redistribution of cells toward the ends of tubular blastocysts. There are also changes in the actin cytoskeleton in Tr during the transition from spherical to tubular and filamentous forms as follows: (i) early cleavage stage embryos have filamentous actin concentrated at sites of contact between blastomeres; (ii) compacting morulae accumulate actin at the margins of blastomeres; and (iii) Tr cells of expanding blastocysts initially exhibit pericellular distribution of actin that later forms continuous actin-rich lateral borders and stress fibers along their basal surface (Albertini *et al*., 1987; Mattson *et al*., 1990). The actin cytoskeleton is essential for force generation for conceptus elongation as constricted regions along the length of filamentous conceptuses contain polarized Tr cells with a distinct F-actin array. Focal adhesions are macromolecular complexes comprised of heterodimeric transmembrane integrin receptors that connect ECM to the actin cytoskeleton to regulate cell growth, proliferation, survival, migration, gene expression and cell morphology (Erikson *et al*., 2009). Recently, SPP1 was shown to bind directly to the integrin heterodimers αVβ6 on Tr and αVβ3 on uterine LE to induce assembly of focal adhesions that promote migration and attachment of Tr to LE that may be critical to conceptus elongation and implantation (Erikson *et al*., 2009).

Living cells use tensegrity (tensional integrity) architecture to control shape and structure of tissues and cells through changes in stability of cytoskeletal structures that include: (i) microfilaments, self-assembling actin polymers that form relatively rigid but flexible networks that self-assemble into cross-linked bundles, or when associated with myosin II, form 'contractile microfilaments' that generate tension; (ii) intermediate filaments, polymers composed of cytokeratins in epithelial cells that form flexible cables extending from the cell surface to the nucleus to distribute force; and (iii) microtubules, larger hollow polymers of tubulin that extend across the cytoplasm to the cell periphery (Zaidel-Bar *et al*., 2007; Ingber, 2008). Integrins and associated proteins are regarded as 'tensegrity structures' because molecular connections between ECM, integrins, cytoskeletal filaments and nuclear scaffolds provide a discrete path for transfer of mechanical signals through cells as well as a mechanism for producing integrated changes in cell and nuclear structure. The resulting focal adhesion complex or 'integrin adhesome' (Zaidel-Bar *et al*., 2007) physically links integrins to the ends of contractile microfilament bundles ('stress fibers') to form a molecular bridge between ECM and cytoskeleton. Focal adhesions increase in size as tension increases across transmembrane integrin receptors (Riveline *et al*., 2001). Cells are therefore able to respond to both internally generated or externally applied forces and can sense the rigidity and anisotropy of the ECM (Geiger *et al*., 2009). Pulling on ECM tugs on integrins and associated focal adhesion proteins to deform mechanosensory molecules that elicit biochemical signals which change intracellular metabolism and gene expression as our model proposes for elongating ovine conceptus Tr.

Integrin and growth factor associated cell signaling from the extracellular space into the cell (outside-in signaling) regulates multiple cellular processes including survival, proliferation, shape, polarity, adhesion, migration and differentiation of cells (Hehlgans *et al*., 2007). Ligand binding to the extracellular integrin domain induces conformational changes and integrin clustering for activation of signaling cascades and recruitment of multiprotein complexes to focal adhesions. More than 150 different proteins have been identified that either physically reside within these adhesion sites or interact with the adhesion components and affect their activity (Zaidel-Bar *et al*., 2007). Many of these components link integrin-mediated signals with other signaling pathways to promote extensive cross-talk with growth factors, cytokines, G-protein coupled receptors and nutrient signaling pathways.

In mice, leucine or arginine is required for expanded blastocysts to exhibit motility and outgrowth of Tr required for implantation (Lessey *et al*., 1994; Zeng *et al*., 2008). These amino acids regulate motility and outgrowth of Tr through activation of serine/threonine kinase mTOR (FRAP1) cell signaling which activates Rac-1, a member of the Rho GTPase family. Increased FRAP1 signaling also stimulates protein synthesis and expression of *IGF2*, nitric oxide synthase (NOS) and ornithine decarboxylase (ODC) mRNAs (Martin and Sutherland, 2001; Martin *et al*., 2003). Implantation of the human embryo and migration of human extravillous Tr requires multiple Rho GTPase family members in both Tr cells and the endometrial stromal cells into which they invade (Grewal *et al*., 2008; Nicola *et al*., 2008). Rho GTPases including RhoA, Rac1 and Ccd42 are ubiquitous proteins that control cytoskeletal changes by forming actin-containing stress fibers and projecting filopodia and lamellipodia during cell migration through linking ECM molecules with the actin cytoskeleton by forming focal adhesions. Therefore, activation of GTPases are also thought to be controlled by integrin activation, but the mechanism(s) whereby ECM favors activation of individual molecules is not known (Symons and Segall, 2009).

The mTOR signaling pathway has been linked to elongation of conceptus Tr in sheep. For ovine conceptus development during implantation and placentation, integrin activation by SPP1 binding and arginine are proposed to stimulate remodeling of Tr for elongation and adherence to LE/sGE via cytoskeletal reorganization that facilitates cell motility, stabilizes adhesion and collectively activates mTOR signaling pathways mediated by AKT1, TSC1/2 and mTORC1 (cell proliferation and mRNA translation), as well as mTORC2 (cell migration, cell survival and cytoskeletal organization)

in Tr cells. For ovine Tr cells, SPP1 binds αvβ3 and α5β1 integrins to induce focal adhesion assembly, a prerequisite for adhesion and migration of Tr, through activation of: (i) RPS6K via crosstalk between FRAP1/mTOR and mitogen-activated protein kinase (MAPK) pathways; (ii) mTOR, PI3K, MAPK3/MAPK1 (Erk1/2) and MAPK14 (p38) signaling to stimulate Tr cell migration; and (iii) focal adhesion assembly and myosin II motor activity to induce migration of Tr cells. These cell signaling pathways, acting in concert, mediate adhesion, migration and cytoskeletal remodeling of ovine Tr cells essential for expansion and elongation of conceptuses and attachment to uterine LE for implantation (J. Kim *et al.*, unpublished results).

Decidualization

Penetration of the LE barrier by invasive Tr cells triggers a series a stromal responses collectively termed decidualization (Salamonsen, 1999). During decidualization, hyperplasia and hypertrophy transforms small spindle-like endometrial stromal cells into enlarged polygonal epithelial-like cells with extensive cell–cell contacts (Salamonsen *et al.*, 1995; Afonso *et al.*, 1997). As they differentiate, these cells express additional or different arrays of cytoskeletal proteins (Gard and Lazarides, 1980) and exhibit marked accumulation of filamentous proteins which include microtubules, microfilaments, intermediate filaments and the microtubular lattice (Sananes *et al.*, 1978). Two cytoskeletal proteins characteristic of decidual cells are desmin (Oliveira *et al.*, 2000; Glasser and Julian, 1986) and α-smooth muscle actin (Christensen *et al.*, 1995). These cytoskeletal proteins are believed to be physically involved with changes in growth, shape and protein secretion by stromal cells during the decidualization process (Rao and Cohen, 1991). Functionally, decidualized stromal cells secrete prolactin (Maslar *et al.*, 1986; Tessier *et al.*, 2000) and IGFBP1 (Bell, 1991; Kim *et al.*, 1999) which likely function in complex gene networks that restrain trophoblast invasion (Bell, 1979; Irwin and Giudice, 1998), as well as many other endocrine and paracrine factors (Popovici *et al.*, 2000; Brar *et al.*, 2001; Salamonsen *et al.*, 2002). Decidual cells also accumulate ECM proteins including SPP1, laminin and fibronectin. For SPP1, expression is by decidual natural killer cells in mice (White *et al.*, 2005), but by stromal cells in humans (von Wolff *et al.*, 2004), and SPP1 may be involved in angiogenesis within the decidua. The end result is the formation of a morphologically and functionally distinct tissue that produces hormones, promotes nutrition of conceptuses, prevents fetal allograft rejection and regulates placentation by limiting Tr invasion through generation of a local cytokine environment which promotes Tr attachment over invasion (Lee *et al.*, 1997; Irwin and Giudice, 1999). The decidua constitutes the maternal side of the maternal-fetal interface involved in exchange of molecules between these tissues necessary for successful completion of gestation. It is increasingly apparent that varying degrees of decidualization-like differentiation of stromal cells is common to all implanting species with most extensive transformation accompanying invasive implantation in rodents and primates, moderate transformation with synepitheliochorial placentation, and very minor changes with epitheliochorial placentation (Johnson *et al.*, 2003b, 2009).

Pregnancy recognition signaling

Establishment and maintenance of pregnancy in mammals requires that a functional corpus luteum (CL) be maintained beyond its normal cyclic lifespan for continued production of progesterone required for secretory functions of the endometrium essential for embryonic development, implantation and placentation. The maternal recognition of pregnancy signals from the conceptus may be luteotrophic, if it directly promotes luteal function, or anti-luteolytic, if it prevents uterine release of luteolytic prostaglandin $F_{2\alpha}$ (PGF) which would cause regression of the CL (Bazer *et al.*, 2008). Chorionic gonadotrophin (CG), the luteotrophic signal in primates, acts directly on the CL as does prolactin released from the anterior pituitary in response to mating in rodents. In domestic animals, anti-luteolytic signals from the conceptus include estrogen and prolactin in pigs, interferon-tau (IFNT) in ruminants, and an undetermined factor(s), perhaps estrogen and interferon delta (IFND), in horses (Bazer *et al.*, 2008).

Pregnancy recognition signaling by IFNT in ruminants

IFNT, the pregnancy recognition signal in ruminants, suppresses transcription of *ESR1* and, therefore, estrogen-induced expression of the oxytocin receptor (*OXTR*) gene in uterine LE/sGE to abrogate development of the endometrial luteolytic mechanism involving oxytocin-induced luteolytic pulses of PGF (Bazer *et al.*, 2008). However, basal production of PGF is higher in pregnant than cyclic ewes due to continued expression of prostaglandin endoperoxide synthase 2 (PTGS2). IFNT silencing of *ESR1* expression also prevents estrogens from inducing *PGR* in endometrial epithelia, which is critical as the absence of PGR in uterine epithelia is required for expression of progesterone-induced and IFNT-stimulated genes in ovine uterine LE/sGE (Bazer *et al.*, 2009a, b).

Pregnancy recognition signaling in pigs

The pregnancy recognition signal is estrogen secreted by pig conceptuses on Days 11 and 12 of pregnancy to redirect PGF secretion from the uterine vasculature to the uterine lumen. The theory of estrogen-induced maternal recognition of pregnancy in pigs is based on evidence that: (i) the uterine endometrium secretes luteolytic PGF; (ii) pig conceptuses secrete estrogens which are anti-luteolytic; (iii) PGF is secreted toward the uterine vasculature (endocrine) in cyclic gilts to induce luteolysis; and (iv) secretion of PGF in pregnant gilts is into the uterine lumen (exocrine) where it is sequestered from the corpora lutea and/or metabolized to prevent luteolysis (Bazer and Thatcher, 1977). In addition, PGE2, as well as lysophosphatic acid (LPA) have proposed roles in pregnancy recognition signaling. Expression of PGE2 synthase by trophoblast and endometrium decreases production of PGF in favor of PGE2 to support CL maintenance (Ziecik *et al.*, 2008). In addition LPA increases in uterine luminal fluids of pigs, and its receptor, EDG7, is expressed by pig conceptuses, and its expression is increased by estrogen in endometrial epithelia during early pregnancy (Seo *et al.*, 2008). Indeed, LPA3 is critical for migration of blastocysts as they space themselves equally throughout the two uterine horns. Pig conceptus Tr also secretes interleukin-1 beta (IL1B) during this period, but its role is not known (Ross *et al.*, 2003). Pig conceptus Tr is unique in secreting both IFND, a Type I IFN, and interferon gamma (IFNG), a Type II IFN, during the peri-implantation period (Cencič *et al.*, 2003). Abundant *IFNG* mRNA is detectable in porcine conceptus Tr between Days 13 and 20 of pregnancy, whereas *IFND* mRNA is detectable in Day 14 conceptuses only by RT–PCR analysis (Joyce *et al.*, 2007). On Day 15 of pregnancy,

IFNG and IFND proteins are co-localized to peri-nuclear membranes typically occupied by endoplasmic reticulum and Golgi apparatus, as well as cytoplasmic vesicles within clusters of Tr cells along the endometrial LE. This expression is characterized by *de novo* appearance of zona occludens one, a component of epithelial tight junctions, on their basal aspect, suggesting changes in endometrial polarity (Cencič *et al*., 2003). Although pig conceptus IFNs have no known anti-luteolytic effects for pregnancy recognition, their receptors are expressed on uterine epithelial cells (Cencič *et al*., 2003). Increased secretion of PGE2, expression of several known IFN-responsive genes, and modulation of uterine stromal and GE gene expression in response to IFNs have been demonstrated in preparations of pig conceptus secretory proteins (Johnson *et al*., 2009).

Pregnancy recognition signaling in primates

Pregnancy recognition signaling in primates extends CL function at least until the time of the luteal-placental shift when production of progesterone by the placenta is adequate to support pregnancy (Fazleabas *et al*., 2004; Afshar *et al*., 2007). Primate blastocysts begin implantation following attachment to uterine LE on Days 7–9 post-ovulation in macaques and humans or Days 11–12 in marmoset monkeys. Syncytiotrophoblast cells of human conceptuses secrete CG from Days 8 to 10 for pregnancy recognition and implantation begins on Days 7–9 post-ovulation. CG produced by primate blastocysts signals maternal recognition of pregnancy through its luteotrophic actions via LHCGR on luteal cells. Circulating concentrations of CG, first detected around the time of implantation in all primates, increase to peak values in the first trimester and then decrease during late gestation in humans. Production of CG by the human conceptus may be regulated by gonadotrophin-releasing hormone one from the uterus as gonadotrophin-releasing hormone receptors are detectable in placental tissue. Importantly, gonadotrophin releasing hormone receptor agonists enhance and antagonists suppress CG secretion. In many primates, CG production decreases at the time of the luteal-placental shift in progesterone production. Further, exogenous CG increases progesterone production and extends CL lifespan in both women and monkeys.

Pregnancy recognition signaling in rodents

In rodents, mating induces release of prolactin from the anterior pituitary and it is the initial luteotrophic signal for CL formation and production of progesterone to about Day 12 of pregnancy, and then lactogenic hormones from conceptuses and uterine decidua act on luteal cells to maintain their function and secretion of progesterone (Soares, 2004). The gestation period for rats, mice and hamsters is 20–22 days, and functional CL must produce progesterone through Day 17 (Soares, 2004). Thus, maintenance of functional CL and production of progesterone, requires two endocrine events in rodents: (i) mating-induced diurnal and nocturnal surges of PRL increase LH receptors on luteal cells for formation of CL and suppress 20α-hydroxysteroid dehydrogenase activity to prevent conversion of progesterone to 20α-hydroxy progesterone that will not support pregnancy; and (ii) production of lactogenic hormones by uterine decidua and placentae act via receptors for prolactin on CL to maintain production of progesterone throughout gestation (Soares, 2004).

Uterine biology and conceptus development during the peri-implantation period

Progestamedins, estramedins, corticoids and prostaglandins

Uterine receptivity to implantation is dependent on progesterone which is permissive to actions of interferons, CG, prolactin and placental lactogen (Soares, 2004; Fazleabas, 2007; Joyce *et al*., 2007; Slayden and Keator, 2007; Bazer *et al*., 2009a). 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 by uterine epithelia and selective transport of molecules into the uterine lumen that support conceptus development. In the ewe, we demonstrated that down-regulation of PGR in uterine GE is required for induction of secretory gene expression by progesterone in the same epithelia; however, co-administration of estrogen and progesterone resulted in up-regulation of expression of PGR and decreased expression of serine protease inhibitors and SPP1 mRNAs and protein in uterine GE (Spencer *et al*., 1999). Down-regulation of PGR is also associated with down-regulation of MUC1 on uterine LE which is a prerequisite for uterine receptivity to implantation, as well as up-regulation of galectin 15, SPP1 and IGFBP1 in uterine LE/sGE that stimulate migration and attachment of Tr cells. Further, silencing expression of *PGR* in uterine epithelia allows progesterone to act on PGR-positive uterine stromal cells to increase expression of progestamedins, e.g. fibroblast growth factor-10 (FGF10) and hepatocyte growth factor (HGF) in sheep uteri (Satterfield *et al*., 2008) or FGF7 and HGF in primates (Slayden and Keator, 2007). These progestamedins exert paracrine effects on uterine epithelia and conceptus Tr that express receptors for FGF7 and FGF10 (*FGFR2IIIb*) and HGF (MET; protooncogene *Met*). In sheep, many genes are progesterone-induced and IFN-stimulated; however, a fundamental unanswered question is whether actions of progestamedins and IFNs on uterine epithelia or other uterine cell types involve novel non-classical cell signaling pathways, independent of PGR and STAT1 (Bazer *et al*., 2009b). There is evidence that both progestamedins and IFNT can signal via MAPK and phosphoinositide-3 kinase (PI3K) to affect gene expression and uterine receptivity to implantation (Platanias, 2005). Interestingly, Type I IFNs bind the same receptor, but activate novel cell-specific signaling pathways to differentially affect gene expression in uterine LE/sGE versus GE and stromal cells. Cell-specific gene expression in the ovine uterus is due, at least in part, to expression of interferon regulatory factor 2 (IRF2), a potent inhibitor of transcription, by uterine LE/sGE (Choi *et al*., 2001; Spencer and Bazer, 2002).

Estramedins in pigs

Pig conceptuses secrete estrogens between Days 10 and 15 for pregnancy recognition, but also to increase expression of genes within the uterine LE, which act on conceptus Tr and uterine LE to stimulate proliferation, migration, adhesion and gene expression that supports implantation and development of the conceptus (Bazer *et al*., 2008). The limited number of estrogen-stimulated genes localized in endometrial of pigs include: *AKR1B1*, *B2M*, *CD24*, *FGF7*, *IRF2*, *MX1*, *NMB*, *SLAs 1*, *2*, *3*, *6*, *7*, *8*, *spp1*, *STC1* and *EDG7* (Johnson *et al*.,

2009). IGF1 is expressed by uterine glands of cyclic and pregnant pigs and IGF1 receptors are expressed by cells of the endometrium and conceptuses, suggesting paracrine and autocrine actions of IGFI (Letcher *et al*., 1989). FGF7, an established stromal cell derived paracrine mediator of hormone-regulated epithelial growth and differentiation (Ka *et al*., 2007). However, FGF7 expression is novel in pigs as it is expressed by uterine LE between Days 12 and 15 of the estrous cycle and pregnancy. FGF7 binds to and activates FGF2IIIb expressed by uterine epithelia and conceptus Tr. Estrogen increases *FGF7* expression only after progesterone suppresses expression of PGR by uterine epithelia. The FGF7, in turn, increases cell proliferation, phosphorylated FGFR2IIIb, the mitogen-activated protein kinase cascade and expression of urokinase-type plasminogen activator, a marker for Tr cell differentiation (Ka *et al*., 2007). From about Day 20 of pregnancy, FGF7 is expressed by uterine GE in pigs in response to progesterone and may continue to affect uterine epithelia and conceptus development (G.A. Johnson *et al.*, unpublished results). In addition to the increase in secretion of estrogens between Days 11 and 15 of pregnancy for maternal recognition of pregnancy, increases in estrogens from the placenta between Days 20 and 30 increase expression of endometrial receptors for prolactin, uterine secretory activity and uterine blood (Bazer *et al*., 2008).

Corticoids

Glucocorticoids have positive effects that promote pregnancy. These include stimulation of secretion of CG, suppression of uterine natural killer cells and promotion of trophoblast growth/invasion. There are also potential negative effects that might compromise pregnancy by inhibiting cytokine-prostaglandin signaling, restriction of trophoblast invasion, induction of apoptosis, and inhibition of embryonic and placental growth (Michael and Papageorghiou, 2008). A dialogue initiated by cell surface signaling molecules on conceptus Tr and uterine LE includes integrins and fibronectin that glucocorticoids could suppress to adversely affect implantation (Ryu *et al*., 1999). However, effects of glucocorticoids on fibronectin expression are tissue-specific with dexamethasone suppressing fibronectin in term human cytotrophoblasts and amnion, but acting in synergy with TGFB to up-regulate fibronectin expression in matched samples of chorion and placental mesenchymal cells. Also, during the peri-implantation period of pregnancy, events mediated by pro-inflammatory cytokines, such as IL1B, TNFA and prostaglandins, are modulated by anti-inflammatory effects of glucocorticoids which could otherwise impair the cytokine-prostaglandin signaling required for implantation. Both IL1B and TNFA increase expression and activity of hydroxysteroid dehydrogenase (HSD)11B1 whereas suppressing expression of HSD11B2 in term human chorionic trophoblasts. The result is an increase in conversion of corticosterone to active cortisol and creation of a negative feedback loop at the uterine–conceptus interface between glucocorticoids and cytokines.

In most tissues, the anti-inflammatory effect of glucocorticoids is to inhibit synthesis of prostaglandins and thromboxanes by decreasing the expression and/or actitivity of phospholipase A2 (PLA2) and liberation of arachidonic acid as substrate for prostaglandin-endoperoxide synthase 1 (PTGS1) and PTGS2 (Barnes *et al*., 2006). However, in the placenta, glucocorticoids increase PLA2, PTGS2 and prostaglandin synthases (Zhang *et al*., 2006) and decrease expression of 15-alpha hydroxyprostaglandin dehydrogenase (HPGD) that converts prostaglandins to their inactive forms (Patel *et al*., 2003). Prostaglandins increase expression and activity of HSD11B1 (Alfaidy *et al*., 2001) and decrease activity of HSD11B2 to increase synthesis of cortisol (Hardy *et al*., 2001). Glucocorticoids have been reported to stimulate trophoblast growth and up-regulate expression of pro-matrix metalloproteinase (proMMP)-2 (Mandl *et al*., 2006), but other reports indicate that they inhibit expression of MMP-9 and migration (invasiveness) of cytotrophoblast cells (Librach *et al*., 1994). Further, glucocorticoids affect degradation of ECM during trophoblast invasion by increasing urokinase-type plasminogen activator (uPA) that leads to plasmin-associated degradation of ECM and tissue-type enzyme (tPA) plasmin-dependent breakdown of fibrin for establishment of an efficient vascular exchange in the placenta (Loskutoff *et al*., 1993). The activities of both uPA and tPA are inhibited by plasminogen activator inhibitor (PAI1) secreted by trophoblast and decidual cells (Hofmann *et al*., 1994). Both cortisol and dexamethasone increase expression of PAI1 (Ma *et al*., 2002) which may result in poor placental exchange of nutrients and gases and lead to pre-eclampsia and intrauterine growth retardation (IUGR) (Grancha *et al*., 1996).

Establishment of pregnancy in sheep requires elongation of the conceptus and production of IFNT for pregnancy recognition signaling as discussed previously. Expression of HSD11B1 may be stimulated by progesterone, prostaglandins and/or cortisol which is consistent with findings that *HSD11B1* mRNA is more abundant in ovine uterine LE/sGE between Days 12 and 16 of pregnancy than the estrous cycle and that expression of both HSD11B1 and PTGS2 by uterine LE/sGE is coordinate with conceptus elongation in sheep (Simmons *et al*., 2009b). Physiological levels of cortisol are potent stimulators of both arginase and ornithine decarboxylase expression in cells to increase the synthesis of polyamines essential for cell proliferation and differentiation (Flynn *et al*., 2009; Rhoads and Wu, 2009). Although HSD11B1 is abundant in the uterine LE/sGE, its expression is barely detectable in the conceptus, whereas HSD11B2 expression is barely detectable in uterine endometria, but abundant in the conceptus. Expression of HSD11B1 is induced by progesterone and further stimulated by IFNT in uterine LE/sGE. The glucocorticoid receptor, NR3C1, is present in all ovine uterine cell types. Therefore, HSD11B1 expression in ovine uterine LE/sGE of early pregnancy is regulated by progesterone, IFNT and prostaglandins that generate cortisol that may then act via NR3C1 to regulate ovine endometrial functions during early pregnancy. Prostaglandins such as PGE2, acting via PGE receptors (PTGER1-PTGER3), may also activate p38 MAPK cell signaling (Minamizaki *et al*., 2009). In bovine uteri, IFNT stimulates expression of PTGS2 and PGE synthase to increase the relative abundance of PGE, but also increases expression of EP2 in uterine epithelia (Arosh *et al*., 2004). Therefore, in uterine epithelia, IFNT, progestamedins and prostaglandins may act additively or synergistically via common or complimentary cell signaling pathways to stimulate gene expression in support of elongation, development and implantation of the conceptus.

In rodents, critical spatial and temporal changes in expression of ESR1 and PGR occur in the uterus during the peri-implantation period, i.e. Days 1 to 8 of pregnancy (Tan *et al*., 1999). On Days 1–2, ESR1 are primarily in uterine LE and GE, also in stromal cells by Days 3–4 and, on Day 5 following implantation, ESR1 is in LE and GE, but much lower in stratum compactum stroma. On Days

6–8, ESR1 is primarily in the secondary decidual zone, particularly in the subepithelial cells at the mesometrial pole, but undetectable in the primary decidual zone, whereas the undifferentiated stroma is ESR1 positive. PGR are at very low levels in murine endometrial on Day 1, modest in LE and GE on Day 2, and in LE, GE and stromal cells on Days 3–4. However, PGR are lost from LE and restricted to stromal cells by Days 5–8 and are particularly abundant in the decidua. As for primates and livestock species, cell-specific expression of ESR1 and PGR inform about coordinated effects of estrogen and progesterone in preparation of the uterus for implantation and decidualization during pregnancy.

In an elegant study by Simon *et al*. (2009), uteri from wild-type and PGR null mice were used to produce tissue recombinants in which PGR was present in stroma and/or epithelia or absent in either compartment. Only tissue recombinants involving wild-type stroma and wild-type epithelia responded to progesterone with an increase in expression of Indian hedgehog (*Ihh*) comparable to that in intact uteri. These results, in conjunction with earlier studies of regulation of expression of *Ihh*, *Ptch1* and *Nr2f2* mRNAs and proteins indicate that progesterone binds PGR in stromal cells to induce epithelial IHH which then induces PTCH1 and NR2F2 in stromal cells to initiate a cell signaling cascade critical to expression of genes necessary for implantation and decidualization. As noted by Simon *et al*. (2009), a central remaining question is related to the mechanism whereby progesterone stimulates *Ihh* mRNA expression, i.e. does this require an autocrine or paracrine action of a progestamedin?

Progesterone may also signal in the endometrium through a mechanism(s) that is independent of the classical PGR pathway (Pru, 2009). Indeed, studies of *Pgr*-null mice revealed that some actions of progesterone are not accounted for by activation of PGRs (Losel *et al*., 2003). For instance, it is accepted that classical PGRs mediate genomic responses to progesterone in the uterus, but *Ihh*, a gene essential for uterine receptivity and fertility in mice, is transiently up-regulated in endometrial epithelia of PGR-deficient female mice in response to progesterone treatment *in vivo*, indicating that even progesterone-dependent genomic responses in the uterus are not exclusively coordinated by classical PGRs (Matsumoto *et al*., 2002). There are two families of non-classical progestin receptors in the uterus which may play a role in non-classical progesterone signaling that is complimentary to or independent of classical effects of progesterone mediated via PGR (Fernandes *et al*., 2005; Zhang *et al*., 2008).

Progesterone, estrogen and interferons

A common feature of the peri-implantation period of pregnancy in domestic animals, rodents and primates is production of a Type I and/or a Type II IFN by conceptus Tr that induces and/or stimulates 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, other IFNs may affect uterine receptivity to implantation, decidualization and placental growth and development in primates, ruminants, pigs and rodents (Bazer *et al*., 2008, 2009a). All Type I IFNs bind a common receptor composed of two subunits, IFNAR1 and IFNAR2, to induce cell signaling via the Janus activated kinases (JAKs) and tyrosine kinase 2 (TYK2) pathways, respectively (Darnell *et al*., 1994). Signaling by Type II IFNG involves activation of JAK1 and JAK2 associated with IFNGR1 and IFNGR2 subunits of Type II IFNR, respectively. IFNG stimulates autophosphorylation and subsequent tyrosine phosphorylation and homodimerization of STAT1 which translocates to the nucleus and bind GAS elements in promoter regions of IFNG-regulated genes (Leanza *et al*., 2007). IFNs are expressed by human placentae (IFNA, IFNB, IFNG), decidua (IFNA, IFNB and IFNG) and fetal membranes (IFNA, IFNG), as well as conceptus Tr of sheep (IFNT), pig (IFND and IFNG), horse (IFND) and rodent uteri and/or conceptuses (IFNA, IFNB) (Bazer *et al*., 2008, 2009b). These IFNs have classical antiviral, anti-proliferative and immunosuppressive effects, as well as unique biological activities.

It is now clear that Type I IFNs activate unique cell-specific signaling pathways to differentially affect gene expression in LE/sGE, GE and stromal cells of the uterus (Spencer *et al*., 2008; Bazer *et al*., 2009b). In the ovine uterus limited expression of IFNT-stimulate genes is due, at least in part, to expression of IRF2, a potent inhibitor of transcription, in uterine LE/sGE (Choi *et al*., 2001). In spite of expression of IRF2 in ovine uterine LE/sGE, many ISGs are progesterone-induced and further stimulated by IFNT in these cells, perhaps due to both progestamedins and IFNT activating MAPK and PI3K cell signaling (Platanias, 2005; Spencer *et al*., 2007; Bazer *et al*., 2008, 2009b). These genes include those for glucose transport (SLC2A1 and SLC5A11) and amino acid transport (SLC7A1 and SLC7A2) that increase abundance of glucose, leucine and arginine in that uterine lumen. These nutrients can then stimulate proliferation of Tr cells by activating the glutamine: fructose-6-phosphate amidotransferase (GFAT)-mediated FK506 binding protein 12-rapamycin associated protein 1 (FRAP1, mTOR or 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 *et al*., 2004). Other genes induced by progesterone and further stimulated by IFNT in ovine uterine LE/sGE include LGALS15, cathepsin L (CSTL), cystatin C (CST3), hypoxia inducible factors 2A (HIF2A), gastrin-releasing peptide (GRP), HSD11B1 and IGFBP1. 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. Details on regulation of expression and potential functions of these genes have been reviewed (Spencer *et al*., 2007; Bazer *et al*., 2008, 2009b).

Estrogens and IFNs regulate endometrial genes that affect conceptuses during pregnancy in pigs (Johnson *et al*., 2009). Estrogens secreted by pig conceptuses induce *SPP1* expression in uterine LE, whereas stromal induction of *STAT1* is coordinate with secretion of IFNG and IFND by pig conceptus Tr. Indeed, estradiol induces *SPP1* mRNA in endometrial LE (White *et al*., 2005), although intrauterine delivery of conceptus secretory proteins containing IFND and IFNG in cyclic pigs treated with exogenous estrogen increases expression of *STAT1* (Joyce *et al*., 2007). Up-regulation of *SPP1* within uterine LE and *STAT1* within stroma and GE is unique to uterine LE in close proximity to a conceptus which implies paracrine regulation of these genes by conceptus estrogens and IFNs. 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 is similar in magnitude to *IFNT* production by sheep conceptuses (Joyce *et al*., 2007). Indeed, *STAT1* expression increases universally in the stroma and GE of pregnant ewes, presumably due to secretion of abundant amounts of IFNT by conceptus Tr (Spencer *et al*., 2007). Perhaps the spatial pattern of *STAT1* expression in the pig uterus requires that IFND and IFNG act synergistically to up-regulate expression of ISGs. Interactions between Type I and Type II IFNs on cell signaling (Decker *et al*., 1989) may allow IFNG to act on uterine stroma and GE to increase intracellular IFN-stimulated gene factor 3 and permit low amounts of IFND to up-regulate *STAT1* expression in close proximity to implanting pig conceptuses. Both estrogen- and IFN-stimulated genes have been localized in pig endometrium (Johnson *et al*., 2009). Type I and Type II interferons each induce expression of largely non-overlapping sets of genes and they may also have synergistic interactions to affect physiological responses (Levy *et al*., 1990). Although IFNG may enhance uterine receptivity to implantation in pigs, highly localized and abundant expression of IFNG, TNFA, IL1B and IL1R has been linked to arrested conceptus development between Days 15 and 23 of pregnancy (Wessels *et al*., 2007).

Nutrients, nutrient sensing pathways, growth factors and ECM Molecules affecting growth and development of the conceptus

Conceptus growth and development requires amino acids, glucose, fatty acids, vitamins and minerals. Before placentation, these nutrients are transported from maternal plasma into the uterine lumen. Thereafter, they are supplied to the fetus through the umbilical circulation. Both amino acids and glucose are major sources of energy for the embryo/fetus and amino acids are building blocks of proteins and some of them (e.g. branched-chain amino acids, glutamate, serine and proline) undergo extensive catabolism in the placenta (Wu *et al*., 2009). Glutamine is the most abundant amino acid in fetal plasma and is present at exceedingly high concentrations in fetal fluids (2–20 mM) (Wu *et al*., 1996). Interestingly, the placentae of sheep, pigs and rats have a limited ability to degrade glutamine due to the lack of glutaminase and use arginine in a species-dependent manner. For example, the ovine placentome actively degrades arginine by arginase, but the porcine placenta lacks this pathway (Wu *et al*., 2004). Notably, the ovine conceptus uses citrulline as an effective precursor of arginine to support fetal growth (Lassala *et al*., 2009).

The mTOR/FRAP1 cell signaling pathway is an evolutionarily conserved serine/threonine kinase located downstream of PI3K that is central to control of cell growth and proliferation through regulation of mRNA translation for protein synthesis and cell proliferation (Wullschleger *et al*., 2006). Cellular events directly controlled by the mTOR pathway include mRNA translation, ribosome synthesis, expression of metabolism-related genes, autophagy and cytoskeletal reorganization (Kim *et al*., 2002). During embryonic development, molecules that stimulate mTOR activity may also stimulate translation of mRNAs critical to blastocyst/conceptus development, including IGF2 and actions of selected amino acids as mTOR is a 'nutrient sensing system' (Martin and Sutherland, 2001). Cell signaling via mTOR stimulates cell migration and invasion, as well as cell growth and proliferation in different cell types (Liu *et al*., 2008). In fact, *mTOR/Frap1* null mice die shortly after implantation due to impaired cell proliferation and hypertrophy in both the embryonic disc and trophoblast (Murakami *et al*., 2004).

Nutrients are essential components of histotroph required for development and survival of conceptuses during pregnancy (Bazer *et al*., 2008, 2009b; Wu *et al*., 2009). A systematic study of temporal and cell-specific changes in expression of transporters for glucose and amino acids, their regulation by progesterone and/or IFNT, changes in expression of NOS isoforms, ODC and related proteins, as well as components of mTORC1 and mTORC2 cell signaling in ovine uteri and conceptuses revealed that: (i) total recoverable glucose, Arg, Leu, Gln, glutathione, calcium and sodium are more abundant in uterine fluids of pregnant than cyclic ewes between Days 10 and 16 after onset of estrus or mating; (ii) uteri and conceptuses express tissue and cell-specific facilitative and sodium-dependent transporters for glucose, as well as for cationic, acidic and neutral amino acids, some of which are regulated by progesterone or progesterone and IFNT; (iii) transport of Arg into the uterine lumen and uptake by conceptuses is by System y+ (SLC7A1, 2 and 3) cationic amino acid transporters; (iv) NOS1 and ODC1 are most abundant in uterine LE/sGE although NOS3 is most abundant in Tr and endoderm of conceptuses; (v) expression of GCH1, the key enzyme for synthesis of tetrahydrobiopterin, a cofactor for NO production, ODC1 and NOS1 is more abundant in conceptuses than endometrial cells; and (vi) progesterone stimulates expression of NOS1 and GTP cyclohydrolase (GCH1), although IFNT inhibits expression of NOS1. Further, components of both the mTORC1 and mTORC2 cell signaling pathways (FRAP1, LST8, MAPKAP1, RAPTOR, RICTOR, TSC1, TSC2, RHEB and EIF4EBP1) are localized to uterine LE/sGE, GE and stromal cells, as well as Tr and endoderm of conceptuses between Days 13 and 18 of pregnancy. The abundance of FRAP1, RAPTOR, RICTOR, TSC1 and TSC2 mRNAs in endometria was not affected by pregnancy status or day of the estrous cycle or pregnancy; however, increased expression of LST8, MAPKAP1, RHEB and EIF4EBP1 mRNAs only occurred in endometria during early pregnancy. Further, progesterone and IFNT stimulate expression of RHEB and EIF4EBP1 in uterine endometria. Importantly, FRAP1 was abundant in cytoplasm and phosphorylated FRAP1 was very abundant in nuclei of ovine Tr cells and endoderm, and increases in abundance of RICTOR, RHEB and EIF4EBP1, as well as RHEB protein in endometria were coordinate with rapid conceptus growth and development during the peri-implantation period. These results suggest differential effects of mTORC1 and mTORC2 on elongation of ovine conceptuses.

In related studies (Satterfield *et al*., 2008, 2009b), an early increase in circulating levels of progesterone accelerates blastocyst growth and development in ewes that is associated with increases in total recoverable glucose, aspartate (acidic amino acid), Arg and lysine (basic amino acids), and citrulline, asparagine, serine, Gln, beta-alanine and alanine (neutral amino acids) in uterine flushings on Day 9 of pregnancy compared with control ewes. However, on Day 12 of pregnancy, only Arg and lysine were more abundant in uterine flushings from progesterone-treated ewes as were transporters for glucose (SLC2A1 and SLC5A1) and Arg (SLC7A2B) in uterine LE/sGE on both Days 9 and 12. These novel results indicate that progesterone-induced advances in transport of select nutrients, particularly Arg and glucose, into the uterine lumen on Days 9 and 12 of pregnancy are coordinate with advanced conceptus development.

These findings are supported by unpublished results (J. Kim, G. Wu, G. Johnson, T.E. Spencer and F.W. Bazer) from *in vitro* studies with ovine Tr1 cells indicating novel cell signaling whereby: (i) Arg activates mTOR cell signaling and phosphorylation of ribosomal protein kinase (RPSK); (ii) Arg, Leu and glucose increase phosphorylation of AKT1, GSK3B, FRAP1 and RPS6K proteins; (iii) Arg increases the abundance of pRPS6K and pRPS6 in the cytoplasm of oTr cells; and (iv) cell proliferation was independent of NOS, but dependent on production of polyamines. Both NO and polyamines are critical for implantation and development of conceptuses. Our studies of pathways for their production revealed effects of the estrous cycle, pregnancy, progesterone and IFNT on expression of NO synthases (*NOS1*, *NOS2*, *NOS3*), GTP cyclohydrolase (*GCH1*, the key enzyme in *de novo* synthesis of BH4, a cofactor for NO production), and *ODC1* in uterine endometria from cyclic and pregnant ewes. NOS1 and ODC are most abundant in uterine LE/sGE whereas NOS3 is abundant in Tr and endoderm of ovine conceptuses, as are total NOS1 and NOS3 proteins, inhibitory phosphorylated (p) p-NOS1 protein and stimulatory p-NOS3 protein. GCH1 is abundant in Tr and endoderm of conceptuses between Days 13 and 15 of pregnancy, but decreases whereas ODC1 abundance increases between Days 13 and 18 of pregnancy. Progesterone stimulates NOS1 and GCH1 expression in ovine uterine LE/sGE and GE, although IFNT inhibits NOS1 expression in these cell types. Thus, biosynthesis of NO and polyamines in ovine uterine endometria and conceptuses is regulated at transcriptional, translational and post-translational levels to favor conceptus development and implantation.

Insulin-like Growth Factor 2 (IGF2), an imprinted and paternally expressed gene in the fetus and placenta of mice, humans and sheep, regulates fetal and placental growth and differentiation, extravillous trophoblast migration/invasion, and nutrient transfer through placental exchange mechanisms (Kim *et al*., 2008). In mice, deletion of *Igf2* in the labyrinthine trophoblast decreased fetal-placental growth and placental exchange of nutrients (Constância *et al*., 2002). Further, altered expression of IGF2 in human trophoblast cells during early pregnancy is associated with intrauterine growth restriction, premature delivery and pre-eclampsia (Smith *et al*., 2002).

In pregnant ewes, *IGF2* mRNA is most abundant in caruncular endometrial stroma; however, in intercaruncular endometrium, its expression transitions from uterine stroma to LE between Days 14 and 20 of pregnancy (Kim *et al*., 2008). IGF2 is present in all cells of the conceptus, but particularly abundant in primitive endoderm and yolk sac early in pregnancy and in the chorioallantois and LE of ewes during mid- to late-pregnancy. Abundant amounts of IGF2 at the interface between cotyledonary and caruncular sides of placentomes suggest a role in conceptus development and fetal-placental development. For example, IGF2 regulates nutrient transport (glucose transporters and amino acid transporters) by the placenta to meet fetal demands, perhaps via a positive regulatory feedback loop between IGF2 and mTOR (Gingras *et al*., 2004). In support of this, IGF2 increases abundance of p-PDK1, p-AKT1, p-GSK3B, p-FRAP1 and p-RPS6K proteins in ovine Tr cells that is coordinate with rapid increases in p-ERK1/2 and p-P38 MAPK proteins, as well as proliferation and migration of ovine Tr1 cells (Kim *et al*., 2008). Thus, IGF2 may coordinately activate multiple cell signaling pathways critical to survival, growth and differentiation of mammalian conceptuses during early pregnancy. Further, available evidence suggests novel converging pathways whereby Arg, SPP1 and IGF2 may activate both mTORC1 (cell proliferation and mRNA translation) and mTORC2 (cytoskeletal alterations, cell migration and cell survival) in ovine Tr cells (Bazer *et al*., 2009b).

Transport of nutrients into the uterine lumen and conceptus

Research with domestic and laboratory animals has revealed that many components of uterine luminal fluid are required for conceptus development. In fact, conceptuses fail to develop beyond Day 14 of pregnancy in ewes that lack uterine glands and their secretions (Gray *et al*., 2002) that include nutrients (amino acids, glucose, essential fatty acids, vitamins and minerals), proteases, protease inhibitors, transport proteins for nutrients and minerals, cytokines, lymphokines and growth factors (Bazer, 1975; Roberts and Bazer, 1988; Mahan and Vallet, 1997; Spencer *et al*., 2008; Satterfield *et al*., 2009a). Vitamins and/or their transport proteins identified in uterine secretions, allantoic fluid and blood of fetuses include those for riboflavin, thiamin, niacin, biotin, cobalamin, retinol and retinoic acid, Vitamin E, ascorbic acid, folic acid, vitamin D and vitamin K (Mahan and Vallet, 1997; Vallet *et al*., 1998). Folic acid has received considerable attention as it is important in reproductive health of humans and animals by participating in the metabolism of one-carbon units and amino acids including homocysteine, methionine, glycine, serine and histidine (Tamura and Picciano, 2006; Forges *et al*., 2007). Folic acid is a required cofactor in the transfer of methyl groups and metabolites of folic acid are required for synthesis of the purine ring, methionine (from homocysteine) and thymidine essential for cell division, fetal growth and erythropoiesis (Vallet *et al*., 1998; Kim and Vallet, 2004). In pigs, both secreted and placental membrane forms of folate-binding protein are expressed in the intrauterine environment during pregnancy (Kim and Vallet, 2004). Deficiencies in folic acid are associated with birth defects. Mice in which the folate binding protein has been deleted die *in utero* during the peri-implantation period and are resorbed (Spiegelstein *et al*., 2004). Treatment of dams deficient in folate binding protein before and throughout gestation with folinic acid (N^5-formyl-tetrahydrofolate) prevented premature death of most embryos. For folate binding protein null embryos, maternal supplementation with various forms of folic acid could rescue the phenotype to some extent, but surviving fetuses had a high frequency of neural tube defects, as well as malformations of craniofacial structures, eyes and abdominal wall.

Conceptus Tr development and and endogenous retroviruses: cellular and molecular regulators of mononuclear Tr proliferation and differentiation into trophoblast binucleate cells

The endogenous retroviruses (ERVs) are now implicated in development and differentiation of conceptus Tr in humans, rodents and sheep (Spencer *et al*., 2007). During the course of evolution, all vertebrates have been exposed to multiple waves of cross-species infection by exogenous retroviruses. Some of those viruses infected germ cells and are inherited in an integrated, proviral form (Boeke and Stoye, 1997). These ERVs have undergone further amplification and now make up a greater fraction of our DNA than do normal coding sequences (Jern and Coffin, 2008). Although once considered junk

DNA, it is now clear that ERVs have important biological roles in protection against retroviral infection (Best *et al*., 1997) and placental development (Harris, 1998; Rawn and Cross, 2008).

Syncytin-1 and -2, a product of the two human ERV *envelope* (*env*) genes that entered the primate lineage 25–40 million years ago, was discovered in 2000 as a captive retroviral protein expressed in human placental cells (Knerr *et al*., 2004). Both of the human ERV *env* genes encode highly fusogenic retroviral Env proteins (syncytin-1 and -2), possibly involved in the formation of the placenta syncytiotrophoblast layer generated by trophoblast cell fusion (Blond *et al*., 2000; Mi *et al*., 2000). Similarly, mice have two ERVs, syncytin-A and -B, that are expressed in syncytiotrophoblast and also elicit cell–cell fusion *in vitro* (Dupressoir *et al*., 2005). Syncytin-A plays an important biological role in syncytiotrophoblast development, because *syncytin-A* null mice die *in utero*, apparently as a result of the failure of trophoblast cells to fuse and form one of the two syncytiotrophoblast layers present in the placenta (Dupressoir *et al*., 2009). The syncytiotrophoblast layers of mammalian placentae play a key role in transport of nutrients for the developing conceptus (Watson and Cross, 2005).

The ERVs also appear to play an essential role in placental development in sheep. Jaagsiekte sheep retrovirus (JSRV) is a pathogenic exogenous retrovirus and the causative agent of ovine pulmonary adenocarcinoma (Dunlap *et al*., 2006a; Arnaud *et al*., 2008). The sheep genome contains at least 27 copies of endogenous retroviruses (enJSRVs) highly related to JSRV. The earliest hints that enJSRVs could participate in some aspect of uteroplacental biology came from the observation that their RNA was particularly abundant in organs of the reproductive tract (Palmarini *et al*., 2004). The highest levels of enJSRVs RNA are expressed in uterine LE and GE, as well as in the epithelia of the oviducts and cervix. Lower levels of enJSRVs RNA are also detected in vaginal epithelia. In the conceptus, enJSRVs RNA is detectable in mononuclear Tr, but more abundant in trophoblast giant binucleate cells (BNCs) and multinucleated syncytia that form the fetal part of placentomes for nutrition of the conceptus (Dunlap *et al*., 2006b). Of particular note, hyaluronidase 2 (HYAL2), a cell-surface receptor for the exogenous JSRV and enJSRVs envelope proteins is expressed by BNC and syncytial plaques in sheep placentae, but not uterine epithelia, stroma or myometrium.

Expression of enJSRV *env* in conceptus Tr begins on Day 12 of pregnancy which is coincident with the onset of conceptus elongation and production of IFNT for pregnancy recognition signaling (Dunlap *et al*., 2006a). Most interestingly, inhibition of enJSRVs Env production by morpholino antisense oligonucleotides *in utero* retards conceptus growth and elongation and inhibits trophoblast giant BNC differentiation, which culminates in loss of pregnancy (Dunlap *et al*., 2006a). These results, together with the fact that *HYAL2* mRNA, which functions as a cellular receptor for both JSRV and enJSRVs Env, is detected in the trophoblast giant BNC and multinucleated syncytia of the conceptus, suggest that expression of enJSRVs Env and HYAL2 is important for growth and differentiation of conceptus Tr in sheep (Fig. 1). The abundant expression of ERVs in human and mouse placenta, in particular the presence of intact *env* genes in the syncytiotrophoblast, which have been preserved over millions of years, together with the observation that they elicit fusion of cells *in vitro*, suggests that independent ERVs were positively selected for a convergent biological role in placental morphogenesis during evolution (Harris, 1998; Knerr *et al*., 2004; Rawn and Cross, 2008).

In the endometrium, enJSRV expression fluctuates during the estrous cycle and early pregnancy, but increases in abundance between Days 1 and 13 as concentrations of progesterone in plasma increase (Palmarini *et al*., 2001). Moreover, long terminal repeats of some enJSRV loci respond moderately to progesterone in transient transfection assays. Given that five of the enJSRV loci have intact open reading frames for all their genes, it is plausible that enJSRV-derived viral particles are shed into the uterine lumen. Indeed, viral particles have been observed in uterine epithelia and ovine conceptus Tr, but it is not known whether these particles have any biological function (Kalter *et al*., 1975).

Thus, evidence from studies of primates, sheep and rodents suggests that ERVs influence mammalian evolution through effects on placental morphogenesis as retroviral *env* genes were co-opted to influence placental development. Given the structural diversity among the placentae of different orders of mammals, one might speculate that specific roles played by these viral genes may differ, perhaps depending on cellular expression patterns of ERV *env* and their specific receptors. Indeed, proviral inheritance may provide a better predictor of the diversities among syncytial morphologies than taxonomy (Stoye, 2009).

Vascular regulation and angiogenesis

The mammalian placenta undergoes rapid formation of new blood vessels from existing ones (angiogenesis) (Reynolds *et al*., 2006) to increase the supply of nutrients and oxygen from mother to fetus. Successful pregnancy requires complex remodeling of the endometrium to orchestrate implantation and ensuing angiogenesis that provides hematotrophic support for the developing conceptus (Charnock-Jones *et al*., 2004; Kaufmann *et al*., 2004; Red-Horse *et al*., 2004). Placentation facilitates these events and is defined as the expansion and juxtaposition of the microcirculatory systems of the uterus and placenta to optimize exchange of nutrients, gasses and metabolic wastes (Breier, 2000). Dysregulated endometrial angiogenesis underlies infertility in (i) endometriosis (Siristatidis *et al*., 2006); (ii) fetal undernutrition that may lead to adult onset of diseases including cardiovascular disease and type 2 diabetes (Barker, 2004); and (iii) rudimentary endovascular invasion that contributes to pre-eclampsia (Norwitz, 2006). Thus, an understanding of signals that promote uterine and placental angiogenesis in pregnancy is critical.

In ewes, progesterone induces hypoxia inducible factors (HIF1A and HIF2A) and IFNT further stimulates expression of HIF2A in uterine LE/sGE (Song *et al*., 2008). HIF functions to control expression of over 200 genes, including *EPO*, CBP/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (*CITED2*), *VEGF*, solute carrier family 2 (facilitated glucose transporter) member 1 (*SLC2A1*; also termed *GLUT1*), and *IGF2*. Mice deficient in *Hif1a*, *Hif2a*, or aryl hydrocarbon receptor nuclear translocator (*Arnt*) die at mid-gestation from vascular defects primarily involving embryonic and extraembryonic vasculature (Semenza, 2000). In contrast, mice deficient in *Hif2b* or *Hif3b* do not exhibit vascular abnormalities (Cowden and Simon, 2002). These results suggest that *Vegf* is primarily regulated by HIF1A, HIF2A and ARNT during embryonic development.

The placenta and the uterus produce classical angiogenic factors that include VEGF, basic fibroblast growth factor (FGF2), angiopoietins

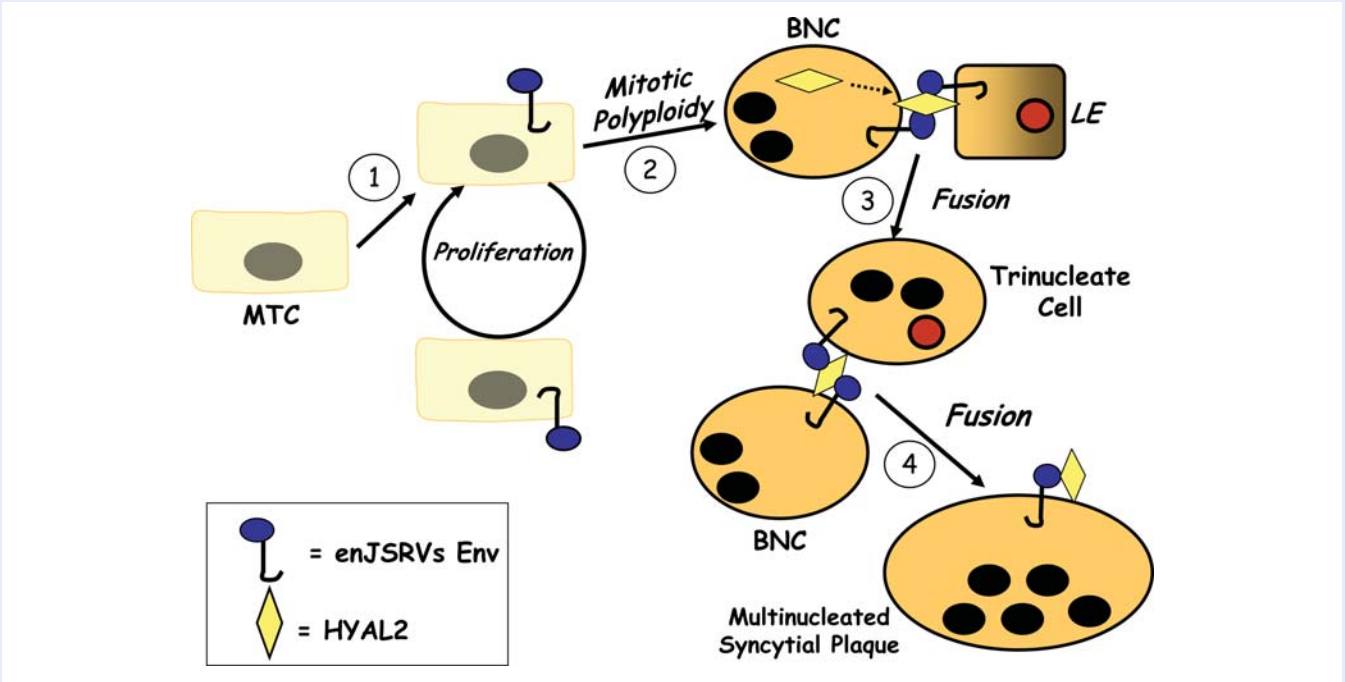

Figure 1 Hypothesis on the biological role of endogenous retroviruses (enJSRVs) Env and HYAL2 in trophoblast differentiation in sheep. Blastocyst growth from a spherical to an ovoid form occurs between Days 9 and 11 after hatching from the zona pellucida on Day 8 in sheep. Beginning on Day 12 of pregnancy, enJSRVs are expressed in the mononuclear trophectoderm cells (MTC) that begin to migrate and proliferate rapidly to support conceptus elongation and outgrowth (Step 1). By Day 14, some of the MTC begin to differentiate into trophoblast giant binucleate cells (BNC) (Step 2). Results interpreted exclusively from microscopy studies support the idea that trophoblast giant BNC are derived from karyokinesis without cytokinesis (endoreduplication). The inception of BNC differentiation coincides with the onset of *HYAL2* expression in the BNC of the conceptus. The newly differentiated BNC begin to migrate and fuse with the endometrial luminal epithelial (LE) cells, forming a trinucleate cell (Step 3). During this period, the trophoblast giant BNC and endometrial LE cells express enJSRVs *env* RNA, whereas only the BNC express *HYAL2*. In fact, HYAL2 is not detected in any uterine cell type. Thus, the fusion of placental BNC and LE cells is hypothesized to involve enJSRVs Env and HYAL2. By Days 20 to 25, virtually all of the endometrial LE cells are fused with the BNC. During most of gestation, the BNC continue to differentiate from the MTC and then fuse with each other to form multinucleated syncytial plaques with 20–25 nuclei (Step 4). The trophoblast giant BNC and multinucleated syncytia line the cotyledons of the placenta that interdigitate with the endometrial caruncles to form the placentomes, which is necessary for hematotrophic nutrition of the fetus.

(ANG), and their receptors that affect vascular development in the reproductive tract of sheep (Borowicz *et al*., 2007). Both VEGF and FGF2 are major angiogenic growth factors of the placenta and uterus that account for most of the heparin-binding angiogenic activity. VEGF stimulates vascular permeability, vascular endothelial cell migration and protease production to enhance angiogenesis. Mice lacking VEGF receptors (VEGFR-1 or flt-1 or VEGFR-2 or flk-1) experience defects in fetal-placental vasculogenesis and angiogenesis resulting in embryonic death by mid-gestation. Basic FGF also stimulates proliferation of both uterine and fetal placental arterial endothelial cells. Both VEGF and FGF2 likely regulate uterine and placental blood flow by stimulating production of NO by endothelial cells and NO, in turn, stimulates expression of VEGF and FGF2. ANG regulate vascular growth and development by activating Tie2-mediated cell signaling. *Ang1* null mice exhibit cardiovascular defects and die by mid-gestation. ANG1 does not stimulate endothelial cell proliferation, but affects microvascular organization, stabilization and endothelial cell survival required for vascular remodeling.

Several other key signaling pathways that mediate angiogenesis are in response to molecules that include prostanoids (Jabbour *et al*., 2006), angiotensin (Ino *et al*., 2006; Sugawara and Ito, 2006), as well as integrins and metalloproteinases (Bayless and Davis, 2003).

Sphingosine-1 phosphate, another potent inducer of angiogenesis (Hla, 2004; Langlois *et al*., 2004) is emerging as a novel target in anticancer therapies (Milstien and Spiegel, 2006; Sabbadini, 2006; Visentin *et al*., 2006). S1P signals through activation of one or more of its five known high-affinity G-protein-coupled receptors, $S1P_1$–$S1P_5$ (Spiegel and Milstien, 2003a). $S1P_1$ and $S1P_3$ are expressed in endothelial cells and mediate angiogenic responses in multiple systems (LaMontagne *et al*., 2006), although the $S1P_2$ receptor functions in an angiostatic capacity (Inoki *et al*., 2006). Sphingosine kinase 1 (SPHK1) phosphorylates sphingosine to biologically active S1P whereas S1P phosphohydrolases (SPP1 and SPP2) and sphingosine lyase (SPPL) dephosphorylate S1P back to sphingosine. Importantly, the S1P pathway is highly evolutionarily conserved (Spiegel and Milstien, 2003b). Results from studies of transgenic mice revealed that expression of S1P receptors and modulating enzymes are important for angiogenesis, endometrial development and placentation (Kono *et al*., 2004). $S1P_1$, $S1P_2$, $S1P_3$ and SPHK1 are up-regulated as pregnancy progresses and up-regulation of S1P synthesis is

coordinate with uterine decidualization between Days 5.5 and 7.5 of pregnancy in mice (Kaneko-Tarui *et al*., 2007; Mizugishi *et al*., 2007). Interestingly, S1P and its metabolites and modifying enzymes exhibit cross-talk with various signaling pathways. Ceramide, a sphingosine metabolite, induces PTGS2 (Ballou *et al*., 1992) which is the rate-limiting enzyme for prostanoid biosynthesis (Simmons *et al*., 2004). Further, S1P induced PTGS2 in human amniotic cells (Kim *et al*., 2003), predecidualized uterine stromal cells (Skaznik-Wikiel *et al*., 2006), smooth muscle cells (Hsieh *et al*., 2006) and human chondrocytes (Masuko *et al*., 2007). Serrano-Sanchez *et al*. (2008) reported a correlation between SPHK activity and PTGS2 induction in rat myometrium. Inside-out signaling of S1P can be induced by receptor tyrosine kinase activation by various growth factors, where growth factor ligation of receptor via VEGF, TGFB and IGF leads to SPHK activation and translocation to the membrane, which induces local production of S1P to stimulate G-protein coupled receptors (Lebman and Spiegel, 2008). Interestingly, S1P prevents HIF degradation independent of oxygen (Michaud *et al*., 2009) and, under hypoxic conditions, SPHK1 can modulate HIF levels (Ader *et al*., 2009). Also, S1P stimulates eNOS translocation to the membrane and production of NO (Rikitake *et al*., 2002). We have shown that S1P synergizes with angiogenic growth factors (Bayless and Davis, 2003; Su *et al*., 2008) and wall shear stress to increase Akt phosphorylation and sprouting responses by endothelial cells (Kang *et al*., 2008). Akt is activated downstream of S1P (Lee *et al*., 2001) and phosphorylates eNOS on Ser1179 (Fulton *et al*., 2002) which leads to NO production, angiogenesis and vasodilation (Morbidelli *et al*., 2003). Thus, the S1P signaling pathway is intricately linked to other key signaling pathways, including growth factors, prostanoids, NO and HIFs, which render the S1P pathway perfectly suited to regulate angiogenic responses.

Following successful elongation of the conceptus, trophoblast outgrowth and implantation, the placentae of ruminants organizes into placentomal and interplacentomal regions. Development of placentomes, although morphologically distinct from humans, is driven by local alterations in integrins and ECM proteins common to the maternal-fetal interface of both primates and ruminants (Johnson *et al*., 2003a; Carson *et al*., 2006; Foulk *et al*., 2007). During placentome development, highly branched villous tree-like placental folds, termed cotyledons form by Day 20 of gestation (Cross *et al*., 2003). Cotyledonary chorionic villi lined by syncytial plaques then protrude into crypts in the maternal endometrial caruncular tissue (a glandular areas of the endometrium consisting of stroma covered by a single layer of LE) resulting in extensive interdigitation of endometrial and placental tissues by Day 40 of gestation to form a placentome that is somewhat analogous to the decidua in humans. Failure of placentomes to develop results in fetal loss because they allow for close proximity of maternal and fetal blood vessel interdigitation for exchange oxygen and nutrients (Wu *et al*., 2004; Reynolds *et al*., 2005). Approximately 90% of the blood from the uterine artery and umbilical vein is directed into the vasculature of maternal caruncules and fetal cotyledons, respectively (Caton *et al*., 1983). The doubling of capillaries per unit tissue in the caruncle from Days 50 to 70 coincides with an initial peak of NO production, whereas VEGF levels are declining (Kwon *et al*., 2004; Vonnahme *et al*., 2005). This suggests that another pro-angiogenic factor, such as S1P, is responsible for NO production and angiogenesis between Days 50 and 70. Treatment of ewes with a specific S1P receptor antagonist, FTY720, inhibits development of caruncles in placentomes (K. Bayless and G.A. Johnson, unpublished results).

Polyamines and NO are also essential to placental growth and angiogenesis as rats fed Arg-free diets experience reduced NO synthesis, increased fetal resorptions, IUGR, increased perinatal mortality and decreased numbers of live pups at birth (Wu *et al*., 2005, 2008, 2009). Also, oral administration of Arg (3 g daily for 4 weeks) to women with pre-eclampsia increased NO synthesis which was associated with reduced blood pressure, prolonged pregnancy, improved fetal well-being, enhanced fetal growth and uterine quiescence to prevent preterm labor (Wu *et al*., 2009). NO is a key regulator of angiogenesis during pregnancy that is derived from cNOS and/or iNOS expressed by placentae of rodents, humans, pigs and sheep. Placental synthesis of NO (the major vasodilator), like that of polyamines (other products of Arg catabolism), is essential for placental angiogenesis and growth) and increases markedly between Days 30 and 60 of gestation when placental growth and placentomal development are most rapid (Kwon *et al*., 2003). Inhibition of NOS or ornithine decarboxylase (a key enzyme in polyamine synthesis) activity during early pregnancy markedly reduces placental size that leads to IUGR in rats. Increases in NO synthesis in sheep placentomes from Day 100 of gestation are coordinate with significant increases in placental-fetal blood flow and rapid fetal growth. Intriguingly, in ovine placentae and endometria, both NADPH and BH4 levels increase markedly between Days 40 and 60 of gestation along with increases in concentrations of citrulline (the precursor of arginine) and arginine in allantoic fluid. Between Days 80 and 100 of gestation, BH4 concentrations also increase in placentomes and endometria, as do concentrations of arginine in allantoic fluid. Arginine is a potential regulator of the pentose cycle activity and a stimulator of endothelial GTP-CH expression critical for regulating the synthesis of NADPH and BH4 and NO production in placenta and endometrium which prevent or ameliorating IUGR and development of hypertension and pre-eclampsia (Wu *et al*., 2009).

In summary, this review addresses novel mechanisms responsible for conceptus-endometrial interactions during pregnancy. However, there are many events for which mechanisms are yet to be discovered. There is a gap in our knowledge about the requirement for loss of expression of PGR by endometrial epithelia as a prerequisite for implantation, expression of genes for secretory proteins, and selective transport of molecules into the uterine lumen to support conceptus growth and development for successful establishment of pregnancy. Thus, there is a clear need to identify the progestamedin or estramedin unique to each species and to understand mechanisms whereby they exert paracrine effects on uterine epithelia individually and in concert with cell signaling pathways activated by secretions from the conceptus such as IFNs, lactogenic hormones, and prostaglandins. Comparative reproductive biology is necessary to advance understanding of these mechanisms. For example, the ewe is a proven model for research to understand the roles of IFNs during the peri-implantation period because Tr or immune cells as the sites of implantation of most, if not all mammals, are now known to express Type I and/or Type II IFNs. Thus, IFN-stimulated genes are among the most highly up-regulated genes in human decidualized stromal cells treated with trophoblast conditioned medium and in uteri of domestic and laboratory animals. Understanding effects of ovine IFNT on gene expression in the uterus will advance our understanding of novel

mechanisms whereby progesterone and IFNs directly or indirectly act on cells of the reproductive system to induce ISGs critical to establishment and maintenance of pregnancy in mammals. Similarly, understanding the roles of novel endogenous retroviruses in reproductive tissues will advance our understanding of their roles in implantation, placentation and the endocrinology of pregnancy. This knowledge is essential for translational research into strategies to enhance reproductive efficiencies and reproductive health in humans and animals.

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