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Biological roles of progesterone, prostaglandins, and interferon tau in endometrial function and conceptus elongation in ruminants

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Abstract

A large majority of pregnancy loss in cattle occurs during the first three weeks after conception, particularly during the peri-implantation period. This review integrates established and new information on the biological role of ovarian progesterone (P4), prostaglandins (PGs) and interferon tau (IFNT) in endometrial function and conceptus elongation during the peri-implantation period of pregnancy in ruminants. Progesterone is secreted by the ovarian corpus luteum (CL) and is the unequivocal hormone of pregnancy. Prostaglandins are produced from both the endometrium as well as conceptus trophectoderm during early pregnancy. Interferon tau is produced solely by the conceptus trophectoderm and is the maternal recognition of pregnancy signal that inhibits production of luteolytic pulses of $PGF_{2\alpha}$ by the endometrium to maintain the CL and thus production of P4. Conceptus-endometrial interactions in ruminants are complex and involve carefully orchestrated temporal and spatial alterations in endometrial gene expression during pregnancy. Available results support the idea that the individual, interactive, and coordinated actions of P4, PGs, and IFNT regulate uterine receptivity to conceptus implantation by controlling expression of genes in the endometrium and that their actions are essential for conceptus elongation. One outcome of gene expression changes in the endometrial epithelia is alterations in lumenal secretions that govern conceptus elongation via effects on the trophectoderm. An increased knowledge of conceptus-endometrial interactions during early pregnancy in ruminants is necessary to understand and elucidate the causes of infertility and recurrent pregnancy loss and to provide a basis for new strategies to improve fertility, pregnancy outcomes and thus reproductive efficiency.

Keywords: conceptus, endometrium, interferon, pregnancy, prostaglandin, ruminant.

Introduction

This review integrates established and new information on the biological role of ovarian progesterone (P4), prostaglandins (PGs) and interferon tau (IFNT) in endometrial function and conceptus elongation during the peri-implantation period of pregnancy in ruminants. This area of reproduction is particularly important due to relatively high levels of pregnancy loss. In cattle, estimates indicate that fertilization rate is 90% with an average calving rate of about 55%, indicating an embryonic-fetal mortality of about 35% (Diskin *et al.*, 2006). Further, 70 to 80% of total embryonic loss occurs during the first 3 weeks after insemination (Diskin *et al.*, 2006; Diskin and Morris, 2008), particularly between days 7 and 16 (Diskin and Sreenan, 1980; Roche *et al.*, 1981; Berg *et al.*, 2010). Embryo mortality is greater in non-lactating cows than heifers (Berg *et al.*, 2010), and early pregnancy loss is even greater in high producing lactating dairy cattle and can approach 70% (Evans and Walsh, 2011; Thatcher *et al.*, 2011). Infertility and subfertility are also major cost factors in the cattle embryo transfer (ET) industry (Looney *et al.*, 2006). Mean survival rate to calving following transfer of *in vivo*-derived embryos from superovulated donors is only 43% with a range from 31 to 60% (Mcmillan, 1998), whereas the mean survival rate after transfer of *in vitro*-produced embryos is lower and ranges from 30 to 40% (Mcmillan, 1998; Hansen and Block, 2004). Although embryonic mortality is certainly a problem in cattle, our knowledge of the complex biological and genetic mechanisms governing endometrial receptivity and conceptus elongation and implantation is limited in domestic ruminants (Ulbrich *et al.*, 2013).

Establishment of pregnancy in domestic ruminants (i.e., sheep, cattle, goats) begins at the conceptus stage and includes pregnancy recognition signaling, implantation, and placentation (Guillomot, 1995; Spencer *et al.*, 2004, 2007a, 2008). The morula-stage embryo enters the uterus on days 4 to 6 post-mating and then forms a blastocyst that contains an inner cell mass and a blastocoele or central cavity surrounded by a monolayer of trophectoderm. After hatching from the zona pellucida (days 8 to 10), the blastocyst slowly grows into a tubular or ovoid form and is then termed a conceptus (embryo-fetus and associated extraembryonic membranes; Guillomot, 1995; Hue *et al.*, 2012). In sheep, the ovoid conceptus of about 1 cm on day 11 begins to elongate on day 12 and forms a filamentous conceptus of 10 to 15 cm or more in length that occupies the entire length of the uterine horn ipsilateral to the corpus luteum (CL). In cattle, the hatched blastocyst forms an ovoid conceptus between days 12 to 14 and is only about 2 mm in length on day13. By day 14, the conceptus is about 6 mm, and

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the elongating bovine conceptus reaches a length of about 60 mm (6 cm) by day 16 and is 20 cm or more by day 19. Indeed, the bovine blastocyst/conceptus doubles in length every day between days 9 and 16 with a significant increase (~10-fold) in growth between days 12 and 15 (Betteridge *et al.*, 1980; Berg *et al.*, 2010). After day 16 in sheep and day 19 in cattle, the elongating conceptus begins the process of implantation and placentation (Guillomot *et al.*, 1981). Conceptus elongation involves exponential increases in length and weight of the trophectoderm (Wales and Cuneo, 1989) and onset of extraembryonic membrane differentiation, including gastrulation of the embryo and formation of the yolk sac and allantois that are vital for embryonic survival and formation of a functional placenta (Guillomot, 1995; Hue *et al.*, 2012). Trophoblast elongation observed in ruminants is not due to the geometrical change of cell shape but is likely the consequence of cell addition associated with peculiar plans of cell division or intercalation (Wang *et al.*, 2009).

Blastocyst growth into an elongated conceptus does not occur *in vitro*, as it is dependent on ovarian P4 and secretions supplied by the endometrium of the uterus (Betteridge and Flechon, 1988; Gray *et al.*, 2001b; Lonergan, 2011). The trophectoderm of the elongating conceptus synthesizes and secretes prostaglandins (PGs) and interferon tau (IFNT) in ruminants (Lewis, 1989; Ulbrich *et al.*, 2009; Forde and Lonergan, 2012; Dorniak *et al.*, 2013b). Interferon tau is the signal for maternal recognition of pregnancy in ruminants and is secreted predominantly by the elongating conceptus (Roberts *et al.*, 2003; Robinson *et al.*, 2006). As a pregnancy recognition signal, IFNT ensures continued production of P4 by the CL (Thatcher *et al.*, 1989; Spencer *et al.*, 2007a). Additionally, IFNT stimulates transcription of a number of genes and activities of several enzymes in a cell-specific manner within the endometrium implicated in establishment of uterine receptivity and conceptus elongation and implantation (Hansen *et al.*, 1999; Spencer *et al.*, 2007a; Dorniak *et al.*, 2013a). The endometrium itself, as well as the ovoid and elongating conceptuses, produces PGs during early pregnancy (Marcus, 1981; Lewis, 1989). The precise role of conceptus-derived PGs remains to be determined in cattle (Ulbrich *et al.*, 2009); however, PGs regulate conceptus growth and elongation in sheep through modulation of endometrial genes important for elongation of the conceptus (Dorniak *et al.*, 2011, 2012b).

The endometrium of the uterus secretes substances, collectively termed histotroph, that govern elongation of the conceptus via effects on trophectoderm proliferation and migration as well as attachment and adhesion to the endometrial luminal epithelium (LE; Spencer *et al.*, 2007b, 2008; Bazer *et al.*, 2010). Histotroph is derived primarily from transport and/or synthesis and secretion of substances by the endometrial LE and glandular epithelia (GE), and it is a complex and rather undefined mixture of proteins, lipids, amino acids, sugars, and ions (Bazer, 1975; Gray *et al.*, 2001a; Koch *et al.*, 2010; Bazer *et al.*, 2012). The recurrent early pregnancy loss observed in uterine gland knockout (UGKO) ewes established the importance of uterine epithelial-derived histotroph for support of conceptus elongation and implantation (Gray *et al.*, 2001b). Available evidence supports the idea that ovarian P4 induces expression of a number of genes, specifically in the endometrial epithelia, that are then further stimulated by factors from the conceptus (e.g., IFNT and PGs) as well as the endometrium itself (e.g., PGs; Dorniak *et al.*, 2013a). The genes and functions regulated by these hormones and factors in the endometrial epithelia elicit specific changes in the intrauterine histotrophic milieu necessary for conceptus elongation (Spencer *et al.*, 2007b, 2008; Bazer *et al.*, 2010; Forde and Lonergan, 2012; Dorniak *et al.*, 2013a).

Progesterone regulation of endometrial function and conceptus elongation

Progesterone stimulates and maintains endometrial functions necessary for conceptus growth, implantation, placentation, and development to term. In cattle, concentrations of P4 in early pregnancy clearly affect embryonic survival during early pregnancy (Mann and Lamming, 2001; Lonergan, 2011). In both lactating dairy cows and heifers, there is a strong positive association between the post-ovulatory rise in P4 and embryonic development. Increasing concentrations of P4 after ovulation enhanced conceptus elongation in beef heifers (Garrett *et al.*, 1988; Carter *et al.*, 2008), dairy cows (Mann *et al.*, 2006), and sheep (Satterfield *et al.*, 2006), while lower P4 concentrations in the early luteal phase retarded embryonic development in sheep and cattle (Nephew *et al.*, 1991; Mann and Lamming, 2001; Forde *et al.*, 2011a). Supplementation of cattle with P4 during early pregnancy has equivocal effects to increase embryonic survival (Beltman *et al.*, 2009a). However, P4 supplementation is unlikely to rescue development of embryos with inherent genetic defects or in high-producing dairy cows (Mann *et al.*, 2006; Lonergan *et al.*, 2007; Wiltbank *et al.*, 2011).

Progesterone predominantly exerts an indirect effect on the conceptus via the endometrium to regulate blastocyst growth and conceptus elongation (Clemente *et al.*, 2009; Forde *et al.*, 2011a; Larson *et al.*, 2011). Similar to the human (Giudice and Ferenczy, 1996; Kao *et al.*, 2002), endometria of both cyclic and pregnant sheep and cattle express genes implicated in uterine receptivity, which can be defined as a physiological state of the uterus when conceptus growth and implantation for establishment of pregnancy is possible. The absence of a sufficiently developed conceptus to signal pregnancy recognition results in those genes being 'turned off' as luteolysis ensues and the animal returns to estrus for another opportunity to mate. The outcome of the P4-induced changes in the cyclic and pregnant uterus

is to modify the intrauterine milieu, such as an increase in select amino acids, glucose, cytokines, and growth factors in histotroph, for support of blastocyst growth into an ovoid conceptus and elongation to form a filamentous conceptus (Spencer *et al.*, 2008; Bazer *et al.*, 2010; Forde and Lonergan, 2012).

Sheep

Actions of ovarian P4 on the uterus are essential for conceptus survival and growth in sheep (Satterfield *et al.*, 2006). Between days 10 and 12 after onset of estrus or mating in cyclic and pregnant ewes (Fig. 1 and Table 1), P4 induces the expression of many conceptus elongation- and implantation-related genes. The initiation of expression of those genes requires P4 action and is temporally associated with the loss of progesterone receptors (PGR) between days 10 and 12 in the endometrial LE and between days 12 and 14 to 16 in the GE after onset of estrus; however, PGR expression is not lost in the stroma or myometrium in the ovine uterus (Spencer and Bazer, 2002). In the endometrial LE and superficial GE (sGE), P4 induces genes that encode secreted attachment and migration factors (galectin-15 [LGALS15], IGFBP1), intracellular enzymes (prostaglandin G/H synthase and cyclooxygenase 2 [PTGS2] and hydroxysteroid (11-beta) dehydrogenase 1 [HSD11B1]), secreted proteases (cathepsin L [CTSL]), secreted protease inhibitors (cystatin C [CST]3 and 6), a secreted cell proliferation factor (gastrin releasing peptide [GRP]), glucose transporters (SLC2A1, SLC2A5, SLC5A1), and a cationic amino acid (arginine, lysine, and ornithine) transporter (SLC7A2; Spencer *et al.*, 2007a, 2008; Bazer *et al.*, 2010). In the endometrial GE, P4 induces genes that encode for a secreted cell proliferation factor (GRP), a glucose transporter (SLC5A11), secreted adhesion protein (secreted phosphoprotein one or SPP1), a regulator of calcium/phosphate homeostasis (stanniocalcin one or STC1), and an immunomodulatory factor (SERPINA14, also known as uterine milk proteins or uterine serpins). Several of the P4-induced epithelia genes are further stimulated by the actions of PGs and/or IFNT.

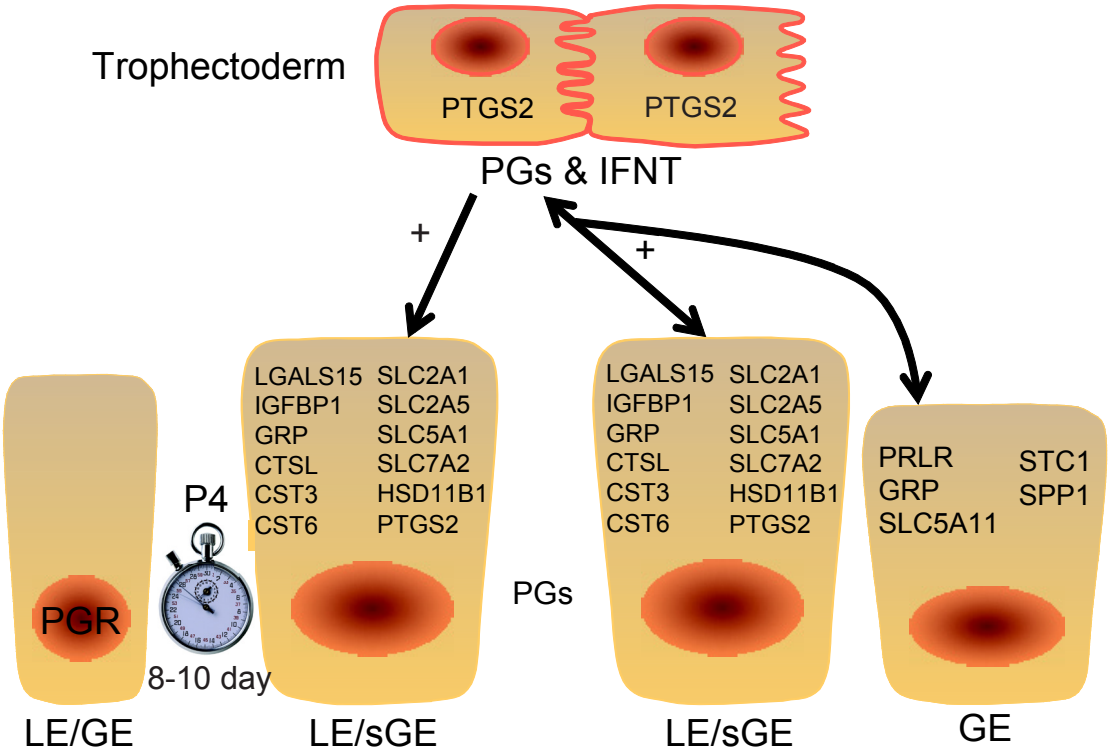

Figure 1. Schematic illustrating the effects of ovarian hormones and factors from the endometrium and conceptus trophectoderm on expression of elongation- and implantation-related genes in the endometrial epithelia of the ovine uterus during early pregnancy. Progesterone action for 8-10 days down-regulate expression of the progesterone receptor (PGR). The loss of PGR is correlated with the induction of many genes in the endometrial LE and sGE, including PTGS2 involved in prostaglandin (PG) production in both cyclic and pregnant ewes. If the ewe is pregnant, the trophectoderm synthesizes and secretes PGs and interferon tau (IFNT) that act on the endometrium in a cell-specific manner to up-regulate the expression of many P4-induced genes that govern endometrial functions and/or elongation of the conceptus. Legend: GE, glandular epithelia; IFNT, interferon tau; LE, luminal epithelium; PG, prostaglandins; PGR, progesterone receptor; sGE, superficial glandular epithelia. Adapted from Dorniak *et al*. (2012).

Table 1. Effects of ovarian progesterone and intrauterine infusions of interferon tau (IFNT) or prostaglandins (PGs) on elongation- and implantation-related genes expressed in the endometrial epithelia of the ovine uterus[1]

Gene symbol	Progesterone	IFNT	PGs[2]
Transport of glucose			
SLC2A1	↑	+	+
SLC2A5	n.d.	n.e.	+
SLC2A12	n.d.	+	n.e. or +
SLC5A1	↑	+	n.e. or +
SLC5A11	↑	+	n.e. or +
Transport of amino acids			
SLC1A5	n.d.	n.d.	+
SLC7A2	↑	+	n.e.
Cell proliferation, migration and(or) attachment			
GRP	↑	+	+
IGFBP1	↑	+	++
LGALS15	↑	++	++
SPP1	↑	+	n.d.
Proteases and their inhibitors			
CTSL	↑	++	n.e. or +
CST3	↑	+	n.e. or +
CST6		+	n.e.
Enzymes			
HSD11B1	↑	+	++
PTGS2	↑	n.e. (+ activity)	n.e. (+ activity)
Transcription factors			
HIF1A	↑	+	n.e. or +
HIF2A	↑	+	n.e. or +

[1]Effect of hormone or factor denoted as induction (↑), stimulation (+), no effect (n.e.), decrease (–) or not determined (n.d.). [2]Summary data for infusion of PGE2, PGF2α, or PGI2 (Dorniak *et al*., 2012).

Cattle

Comparisons of the endometrial transcriptome in cyclic and pregnant heifers (days 5, 7, 12, and 13) found no difference prior to pregnancy recognition (days 15 or 16; Forde *et al.*, 2011b; Bauersachs *et al.*, 2012). Indeed, the major changes required to drive conceptus elongation and establish uterine receptivity to implantation occur between days 7 and 13 in response to ovarian P4, irrespective of whether an appropriately developed embryo/conceptus is present or not (Forde *et al.*, 2009, 2010, 2011a, b, 2012b; Simmons *et al.*, 2009; Forde and Lonergan, 2012). Similar to sheep, PGR protein is lost from the LE by day 13 and in the GE by day 16, and PGR loss is associated with the down- and up-regulation of genes expressed in the endometrial epithelia (Okumu *et al.*, 2010). Using a global gene profiling approach, studies have identified the temporal changes that occur in endometrial gene expression in both cyclic (Forde *et al.*, 2011a) and pregnant (Forde *et al.*, 2009) heifers following an elevation or diminution of post-ovulatory P4 during metestrus that promotes or delays conceptus elongation, respectively (Beltman *et al.*, 2009b; Clemente *et al.*, 2009; Forde *et al.*, 2011a). As summarized in a recent review by Forde and Lonergan (Forde and Lonergan, 2012), the expression of several genes are lost in the LE and GE, including PGR and a protease (alanyl (membrane) aminopeptidase [ANPEP]), and in the GE, including a lipase (lipoprotein lipase [LPL]), protease (matrix metallopeptidase 2 [MMP2]) and immunomodulatory protein with antimicrobial activity (lactotransferrin [LTF]) between days 7 and 13 after onset of estrus or mating in cyclic and pregnant heifers. As expected, many conceptus elongation- and implantation-related genes appear in the endometrial epithelia between days 7 and 13 in cyclic and pregnant heifers. Genes up-regulated in the LE encode a mitogen (connective tissue growth factor [CTGF]) and in the GE encode a transport protein (retinol binding protein 4 [RBP4]), a glucose transporter (SLC5A1), and a protein involved in transport and cell proliferation (fatty acid binding protein 3 [FABP3]). Further, some genes are up-regulated in both the LE and GE that encode secreted attachment and migration factors (lectin, galactoside-binding, soluble, 9 [LGALS9] and IGFBP1) as well as an intracellular enzyme (PTGS2). It is quite clear that substantial differences in gene expression occur between the receptive endometrium of sheep and cattle, as one of the most abundant genes (LGALS15) induced by P4 and stimulated by IFNT in the endometrium of sheep is not expressed in cattle (Lewis *et al.*, 2007). However, PTGS2 and IGFBP1 are common endometrial receptivity markers and regulators of conceptus

elongation in both sheep and cattle (Simmons *et al.*, 2009; Dorniak *et al.*, 2012a).

Interferon tau regulation of endometrial function and conceptus elongation

Maternal recognition of pregnancy is the physiological process whereby the conceptus signals its presence to the maternal system and prolongs the lifespan of the ovarian CL (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 (Spencer *et al.*, 1996, 2007b; Spencer and Bazer, 2004; Bazer *et al.*, 2010). The antiluteolytic effects of IFNT are to inhibit transcription of the *estrogen receptor alpha* (*ESR1*) gene in sheep and *oxytocin receptor* (*OXTR*) gene in both sheep and cattle specifically in the endometrial LE. The absence of OXTR in the endometrium prevents the release of luteolytic pulses of PGF2α, thereby sustaining lifespan of the CL and P4 production. Although IFNT inhibits *OXTR* expression, it does not inhibit expression of *PTGS2*, which is important for the generation of PGs that are critical regulators of conceptus elongation during early pregnancy (Dorniak *et al.*, 2011). In addition to antiluteolytic effects, IFNT acts in a paracrine manner on the endometrium to induce or enhance expression of ISGs that are hypothesized to regulate uterine receptivity and conceptus elongation and implantation (Hansen *et al.*, 1999, 2010; Spencer *et al.*, 2008; Bazer *et al.*, 2009a).

Classical type I IFN-stimulated genes in the endometrium

A number of transcriptional profiling experiments conducted with human cells, ovine endometrium, bovine endometrium, and bovine peripheral blood lymphocytes have elucidated classical ISG induced by IFNT during pregnancy (Spencer *et al.*, 2007a, 2008; Ott and Gifford, 2010; Forde *et al.*, 2011b; Bauersachs *et al.*, 2012). In cattle, comparisons of days 15 to 18 pregnant and non-pregnant or cyclic endometria revealed conceptus effects on endometrial gene expression, particularly the induction or up-regulation of classical IFN-stimulated genes (ISGs; Bauersachs *et al.*, 2006, 2012; Forde *et al.*, 2009, 2011b; Cerri *et al.*, 2012; Forde and Lonergan, 2012). In sheep, *ISG15* (ISG15 ubiquitin-like modifier) is 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 to 13 of pregnancy (Johnson *et al.*, 1999b). In response to IFNT from the elongating conceptus, *ISG15* is induced in the stratum compactum stroma and GE by days 13 to 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 to 16 of pregnancy (Johnson *et al.*, 1999b, 2000). As IFNT production by the conceptus trophectoderm declines, expression of ISG in the stroma and GE also declines, but some remain abundant in endometrial stroma and GE on days 18 to 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).

In vivo studies revealed that the majority of classical ISG (*B2M*, *GBP2, IFI27, IFIT1, ISG15, IRF9, MIC, OAS*, *RSAD2*, *STAT1,* and *STAT2*) are not induced or up-regulated by IFNT in endometrial LE or sGE of the ovine uterus during early pregnancy (Johnson *et al.*, 1999b, 2001; Choi *et al.*, 2001, 2003; Song *et al.*, 2007). This finding was initially surprising, because all endometrial cell types express *IFNAR1* (interferon [alpha, beta, and omega] receptor 1) and *IFNAR2* subunits of the common Type I IFN receptor (Rosenfeld *et al.*, 2002). Further, bovine endometrial, ovine endometrial, and human 2fTGH fibroblast cells were used to determine that IFNT activates the canonical janus kinase-signal transducer and activator of transcription-interferon regulatory factor (JAK-STAT-IRF) signaling pathway used by other Type I IFNs (Stark *et al.*, 1998). About the same time, it was discovered that IRF2, a potent transcriptional repressor of ISG (Taniguchi *et al.*, 2001), is expressed specifically in the endometrial LE and sGE and represses transcriptional activity of IFN-stimulated response element (ISRE)-containing promoters (Spencer *et al.*, 1998; Choi *et al.*, 2001). In fact, all components of the ISGF3 transcription factor complex (*STAT1, STAT2, IRF9*) and other classical ISGs (*B2M, GBP2, IFI27, IFIT1, ISG15, MIC, OAS*) contain one or more ISRE in their promoters. Thus, IRF2 in LE appears to restrict IFNT induction of most classical ISG to stroma and GE of the ovine uterus (Dorniak *et al.*, 2013a). The silencing of *MIC* and *B2M* genes in endometrial LE or sGE during pregnancy may be a critical mechanism preventing immune rejection of the semi-allogeneic conceptus (Choi *et al.*, 2003). As IRF2 is not expressed in other uterine cell types, classical ISG are substantially increased in the endometrial stroma, GE and immune cells by IFNT from the conceptus during early pregnancy by IFNT. Of particular note, several reports indicate induction or increases in ISGs in peripheral blood lymphocytes and the CL during pregnancy of sheep and cattle or in ewes receiving intrauterine injections of IFNT (Hansen *et al.*, 2010; Ott and Gifford, 2010). Recent evidence indicates that IFNT traffics out of the uterus to exert systemic effects that alter maternal physiology, such as function of the CL (Bott *et al.*, 2010; Hansen *et al.*, 2010).

One challenge has been to determine which of the large number of classical ISGs induced in the endometrium by IFNT have a biological role in conceptus-endometrial interactions, given that they have traditionally been associated with cellular antiviral

responses as the main function of Type I IFN is to inhibit viral infection (Pestka, 2007). One classical ISG with reported biological effects on trophectoderm growth and adhesion in ruminants is *CXCL10* [chemokine (C-X-C motif) ligand 10; alias IP-10], 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 (Nagaoka *et al.*, 2003a, b). ISG15 conjugates to intracellular proteins through a ubiquitin-like mechanism (Hansen *et al.*, 1999), and deletion of *Isg15* in mice results in 50% pregnancy loss manifest during early placentation (Ashley *et al.*, 2010). In addition, MX proteins are thought to regulate secretion through an unconventional secretory pathway (Toyokawa *et al.*, 2007). The enzymes which comprise the 2',5'-oligoadenylate synthetase (OAS) family regulate ribonuclease L antiviral responses and may play additional roles in control of cellular growth and differentiation (Johnson *et al.*, 2001).

Non-classical IFNT-stimulated genes in the endometrium

Although IFNT is the only known IFN to act as the pregnancy recognition signal, IFN appear to have a biological role in uterine receptivity, decidualization, and placental growth and development in primates, ruminants, pigs, and rodents (Hansen *et al.*, 1999; Bazer *et al.*, 2009a). Transcriptional profiling of human U3A (STAT1 null) cells and ovine endometrium, as well as candidate gene analyses were used to discover novel 'non-classical' ISG in the endometrial LE during pregnancy such as *WNT7A* (wingless-type MMTV integration site family, member 7A), *LGALS15, CTSL, CST3, HSD11B1,* and *IGFBP1* (Kim *et al.*, 2003a; Song *et al.*, 2005, 2006; Gray *et al.*, 2006; Satterfield *et al.*, 2006).

Subsequently, a series of transcriptomic and candidate gene studies found that IFNT stimulates expression of a number of elongation- and implantation-related genes that are initially induced by P4 (*CST3, CST6, CTSL, GRP, HSD11B1, IGFBP1, LGALS15, SLC2A1, SLC2A5, SLC5A11, SLC7A2*) specifically in the endometrial LE, sGE, and/or GE (Spencer *et al.*, 2007a, 2008; Bazer *et al.*, 2009a, b; Fig. 1). None of those genes are classical Type I ISG, and they are referred to as 'non-classical or novel' ISG. Indeed, IFNT stimulation of those non-classical ISG requires induction by P4 and loss of PR in the epithelia. Importantly, all of the non-classical ISG encode factors that have actions on the trophectoderm (proliferation, migration, attachment and/or adhesion, nutrient transport) important for conceptus elongation (Table 1). The effects of IFNT in the bovine endometrium are not as well understood in terms of non-classical ISGs, but recent studies have started to unravel those effects in cattle (Forde *et al.*, 2011b, 2012a; Bauersachs *et al.*, 2012).

Given that the critical signaling components of the JAK-STAT signaling system (STAT1, STAT2, IRF9) are not expressed in endometrial LE or sGE (Choi *et al.*, 2001), IFNT must utilize a noncanonical, STAT1-independent signaling pathway to regulate expression of genes in endometrial LE and sGE of the ovine uterus. The noncanonical pathway mediating IFNT stimulation of genes in the endometrial LE and sGE has not been entirely elucidated, but other Type I IFN utilize mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) cascades (Platanias, 2005). Recent evidence indicates that IFNT activates distinct epithelial and stromal cell-specific JAK, epidermal growth factor receptor, MAPK (ERK1/2), PI3K-AKT, and/or Jun N-terminal kinase (JNK) signaling modules to regulate expression of PGE_2 receptors in the endometrium of the ovine uterus or in ovine uterine LE cells *in vitro* (Banu *et al.*, 2010; Lee *et al.*, 2012). As discussed subsequently, recent evidence indicates that PTGS2-derived PGs and HSD11B1-derived cortisol are part of the noncanonical pathway of IFNT action on the endometrium in sheep (Dorniak *et al.*, 2011, 2012a, b, 2013b).

Prostaglandin regulation of endometrial function and conceptus elongation

Results of recent studies in sheep clearly support the concept that PGs regulate expression of elongation- and implantation-related genes in the endometrial epithelia of ruminants during early pregnancy and are involved in conceptus elongation (Simmons *et al.*, 2009, 2010; Dorniak *et al.*, 2011; Fig. 1 and 2). The conceptus and endometria synthesize a variety of PGs during early pregnancy in both sheep and cattle (Lewis *et al.*, 1982; Lewis and Waterman, 1983; Lewis and Waterman, 1985; Lewis, 1989; Charpigny *et al.*, 1997a, b). The endometrium and uterine lumen also contains and produces substantially more PG during early pregnancy than during the estrous cycle (Ellinwood *et al.*, 1979; Marcus, 1981; Ulbrich *et al.*, 2009). Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) or PTGS2 is the dominant cyclooxygenase expressed in both the endometrium and trophectoderm of the elongating conceptus (Charpigny *et al.*, 1997a, b). Although the antiluteolytic effects of IFNT are clearly to inhibit expression of the *OXTR* in the endometrial LE and sGE of early pregnant ewes, it does not impede up-regulation of PTGS2, a rate-limiting enzyme in PG synthesis (Charpigny *et al.*, 1997b; Kim *et al.*, 2003b). As illustrated in Fig. 1, *PTGS2* expression appears between days 10 and 12 post-estrus and mating in the endometrial LE and sGE and is induced by ovarian P4 (Charpigny *et al.*, 1997b; Simmons *et al.*, 2010). In the bovine uterus, PTGS2 is also not down-regulated in endometria of early pregnant cattle, but rather is up-regulated by IFNT (Arosh *et al.*, 2004; Emond *et al.*, 2004); indeed, IFNT acts as a molecular switch that

stimulates PGE_2 production in the bovine endometrium (Krishnaswamy *et al.*, 2009). Further, Type I IFNs were found to stimulate phospholipase A2 (PLA2) and synthesis of PGE2 and PGF2α in several different cell types over 25 years ago (Fitzpatrick and Stringfellow, 1980; Fuse *et al.*, 1982).

Prostaglandins clearly regulate endometrial functions and conceptus elongation during early pregnancy (Simmons *et al.*, 2010; Dorniak *et al.*, 2011, 2012a, b; Table 1 and Fig. 2). In sheep, PTGS2 activity in the endometrium is stimulated by IFNT, and PTGS2-derived PG were found to mediate, in part, the effects of P4 and IFNT on the endometrium of the ovine uterus. In those studies, the abundance of *HSD11B1* and *IGFBP1* mRNA in the endometrium was considerably reduced by intrauterine infusion of meloxicam, a selective PTGS2 inhibitor. Both *HSD11B1* and *IGFBP1* are upregulated by PGs in the ovine placenta and human uterine decidua, respectively (Strakova *et al.*, 2000; Michael *et al.*, 2003; Michael and Papageorghiou, 2008).

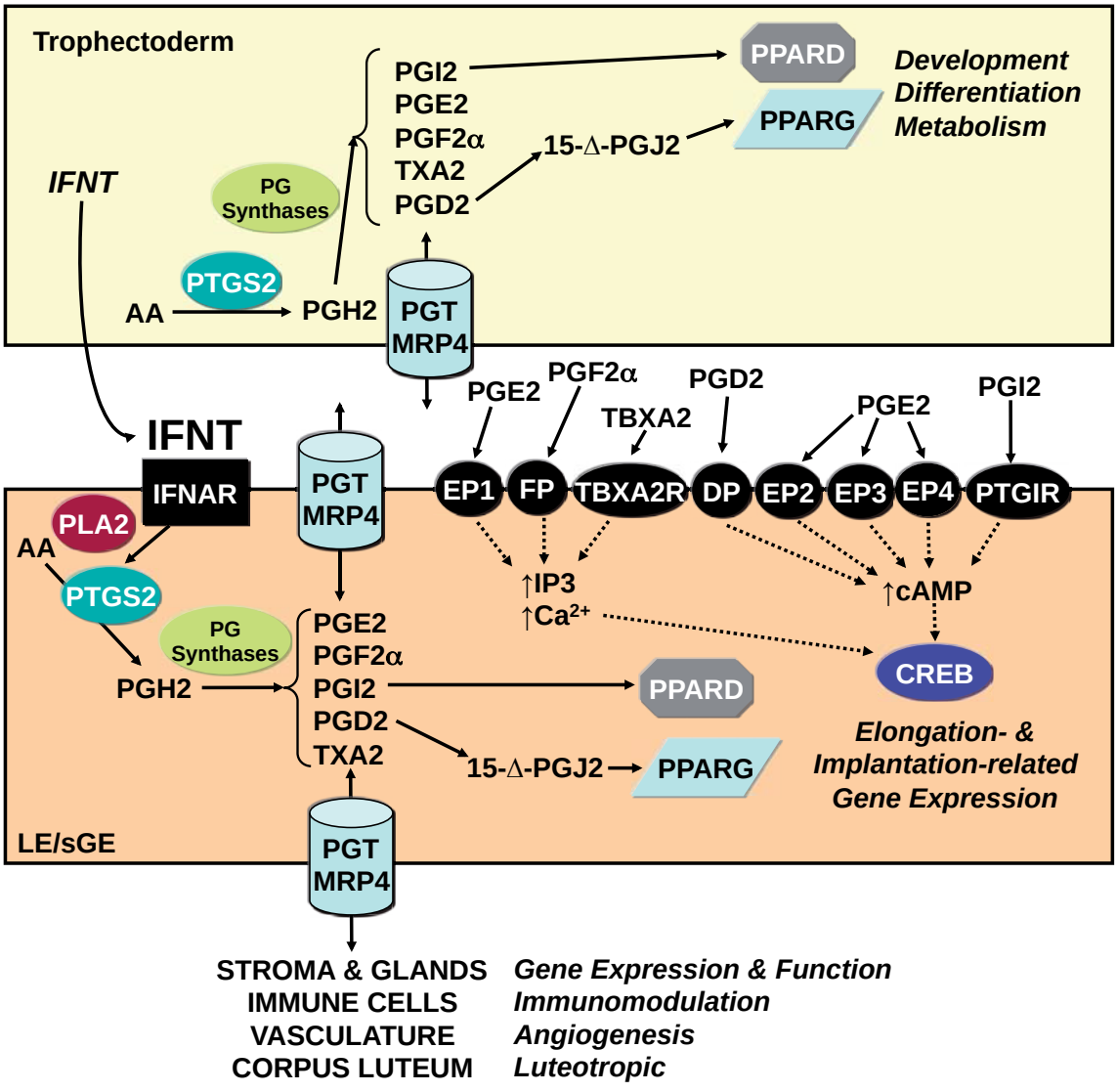

Figure 2. Schematic illustrating working hypothesis of the biological role of interferon tau (IFNT) and prostaglandins (PGs) in uterine function and conceptus elongation during early pregnancy in sheep. See text for detailed description. Legend: ABCC4, ATP-binding cassette, sub-family C (CFTR/MRP), member 4; CREB, cAMP responsive element binding protein; IFNAR, interferon (alpha, beta, and omega) receptor; DP, prostaglandin D receptor (PTGDR); EP, prostaglandin E receptor (PTGER); FP, prostaglandin F receptor (PTGFR); IP, prostaglandin I receptor (PTGIR); PLA2, phospholipase A2; PPARD, peroxisome proliferator-activated receptor delta; PPARG, peroxisome proliferator-activated receptor gamma; PTGS2, prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase); PG Synthases, prostaglandin synthases (AKR1C3, PTGDS, PTGES, PTGFS, PTGIS, TBXAS); SLCO2A1, solute carrier organic anion transporter family, member 2A1 (prostaglandin transporter); TBXA2R, thromboxane A2 receptor.

Prostaglandins are essential for conceptus elongation, as intrauterine infusions of meloxicam prevented conceptus elongation in early pregnant sheep (Simmons *et al.*, 2010; Dorniak *et al.*, 2011). The elongating conceptuses of both sheep and cattle synthesize and secrete more PG than the underlying endometrium (Lewis *et al.*, 1982; Lewis and Waterman, 1983; Lewis, 1989). Thus, PG levels are much greater in the uterine lumen of pregnant as compared with cyclic or nonpregnant cattle (Ulbrich *et al.*, 2009). Day 14 sheep conceptuses *in vitro* release mainly cyclooxygenase metabolites including PGF2α, 6-keto-PGF1α (i.e., a stable metabolite of PGI2), and PGE2 (Charpigny *et al.*, 1997a), and day 16 conceptuses produce substantially more of those PG than day 14 conceptuses (Lewis and Waterman, 1985). Given that membrane and nuclear receptors for PGs are present in all cell types of the endometrium and conceptus during early pregnancy (Cammas *et al.*, 2006; Dorniak *et al.*, 2011), PTGS2-derived PGs from the conceptus likely have paracrine, autocrine, and perhaps intracrine effects on endometrial function and conceptus development during early pregnancy. Indeed, expression of *PTGS2* in biopsies of day 7 bovine blastocysts is a predictor of the successful development of that blastocyst to term and delivery of a live calf (El-Sayed *et al.*, 2006). Recently, Dorniak and coworkers (Dorniak *et al.*, 2012a) infused PGE2, PGF2α, PGI2, or IFNT at the levels produced by the day 14 conceptus into the uterus of cyclic ewes. In that study, expression of *GRP*, *IGFBP1*, and *LGALS15* were increased by PGE2, PGI2, and IFNT, but only IFNT increased *CST6* (Table 1). Differential effects of PG were also observed for *CTSL* and its inhibitor *CST3*. For glucose transporters, IFNT and all PG increased *SLC2A1*, but only PG increased *SLC2A5* expression, whereas *SLC2A12* and *SLC5A1* were increased by IFNT, PGE2, and PGF2α. Infusions of all PG and IFNT increased the amino acid transporter *SLC1A5*, but only IFNT increased *SLC7A2*. In the uterine lumen, only IFNT increased glucose levels, and only PGE2 and PGF2α increased total amino acids (Dorniak *et al.*, 2012a). Thus, available results support the idea that PG and IFNT from the conceptus coordinately regulate endometrial functions important for growth and development of the conceptus during the peri-implantation period of pregnancy (Dorniak *et al.*, 2013a). In fact, pregnancy rates were substantially reduced in heifers that received meloxicam, a partially selective inhibitor of PTGS2, on day 15 after insemination (Erdem and Guzeloglu, 2010). Thus, PGs are critical regulators of conceptus elongation and implantation in ruminants, as they are for blastocyst implantation and decidualization during pregnancy in mice, rats, hamsters, mink, and likely humans (Dey *et al.*, 2004; Wang and Dey, 2006; Kennedy *et al.*, 2007).

Conclusions

The individual, additive and synergistic actions of P4, IFNT, and PGs regulate expression of elongation- and implantation-related genes in the endometrial epithelia and that P4 and PGs are essential regulators of conceptus elongation in ruminants. The outcome of these carefully orchestrated changes in gene expression is secretion or transport of substances (e.g., glucose, amino acids, proteins) from the endometrium into the uterine lumen that govern conceptus survival and elongation via effects on trophectoderm proliferation, migration, attachment, and adhesion. Recent studies indicate that some, but not all, of the same mechanisms, pathways and factors regulate conceptus elongation in cattle are conserved between cattle and sheep (Bauersachs *et al.*, 2008; Spencer *et al.*, 2008; Forde *et al.*, 2011b; Forde and Lonergan, 2012). One important area of future research is determining which endometrial genes and products are critical determinants of uterine receptivity and early pregnancy success. This knowledge should be useful to develop genetic tools essential to select animals for enhanced fertility. Improvement of functional traits using conventional approaches of quantitative genetics is difficult, because most reproductive traits are complex (polygenic) with low heritability (Weigel, 2006; Veerkamp and Beerda, 2007). McMillan and Donnison (1999) summarized a novel approach for experimentally identifying high and low fertility heifers based on early pregnancy success using serial transfer of *in vitro*-produced embryos. Of note, those investigators suggested that a failure in the mechanism involved in conceptus elongation and maternal recognition of pregnancy was a major cause of early pregnancy loss in low fertility heifers (Mcmillan and Donnison, 1999; Peterson and Lee, 2003). Accordingly, the selected high fertility heifers would have a uterus that was superior in the ability to support growth and development of the conceptus. Thus, natural variation in early pregnancy rates in cattle can be used to define genes and pathways important for endometrial receptivity and essential for early pregnancy loss and success. Other ruminant models to understand endometrial receptivity and pregnancy loss include: (a) the UGKO ewe (Gray *et al.*, 2002); (b) heifers versus cows (Berg *et al.*, 2010); (c) non-lactating versus lactating cows (Cerri *et al.*, 2012); (d) advanced versus delayed post-ovulatory rise in P4 (Lonergan, 2011; Forde and Lonergan, 2012); and (e) recessive lethal mutations that manifest in defective conceptus elongation and/or epiblast formation (Charlier *et al.*, 2012). A systems biology approach is necessary to understand the multifactorial phenomenon of recurrent pregnancy loss and provide a basis for new strategies to improve pregnancy outcomes, fertility, and reproductive efficiency in ruminant livestock.

References

Arosh JA, Banu SK, Kimmins S, Chapdelaine P, Maclaren LA, Fortier MA. 2004. Effect of interferon-tau 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.

Ashley RL, Henkes LE, Bouma GJ, Pru JK, Hansen TR. 2010. Deletion of the Isg15 gene results in up-regulation of decidual cell survival genes and down-regulation of adhesion genes: implication for regulation by IL-1beta. *Endocrinology*, 151:4527-4536.

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.

Banu SK, Lee J, Stephen SD, Nithy TK, Arosh JA. 2010. Interferon tau regulates PGF2alpha release from the ovine endometrial epithelial cells via activation of novel JAK/EGFR/ERK/EGR-1 pathways. *Mol Endocrinol*, 24:2315-2330.

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, Ulbrich SE, Blum H, Wolf E. 2008. Transcriptome studies of bovine endometrium reveal molecular profiles characteristic for specific stages of estrous cycle and early pregnancy. *Exp Clin Endocrinol Diabetes*, 116:371-384.

Bauersachs S, Ulbrich SE, Reichenbach HD, Reichenbach M, Buttner M, Meyer HH, Spencer TE, Minten M, Sax G, Winter G, Wolf E. 2012. Comparison of the effects of early pregnancy with human interferon, alpha 2 (IFNA2), on gene expression in bovine endometrium. *Biol Reprod*, 86:46. (abstract)

Bazer FW. 1975. Uterine protein secretions: Relationship to development of the conceptus. *J Anim Sci*, 41:1376-1382.

Bazer FW, Thatcher WW, Hansen PJ, Mirando MA, Ott TL, Plante C. 1991. Physiological mechanisms of pregnancy recognition in ruminants. *J Reprod Fertil Suppl*, 43:39-47.

Bazer FW, Spencer TE, Johnson GA. 2009a. Interferons and uterine receptivity. *Semin Reprod Med*, 27:90-102.

Bazer FW, Spencer TE, Johnson GA, Burghardt RC, Wu G. 2009b. Comparative aspects of implantation. *Reproduction*, 138:195-209.

Bazer FW, Wu G, Spencer TE, Johnson GA, Burghardt RC, Bayless K. 2010. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. *Mol Hum Reprod*, 16:135-152.

Bazer FW, Song G, Kim J, Erikson DW, Johnson GA, Burghardt RC, Gao H, Carey Satterfield M, Spencer TE, Wu G. 2012. Mechanistic mammalian target of rapamycin (MTOR) cell signaling: effects of select nutrients and secreted phosphoprotein 1 on development of mammalian conceptuses. *Mol Cell Endocrinol*, 354:22-33.

Beltman ME, Lonergan P, Diskin MG, Roche JF, Crowe MA. 2009a. Effect of progesterone supplementation in the first week post conception on embryo survival in beef heifers. *Theriogenology*, 71:1173-1179.

Beltman ME, Roche JF, Lonergan P, Forde N, Crowe MA. 2009b. Evaluation of models to induce low progesterone during the early luteal phase in cattle. *Theriogenology*, 72:986-992.

Berg DK, Van Leeuwen J, Beaumont S, Berg M, Pfeffer PL. 2010. Embryo loss in cattle between days 7 and 16 of pregnancy. *Theriogenology*, 73:250-260.

Betteridge KJ, Eaglesome MD, Randall GC, Mitchell D. 1980. Collection, description and transfer of embryos from cattle 10--16 days after oestrus. *J Reprod Fertil*, 59:205-216.

Betteridge KJ, Flechon JE. 1988. The anatomy and physiology of pre-attachment bovine embryos. *Theriogenology*, 29:155-187.

Bott RC, Ashley RL, Henkes LE, Antoniazzi AQ, Bruemmer JE, Niswender GD, Bazer FW, Spencer TE, Smirnova NP, Anthony RV, Hansen TR. 2010. Uterine vein infusion of interferon tau (IFNT) extends luteal life span in ewes. *Biol Reprod*, 82:725-735.

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.

Carter F, Forde N, Duffy P, Wade M, Fair T, Crowe MA, Evans AC, Kenny DA, Roche JF, Lonergan P. 2008. Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. *Reprod Fertil Dev*, 20:368-375.

Cerri RL, Thompson IM, Kim IH, Ealy AD, Hansen PJ, Staples CR, Li JL, Santos JE, Thatcher WW. 2012. Effects of lactation and pregnancy on gene expression of endometrium of Holstein cows at day 17 of the estrous cycle or pregnancy. *J Dairy Sci*, 95:5657-5675.

Charlier C, Agerholm JS, Coppieters W, Karlskov-Mortensen P, Li W, De Jong G, Fasquelle C, Karim L, Cirera S, Cambisano N, Ahariz N, Mullaart E, Georges M, Fredholm M. 2012. A deletion in the bovine FANCI gene compromises fertility by causing fetal death and brachyspina. *PLoS One*, 7:e43085.

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. *Biol Reprod*, 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.

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. *Biol Reprod*, 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. *Biol Reprod*, 68:1703-1710.

Clemente M, De La Fuente J, Fair T, Al Naib A, Gutierrez-Adan A, Roche JF, Rizos D, Lonergan P. 2009. Progesterone and conceptus elongation in cattle: a direct effect on the embryo or an indirect effect via the endometrium? *Reproduction*, 138:507-517.

Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. 2004. Molecular cues to implantation. *Endocr Rev*, 25:341-373.

Diskin MG, Sreenan JM. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. *J Reprod Fertil*, 59:463-468.

Diskin MG, Murphy JJ, Sreenan JM. 2006. Embryo survival in dairy cows managed under pastoral conditions. *Anim Reprod Sci*, 96:297-311.

Diskin MG, Morris DG. 2008. Embryonic and early foetal losses in cattle and other ruminants. *Reprod Domest Anim*, 43(suppl. 2):260-267.

Dorniak P, Bazer FW, Spencer TE. 2011. Prostaglandins regulate conceptus elongation and mediate effects of interferon tau on the ovine uterine endometrium. *Biol Reprod*, 84:1119-1127.

Dorniak P, Bazer FW, Wu G, Spencer TE. 2012a. Conceptus-derived prostaglandins regulate endometrial function in sheep. *Biol Reprod*, 87:9. (abstract).

Dorniak P, Welsh TH, Jr, Bazer FW, Spencer TE. 2012b. Endometrial HSD11B1 and cortisol regeneration in the ovine uterus: effects of pregnancy, interferon tau, and prostaglandins. *Biol Reprod*, 86:124. abstract.

Dorniak P, Bazer FW, Spencer TE. 2013a. Biological role of interferon tau in endometrial function and conceptus elongation. *J Anim Sci*, 91:1627-1638.

Dorniak P, Welsh TH Jr, Bazer FW, Spencer TE. 2013b. Cortisol and interferon tau regulation of endometrial function and conceptus development in female sheep. *Endocrinology*, 154:931-941.

El-Sayed A, Hoelker M, Rings F, Salilew D, Jennen D, Tholen E, Sirard M-A, Schellander K, Tesfaye D. 2006. Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. *Physiol Genomics*, 28:84-96.

Ellinwood WE, Nett TM, Niswender GD. 1979. Maintenance of the corpus luteum of early pregnancy in the ewe. II. Prostaglandin secretion by the endometrium in vitro and in vivo. *Biol Reprod*, 21:845-856.

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-tau. *Biol Reprod*, 70:54-64.

Erdem H, Guzeloglu A. 2010. Effect of meloxicam treatment during early pregnancy in Holstein heifers. *Reprod Domest Anim*, 45:625-628.

Evans AC, Walsh SW. 2011. The physiology of multifactorial problems limiting the establishment of pregnancy in dairy cattle. *Reprod Fertil Dev*, 24:233-237.

Fitzpatrick FA, Stringfellow DA. 1980. Virus and interferon effects on cellular prostaglandin biosynthesis. *J Immunology*, 125:431-437.

Forde N, Carter F, Fair T, Crowe MA, Evans AC, Spencer TE, Bazer FW, McBride R, Boland MP, O'Gaora P, Lonergan P, Roche JF. 2009. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. *Biol Reprod*, 81:784-794.

Forde N, Spencer TE, Bazer FW, Song G, Roche JF, Lonergan P. 2010. Effect of pregnancy and progesterone concentration on expression of genes encoding for transporters or secreted proteins in the bovine endometrium. *Physiol Genomics*, 41:53-62.

Forde N, Beltman ME, Duffy GB, Duffy P, Mehta JP, O'Gaora P, Roche JF, Lonergan P, Crowe MA. 2011a. Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. *Biol Reprod*, 84:266-278.

Forde N, Carter F, Spencer TE, Bazer FW, Sandra O, Mansouri-Attia N, Okumu LA, McGettigan PA, Mehta JP, McBride R, O'Gaora P, Roche JF, Lonergan P. 2011b. Conceptus-induced changes in the endometrial transcriptome: how soon does the cow know she is pregnant? *Biol Reprod*, 85:144-156.

Forde N, Duffy GB, McGettigan PA, Browne JA, Mehta JP, Kelly AK, Mansouri-Attia N, Sandra O, Loftus BJ, Crowe MA, Fair T, Roche JF, Lonergan P, Evans AC. 2012a. Evidence for an early endometrial response to pregnancy in cattle: both dependent upon and independent of interferon tau. *Physiol Genomics*, 44:799-810.

Forde N, Lonergan P. 2012. Transcriptomic analysis of the bovine endometrium: what is required to establish uterine receptivity to implantation in cattle? *J Reprod Dev*, 58:189-195.

Forde N, Mehta JP, Minten M, Crowe MA, Roche JF, Spencer TE, Lonergan P. 2012b. Effects of low progesterone on the endometrial transcriptome in cattle. *Biol Reprod*, 87:124, 1-11.

Fuse A, Mahmud I, Kuwata T. 1982. Mechanism of stimulation by human interferon of prostaglandin

synthesis in human cell lines. *Cancer Res*, 42:3209-3214.

Garrett JE, Geisert RD, Zavy MT, Gries LK, Wettemann RP, Buchanan DS. 1988. Effect of exogenous progesterone on prostaglandin F2 alpha release and the interestrous interval in the bovine. *Prostaglandins*, 36:85-96.

Giudice LC, Ferenczy A. 1996. The endometrial cycle. *In*: Adashi EY, Rock J, Rosenwaks Z (Ed.). *Reproductive Endocrinology, Surgery and Technology*. Philadelphia: Lippencott-Raven. pp. 271-300.

Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW, Spencer TE. 2001a. Developmental biology of uterine glands. *Biol Reprod*, 65:1311-1323.

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. *Biol Reprod*, 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, 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. *Biol Reprod*, 74:383-394.

Guillomot M, Flechon JE, Wintenberger-Torres S. 1981. Conceptus attachment in the ewe: an ultrastructural study. *Placenta*, 2:169-182.

Guillomot M. 1995. Cellular interactions during implantation in domestic ruminants. *J Reprod Fertil Suppl*, 49:39-51.

Hansen PJ, Block J. 2004. Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. *Reprod Fertil Dev*, 16:1-14.

Hansen TR, Austin KJ, Perry DJ, Pru JK, Teixeira MG, Johnson GA. 1999. Mechanism of action of interferon-tau in the uterus during early pregnancy. *J Reprod Fertil*, 54:329-339.

Hansen TR, Henkes LK, Ashley RL, Bott RC, Antoniazzi AQ, Han H. 2010. Endocrine actions of interferon-tau in ruminants. *Soc Reprod Fertil Suppl*, 67:325-340.

Hue I, Degrelle SA, Turenne N. 2012. Conceptus elongation in cattle: genes, models and questions. *Anim Reprod Sci*, 134:19-28.

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. *Biol Reprod*, 61:312-318.

Johnson GA, Spencer TE, Burghardt RC, Joyce MM, Bazer FW. 2000. Interferon-tau and progesterone regulate ubiquitin cross-reactive protein expression in the ovine uterus. *Biol Reprod*, 62:622-627.

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. *Biol Reprod*, 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.

Kennedy TG, Gillio-Meina C, Phang SH. 2007. Prostaglandins and the initiation of blastocyst implantation and decidualization. *Reproduction*, 134:635-643.

Kim S, Choi Y, Bazer FW, Spencer TE. 2003a. Identification of genes in the ovine endometrium regulated by interferon tau independent of signal transducer and activator of transcription 1. *Endocrinology*, 144:5203-5214.

Kim S, Choi Y, Spencer TE, Bazer FW. 2003b. Effects of the estrous cycle, pregnancy and interferon tau on expression of cyclooxygenase two (COX-2) in ovine endometrium. *Reprod Biol Endocrinol*, 1:58.

Koch JM, Ramadoss J, Magness RR. 2010. Proteomic profile of uterine luminal fluid from early pregnant ewes. *J Proteome Res*, 9:3878-3885.

Krishnaswamy N, Chapdelaine P, Tremblay JP, Fortier MA. 2009. Development and characterization of a simian virus 40 immortalized bovine endometrial stromal cell line. *Endocrinology*, 150:485-491.

Larson JE, Krisher RL, Lamb GC. 2011. Effects of supplemental progesterone on the development, metabolism and blastocyst cell number of bovine embryos produced in vitro. *Reprod Fertil Dev*, 23:311-318.

Lee J, Banu SK, Nithy TK, Stanley JA, Arosh JA. 2012. Early pregnancy induced expression of prostaglandin E2 receptors EP2 and EP4 in the ovine endometrium and regulated by interferon tau through multiple cell signaling pathways. *Mol Cell Endocrinol*, 348:211-223.

Lewis GS, Thatcher WW, Bazer FW, Curl JS. 1982. Metabolism of arachidonic acid in vitro by bovine blastocysts and endometrium. *Biol Reprod*, 27:431-439.

Lewis GS, Waterman RA. 1983. Effects of endometrium on metabolism of arachidonic acid by bovine blastocysts in vitro. *Prostaglandins*, 25:881-889.

Lewis GS, Waterman RA. 1985. Metabolism of arachidonic acid in vitro by ovine conceptuses recovered during early pregnancy. *Prostaglandins*, 30:263-283.

Lewis GS. 1989. Prostaglandin secretion by the blastocyst. *J Reprod Fertil Suppl*, 37:261-267.

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. *Biol Reprod*, 77:1027-1036.

Lonergan P, Woods A, Fair T, Carter F, Rizos D, Ward F, Quinn K, Evans A. 2007. Effect of embryo source and recipient progesterone environment on embryo development in cattle. *Reprod Fertil Dev*, 19:861-868.

Lonergan P. 2011. Influence of progesterone on oocyte quality and embryo development in cows. *Theriogenology*.

Looney CR, Nelson JS, Schneider HJ, Forrest DW. 2006. Improving fertility in beef cow recipients. *Theriogenology*, 65:201-209.

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, Fray MD, Lamming GE. 2006. Effects of time of progesterone supplementation on embryo development and interferon-tau production in the cow. *Vet J*, 171:500-503.

Marcus GJ. 1981. Prostaglandin formation by the sheep embryo and endometrium as an indication of maternal recognition of pregnancy. *Biol Reprod*, 25:56-64.

Mcmillan WH. 1998. Statistical models predicting embryo survival to term in cattle after embryo transfer. *Theriogenology*, 50:1053-1070.

Mcmillan WH, Donnison MJ. 1999. Understanding maternal contributions to fertility in recipient cattle: development of herds with contrasting pregnancy rates. *Anim Reprod Sci*, 57:127-140.

Michael AE, Thurston LM, Rae MT. 2003. Glucocorticoid metabolism and reproduction: a tale of two enzymes. *Reproduction*, 126:425-441.

Michael AE, Papageorghiou AT. 2008. Potential significance of physiological and pharmacological glucocorticoids in early pregnancy. *Hum Reprod Update*, 14:497-517.

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. *J Biol Chem*, 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. *Biol Reprod*, 68:1413-1421.

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. *Biol Reprod*, 44:536-539.

Okumu LA, Forde N, Fahey AG, Fitzpatrick E, Roche JF, Crowe MA, Lonergan P. 2010. The effect of elevated progesterone and pregnancy status on mRNA expression and localisation of progesterone and oestrogen receptors in the bovine uterus. *Reproduction*, 140:143-153.

Ott TL, Gifford CA. 2010. Effects of early conceptus signals on circulating immune cells: lessons from domestic ruminants. *Am J Reprod Immunol*, 64:245-254.

Pestka S. 2007. The interferons: 50 years after their discovery, there is much more to learn. *J Biol Chem*, 282:20047-20051.

Peterson AJ, Lee RS. 2003. Improving successful pregnancies after embryo transfer. *Theriogenology*, 59:687-697.

Platanias LC. 2005. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol*, 5:375-386.

Roberts RM, Ezashi T, Rosenfeld CS, Ealy AD, Kubisch HM. 2003. Evolution of the interferon tau genes and their promoters, and maternal-trophoblast interactions in control of their expression. *Reproduction Suppl*, 61:239-251.

Robinson RS, Fray MD, Wathes DC, Lamming GE, Mann GE. 2006. In vivo expression of interferon tau mRNA by the embryonic trophoblast and uterine concentrations of interferon tau protein during early pregnancy in the cow. *Mol Reprod Dev*, 73:470-474.

Roche JF, Bolandl MP, Mcgeady TA. 1981. Reproductive wastage following artificial insemination of heifers. *Vet Rec*, 109:401-404.

Rosenfeld CS, Han CS, Alexenko AP, Spencer TE, Roberts RM. 2002. Expression of interferon receptor subunits, IFNAR1 and IFNAR2, in the ovine uterus. *Biol Reprod*, 67:847-853.

Satterfield MC, Bazer FW, Spencer TE. 2006. Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. *Biol Reprod*, 75:289-296.

Simmons RM, Erikson DW, Kim J, Burghardt RC, Bazer FW, Johnson GA, Spencer TE. 2009. Insulin-like growth factor binding protein-1 in the ruminant uterus: potential endometrial marker and regulator of conceptus elongation. *Endocrinology*, 150:4295-4305.

Simmons RM, Satterfield MC, Welsh TH, Jr, Bazer FW, Spencer TE. 2010. HSD11B1, HSD11B2, PTGS2, and NR3C1 expression in the peri-implantation ovine uterus: effects of pregnancy, progesterone, and interferon tau. *Biol Reprod*, 82:35-43.

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. 2006. Progesterone and interferon tau regulate Cystatin C (CST3) in the endometrium. *Endocrinology*, 147:3478-3483.

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, Ott TL, Bazer FW. 1996. tau-Interferon: pregnancy recognition signal in ruminants. *Proc Soc Exp Biol Med*, 213:215-229.

Spencer TE, Ott TL, Bazer FW. 1998. Expression of interferon regulatory factors one and two in the ovine endometrium: effects of pregnancy and ovine interferon tau. *Biol Reprod*, 58:1154-1162.

Spencer TE, Bazer FW. 2002. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Front Biosci*, 7:d1879-d1898.

Spencer TE, Bazer FW. 2004. Conceptus signals for establishment and maintenance of pregnancy. *Reprod Biol Endocrinol*, 2:49.

Spencer TE, Johnson GA, Bazer FW, Burghardt RC. 2004. 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. *Soc Reprod Fertil Suppl*, 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. *Reprod Fertil Dev*, 19:65-78.

Spencer TE, Sandra O, Wolf E. 2008. Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. *Reproduction*, 135:165-179.

Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. 1998. How cells respond to interferons. *Annu Rev Biochem*, 67:227-264.

Strakova Z, Srisuparp S, Fazleabas AT. 2000. Interleukin-1β induces the expression of insulin-like growth factor binding protein-1 during decidualization in the primate. *Endocrinology*, 141:4664-4670.

Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. 2001. IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol*, 19:623-655.

Thatcher WW, Hansen PJ, Gross TS, Helmer SD, Plante C, Bazer FW. 1989. Antiluteolytic effects of bovine trophoblast protein-1. *J Reprod Fertil Suppl*, 37:91-99

Thatcher WW, Santos JE, Staples CR. 2011. Dietary manipulations to improve embryonic survival in cattle. *Theriogenology*, 76:1619-1631.

Toyokawa K, Leite F, Ott TL. 2007. Cellular localization and function of the antiviral protein, ovine Mx1 (oMx1): II. The oMx1 protein is a regulator of secretion in an ovine glandular epithelial cell line. *Am J Reprod Immunol*, 57:23-33.

Ulbrich SE, Schulke K, Groebner AE, Reichenbach HD, Angioni C, Geisslinger G, Meyer HH. 2009. Quantitative characterization of prostaglandins in the uterus of early pregnant cattle. *Reproduction*, 138:371-382

Ulbrich SE, Groebner AE, Bauersachs S. 2013. Transcriptional profiling to address molecular determinants of endometrial receptivity--lessons from studies in livestock species. *Methods*, 59:108-115.

Veerkamp RF, Beerda B. 2007. Genetics and genomics to improve fertility in high producing dairy cows. *Theriogenology*, 68(suppl, 1):S266-S273.

Wales RG, Cuneo CL. 1989. Morphology and chemical analysis of the sheep conceptus from the 13th to the 19th day of pregnancy. *Reprod Fertil Dev*, 1:31-39.

Wang H, Dey SK. 2006. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet*, 7:185-199.

Wang J, Guillomot M, Hue I. 2009. Cellular organization of the trophoblastic epithelium in elongating conceptuses of ruminants. *C R Biol*, 332:986-997.

Weigel KA. 2006. Prospects for improving reproductive performance through genetic selection. *Anim Reprod Sci*, 96:323-330.

Wiltbank MC, Souza AH, Carvalho PD, Bender RW, Nascimento AB. 2011. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. *Reprod Fertil Dev*, 24:238-243.