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Genes involved in conceptus–endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches

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Abstract

This review summarizes new knowledge on expression of genes and provides insights into approaches for study of conceptus–endometrial interactions in ruminants with emphasis on the peri-implantation stage of pregnancy. Conceptus–endometrial interactions in ruminants are complex and involve carefully orchestrated temporal and spatial alterations in gene expression regulated by hormones from the ovary and conceptus. Progesterone is the hormone of pregnancy and acts on the uterus to stimulate blastocyst survival, growth, and development. Inadequate progesterone levels or a delayed rise in progesterone is associated with pregnancy loss. The mononuclear trophoblast cells of the elongating blastocyst synthesize and secrete interferon-τ (IFNT), the pregnancy recognition signal. Trophoblast giant binucleate cells begin to differentiate and produce hormones including chorionic somatomammotropin 1 (CSH1 or placental lactogen). A number of genes, induced or stimulated by progesterone, IFNT, and/or CSH1 in a cell-specific manner, are implicated in trophoblast adhesion to the endometrial luminal epithelium and regulation of conceptus growth and differentiation. Transcriptional profiling experiments are beginning to unravel the complex dynamics of conceptus–endometrial interactions in cattle and sheep. Future experiments should incorporate physiological models of pregnancy loss and be complemented by metabolomic studies of uterine lumen contents to more completely define factors required for blastocyst survival, growth, and implantation. Both reduction and holistic approaches will be important to understand the multifactorial phenomenon of recurrent pregnancy loss and provide a basis for new strategies to improve pregnancy outcome and reproductive efficiency in cattle and other domestic animals.


Introduction

The conceptus (embryo/fetus and associated extraembryonic membranes) and endometrium reciprocally interact throughout pregnancy. During the peri-implantation period, conceptus–endometrial interactions are influenced by progesterone and placental hormones required for establishment and maintenance of pregnancy. This review integrates established and new information on genes involved in conceptus–endometrial interactions with emphasis on knowledge generated from reductionist transcriptional profiling experiments conducted in cattle and sheep. The review also offers insights into holistic approaches using physiological models and systems biology for studying conceptus–endometrial interactions in ruminants. This area of reproductive biology is particularly important in ruminants due to relatively high levels of pregnancy loss during the peri-implantation period. In cattle, estimates indicate that fertilization rate is 90% with an average calving rate of about 55%, suggesting an embryonic/fetal mortality of about 35%; further, 70–80% of total embryonic loss occurs between days 8 and 16 after insemination (Diskin et al. 2006). Early
pregnancy loss is even greater in the high-yielding dairy cattle, which is a major impediment to milk production efficiency (Moore & Thatcher 2006).

Overview of peri-implantation conceptus–endometrial interactions in ruminants

Establishment of pregnancy in domestic ruminants (sheep, cattle, and goats) begins at the blastocyst stage and includes pregnancy recognition signaling, conceptus implantation, and placentation (for reviews, see Guillomot et al. 1993, Guillomot 1995, Spencer et al. 2004b, 2007a). Morula-stage embryos enter the uterus on days 4–6 post-mating and then form a blastocyst that contains an inner cell mass and a blastocoel or central cavity surrounded by a monolayer of trophoderm (Guillomot 1995). After hatching from the zona pellucida, blastocysts develop into a tubular form, and then elongate on day 12 (sheep) or day 15 (cattle) to filamentous conceptuses that occupy the entire length of the uterine horn. The elongated or filamentous conceptus is composed mainly of extraembryonic trophoderm lined with endoderm. Hatched blastocysts and trophoblastic vesicles do not elongate in vitro, but do so when transferred into the uterus of either sheep or cows (Heyman et al. 1984, Flechon et al. 1986). Progesterone acts on the uterus to indirectly stimulate pre-implantation blastocyst growth and elongation (Garrett et al. 1988a, Mann & Lamming 2001, Mann et al. 2006, Satterfield et al. 2006). Elongation of the blastocyst is critical for developmentally regulated production of interferon τ (IFNT), the pregnancy recognition signal, and for implantation (Farin et al. 1989, Guillomot et al. 1990, Gray et al. 2002). IFNT acts in a paracrine manner on the endometrium to inhibit development of the endometrial lutecyotic mechanism required for pulsatile release of prostaglandin F2α (PGF2α), thereby ensuring continued production of progesterone by the ovarian corpus luteum (Thatcher et al. 1989, Spencer et al. 2007a). Additionally, IFNT stimulates a number of genes in a cell-specific manner within the endometrium that are implicated in uterine receptivity and conceptus development (Hansen et al. 1999, Spencer et al. 2007a).

Coincident with apposition and adhesion of mononuclear cells of the trophoderm to the endometrial luminal epithelium (LE), trophoblast giant binucleate cells (BNC) differentiate within the trophoderm (Wooding 1984) from mononucleate stem cells by consecutive nuclear divisions without cytokinesis, a phenomenon termed mitotic polyploidy (Wooding 1992). Migration of BNC to the microvillar junction and then fusion with individual LE cells produce trinucleate fetomaternal hybrid cells (Wooding 1984). Continued BNC migration and fusion with trinucleate cells, together with displacement and/or death of the remaining uterine LE, apparently produces multinucleated syncytial plaques, linked by tight junctions and limited in size to 20–25 nuclei that cover the caruncles (Wooding 1982, 1984, 1992). This caruncular syncytium, in which no nuclear division has been reported, expands in area during formation and maintenance of cotyledons, presumably deriving nuclei from continued BNC migration and fusion. The BNC have at least two main functions: (1) formation of a hybrid fetomaternal syncytium for successful implantation and subsequent cotyledonary growth in the placentome and (2) synthesis and secretion of protein and steroid hormones such as chorionic somatomammotropin hormone 1 (CSH1; alias placental lactogen) and progesterone (Wooding 1992). CSH1 acts on the endometrial glands of the uterus to stimulate their development and expression of genes that encode secreted proteins (Spencer et al. 2004a). In addition to trophoderm differentiation, several other events occur during blastocyst elongation, including gastrulation of the embryo and formation of the yolk sac and allantois (Guillomot 1995), which are vital for embryonic survival and formation of a functional placenta.

Hormonal regulation of endometrial function and conceptus development

Progesterone regulation of blastocyst survival and growth

Progesterone stimulates and maintains endometrial functions necessary for conceptus growth, implantation, placentation, and development to term (see Bazer 1975, Bazer et al. 1979, Spencer & Bazer 2002, Spencer et al. 2004a). Although blastocysts can develop entirely in vitro, the overall success of this process and quality of the blastocysts is markedly lower than in vivo (Hasler et al. 1995). Moreover, blastocysts must be transferred into a receptive uterus for growth and development into an elongated filamentous conceptus (Heyman et al. 1984, Flechon et al. 1986, Maddox-Hyttell et al. 2003). In cattle, concentrations of progesterone in early pregnancy clearly affect embryonic survival during early pregnancy (Mann & Lamming 1999). In both lactating dairy cows and heifers, there is a strong positive association between the time that concentrations of progesterone increase in plasma and embryonic survival (Villa-Godoy et al. 1988, Larson et al. 1997, Starbuck et al. 1999). Heifers and ewes with lower concentrations of progesterone in the early luteal phase had retarded conceptuses that secreted less IFNT (Nephew et al. 1991, Mann & Lamming 2001). Increasing concentrations of progesterone from days 2 to 5 or days 5 to 9 enhanced conceptus development and size on day 14 in heifers (Garrett et al. 1988b) and day 16 in cows (Mann et al. 2006), while animals with lower concentrations in the early luteal phase had retarded embryonic development (Nephew et al. 1991, Mann & Lamming 2001) and
less IFNT produced by the conceptuses (Mann & Lamming 2001). Advancement of conceptus development by exogenous progesterone during metestrus and early diestrus has also been reported in sheep (Kleemann et al. 1994, Satterfield et al. 2006). Increasing progesterone in cattle after artificial insemination or mating increased embryonic survival (Wiltbank et al. 1956, Johnson et al. 1958, Robinson et al. 1989, van Cleef et al. 1991, Thatcher et al. 2001, Larson et al. 2007), although other studies found that progesterone augmentation had no effect on pregnancy rates in cattle and sheep (Funston et al. 2005, Howard et al. 2006). Progesterone supplementation is unlikely to rescue development of embryos with inherent genetic defects, and its efficacy may be different across breeds (beef cattle versus dairy cattle), overall fertility, and metabolic state (lactating versus dry; Mann & Lamming 1999, Mann et al. 2001).

Although progesterone acts via the uterus to stimulate blastocyst survival and growth, the specific genes and physiological mechanisms regulated by progesterone are only now being elucidated. As summarized in Supplementary Table 1, which can be viewed online at http://www.reproduction-online.org/supplemental/; a combination of candidate gene analyses and transcriptional profiling experiments has defined a number of genes and pathways regulated by pregnancy, progesterone, and IFNT in sheep and cattle (Bauersachs et al. 2005, 2006, Chen et al. 2006, Gray et al. 2006, Klein et al. 2006). Indeed, progesterone both positively and negatively regulates expression of genes in the endometrium, and progesterone and IFNT costimulate a number of genes, particularly in the endometrial epithelium (Supplementary Table 1 and Fig. 1). As depicted in Fig. 1, temporal and spatial analyses of gene expression revealed that galec15 (LGALS15), cystatin C (CST3), and cathepsin L (CTSL) expression is initiated in the endometrial LE by day 12 and maintained to day 14 in both cyclic and pregnant ewes (Gray et al. 2004, Song et al. 2005, 2006a). Those genes are no longer expressed by day 16 in cyclic ewes, but their expression is further stimulated by IFNT from the conceptus in pregnant ewes. LGALS15 is implicated in blastocyst attachment and elongation (Gray et al. 2004), because functional studies of LGALS15 and other galectins have implicated these proteins in cell growth, differentiation and apoptosis, as well as in cell adhesion, chemotraction, and migration (Yang & Liu 2003, Farmer et al. 2007, Lewis et al. 2007). Cathepsins are peptidases that can degrade extracellular matrix, catalyze intracellular proteins, process prohormones, and regulate uterine receptivity for implantation and trophoblast invasion in several mammals (Salamonsen 1999). CST3 is an inhibitor of CTSL. A balance of proteases and their inhibitors is likely required to modify the glycoalyx on endometrial LE and trophoblast during apposition and adhesion phases of implantation (Carson et al. 2000). Similar to the human (Giudice & Ferenczy 1996, Kao et al. 2002), endometria of both cyclic and pregnant ewes express genes implicated in uterine receptivity and blastocyst development and implantation. However, the absence of a sufficiently developed blastocyst to signal pregnancy recognition results in those genes being ‘turned off’ as luteolysis ensues and the ewe returns to estrus for another opportunity to mate. In addition to the upregulating genes, progesterone also downregulates a number of genes in the endometrium that can be revealed by treatment of animals with an antiprogestin (Gray et al. 2006; Supplementary Table 1).

PGR regulation and endometrial gene expression

Recent studies found that LGALS15, CTSL, and CST3 are induced by progesterone and stimulated further by IFNT (Gray et al. 2004, Song et al. 2005, 2006a, Satterfield et al. 2006). In most mammalian uteri, progesterone receptors (PGR) are expressed in endometrial epithelia and stroma during the early to mid-luteal phase, allowing direct regulation (induction or repression) of genes by progesterone. However, continuous exposure of the endometrium to progesterone negatively regulates PGR expression in the LE and then glandular epithelium (GE), and the downregulation of PGR is temporally associated with the induction of many progesterone-stimulated genes (Supplementary Table 1; Fig. 1). Indeed, the paradigm of loss of PGR in uterine epithelia immediately prior to implantation is common across mammals (Carson et al. 2000). In the ovine uterus, PGR protein is not detectable in LE and GE after days 11 and 13 of pregnancy respectively but can be detected in the uterine stroma and myometrium throughout gestation (Wathes & Hamon 1993, Spencer & Bazer 1995), suggesting that progestamedins, such as fibroblast growth factor 7 (FGF7), FGF10, and hepatocyte growth factor, from stromal cells regulate epithelial functions during most of pregnancy (Chen et al. 2000a, 2000b). In sheep, PGR loss from the uterine epithelium is determined by timing of the post-ovulatory rise in progesterone and requires continuous exposure to progesterone for at least 8 days (Spencer et al. 1995, Satterfield et al. 2006). Thus, an early increase in circulating progesterone apparently advances the timing of PGR loss from uterine epithelia (Satterfield et al. 2006). In cyclic ewes, loss of the PGR allows for induction of estrogen receptor-α, expression of oxytocin receptors (OXTR), and development of the endometrial luteolytic mechanism (see Spencer et al. 2007b). In pregnant ewes (Fig. 1), loss of PGR in endometrial epithelia is associated with a reduction in anti-adhesive MUC1 (mucin glycoprotein 1) and onset LGALS15, CTSL, and CST3 expression in the LE (Gray et al. 2004, Song et al. 2005, 2006a, Satterfield et al. 2006) and SPP1 (secreted phosphoprotein 1 or osteopontin), STC1 (stanniocalcin), and UTMP (uterine milk proteins or serpins) expression in the GE (Johnson et al. 2000).
One understudied area is what controls expression of the PGR and how PGR regulates transcription of target genes in a cell-specific manner within the uterus. Although PGR expression is absent from endometrial LE of cattle by day 16 post-estrus/mating (Robinson et al. 2001), comparative studies on temporal and spatial alterations in gene expression in bovine uteri during the estrous cycle and early pregnancy are not available to determine whether the same genes associated with uterine receptivity and conceptus implantation in sheep are present and operative in cattle. Recent studies suggest that uterine gene expression may be spatially different from sheep. For instance, Bauersachs et al. (2005) found that UTMP mRNA levels were markedly increased at estrus when compared with diestrus with highest levels in the cranial part of the ipsilateral uterine horn. Moreover, LGALS15 was not identified in transcriptional profiling studies of early pregnancy in cattle (Bauersachs et al. 2005, 2006, Klein et al. 2006). Although the LGALS15 gene is present in sheep, cattle, and goats, it is only expressed in endometria of sheep and goats (Lewis et al. 2007). However, other lectins (LGALS9 and LGALS3BP) were identified in the uteri of cattle (Supplementary Table 1), and their transcripts were upregulated mainly in LE of pregnant cows (Bauersachs et al. 2006). Thus, one possibility is that common pathways and gene families regulate conceptus–endometrial interactions during early pregnancy across mammals, but specific genes may be different in each species (Bauersachs et al. 2006). Understanding the commonalities and differences in progesterone actions on the endometrium will require carefully conducted longitudinal and cell-specific transcriptional profiling studies.

Conceptus regulation of uterine function: IFNT and placental lactogen

Maternal recognition of pregnancy is the physiological process whereby the conceptus signals its presence to the maternal system and prolongs the lifespan of the corpus luteum (Bazer et al. 1991). In ruminants, IFNT is the pregnancy recognition signal secreted by the elongating conceptus that acts on the endometrium to inhibit development of the luteolytic mechanism (see Thatcher et al. 1989, Bazer 1992, Mann et al. 1999, 2000b, Stewart et al. 2000, Song et al. 2006b). The relative abundance of mRNA or protein across days of pregnancy is indicated within the uterus as well as regulation of genes by progesterone and/or IFNT. Red, downregulated by progesterone; orange, induced or stimulated by progesterone; brown, induced or stimulated by IFNT; blue, induced by progesterone and stimulated by IFNT; n.d., not determined due to inability to flush intact conceptuses from the uterine lumen on days 18–20 of pregnancy.

![Figure 1 Temporal and spatial roadmap of progesterone- and IFNT-stimulated genes in the uterus during establishment of pregnancy in sheep.](image-url)
Spencer & Bazer 2004, Spencer et al. 2007b). Although IFNT inhibits OXTR expression, it does not inhibit PTGS2 (prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) prostaglandin or COX2), which is important for the generation of prostaglandins, such as PGE2 and PGI2, considered to be critical regulators of uterine receptivity and conceptus-endometrial interactions during early pregnancy (Charpigny et al. 1997a, 1997b, Arosh et al. 2004, Emond et al. 2004, Cammas et al. 2006, Critchley et al. 2006). In addition to its antiluteolytic actions, IFNT acts on the endometrium to induce or enhance expression of a number of genes (IFNT-stimulated genes or ISGs) that are hypothesized to regulate uterine receptivity and conceptus development during implantation (Hansen et al. 1999, Spencer et al. 2004b, 2007a). Given that IFNT is a member of the family of Type 1 IFN family (Roberts et al. 1997), one challenge is to determine which of the large number of ISGs have a specific function in conceptus-endometrial interactions given that they have traditionally been associated with cellular antiviral responses; the main function of type I IFNs is to inhibit viral infection (Pestka 2007).

**ISGs in the endometrium**

IFNT induces or increases expression of several ISGs in endometria that are hypothesized to be important for conceptus implantation (Hansen et al. 1999, Spencer et al. 2004b, 2007a). Since expression of ISGs increases in a stage-specific manner within endometria of diverse species, including domestic animals, laboratory rodents, primates, and humans during early pregnancy, they may be universally important in establishment of uterine receptivity to conceptus implantation (Li et al. 2001, Austin et al. 2003, Cheon et al. 2003, Bany & Cross 2006, Hess et al. 2007, Kashiwagi et al. 2007). A number of transcriptional profiling experiments conducted with human cells, ovine endometrium, and bovine endometrium have elucidated genes regulated by IFNT during pregnancy (Kim et al. 2003, Sandra et al. 2005, Bauersachs et al. 2006, Chen et al. 2006, Gray et al. 2006, Klein et al. 2006; see Supplementary Table 1 and Figs 1 and 2 for summary and interpretation).

**IFNT and the JAK–STAT–IRF pathway**

As illustrated in Fig. 2, IFNT activates the classical JAK–STAT–IRF (janus kinase–signal transducer and activator of transcription–interferon regulatory factor) signaling pathway used by other type I IFNs (Stark et al. 1998) in a uterine cell-specific manner. As illustrated in Figs 1–3 and summarized in Supplementary Table 1, numerous ISGs are induced or stimulated in the endometrium during conceptus elongation in both cattle and sheep. Curiously, in vivo studies revealed that many classical ISGs (β-2-microglobulin (B2M), guanylate-binding protein 2 (GBP2), interferon, α-inducible protein 27 (IFI27), interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), interferon-stimulated gene 15 (ISG15), IRF9, MHC class I polypeptide (MIC), 2′,5′-oligoadenylate synthetase (OAS), radical S-adenosyl methionine domain containing 2 (RSAD2), STAT1, and STAT2) are not induced or upregulated by IFNT in endometrial LE of the ovine uterus (Johnson et al. 1999b, 2001, Choi et al. 2001, 2003, Kim et al. 2003, Song et al. 2007). This finding was initially surprising, because all endometrial cell types express IFNAR1 (interferon (α, β, and ω) receptor 1) and IFNAR2 subunits of the common type I IFNAR (Rosenfeld et al. 2002). However, it was discovered that IRF2, a potent transcriptional repressor of ISGs, is expressed specifically in uterine LE to repress transcriptional activity of IFN-stimulated response element (ISRE)-containing promoters (Choi et al. 2001). Thus, IRF2 in LE appears to restrict IFNT induction of many ISGs to stroma and GE of the ovine uterus (Figs 1 and 2). In fact, all components of the ISGF3 transcription factor complex (STAT1, STAT2, and IRF9) and other classical ISGs (B2M, GBP2, IFI27, IFIT1, ISG15, MIC, and OAS) contain one or more ISREs in their promoters. Further, suppressor of cytokine signaling (SOCS1–3) are also upregulated in endometria by pregnancy and IFNT (Sandra et al. 2005). Depending on their cell-specific expression in the uterus, SOCS1–3 may be involved in negative regulation of the JAK–STAT pathway activated by IFNT (Kile et al. 2002). The silencing of MIC and B2M genes in endometrial LE during pregnancy may be a critical mechanism preventing immune rejection of the conceptus semi-allograft (Choi et al. 2003). Of particular note, several reports indicate induction or increases in ISGs in peripheral blood lymphocytes and the corpus luteum during pregnancy or in ewes receiving intrauterine injections of IFNT (Spencer et al. 1999a, Yankey et al. 2001, Gifford et al. 2007). Thus, IFNT or IFN-stimulated immune cells may traffic out of the uterus to exert systemic effects that alter maternal physiology, particularly the corpus luteum of pregnancy.

Given that the critical signaling components of the JAK-STAT signaling system (STAT1, STAT2, IRF9) are not expressed in endometrial LE, IFNT must utilize a non-classical STAT1-independent cell signaling pathway to regulate expression of genes in endometrial LE of the ovine uterus (Fig. 2). Transcriptional profiling of human U3A (STAT1 null) cells and ovine endometrium treated with IFNT were used to discover novel ISGs in the endometrial LE during pregnancy including WNT7A (wingless-type MMTV integration site family, member 7A), LGALS15, CSTL, and CST3 (Kim et al. 2003, Song et al. 2005, 2006a, Gray et al. 2006). The expression patterns of novel and classical ISGs are summarized in Figs 1 and 2. Functional aspects of a few IFN-stimulated genes will be discussed, and the reader is referred to several reviews with additional information on other
Figure 2 Schematic of current working hypothesis on cell-specific IFNT signaling in the endometrium of the ovine uterus. IFNT, produced by developing conceptuses of ruminants, binds to IFNAR present on cells of the ovine endometrium. (A) In the uterine luminal and superficial glandular epithelia (LE/sGE), IRF2, a potent and stable transcriptional repressor, increases during early pregnancy. The continual presence of IRF2 inhibits classical IFN-stimulated genes (ISGs) such as STAT1, STAT2, IRF9, B2M, ISG15, MHC, and OAS through direct ISRE and IRFE binding and coactivator repulsion. Thus, critical factors in the classical JAK-STAT-IRF pathway (STAT1, STAT2, and IRF9) are not present, resulting in the absence of ISGF3 or IRF1 transcription factors necessary to transactivate ISGs. However, IFNT does activate an unknown cell signaling pathway that results in induction of WNT7A and stimulation of non-classical IFNT-stimulated genes (CST3, CTSL, and LGALS15) specifically in LE/sGE. (B) In cells of the stroma (ST) and middle to deep GE, the IRF2 repressor is not expressed. Thus, IFNT-mediated association of IFNAR subunits facilitates cross-phosphorylation and activation of JAK1 and Tyk2, which in turn phosphorylates the receptor and creates a docking site for STAT2. STAT2 is then phosphorylated, thus creating a docking site for STAT1 which is then phosphorylated. STAT1 and STAT2 are then released from the receptor and can form two transcription factor complexes, GAF and ISGF3. ISGF3 is formed by association of a STAT1-2 heterodimer and IRF9 in the cytoplasm, translocates to the nucleus, and transactivates genes containing an ISRE(s), such as STAT1, STAT2, IRF9, B2M, ISG15, MHC, and OAS. GAF is formed by STAT1 homodimers, which translocates to the nucleus and transactivates genes containing a GAS element(s) such as IRF1. IRF1 can also bind and transactivate ISRE-containing genes as well as IRFE-containing genes. The simultaneous induction of STAT2 and IRF9 by IFNT appears to shift transcription factor formation from GAF towards predominantly ISGF3. Therefore, IFNT activation of the JAK-STAT-IRF signal transduction pathway allows for constant formation of ISGF3 and GAF transcription factor complexes and hyperactivation of ISG expression in the ST and GE. B2M, β-2-microglobulin; CST3, cystatin C; CTSL, cathepsin L; GAF, γ-activated factor; GAS, γ activation sequence; IFNAR, type 1 IFN receptor; IFNT, interferon τ; IRF1, interferon regulatory factor 1; IRF2, interferon regulatory factor 2; IRF3, IRF-response element; IRF9, IFN regulatory factor 9 (alias ISGF3G); ISG15, ISG15 ubiquitin-like modifier; alias IFI15 or UCRP; ISRE, IFN-stimulated response element; JAK, janus kinase; LGALS15, galectin 15; MHC, MHC class I polypeptide-related sequence; OAS, 2′,5′-oligoadenylate synthetases; STAT1, signal transducer and activator of transcription 1, 91 kDa; STAT2, signal transducer and activator of transcription 2, 113 kDa; WNT7A, wingless-type MMVM integration site family, member 7A.

**ISG15**

Many classical ISGs, such as *ISG15*, are expressed in LE of the ovine uterus on days 10 or 11 of the estrous cycle and pregnancy, but are undetectable in LE by days 12–13 (Johnson et al. 1999b; see Figs 1 and 2). In response to IFNT from elongating conceptuses, *ISG15* is induced in the stratum compactum stroma and GE by days 13–14, and expression extends to the stratum spongiosum stroma, deep glands, and myometrium as well as resident immune cells of the ovine uterus by days 15–16 of pregnancy (Johnson et al. 1999b, 2000a). As IFNT production by the conceptus declines, expression of ISGs also declines, but some remain abundant in endometrial stroma and GE on days 18–20 of pregnancy. Similar temporal and spatial alterations in *ISG15* expression occur in the bovine uterus during early pregnancy (Johnson et al. 1999a, Austin et al. 2004).

**WNT7A**

During early pregnancy in sheep (Fig. 1), WNT7A is present on day 10, downregulated on day 12, and then induced by IFNT between days 12 and 14 of pregnancy specifically in LE (Kim et al. 2003). The WNT family (19 genes in human) includes many highly conserved and secreted glycoproteins that regulate cell and tissue growth and differentiation during embryonic development and play a central role in coordinating uterine–conceptus interactions required for implantation in mice and perhaps humans (Mohamed et al. 2005). WNT7A activates the canonical WNT signaling pathway in ovine trophoectoderm cells and likely plays a role in regulating gene expression, proliferation, and perhaps BNC differentiation (Nakano et al. 2005, Hayashi et al. 2007). Further, WNT7A may have autocrine effects on LE to regulate expression of target genes important for uterine receptivity and conceptus implantation, such as *LGALS15*, *CST3*, and *CTSL*.

**LGALS15**

Similar to CTS and CTS3 (Fig. 1), LGALS15 is induced by progesterone in LE between days 10 and 14 and is further increased by IFNT (Gray et al. 2004). Galectins are proteins with a conserved carbohydrate recognition domain that bind β-galactosides, thereby cross-linking glycoproteins as well as glycolipid receptors on the surface of cells, such as integrins, and initiating biological responses (Yang & Liu 2003).
originally termed ovgal11, was originally identified in ovine intestinal epithelium as being induced in response to infection by the nematode parasite Haemonchus contortus (Dunphy et al. 2000). Interestingly, LGALS15 is the 14 K protein from sheep endometrium initially characterized as a progesterone-induced protein associated with crystalline inclusion bodies in uterine epithelia and conceptus trophoderm (Kazemi et al. 1990). LGALS15 is implicated in conceptus implantation (Spencer et al. 2004b, 2007a), because functional studies of other galectins have implicated these proteins in cell growth, differentation, and apoptosis as well as in cell adhesion, chemoattraction, and migration (Yang & Liu 2003). Recently, Farmer et al. (2007) found that LGALS15 stimulates migration and adhesion of ovine trophoderm cells via activation of Jun N-terminal kinase and integrin signaling respectively. Of particular relevance to this review paper, the LGALS15 gene is present in both sheep and cattle but not humans, mice, or other sequenced species, and the gene is only expressed in the endometrium of sheep and goats (Lewis et al. 2007).

**CXCL10**

A classical ISG with reported biological effects on trophoderm growth and adhesion in ruminants is **CXCL10** (chemokine (C-X-C motif) ligand 10; alias IP-10; Nagaoka et al. 2003a, 2003b). CXCL10 is a member of the C-X-C chemokine family that regulates multiple aspects of inflammatory and immune responses primarily through chemotactic activity toward subsets of leukocytes. CXCL10 mRNA was localized to monocytes in the subepithelial stroma of uteri from pregnant, but not cyclic ewes. Whether IFNT directly regulates CXCL10 in the monocytes or simply attract monocytes to the endometrium remains to be determined. In the ovine uterus, CXCL10 appeared on day 17 in the uterine lumen, and the CXCR3 receptor was localized to trophoderm (Fig. 1). Subsequently, recombinant CXCL10 was shown to stimulate migration of trophoderm cells and promote their adhesion to fibronectin, as well as increase expression of integrins α5, αV, and β3 subunit mRNAs (Nagaoka et al. 2003a, Imakawa et al. 2006). Integrins are essential for conceptus implantation (see Burghardt et al. 2002). In the human, chemokines are expressed by maternal and embryonic cells during implantation, whereas corresponding receptors are on trophoblast cells (Hannan et al. 2006). Further, trophoblast migration is promoted by chemokines and endometrial cell-conditioned medium indicating an important involvement of chemokines in maternal–fetal communication (Lea & Sandra 2007).

**ISG differences in bovine and sheep uteri**

Many ISGs are induced or upregulated in endometria of pregnant cattle and sheep; however, there may be cell-specific differences in expression between these species. For instance (Supplementary Table 1), mRNAs of several pregnancy- and IFN-stimulated genes (AGRN (agrin), BST2 (bone marrow stromal antigen 2), C1R, C1S, IFITM3, LGALS3BP, LGALS9, PARP12, SERPING1, ubiquitin-activating enzyme-1-like protein (UBE1L), USP18, and XAF1) are found in endometrial LE of day 18 pregnant cattle (Bauersachs et al. 2006, Klein et al. 2006). However, ISG15 is not stimulated or present in endometrial LE of early pregnant cows or ewes, but is markedly upregulated in the stroma and GE (Johnson et al. 1999a, 1999b, 2000a). ISG15 encodes a ubiquitin-like protein that can be conjugated to intracellular proteins, such as phospholipase C-γ1, JAK1, extracellular regulated kinase 1, and STAT1. Austin et al. (2004) postulated that one function of ISG15 is to stabilize proteins rather than target them to degradation as described for polyubiquitination. This is consistent with the fact that conjugated ISG15 remains in the uterus as late as day 45 of pregnancy. Interestingly, the gene for bovine **UBE1L**, the initiating enzyme for ISG15ylation, was also identified as an upregulated gene in endometrium from day 18 pregnant cows (Rempel et al. 2005). Furthermore, the mRNAs of **IFITM1** and **IFITM3**, encoding proteins hypothesized to possess E2 ubiquitin-conjugating enzyme activity that is involved in protein ubiquitylation, were also upregulated in endometrium from pregnant cattle, supporting an important role for ISG15ylation in establishment and maintenance of pregnancy (Fig. 2; Klein et al. 2006). Comparative studies on temporal and spatial alterations in gene expression in bovine uteri during the estrous cycle and early pregnancy are underway to determine whether the genes associated with uterine receptivity and conceptus implantation in sheep are present and operative in cattle.

**CSH1 regulation of uterine gland morphogenesis and secretory function**

During early pregnancy, ovine and bovine uteri are exposed sequentially to estrogen, progesterone, IFNT, and CSH1, which is proposed to initiate and maintain endometrial gland morphogenesis and differentiated secretory functions (for review see Spencer et al. 1999b, 2004a, Spencer & Bazer 2002). Placentae of many species, including rodents, humans, nonhuman primates, and ruminants, secrete hormones structurally related to pituitary prolactin (PRL) and growth hormone (GH) that are termed CSH1 (alias placental lactogen; Soares 2004). Ovine CSH1 is produced by trophoblast giant BNC from days 15 to 16 of pregnancy, which is coordinated with onset of expression of **UTMP**, **SPP1**, gastrin-releasing peptide (GRP), and **STC1** (Ing et al. 1989, Whitley et al. 1998, Stewart et al. 2000, Song et al. 2006b), which are excellent markers for GE differentiation and secretory function during pregnancy in sheep (Fig. 1). Surprisingly, bovine **STC1** and **GRP** mRNA levels...
were higher in endometrium from day 18 cyclic than day 18 pregnant cows (Bauersachs et al. 2006), suggesting species-specific control of expression of these genes. A homodimer of the PRL receptor (PRLR), as well as a heterodimer of PRLR and GH receptor, transduce signals by ovine CSH1/placental lactogen (Gertler & Djiane 2002). In the ovine uterus, PRLR gene expression is unique to GE (Cassy et al. 1999, Stewart et al. 2000). Temporal changes in circulating levels of CSH1 are correlated with endometrial gland hyperplasia and hypertrophy and increased production of SPP1 and UTMP during pregnancy (Supplementary Table 1). Sequential exposure of the pregnant ovine endometrium to progesterone, IFNT, and CSH1 appears to be required to activate and maintain endometrial remodeling, secretory function of GE, and perhaps uterine growth during gestation. Chronic treatment of ovariectomized ewes with progesterone induces SPP1, UTMP, and STC1 expression by GE (Moffatt et al. 1987, Spencer et al. 1999b, Johnson et al. 2000b, Song et al. 2006b). However, intrauterine infusions of CSH1 further increases endometrial SPP1, STC1, and UTMP gene expression, but only when ewes receive progesterone and intrauterine infusions of IFNT (Spencer et al. 1999b, Noel et al. 2003). The effects of IFNT may be attributed, in part, to increasing PRLR in the endometrial glands (Martin et al. 2004). Available evidence indicates that placental hormones play key roles in stimulating endometrial gland morphogenesis and differentiated functions during pregnancy that are required for conceptus development in ruminants.

Models and approaches for studying conceptus–endometrial interactions

Genomics

Several groups have used differential display and subtractive hybridization to identify differentially expressed genes in the endometrium that were subsequently arrayed for transcriptional profiling experiments (Spencer et al. 1999c, Bauersachs et al. 2005, 2006, Klein et al. 2006). Further, custom arrays from the endometrium have been made for transcriptional profiling experiments in sheep (Chen et al. 2006, Gray et al. 2006) and cattle (Bauersachs et al. 2007). The advent of commercially available bovine arrays from the bovine oligo microarray consortium (www.bovineoligo.org) and commercial companies should enhance research profiling gene expression in ruminant embryos and endometria. Given that endometrial gene expression changes in response to progesterone and the conceptus in a temporal and cell-specific manner (Fig. 1), days of the estrus cycle and pregnancy status should be chosen carefully. Further, the ruminant endometrium has caruncular and intercaruncular areas with varying amounts of different cell types, including LE, GE, stroma, blood and lymph vessels, and immune cells. Therefore, transcriptional profiling of individual cell populations may be warranted and accomplished using laser capture microdissection (Niklaus & Pollard 2006). These studies should elucidate temporal and spatial changes in gene expression that occur in specific endometrial and conceptus cell types during pregnancy. Another important point is the developmental potential of the conceptus, which will have important effects on the molecular responses of the maternal environment, but is difficult to predict. Recently, it was proposed to develop transgenic reporter systems which facilitate monitoring of important steps in development in living embryos (Habermann et al. 2007). For instance, a Pou5f1-EGFP reporter gene allows quantitative monitoring of pluripotency gene activation after somatic cell nuclear transfer (SCNT) in cattle (Wuensch et al. 2007). This system will be an important tool to evaluate whether placentation abnormalities, which are a major reason for pregnancy loss after transfer of SCNT embryos, result from disturbed embryo–maternal communication. Advanced bioinformatic analyses of these data should reveal gene networks and biological pathways involved in physiological and pathological conceptus–endometrial interactions. Further, they are critical to understanding the bases for polygenic traits such as uterine capacity.

Proteomics and metabolomics

In addition to genomics, proteomic and metabolomic analyses are required to understand conceptus–endometrial interactions due to the nature of histotroph, defined as tropic substances of tissue origin, present in the uterine lumen that impacts conceptus growth throughout pregnancy. All mammalian uteri contain endometrial epithelia that synthesize and secrete or transport a complex and rather undefined mixture of amino acids, ions, glucose, enzymes, growth factors, hormones, transport proteins, and other substances termed histotroph (Bazer 1975). The epithelial cells of the uterine lumen are highly secretory during implantation, and the trophectoderm exhibits intense pinocytotic activity which increases as the conceptus develops (Guillomot 1995). Therefore, factors supporting growth of pre- and peri-implantation blastocysts and elongating conceptuses are likely obtained from uterine histotroph (Lee et al. 1998). This hypothesis is supported by results from studies of asynchronous uterine transfer of embryos and trophoblast vesicles (Lawson et al. 1983, Flechon et al. 1986) and from studies of uterine gland knockout (UGKO) ewes (Gray et al. 2001b, 2002).

The UGKO ewe model is produced by continuous administration of a synthetic, non-metabolizable progesterin to neonatal ewes from birth to postnatal day 56 (Gray et al. 2000a). This inappropriate exposure of ewe lambs to a progesterin permanently ablates differentiation and development of the endometrial glands from
LE and produces an UGKO phenotype without apparent alterations in development of myometrium, or other Müllerian duct-derived female reproductive tract structures, or function of the hypothalamic–pituitary–ovarian axis (Gray et al. 2000b, 2001a). The UGKO endometrium is devoid of glands and has markedly reduced LE surface area. UGKO ewes exhibit recurrent early pregnancy loss as blastocyst fails to elongate, and transfer of blastocysts from uteri of control ewes into uteri of timed recipient UGKO ewes does not ameliorate this defect (Gray et al. 2001b). Morphologically normal blastocysts are present in uterine flushes of bred UGKO ewes on days 6 and 9 post-mating, but not on day 14 (Gray et al. 2001b, 2002) when uteri contain either no conceptus or a severely growth-retarded tubular conceptus. Similarly, exposure of neonatal heifers to progestins alters uterine development and reduces pregnancy success in adult cows (Bartol et al. 1995). These results demonstrate that histotroph from endometrial epithelia are required for peri-implantation blastocyst survival and elongation in sheep and likely cattle.

Defects in blastocyst survival and elongation in UGKO ewes are not due to alterations in expression of steroid receptors, anti-adhesive MUC1, adhesive integrins on the endometrial LE, or responsiveness of the endometrium to IFN-γ (Gray et al. 2000a, 2002). However, uterine flushes from day 14 bred UGKO ewes contain either very low or undetectable amounts of LGALS15, glycosylated cell adhesion molecule 1, and SPP1, which are adhesion proteins secreted by the uterine LE and GE that are abundant in uterine luminal histotroph of control ewes (Gray et al. 2002, 2004, 2006). Therefore, the reduction or absence of adhesion proteins from endometrial GE is a likely cause of recurrent pregnancy loss in UGKO ewes. Other essential, but as yet undefined, components of histotroph are undoubtedly absent or reduced in the uteri of infertile UGKO ewes.

The complex nature of histotroph in the uterine lumen makes it very amenable to analyses by proteomics and metabolomics. Indeed, components of histotroph come from genes expressed in the endometrium and conceptus, as well as components of serum that are selectively and specifically transported by the endometrium into the uterine lumen. Proteomic analyses of uterine histotroph from ewes and cows identified a number of proteins (Bartol et al. 1985a, 1985b, Lee et al. 1998). Pregnancy and progesterone also alter tight and adherens junctions that likely impact histotroph transport and sequestration in the uterine lumen (Satterfield et al. 2007). Increased knowledge of the bovine proteome coupled with non-gel-based qualitative and quantitative proteomic approaches utilizing mass spectrometry should enable researchers to rapidly identify proteins in uterine histotroph. Of particular interest, Berendt et al. (2005) used this approach to identify several endometrial proteins (pGDP dissociation inhibitor β (ARHGDIIB); α-hydroxysteroid dehydrogenase (AKR1C1); soluble NADP(+)−dependent isocitrate dehydrogenase 1; and acyl-CoA-binding protein) involved in conceptus–maternal interactions. Interestingly, monozygotic twins in cattle (generated by embryo splitting) were used as a model to eliminate genetic variability as a source for proteome differences. Indeed, proteomics approaches have identified biomarkers of uterine receptivity, such as the immunophilin FK506-binding protein 4, and reproductive tract dysfunction and disease in other species (Daikoku et al. 2005, Dasari et al. 2007).

In addition to proteins, uterine histotroph contains amino acids, sugars, and lipids which have not been well studied in ruminants. In pigs, distinct changes in amino acids, lipids, and sugars occur in the uterine lumen and fetal fluids (Geisert et al. 1982, Kwon et al. 2003a, 2003b). Glucose and amino acids have a recognized role in pre-implantation embryo development (Leese 1995, Devreker & Englert 2000), and recently amino acid transport and amino acids have been implicated in blastocyst implantation and trophectoderm differentiation via modulation of nutrient-sensing pathways (Martin & Sutherland 2001, Martin et al. 2003). Further, lipids and their metabolites are involved endometrial function and placental development in mice and humans (Schaiff et al. 2006, 2007). The accessibility of the ruminant reproductive tract and size of the uterus should make it an attractive model for proteomic and metabolomic studies of conceptus–endometrial interactions that is not as feasible in mice and humans.

**Integrative systems biology and holistic approaches**

For the past several decades, reproductive biologists have endeavored to dissect out the individual genes and pathways regulating certain facets of endometrial function, conceptus development, and influences of the conceptus on endometrial function. This reductionist approach has yielded much knowledge about pregnancy recognition in which the conceptus signals its presence to the mother in order to ensure survival of the corpus luteum. However, conceptus–endometrial interactions are complex and not determined by a single gene. Therefore, a systems biology approach integrating multiple levels of information (genome, epigenome, transcriptome, proteome, interactome, etc.) is necessary to understand the biology of complex physiological systems that dictate uterine capacity, conceptus development, and maternal adaptations to pregnancy.

Holism (from Ολος holos, a Greek word meaning all, entire, total) is a theory that the universe and especially living nature is correctly seen in terms of interacting whole systems that are more than the mere sum of elementary parts (http://en.wikipedia.org/wiki/Holism). Thus, the properties of a given system cannot be determined or explained by the sum of its component parts alone. Instead, the system as a whole determines in...
an important way how the parts behave. Holistic approaches relate to or are concerned with whole or complete systems rather than with the analysis of, treatment of, or dissection into parts. Reductionism is sometimes seen as the opposite of holism. Reductionism in science says that a complex system can be explained by reduction to its fundamental parts. Holism and reductionism can also be regarded as complementary viewpoints, in which case both are needed to understand a given system. Integration of reductionistic and holistic approaches using systems biology and bioinformatics is expected to reveal truly novel insights into conceptus-endometrial interactions.

Conclusions and future directions
During the past decade, knowledge of mechanisms and factors regulating conceptus implantation in mammals has benefited from studies of sheep, cattle, and domestic animals. However, much remains to be discovered about interactions that regulate blastocyst implantation. Our knowledge of the cellular and molecular mechanisms of blastocyst and conceptus differentiation is very limited. As illustrated in Figs 1–3 and summarized in Supplementary Table 1, a number of potential adhesion factors and cascades are proposed to regulate implantation in cattle and sheep. These adhesion systems probably function only if in the correct spatio-temporal sequence (Aplin 1997). Results from studies of rodents firmly support the hypothesis that implantation involves a multiplicity of receptor–ligand interactions that are organized into a cascade. Therefore, the individual and integrative roles of adhesion factors will need to be mechanistically determined using in vivo, ex vivo, and in vitro experiments. Although gene knockouts and transgenics are technically possible in domestic animals, these techniques are not routinely feasible as research tools to understand the specific roles of factors proposed to regulate implantation. Therefore, other strategies must be employed to conduct hypothesis-based research to determine the specific roles of candidate genes regulating implantation in domestic animals. Promising technologies include the use of lentiviral vectors, antisense oligodeoxynucleotides, morpholinos, and small inhibitory RNAs that could be used in vivo and ex vivo to perform gain-of-function and loss-of-function studies of specific gene(s) in endometrial epithelia and trophoblast (Hofmann et al. 2004, Dunlap et al. 2006, Golding et al. 2006). Future research with sheep and other domestic animals must incorporate these types of approaches to determine mechanistic roles of specific factors hypothesized to mediate implantation. The sequencing of genomes of domestic animals is expected to generate knowledge and reagents useful to understand the bases of enhanced fertility in specific breeds of domestic animals (Finnish Landrace sheep and Meishan pigs) as well as infertility (Holstein dairy cattle). Understanding of key signals that regulate uterine receptivity and implantation can be used to diagnose and identify the cause(s) of recurrent pregnancy loss and improve pregnancy rates in mammals.

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

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