Focus on Mammalian Embryogenomics

Genes involved in conceptus–endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approachesThomas E Spencer, Olivier Sandra¹ and Eckhard Wolf^{2,3}

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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.

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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

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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 blastocoele or central cavity surrounded by a monolayer of trophectoderm (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 trophectoderm 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 luteolytic mechanism required for pulsatile release of prostaglandin F $_{2\alpha}$ (PGF $_{2\alpha}$), 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 trophectoderm to the endometrial luminal epithelium (LE), trophoblast giant binucleate cells (BNC) differentiate within the trophectoderm (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 trophectoderm 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 galectin 15 (*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, chemoattraction, and migration (Yang & Liu 2003, Farmer *et al.* 2007, Lewis *et al.* 2007). Cathepsins are peptidases that can degrade extracellular matrix, catabolize 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 glycocalyx 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 antiprogesterin (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 progestagens, 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 epithelia 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.*

2000b, Stewart *et al.* 2000, Song *et al.* 2006b). 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,

Figure 1 Temporal and spatial roadmap of progesterone- and IFNT-stimulated genes in the uterus during establishment of pregnancy in sheep. 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.

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 I 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 IFNT-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*, *CTSL*, 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 IFNT-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 or ISGs (*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*, *MIC*, 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 I IFN receptor; IFNT, interferon τ ; IRF1, interferon regulatory factor 1; IRF2, interferon regulatory factor 2; IRFE, 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; MIC, 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 MMTV integration site family, member 7A.

Figure 3 Model for conceptus–endometrium interactions in the bovine preattachment period. Differential transcriptome analysis of endometrium samples from day 18 pregnant as compared with non-pregnant heifers identified biological processes which are likely to be important for implantation and maintenance of pregnancy. These include IFNT signaling, embryo–maternal immune modulation, cell adhesion, and remodeling of the endometrium to prepare for implantation of the embryo. Genes in solid boxes, upregulated in the endometrium of pregnant animals; genes in dashed boxes, downregulated in the endometrium of pregnant animals. STAT1-1, STAT1 homodimers; ISGF3, transcription factor complex consisting of STAT1/STAT2 heterodimer and IRF9 (from Bauersachs *et al.* 2006).

genes (Spencer *et al.* 2004a, 2004b, 2007a, 2007b, Bauersachs *et al.* 2005, 2006).

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 trophoblast 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 *CTSL* and *CST3* (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). *LGALS15*,

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 trophoctoderm (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, differentiation, 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 trophoctoderm 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 trophoctoderm 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 trophoctoderm (Fig. 1). Subsequently, recombinant *CXCL10* was shown to stimulate migration of trophoctoderm cells and promote their adhesion to fibronectin, as well as increase expression of integrins $\alpha 5$, αV , and $\beta 3$ 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 cell antigen 2), *C1R*, *C1S*, *IFITM3*, *LGALS3BP*, *LGALS9*, *PARP12*, *SERPINC1*, 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- $\gamma 1$, 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 *ISG15*ylation, 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 *ISG15*ylation 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*, *SPPI*, 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 placental 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 trophoctoderm 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 progestin to neonatal ewes from birth to postnatal day 56 (Gray *et al.* 2000a). This inappropriate exposure of ewe lambs to a progestin 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 IFNT (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 (ARHGDI β); 20

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 trophoblast differentiation via modulation of nutrient-sensing pathways (Martin & Sutherland 2001, Martin *et al.* 2003). Further, lipids and their metabolites are involved in 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 ὅλος *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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References

- Aplin JD 1997 Adhesion molecules in implantation. *Reviews of Reproduction* **2** 84–93.
- Arosh JA, Banu SK, Kimmins S, Chapdelaine P, MacLaren LA & Fortier MA 2004 Effect of interferon- τ on prostaglandin biosynthesis, transport, and signaling at the time of maternal recognition of pregnancy in cattle: evidence of polycrine actions of prostaglandin E2. *Endocrinology* **145** 5280–5293.
- Austin KJ, Bany BM, Belden EL, Rempel LA, Cross JC & Hansen TR 2003 Interferon-stimulated gene-15 (ISG15) expression is up-regulated in the mouse uterus in response to the implanting conceptus. *Endocrinology* **144** 3107–3113.
- Austin KJ, Carr AL, Pru JK, Hearne CE, George EL, Belden EL & Hansen TR 2004 Localization of ISG15 and conjugated proteins in bovine endometrium using immunohistochemistry and electron microscopy. *Endocrinology* **145** 967–975.
- Bany BM & Cross JC 2006 Post-implantation mouse conceptuses produce paracrine signals that regulate the uterine endometrium undergoing decidualization. *Developmental Biology* **294** 445–456.
- Bartol FF, Roberts RM, Bazer FW & Thatcher WW 1985a Characterization of proteins produced *in vitro* by bovine endometrial explants. *Biology of Reproduction* **33** 745–759.
- Bartol FF, Roberts RM, Bazer FW, Lewis GS, Godkin JD & Thatcher WW 1985b Characterization of proteins produced *in vitro* by periattachment bovine conceptuses. *Biology of Reproduction* **32** 681–693.

- Bartol FF, Johnson LL, Floyd JG, Wiley AA, Spencer TE, Buxton DF & Coleman DA 1995 Neonatal exposure to progesterone and estradiol alters uterine and morphology and luminal protein content in adult beef heifers. *Theriogenology* **43** 835–844.
- Bauersachs S, Ulbrich SE, Gross K, Schmidt SE, Meyer HH, Einspanier R, Wenigerkind H, Vermehren M, Blum H, Sinowatz F *et al.* 2005 Gene expression profiling of bovine endometrium during the oestrous cycle: detection of molecular pathways involved in functional changes. *Journal of Molecular Endocrinology* **34** 889–908.
- Bauersachs S, Ulbrich SE, Gross K, Schmidt SE, Meyer HH, Wenigerkind H, Vermehren M, Sinowatz F, Blum H & Wolf E 2006 Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. *Reproduction* **132** 319–331.
- Bauersachs S, Mitko K, Blum H & Wolf E 2007 BOE Array Version 1: a tailored tool for studying bovine endometrium biology and pathophysiology. *Journal of Dairy Science* **90** 4420–4423.
- Bazer FW 1975 Uterine protein secretions: relationship to development of the conceptus. *Journal of Animal Science* **41** 1376–1382.
- Bazer FW 1992 Mediators of maternal recognition of pregnancy in mammals. *Proceedings of the Society for Experimental Biology and Medicine* **199** 373–384.
- Bazer FW, Roberts RM & Thatcher WW 1979 Actions of hormones on the uterus and effect on conceptus development. *Journal of Animal Science* **49** 35–45.
- Bazer FW, Thatcher WW, Hansen PJ, Mirando MA, Ott TL & Plante C 1991 Physiological mechanisms of pregnancy recognition in ruminants. *Journal of Reproduction and Fertility* **43** 39–47.
- Berend FJ, Frohlich T, Schmidt SE, Reichenbach HD, Wolf E & Arnold GJ 2005 Holistic differential analysis of embryo-induced alterations in the proteome of bovine endometrium in the preattachment period. *Proteomics* **5** 2551–2560.
- Burghardt RC, Johnson GA, Jaeger LA, Ka H, Garlow JE, Spencer TE & Bazer FW 2002 Integrins and extracellular matrix proteins at the maternal–fetal interface in domestic animals. *Cells, Tissues, Organs* **171** 202–217.
- Cammas L, Reinaud P, Bordas N, Dubois O, Germain G & Charpigny G 2006 Developmental regulation of prostacyclin synthase and prostacyclin receptors in the ovine uterus and conceptus during the peri-implantation period. *Reproduction* **131** 917–927.
- Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA & Yoshinaga K 2000 Embryo implantation. *Developmental Biology* **223** 217–237.
- Cassy S, Charlier M, Guillomot M, Pessemesse L & Djiane J 1999 Cellular localization and evolution of prolactin receptor mRNA in ovine endometrium during pregnancy. *FEBS Letters* **445** 207–211.
- Charpigny G, Reinaud P, Tamby JP, Creminon C & Guillomot M 1997a Cyclooxygenase-2 unlike cyclooxygenase-1 is highly expressed in ovine embryos during the implantation period. *Biology of Reproduction* **57** 1032–1040.
- Charpigny G, Reinaud P, Tamby JP, Creminon C, Martal J, Maclouf J & Guillomot M 1997b Expression of cyclooxygenase-1 and -2 in ovine endometrium during the estrous cycle and early pregnancy. *Endocrinology* **138** 2163–2171.
- Chen C, Spencer TE & Bazer FW 2000a Expression of hepatocyte growth factor and its receptor c-met in the ovine uterus. *Biology of Reproduction* **62** 1844–1850.
- Chen C, Spencer TE & Bazer FW 2000b Fibroblast growth factor-10: a stromal mediator of epithelial function in the ovine uterus. *Biology of Reproduction* **63** 959–966.
- Chen Y, Green JA, Antoniou E, Ealy AD, Mathialagan N, Walker AM, Avasle MP, Rosenfeld CS, Hearne LB & Roberts RM 2006 Effect of interferon- τ administration on endometrium of nonpregnant ewes: a comparison with pregnant ewes. *Endocrinology* **147** 2127–2137.
- Cheon YP, Xu X, Bagchi MK & Bagchi IC 2003 Immune-responsive gene 1 is a novel target of progesterone receptor and plays a critical role during implantation in the mouse. *Endocrinology* **144** 5623–5630.
- Choi Y, Johnson GA, Burghardt RC, Berghman LR, Joyce MM, Taylor KM, Stewart MD, Bazer FW & Spencer TE 2001 Interferon regulatory factor-two restricts expression of interferon-stimulated genes to the endometrial stroma and glandular epithelium of the ovine uterus. *Biology of Reproduction* **65** 1038–1049.
- Choi Y, Johnson GA, Spencer TE & Bazer FW 2003 Pregnancy and interferon tau regulate MHC class I and beta-2-microglobulin expression in the ovine uterus. *Biology of Reproduction* **68** 1703–1710.
- van Cleef J, Drost M & Thatcher WW 1991 Effects of post-insemination progesterone supplementation on fertility and subsequent oestrus responses of dairy heifers. *Theriogenology* **36** 795–807.
- Critchley HO, Osei J, Henderson TA, Boswell L, Sales KJ, Jabbour HN & Hirani N 2006 Hypoxia-inducible factor-1 α expression in human endometrium and its regulation by prostaglandin E-series prostanoid receptor 2 (EP2). *Endocrinology* **147** 744–753.
- Daikoku T, Tranguch S, Friedman DB, Das SK, Smith DF & Dey SK 2005 Proteomic analysis identifies immunophilin FK506 binding protein 4 (FKBP52) as a downstream target of Hoxa10 in the periimplantation mouse uterus. *Molecular Endocrinology* **19** 683–697.
- Dasari S, Pereira L, Reddy AP, Michaels JE, Lu X, Jacob T, Thomas A, Rodland M, Roberts CT Jr, Gravett MG *et al.* 2007 Comprehensive proteomic analysis of human cervical–vaginal fluid. *Journal of Proteome Research* **6** 1258–1268.
- Devreker F & Englert Y 2000 *In vitro* development and metabolism of the human embryo up to the blastocyst stage. *European Journal of Obstetrics and Gynecology* **92** 51–56.
- Diskin MG, Murphy JJ & Sreenan JM 2006 Embryo survival in dairy cows managed under pastoral conditions. *Animal Reproduction Science* **96** 297–311.
- Dunlap KA, Palmarini M, Varela M, Burghardt RC, Hayashi K, Farmer JL & Spencer TE 2006 Endogenous retroviruses regulate periimplantation placental growth and differentiation. *PNAS* **103** 14390–14395.
- Dunphy JL, Balic A, Barcham GJ, Horvath AJ, Nash AD & Meeusen EN 2000 Isolation and characterization of a novel inducible mammalian galectin. *Journal of Biological Chemistry* **275** 32106–32113.
- Emond V, MacLaren LA, Kimmins S, Arosh JA, Fortier MA & Lambert RD 2004 Expression of cyclooxygenase-2 and granulocyte-macrophage colony-stimulating factor in the endometrial epithelium of the cow is up-regulated during early pregnancy and in response to intrauterine infusions of interferon- τ . *Biology of Reproduction* **70** 54–64.
- Farin CE, Imakawa K & Roberts RM 1989 *In situ* localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. *Molecular Endocrinology* **3** 1099–1107.
- Farmer JL, Burghardt RC, Jousan FD, Hansen PJ, Bazer FW & Spencer TE 2007 Galectin 15 (LGALS15) functions in trophoblast migration and attachment. *FASEB Journal* [in press].
- Flechon JE, Guillomot M, Charlier M, Flechon B & Martal J 1986 Experimental studies on the elongation of the ewe blastocyst. *Reproduction, Nutrition, Development* **26** 1017–1024.
- Funston RN, Lipsey RJ, Geary TW & Roberts AJ 2005 Effect of administration of human chorionic gonadotropin after artificial insemination on concentrations of progesterone and conception rates in beef heifers. *Journal of Animal Science* **83** 1403–1405.
- Garrett JE, Geisert RD, Zavy MT & Morgan GL 1988a Evidence for maternal regulation of early conceptus growth and development in beef cattle. *Journal of Reproduction and Fertility* **84** 437–446.
- Garrett JE, Geisert RD, Zavy MT, Gries LK, Wettemann RP & Buchanan DS 1988b Effect of exogenous progesterone on prostaglandin F2 alpha release and the interestrus interval in the bovine. *Prostaglandins* **36** 85–96.
- Geisert RD, Renegar RH, Thatcher WW, Roberts RM & Bazer FW 1982 Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. *Biology of Reproduction* **27** 925–939.
- Gertler A & Djiane J 2002 Mechanism of ruminant placental lactogen action: molecular and *in vivo* studies. *Molecular Genetics and Metabolism* **75** 189–201.
- Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, Davies CJ & Ott TL 2007 Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. *Journal of Dairy Science* **90** 274–280.
- Giudice LC & Ferenczy A 1996 The endometrial cycle. In *Reproductive Endocrinology, Surgery and Technology*, pp 271–300. Eds EY Adashi, J Rock & Z Rosenwaks. Philadelphia: Lippincott-Raven.
- Golding MC, Long CR, Carmell MA, Hannon GJ & Westhusin ME 2006 Suppression of prion protein in livestock by RNA interference. *PNAS* **103** 5285–5290.

- Gray C, Bartol FF, Taylor KM, Wiley AA, Ramsey WS, Ott TL, Bazer FW & Spencer TE 2000a Ovine uterine gland knock-out model: effects of gland ablation on the estrous cycle. *Biology of Reproduction* **62** 448–456.
- Gray CA, Taylor KM, Bazer FW & Spencer TE 2000b Mechanisms regulating norgestomet inhibition of endometrial gland morphogenesis in the neonatal ovine uterus. *Molecular Reproduction and Development* **57** 67–78.
- Gray CA, Bazer FW & Spencer TE 2001a Effects of neonatal progesterone exposure on female reproductive tract structure and function in the adult ewe. *Biology of Reproduction* **64** 797–804.
- Gray CA, Taylor KM, Ramsey WS, Hill JR, Bazer FW, Bartol FF & Spencer TE 2001b Endometrial glands are required for preimplantation conceptus elongation and survival. *Biology of Reproduction* **64** 1608–1613.
- Gray CA, Burghardt RC, Johnson GA, Bazer FW & Spencer TE 2002 Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. *Reproduction* **124** 289–300.
- Gray CA, Adelson DL, Bazer FW, Burghardt RC, Meeusen EN & Spencer TE 2004 Discovery and characterization of an epithelial-specific galectin in the endometrium that forms crystals in the trophoctoderm. *PNAS* **101** 7982–7987.
- Gray CA, Abbey CA, Beremand PD, Choi Y, Farmer JL, Adelson DL, Thomas TL, Bazer FW & Spencer TE 2006 Identification of endometrial genes regulated by early pregnancy, progesterone, and interferon tau in the ovine uterus. *Biology of Reproduction* **74** 383–394.
- Guillomot M 1995 Cellular interactions during implantation in domestic ruminants. *Journal of Reproduction and Fertility* **49** 39–51.
- Guillomot M, Michel C, Gaye P, Charlier N, Trojan J & Martal J 1990 Cellular localization of an embryonic interferon, ovine trophoblastin and its mRNA in sheep embryos during early pregnancy. *Biology of the Cell* **68** 205–211.
- Guillomot M, Flechon JE & Leroy F 1993 Blastocyst development and implantation. In *Reproduction in Mammals and Man*, pp 387–411. Eds C Thibault, MC Levasseur & RHF Hunter. Paris: Ellipses.
- Habermann FA, Wuensch A, Sinowatz F & Wolf E 2007 Reporter genes for embryogenesis research in livestock species. *Theriogenology* **68** (Supplement 1) S116–S124.
- Hannan NJ, Jones RL, White CA & Salamonsen LA 2006 The chemokines, CX3CL1, CCL14, and CCL4, promote human trophoblast migration at the feto-maternal interface. *Biology of Reproduction* **74** 896–904.
- Hansen TR, Austin KJ, Perry DJ, Pru JK, Teixeira MG & Johnson GA 1999 Mechanism of action of interferon- τ in the uterus during early pregnancy. *Journal of Reproduction and Fertility* **54** 329–339.
- Hasler JF, Henderson WB, Hurtgen PJ, Jin ZQ, McCauley AD, Mower SA, Neely B, Shuey LS, Stokes JE & Trimmer SA 1995 Production, freezing and transfer of bovine IVF embryos and subsequent calving results. *Theriogenology* **43** 141–152.
- Hayashi K, Burghardt RC, Bazer FW & Spencer TE 2007 WNTs in the ovine uterus: potential regulation of periimplantation ovine conceptus development. *Endocrinology* **148** 3496–3506.
- Hess AP, Hamilton AE, Talbi S, Dosiou C, Nyegaard M, Nayak N, Genbecev-Krtolica O, Mavrogianis P, Ferrer K, Kruessel J *et al.* 2007 Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. *Biology of Reproduction* **76** 102–117.
- Heyman Y, Camous S, Fevre J, Meziou W & Martal J 1984 Maintenance of the corpus luteum after uterine transfer of trophoblastic vesicles to cyclic cows and ewes. *Journal of Reproduction and Fertility* **70** 533–540.
- Hofmann A, Zakhartchenko V, Weppert M, Sebald H, Wenigerkind H, Brem G, Wolf E & Pfeifer A 2004 Generation of transgenic cattle by lentiviral gene transfer into oocytes. *Biology of Reproduction* **71** 405–409.
- Howard JM, Manzo R, Dalton JC, Frago F & Ahmadzadeh A 2006 Conception rates and serum progesterone concentration in dairy cattle administered gonadotropin releasing hormone 5 days after artificial insemination. *Animal Reproduction Science* **95** 224–233.
- Imakawa K, Imai M, Sakai A, Suzuki M, Nagaoka K, Sakai S, Lee SR, Chang KT, Echternkamp SE & Christenson RK 2006 Regulation of conceptus adhesion by endometrial CXC chemokines during the implantation period in sheep. *Molecular Reproduction and Development* **73** 850–858.
- Ing NH, Francis H, McDonnell JJ, Amann JF & Roberts RM 1989 Progesterone induction of the uterine milk proteins: major secretory proteins of sheep endometrium. *Biology of Reproduction* **41** 643–654.
- Johnson KR, Ross RH & Fourt DL 1958 Effect of progesterone administration on reproductive efficiency. *Journal of Animal Science* **17** 386–390.
- Johnson GA, Austin KJ, Collins AM, Murdoch WJ & Hansen TR 1999a Endometrial ISG17 mRNA and a related mRNA are induced by interferon-tau and localized to glandular epithelial and stromal cells from pregnant cows. *Endocrine* **10** 243–252.
- Johnson GA, Spencer TE, Hansen TR, Austin KJ, Burghardt RC & Bazer FW 1999b Expression of the interferon tau inducible ubiquitin cross-reactive protein in the ovine uterus. *Biology of Reproduction* **61** 312–318.
- Johnson GA, Spencer TE, Burghardt RC, Joyce MM & Bazer FW 2000a Interferon-tau and progesterone regulate ubiquitin cross-reactive protein expression in the ovine uterus. *Biology of Reproduction* **62** 622–627.
- Johnson GA, Spencer TE, Burghardt RC, Taylor KM, Gray CA & Bazer FW 2000b Progesterone modulation of osteopontin gene expression in the ovine uterus. *Biology of Reproduction* **62** 1315–1321.
- Johnson GA, Stewart MD, Gray CA, Choi Y, Burghardt RC, Yu-Lee LY, Bazer FW & Spencer TE 2001 Effects of the estrous cycle, pregnancy, and interferon tau on 2',5'-oligoadenylate synthetase expression in the ovine uterus. *Biology of Reproduction* **64** 1392–1399.
- Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, Osteen K, Taylor RN, Lessey BA & Giudice LC 2002 Global gene profiling in human endometrium during the window of implantation. *Endocrinology* **143** 2119–2138.
- Kashiwagi A, Digirolamo CM, Kanda Y, Niikura Y, Esmon CT, Hansen TR, Shioda T & Pru JK 2007 The postimplantation embryo differentially regulates endometrial gene expression and decidualization. *Endocrinology* **148** 4173–4184.
- Kazemi M, Amann JF, Keisler DH, Ing NH, Roberts RM, Morgan G & Wooding FB 1990 A progesterone-modulated, low-molecular-weight protein from the uterus of the sheep is associated with crystalline inclusion bodies in uterine epithelium and embryonic trophoctoderm. *Biology of Reproduction* **43** 80–96.
- Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HME & Hilton DJ 2002 The SOC box: a tale of destruction and degradation. *Trends in Biochemical Sciences* **27** 235–241.
- Kim S, Choi Y, Bazer FW & Spencer TE 2003 Identification of genes in the ovine endometrium regulated by interferon tau independent of signal transducer and activator of transcription 1. *Endocrinology* **144** 5203–5214.
- Kleemann DO, Walker SK & Seamark RF 1994 Enhanced fetal growth in sheep administered progesterone during the first three days of pregnancy. *Journal of Reproduction and Fertility* **102** 411–417.
- Klein C, Bauersachs S, Ulbrich SE, Einspanier R, Meyer HH, Schmidt SE, Reichenbach HD, Vermehren M, Sinowatz F, Blum H *et al.* 2006 Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. *Biology of Reproduction* **74** 253–264.
- Kwon H, Wu G, Bazer FW & Spencer TE 2003a Developmental changes in polyamine levels and synthesis in the ovine conceptus. *Biology of Reproduction* **69** 1626–1634.
- Kwon H, Spencer TE, Bazer FW & Wu G 2003b Developmental changes of amino acids in ovine fetal fluids. *Biology of Reproduction* **68** 1813–1820.
- Larson SF, Butler WR & Currie WB 1997 Reduced fertility associated with low progesterone postbreeding and increased milk urea nitrogen in lactating cows. *Journal of Dairy Science* **80** 1288–1295.
- Larson SF, Butler WR & Currie WB 2007 Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. *Animal Reproduction Science* **102** 172–179.
- Lawson RA, Parr RA & Cahill LP 1983 Evidence for maternal control of blastocyst growth after asynchronous transfer of embryos to the uterus of the ewe. *Journal of Reproduction and Fertility* **67** 477–483.
- Lea RG & Sandra O 2007 Immunoendocrine aspects of endometrial function and implantation. *Reproduction* **134** 389–404.
- Lee RS, Wheeler TT & Peterson AJ 1998 Large-format, two-dimensional polyacrylamide gel electrophoresis of ovine periimplantation uterine luminal fluid proteins: identification of aldose reductase, cytoplasmic actin, and transferrin as conceptus-synthesized proteins. *Biology of Reproduction* **59** 743–752.

- Leese HJ 1995 Metabolic control during preimplantation mammalian development. *Human Reproduction Update* **1** 63–72.
- Lewis SK, Farmer JL, Burghardt RC, Newton GR, Johnson GA, Adelson DL, Bazer FW & Spencer TE 2007 Galectin 15 (LGALS15): a gene uniquely expressed in the uteri of sheep and goats that functions in trophoblast attachment. *Biology of Reproduction* **77** 1027–1036.
- Li Q, Zhang M, Kumar S, Zhu LJ, Chen D, Bagchi MK & Bagchi IC 2001 Identification and implantation stage-specific expression of an interferon- α -regulated gene in human and rat endometrium. *Endocrinology* **142** 2390–2400.
- Maddox-Hyttell P, Gjørret JO, Vajta G, Alexopoulos NI, Lewis I, Trounson A, Viuff D, Laurincik J, Muller M, Tveden-Nyborg P *et al.* 2003 Morphological assessment of preimplantation embryo quality in cattle. *Reproduction Supplement* **61** 103–116.
- Mann GE & Lamming GE 1999 The influence of progesterone during early pregnancy in cattle. *Reproduction in Domestic Animals* **34** 269–274.
- Mann GE & Lamming GE 2001 Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* **121** 175–180.
- Mann GE, Lamming GE, Robinson RS & Wathes DC 1999 The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. *Journal of Reproduction and Fertility* **54** 317–328.
- Mann GE, Merson P, Fray MD & Lamming GE 2001 Conception rate following progesterone supplementation after second insemination in dairy cows. *Veterinary Journal* **162** 161–162.
- Mann GE, Fray MD & Lamming GE 2006 Effects of time of progesterone supplementation on embryo development and interferon-tau production in the cow. *Veterinary Journal* **171** 500–503.
- Martin PM & Sutherland AE 2001 Exogenous amino acids regulate trophoblast differentiation in the mouse blastocyst through an mTOR-dependent pathway. *Developmental Biology* **240** 182–193.
- Martin PM, Sutherland AE & Van Winkle LJ 2003 Amino acid transport regulates blastocyst implantation. *Biology of Reproduction* **69** 1101–1108.
- Martin C, Pessemesse L, de la Llosa-Hermier MP, Martal J, Djiane J & Charlier M 2004 Interferon- τ upregulates prolactin receptor mRNA in the ovine endometrium during the peri-implantation period. *Reproduction* **128** 99–105.
- Moffatt J, Bazer FW, Hansen PJ, Chun PW & Roberts RM 1987 Purification, secretion and immunocytochemical localization of the uterine milk proteins, major progesterone-induced proteins in uterine secretions of the sheep. *Biology of Reproduction* **36** 419–430.
- Mohamed OA, Jonnaert M, Labelle-Dumais C, Kuroda K, Clarke HJ & Dufort D 2005 Uterine Wnt/ β -catenin signaling is required for implantation. *PNAS* **102** 8579–8584.
- Moore K & Thatcher WW 2006 Major advances associated with reproduction in dairy cattle. *Journal of Dairy Science* **89** 1254–1266.
- Nagaoka K, Nojima H, Watanabe F, Chang KT, Christenson RK, Sakai S & Imakawa K 2003a Regulation of blastocyst migration, apposition, and initial adhesion by a chemokine, interferon gamma-inducible protein 10 kDa (IP-10), during early gestation. *Journal of Biological Chemistry* **278** 29048–29056.
- Nagaoka K, Sakai A, Nojima H, Suda Y, Yokomizo Y, Imakawa K, Sakai S & Christenson RK 2003b A chemokine, interferon (IFN)-gamma-inducible protein 10 kDa, is stimulated by IFN-tau and recruits immune cells in the ovine endometrium. *Biology of Reproduction* **68** 1413–1421.
- Nakano H, Shimada A, Imai K, Takahashi T & Hashizume K 2005 The cytoplasmic expression of E-cadherin and beta-catenin in bovine trophoblasts during binucleate cell differentiation. *Placenta* **26** 393–401.
- Nephew KP, McClure KE, Ott TL, Dubois DH, Bazer FW & Pope WF 1991 Relationship between variation in conceptus development and differences in estrous cycle duration in ewes. *Biology of Reproduction* **44** 536–539.
- Niklaus AL & Pollard JW 2006 Mining the mouse transcriptome of receptive endometrium reveals distinct molecular signatures for the luminal and glandular epithelium. *Endocrinology* **147** 3375–3390.
- Noel S, Herman A, Johnson GA, Gray CA, Stewart MD, Bazer FW, Gertler A & Spencer TE 2003 Ovine placental lactogen specifically binds to endometrial glands of the ovine uterus. *Biology of Reproduction* **68** 772–780.
- Pestka S 2007 The interferons: 50 years after their discovery, there is much more to learn. *Biology of Reproduction* **282** 20047–20051.
- Rempel LA, Francis BR, Austin KJ & Hansen TR 2005 Isolation and sequence of an interferon- τ -inducible, pregnancy- and bovine interferon-stimulated gene product 15 (ISG15)-specific, bovine ubiquitin-activating E1-like (UBE1L) enzyme. *Biology of Reproduction* **72** 365–372.
- Roberts RM, Liu L & Alexenko A 1997 New and atypical families of type I interferons in mammals: comparative functions, structures, and evolutionary relationships. *Progress in Nucleic Acid Research and Molecular Biology* **56** 287–325.
- Robinson NA, Leslie KE & Walton JS 1989 Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. *Journal of Dairy Science* **72** 202–207.
- Robinson RS, Mann GE, Lamming GE & Wathes DC 2001 Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. *Reproduction* **122** 965–979.
- Rosenfeld CS, Han CS, Alexenko AP, Spencer TE & Roberts RM 2002 Expression of interferon receptor subunits, IFNAR1 and IFNAR2, in the ovine uterus. *Biology of Reproduction* **67** 847–853.
- Salamonsen LA 1999 Role of proteases in implantation. *Reviews of Reproduction* **4** 11–22.
- Sandra O, Bataillon I, Roux P, Martal J, Charpigny G, Reinaud P, Bolifraud P, Germain G & Al-Gubory KH 2005 Suppressor of cytokine signalling (SOCS) genes are expressed in the endometrium and regulated by conceptus signals during early pregnancy in the ewe. *Journal of Molecular Endocrinology* **34** 637–644.
- Satterfield MC, Bazer FW & Spencer TE 2006 Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. *Biology of Reproduction* **75** 289–296.
- Satterfield MC, Dunlap KA, Hayashi K, Burghardt RC, Spencer TE, Bazer FW 2007 Tight and adherens junctions in the ovine uterus: differential regulation by pregnancy and progesterone, pp. en.2007-0321.
- Schaff WT, Barak Y & Sadovsky Y 2006 The pleiotropic function of PPAR gamma in the placenta. *Molecular and Cellular Endocrinology* **249** 10–15.
- Schaff WT, Knapp FF Jr, Barak Y, Biron-Shental T, Nelson DM & Sadovsky Y 2007 Ligand-activated PPAR{gamma} alters placental morphology and placental fatty acid uptake in mice. *Endocrinology* **148** 3625–3634.
- Soares MJ 2004 The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. *Reproductive Biology and Endocrinology* **2** 51.
- Song G, Spencer TE & Bazer FW 2005 Cathepsins in the ovine uterus: regulation by pregnancy, progesterone, and interferon tau. *Endocrinology* **146** 4825–4833.
- Song G, Spencer TE & Bazer FW 2006a Progesterone and interferon-tau regulate cystatin C in the endometrium. *Endocrinology* **147** 3478–3483.
- Song G, Bazer FW, Wagner GF & Spencer TE 2006b Stanniocalcin (STC) in the endometrial glands of the ovine uterus: regulation by progesterone and placental hormones. *Biology of Reproduction* **74** 913–922.
- Song G, Bazer FW & Spencer TE 2007 Pregnancy and interferon tau regulate RSAD2 and IFIH1 expression in the ovine uterus. *Reproduction* **133** 285–295.
- Spencer TE & Bazer FW 1995 Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. *Biology of Reproduction* **53** 1527–1543.
- Spencer TE & Bazer FW 2002 Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Frontiers in Bioscience* **7** d1879–d1898.
- Spencer TE & Bazer FW 2004 Conceptus signals for establishment and maintenance of pregnancy. *Reproductive Biology and Endocrinology* **2** 49.
- Spencer TE, Becker WC, George P, Miranda MA, Ogle TF & Bazer FW 1995 Ovine interferon-tau regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. *Biology of Reproduction* **53** 732–745.
- Spencer TE, Stagg AG, Ott TL, Johnson GA, Ramsey WS & Bazer FW 1999a Differential effects of intrauterine and subcutaneous administration of recombinant ovine interferon tau on the endometrium of cyclic ewes. *Biology of Reproduction* **61** 464–470.
- Spencer TE, Gray A, Johnson GA, Taylor KM, Gertler A, Gootwine E, Ott TL & Bazer FW 1999b Effects of recombinant ovine interferon tau, placental lactogen, and growth hormone on the ovine uterus. *Biology of Reproduction* **61** 1409–1418.

- Spencer TE, Stagg AG, Joyce MM, Jenster G, Wood CG, Bazer FW, Wiley AA & Bartol FF 1999c Discovery and characterization of endometrial epithelial messenger ribonucleic acids using the ovine uterine gland knockout model. *Endocrinology* **140** 4070–4080.
- Spencer TE, Johnson GA, Burghardt RC & Bazer FW 2004a Progesterone and placental hormone actions on the uterus: insights from domestic animals. *Biology of Reproduction* **71** 2–10.
- Spencer TE, Johnson GA, Bazer FW & Burghardt RC 2004b Implantation mechanisms: insights from the sheep. *Reproduction* **128** 657–668.
- Spencer TE, Johnson GA, Bazer FW & Burghardt RC 2007a Fetal–maternal interactions during the establishment of pregnancy in ruminants. *Society of Reproduction and Fertility* **64** 379–396.
- Spencer TE, Johnson GA, Bazer FW, Burghardt RC & Palmarini M 2007b Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. *Reproduction, Fertility, and Development* **19** 65–78.
- Starbuck GR, Darwash AO & Lamming GE 1999 The importance of progesterone during early pregnancy in the dairy cow. *Cattle Practice* **7** 397–399.
- Stark GR, Kerr IM, Williams BR, Silverman RH & Schreiber RD 1998 How cells respond to interferons. *Annual Review of Biochemistry* **67** 227–264.
- Stewart MD, Johnson GA, Gray CA, Burghardt RC, Schuler LA, Joyce MM, Bazer FW & Spencer TE 2000 Prolactin receptor and uterine milk protein expression in the ovine endometrium during the estrous cycle and pregnancy. *Biology of Reproduction* **62** 1779–1789.
- Thatcher WW, Hansen PJ, Gross TS, Helmer SD, Plante C & Bazer FW 1989 Antiluteolytic effects of bovine trophoblast protein-1. *Journal of Reproduction and Fertility* **37** 91–99.
- Thatcher WW, Moreira F, Santos JE, Mattos RC, Lopes FL, Pancarci SM & Risco CA 2001 Effects of hormonal treatments on reproductive performance and embryo production. *Theriogenology* **55** 75–89.
- Villa-Godoy A, Hughes TL, Emery RS, Chapin LT & Fogwell RL 1988 Association between energy balance and luteal function in lactating dairy cows. *Journal of Dairy Science* **71** 1063–1072.
- Wathes DC & Hamon M 1993 Localization of oestradiol, progesterone and oxytocin receptors in the uterus during the oestrous cycle and early pregnancy of the ewe. *Journal of Endocrinology* **138** 479–492.
- Whitley JC, Shulkes A, Salamonsen LA, Vogliagis D, Familari M & Giraud AS 1998 Temporal expression and cellular localization of a gastrin-releasing peptide-related gene in ovine uterus during the oestrous cycle and pregnancy. *Journal of Dairy Science* **157** 139–148.
- Wiltbank JN, Hawk HW, Kidder HE, Black WG, Ulberg LC & Casida LE 1956 Effect of progesterone therapy on embryo survival in cows of lowered fertility. *Journal of Dairy Science* **39** 456–461.
- Wooding FB 1982 The role of the binucleate cell in ruminant placental structure. *Journal of Reproduction and Fertility* **31** 31–39.
- Wooding FB 1984 Role of binucleate cells in fetomaternal cell fusion at implantation in the sheep. *American Journal of Anatomy* **170** 233–250.
- Wooding FB 1992 Current topic: the synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. *Placenta* **13** 101–113.
- Wuensch A, Habermann FA, Kurosaka S, Klose R, Zakhartchenko V, Reichenbach HD, Sinowatz F, McLaughlin KJ & Wolf E 2007 Quantitative monitoring of pluripotency gene activation after somatic cloning in cattle. *Biology of Reproduction* **76** 983–991.
- Yang RY & Liu FT 2003 Galectins in cell growth and apoptosis. *Cellular and Molecular Life Sciences* **60** 267–276.
- Yankey SJ, Hicks BA, Carnahan KG, Assiri AM, Sinor SJ, Kodali K, Stellflug JN & Ott TL 2001 Expression of the antiviral protein Mx in peripheral blood mononuclear cells of pregnant and bred, non-pregnant ewes. *Journal of Endocrinology* **170** R7–R11.

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