The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants¹

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ABSTRACT

This review integrates established and new information on the role of progesterone, interferon tau (IFNT), and prostaglandins in uterine biology of ruminants. Establishment of pregnancy in ruminants encompasses growth of the posthatching blastocyst, elongation of the conceptus (embryo and extraembryonic membranes), and suppression of the endometrial luteolytic mechanism to maintain progesterone production by the ovary. Conceptus elongation involves exponential increases in length of the trophectoderm for pregnancy recognition signaling, implantation, and establishment of pregnancy. Pregnancy recognition signaling is accomplished by IFNT from the trophectoderm that has a paracrine antiluteolytic effect to inhibit upregulation of oxytocin receptors in the endometrial epithelia, thereby inhibiting production of luteolytic PGF_{2α} pulses by the uterus. Survival and growth of the preimplantation blastocyst and elongating conceptus clearly requires embryotrophic factors (AA, carbohydrates, proteins, lipids, and other substances) in the uterine lumen. Individual, interactive, and coordinated actions of progesterone, IFNT, and prostaglandins regulate expression of elongation- and implantation-related genes in the endometrial epithelia that, in turn alter the uterine luminal histotroph and govern conceptus survival and growth. An increased knowledge of progesterone biology and conceptus-endometrial interactions is necessary to understand and elucidate the causes of pregnancy loss and provide a basis for new strategies to improve pregnancy outcome and reproductive efficiency in ruminants.

Key words: progesterone, interferon, prostaglandin, uterus, pregnancy

INTRODUCTION

This review integrates established and new information on the biological role of ovarian progesterone (**P4**), conceptus interferon tau (**IFNT**), and prostaglandins (**PG**) in uterine biology of ruminants during early pregnancy (Spencer et al., 2008; Ulbrich et al., 2013; Lonergan and Forde, 2014; Bauersachs and Wolf, 2015). Establishment of pregnancy in domestic ruminants begins at the conceptus (embryo or fetus and associated extraembryonic membranes) stage and encompasses pregnancy recognition signaling, implantation, and onset of placentation (Guillomot, 1995; Spencer et al., 2007a, 2008; Hue et al., 2012). The morula-stage embryo enters the uterus on d 4 to 6 postmating and then forms a blastocyst that contains an inner cell mass and a blastocoele or central cavity surrounded by a single layer of trophectoderm. After hatching from the zona pellucida (d 8 to 10), the blastocyst slowly grows into an ovoid then tubular and filamentous form and is then termed a conceptus.

In sheep, the ovoid conceptus of about 1 mm in length on d 11 begins to elongate on d 12 and forms a filamentous conceptus of 15 to 19 cm or more in length by d 15 that occupies the entire length of the uterine horn ipsilateral to the corpus luteum (**CL**) with extraembryonic membranes extending into the contralateral uterine horn. In cattle, the hatched blastocyst forms an ovoid conceptus between d 12 and 14 and is only about 2 mm in length on d 13. By d 14, the conceptus is about 6 mm, and the elongating bovine conceptus reaches a length of about 60 mm (6 cm) by d 16 and is 20 cm or more by d 19. Thus, the blastocyst or conceptus doubles in length every day between d 9 and 16 with a significant increase (~30 fold) in length between d 12 and 15 (Betteridge et al., 1980; Berg et al., 2010). Conceptus elongation involves exponential increases in length and weight of the trophectoderm (Wales and Cuneo, 1989), trophectoderm cell proliferation (Wang et al., 2009), and onset of extraembryonic membrane differentiation, including gastrulation of the embryo and formation of the yolk sac and allantois that are vi-

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tal for embryonic survival and formation of a functional placenta (Guillomot, 1995; Hue et al., 2012). Successively, the elongated conceptus begins the process of central implantation and placentation around d 16 in sheep and d 19 in cattle.

Blastocyst growth into an elongated conceptus does not occur in vitro, as it requires secretions supplied by the endometrium of the uterus (Fléchon et al., 1986; Betteridge and Flechon, 1988; Gray et al., 2001b; Brandão et al., 2004; Alexopoulos et al., 2005). Uterine luminal fluid (**ULF**) contains embryotrophic substances, collectively termed histotroph, that drive elongation of the conceptus via effects on trophectoderm proliferation and migration as well as attachment and adhesion to the endometrial luminal epithelium (**LE**) (Gray et al., 2001a; Spencer et al., 2008; Bazer et al., 2010). The ULF is derived primarily from transport and 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, AA, sugars (glucose, fructose), ions, and exosomes or microvesicles (Bazer, 1975, 2012; Gray et al., 2001a; Burns et al., 2014). The recurrent early pregnancy loss observed in uterine gland knockout ewes established the importance of uterine epithelial-derived histotroph for support of conceptus elongation and implantation (Gray et al., 2001b, 2002; Spencer and Gray, 2006). Available evidence in sheep and cattle supports the idea that ovarian P4 induces expression of several genes, specifically in the endometrial epithelia, that are then further stimulated by factors from the conceptus (e.g., IFNT and PG) and endometrium itself (PG; Dorniak et al., 2013a; Brooks et al., 2014; Lonergan and Forde, 2014). In turn, the genes and functions regulated by these hormones and factors in the endometrial epithelia cause specific changes in the uterine histotroph that govern conceptus survival and elongation.

P4 REGULATION OF ENDOMETRIAL FUNCTION AND CONCEPTUS ELONGATION

Progesterone stimulates and maintains endometrial functions necessary for embryonic survival, conceptus growth, implantation, placentation, and development to term. A strong positive association exists between the postovulatory rise in concentrations of P4 and embryonic development in sheep and cattle. The effect of P4 on conceptus growth and elongation in sheep and cattle is indirectly mediated by the endometrium (Spencer et al., 2008; Bazer et al., 2010; Lonergan, 2011; Dorniak et al., 2013a; Lonergan and Forde, 2014). During the estrous cycle and pregnancy, P4 induces a set of genes in the endometrium that establish uterine receptivity, which is the physiological state of the uterus when

conceptus growth and implantation 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 P4 actions on the endometrium is modification of the composition of the ULF, including an increase in select AA, glucose, cytokines, and growth factors, for support of blastocyst and conceptus survival and growth.

Sheep

Actions of ovarian P4 on the uterus are essential for peri-implantation conceptus survival and growth in sheep (Satterfield et al., 2006). Increasing concentrations of P4 after ovulation enhance conceptus elongation in sheep (Satterfield et al., 2006), whereas lower P4 concentrations in the early luteal phase retard embryonic development (Nephew et al., 1991). Between d 10 and 12 after onset of estrus or mating in cyclic and pregnant ewes, P4 induces the expression of many conceptus elongation- and implantation-related genes in the endometrial epithelia of the uterus (Spencer et al., 2007a, 2008; Bazer et al., 2010; Dorniak et al., 2013a; Figure 1 and Table 1). The initiation of expression of those genes requires P4 action and is temporally associated with the loss of progesterone receptors (**PGR**) between d 10 and 12 in the endometrial LE and between d 12 and 14 to 16 in the GE after onset of estrus (Spencer and Bazer, 2002); however, PGR remain present in the stroma and myometrium in the ovine uterus throughout pregnancy. In the endometrial LE and superficial GE (**sGE**), P4 induces genes that encode secreted attachment and migration factors [galectin-15 (*LGALS15*) and insulin-like growth factor binding protein one (*IGFBP1*)], intracellular enzymes [prostaglandin G/H synthase and cyclooxygenase 2 (*PTGS2*) and hydroxysteroid (11- β) dehydrogenase 1 (*HSD11B1*)], secreted proteases [cathepsin L1 (*CTSL1*)], secreted protease inhibitors [cystatin C (*CST3*) and 6 (*CST6*)], a secreted candidate cell proliferation factor [gastrin releasing peptide (*GRP*)], glucose transporters (*SLC2A1*, *SLC2A5*, *SLC5A1*), and a cationic AA (arginine, lysine, and ornithine) transporter (*SLC7A2*). In the endometrial GE, P4 induces genes that encode for a secreted candidate cell proliferation factor (*GRP*), a glucose transporter (*SLC5A11*), secreted adhesion protein (secreted phosphoprotein one or *SPP1*), a candidate regulator of calcium or phosphate homeostasis (*STC1*), and a potential immunomodulatory factor (*SERPINA14*, also known as uterine milk proteins or uterine serpins). Many of those P4-induced genes in the epithelia are further stimulated by the actions of IFNT and PG, resulting in changes in components of the ULF

Figure 1. Schematic illustrating the effects of ovarian hormones and factors from the endometrium and conceptus trophoblast on expression of elongation- and implantation-related genes in the endometrial epithelia of the ovine uterus during early pregnancy. Progesterone (P4) action for 8 to 10 d downregulates expression of the progesterone receptor (PGR). The loss of PGR is correlated with the induction of many genes in the endometrial luminal epithelium (LE) and superficial glandular epithelia (sGE), including *PTGS2* involved in prostaglandin (PG) production in both cyclic and pregnant ewes. If the ewe is pregnant, the trophoblast synthesizes and secretes PG and interferon tau (IFNT) that act on the endometrium in a cell-specific manner to upregulate the expression of many P4-induced genes that govern endometrial functions or elongation of the conceptus. GE = glandular epithelia. Color version available online.

histotroph that affect trophoblast proliferation and migration (Figure 1 and Table 1).

Cattle

Due to the extensive use of AI and associated databases in cattle (compared with sheep) it is possible to examine correlations between pregnancy rates and various physiological factors in large sample sizes. Substantial evidence links circulating concentrations of P4 during the cycle immediately before insemination and especially in the early luteal phase of the cycle following insemination with low conception rates and greater rates of early pregnancy loss (Diskin and Morris, 2008; Salilew-Wondim et al., 2010; Lonergan, 2011). Potential mechanisms by which low concentrations of P4 during

the preceding estrous cycle might reduce fertilization or embryo survival rates include effects on oocyte quality or alterations in the oviductal and uterine environment (Pursley and Martins, 2011; Rivera et al., 2011).

A combination of in vitro embryo production and in vivo embryo transfer techniques determined that P4 effects on conceptus development in cattle is indirect and mediated exclusively by the endometrium (Clemente et al., 2009). Addition of P4 to culture medium has no effect on blastocyst formation (Clemente et al., 2009; Larson et al., 2011) or elongation after transfer to synchronized recipients (Clemente et al., 2009). In fact, the embryo does not need to be present in the uterus during the period of P4 elevation to benefit from it (Clemente et al., 2009; O'Hara et al., 2014). Likewise, reducing the output of P4 from the CL delayed

temporal changes in the endometrial transcriptome and compromised conceptus elongation in vivo (Forde et al., 2011a,b; O'Hara et al., 2012). Thus, available results strongly support the idea that P4 governs changes in the endometrial transcriptome (Forde et al., 2009) that govern conceptus elongation (Carter et al., 2008; Clemente et al., 2009).

Similar to findings in sheep, an adequate rise in P4 after ovulation drives the normal temporal changes that occur in the endometrial transcriptome of cattle that are necessary to establish uterine receptivity and promote blastocyst growth into an elongated conceptus (Lonergan and Forde, 2014). Comparisons of the endometrial transcriptome in cyclic and pregnant heifers (d 5, 7, 12, and 13) showed no difference before pregnancy recognition (d 15 or 16; Forde et al., 2011b; Bauersachs et al., 2012). The major changes required to drive conceptus elongation and establish uterine receptivity to implantation occur between d 7 and 13 in response to ovarian P4, irrespective of whether an appropriately developed embryo or conceptus is present (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 d 13 and in the GE by d 16 of the estrous cycle or pregnancy in the bovine uterus, and PGR loss is associated with the down- and upregulation of genes expressed in the endometrial epithelia (Okumu et al., 2010). Global gene expression profiling studies have identified the temporal changes that occur in endome-

trial gene expression in both cyclic (Forde et al., 2011a) and pregnant (Forde et al., 2009) heifers following an elevation or reduction of postovulatory P4 during metestrus that promotes or delays conceptus elongation, respectively (Beltman et al., 2009b; Clemente et al., 2009; Forde et al., 2011a). Expression of several genes is lost in the LE and GE, including PGR and a protease [alanyl (membrane) aminopeptidase (*ANPEP*)], a lipase [lipoprotein lipase (*LPL*)], protease [matrix metalloproteinase 2 (*MMP2*)], and immunomodulatory protein with antimicrobial activity [lactotransferrin (*LTF*)], between d 7 and 13 after onset of estrus or mating in cyclic and pregnant heifers (Forde and Lonergan, 2012; Lonergan and Forde, 2014). Between d 7 and 13 postestrus or postmating, genes upregulated in the LE encode a mitogen [connective tissue growth factor (*CTGF*)] and a serine protease [protease serine 23 (*PRSS23*)], and in the GE encode a transport protein [retinol binding protein 4 (*RBP4*)], a glucose transporter [*SLC5A1*], a zinc metalloendopeptidase [meprin A, B (*MEP1B*)], a gene involved in extracellular matrix formation [nidogen 2 (*NID2*)], and a protein involved in transport and cell proliferation [fatty acid binding protein 3 (*FABP3*)]. Further, some genes are upregulated 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*). Those gene expression changes in the endometrium elicit changes in the ULF

Table 1. Effects of ovarian progesterone (P4) and intrauterine infusion of interferon tau (IFNT) or prostaglandins (PG) on elongation- and implantation-related genes expressed in the endometrial epithelia of the ovine uterus¹

Gene symbol	P4	IFNT	PG ²
Transport of glucose			
<i>SLC2A1</i>	↑	+	+
<i>SLC2A5</i>	n.d.	n.e.	+
<i>SLC2A12</i>	n.d.	+	n.e. or +
<i>SLC5A1</i>	↑	+	n.e. or +
<i>SLC5A11</i>	↑	+	n.e. or +
Transport of AA			
<i>SLC1A5</i>	n.d.	n.d.	+
<i>SLC7A2</i>	↑	+	n.e.
Cell proliferation, migration, attachment			
<i>GRP</i>	↑	+	+
<i>IGFBP1</i>	↑	+	++
<i>LGALS15</i>	↑	++	++
<i>SPP1</i>	↑	+	n.d.
Proteases and their inhibitors			
<i>CTSL1</i>	↑	++	n.e. or +
<i>CST3</i>	↑	+	n.e. or +
<i>CST6</i>		+	n.e.
Intracellular enzymes			
<i>HSD11B1</i>	↑	+	++
<i>PTGS2</i>	↑	n.e. (+ activity)	n.e. (+ activity)

¹Effect of hormone or factor denoted as induction (↑), stimulation (+), no effect (n.e.), or not determined (n.d.).
²Summary data for infusion of PGE₂, PGF_{2α}, or PGI₂ (Dorniak et al., 2011).

histotroph that are hypothesized to support conceptus elongation (Forde and Lonergan, 2012; Forde et al., 2013, 2014; Lonergan and Forde, 2014). It is quite clear that differences in gene expression exist 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, common markers of uterine receptivity and regulators of conceptus elongation also exist, such as *PTGS2* and *IGFBP1* (Simmons et al., 2009; Dorniak et al., 2012).

Clearly, increasing concentrations of P4 after ovulation boosts conceptus elongation in beef heifers, dairy cows, and sheep (Diskin and Morris, 2008; Spencer et al., 2008; Lonergan, 2011). However, supplementation of cattle with P4 during early pregnancy has equivocal efficacy in increasing embryonic survival (Beltman et al., 2009a; Parr et al., 2014) and is unlikely to rescue development of embryos with inherent genetic defects or gestated in high-producing dairy cows (Mann et al., 2006; Lonergan et al., 2007; Wiltbank et al., 2011). In fact, a recent meta-analysis of P4 supplementation during timed AI programs in dairy cows found that it only benefits cows without a CL and has no benefits if the cows are in estrus during the timed program (Bisinotto et al., 2015). A potential solution to low postovulatory concentrations of P4 in dairy cattle may be to enhance CL function via genetic strategies (Lonergan, 2011; Wiltbank et al., 2014).

IFNT 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 and Bazer, 2004; Spencer et al., 2007b; Bazer et al., 2010). The trophoblast cells of the elongating conceptus secrete IFNT predominantly before implantation (Roberts et al., 2003). The antiluteolytic effects of IFNT inhibit transcription of the estrogen receptor α (*ESR1*) gene in sheep and oxytocin receptor (*OXT*) gene in sheep and cattle, specifically in the endometrial LE. The absence of *OXT* in the endometrium prevents the release of luteolytic pulses of $\text{PGF}_{2\alpha}$, thereby ensuring maintenance of the CL and continued production of P4 (Thatcher et al., 1989; Spencer et al., 2007a). Although IFNT inhibits *OXT* expression in the LE and sGE, it does not inhibit expression of *PTGS2*, which is important for the generation of PG

that are critical regulators of endometrial function and conceptus elongation during early pregnancy in sheep (Dorniak et al., 2011) and perhaps cattle (Spencer et al., 2013). In addition to antiluteolytic effects, IFNT acts in a paracrine manner on the endometrium to induce or enhance expression of IFN-stimulated genes (ISG) that are hypothesized to regulate uterine receptivity and conceptus elongation and implantation (Hansen et al., 1999, 2010; Spencer et al., 2008; Bazer et al., 2009). Recent studies in sheep provide in vivo evidence that IFNT is a critical regulator of conceptus elongation, and the embryotrophic actions of IFNT are principally mediated by the endometrium (Dorniak et al., 2013b; Brooks and Spencer, 2015).

Classical Type I IFN-Stimulated Genes in the Endometrium

Several experiments conducted with human cells, ovine endometrium, bovine endometrium, and bovine peripheral blood lymphocytes have elucidated genes 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 d 15 to 18 pregnant and nonpregnant or cyclic endometria revealed that the conceptus induces or upregulates a large number of classical ISG (Bauersachs et al., 2006, 2012; Forde et al., 2009, 2011b; Mansouri-Attia et al., 2009; Cerri et al., 2012; Forde and Lonergan, 2012). In sheep, *ISG15* (ISG15 ubiquitin-like modifier) is expressed in LE of the ovine uterus on d 10 or 11 of the estrous cycle and pregnancy, but is not detected in the LE by d 12 to 13 of pregnancy (Johnson et al., 1999b). As the conceptus begins to elongate and secrete IFNT, *ISG15* is induced in the stratum compactum stroma and GE by d 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 d 15 to 16 of pregnancy (Johnson et al., 1999b, 2000). As IFNT production by the conceptus trophoblast declines, expression of ISG in the stroma and GE also declines, but some remain abundant in endometrial stroma and GE on d 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 studied classical ISG, such as major histocompatibility class I (*MHC*) and β -2-microglobulin (*B2M*), are not induced or upregulated 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). The lack of ISG induction and expression of *MHC* and *B2M* genes in endometrial LE or sGE during pregnancy

may be a critical mechanism preventing immune rejection of the semiallogeneic conceptus (Choi et al., 2003). Of particular note, several reports indicate induction or increases in ISG 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 exits the uterus to exert systemic effects that alter maternal physiology, including function of the CL (Hansen et al., 2010; Antoniazzi et al., 2013).

One challenge is to determine which of the large number of classical ISG induced by IFNT in the endometrium have a biological role in uterine receptivity and conceptus elongation and implantation, as ISG are traditionally implicated in the ability of Type I IFN to inhibit viral infection via cellular antiviral responses (Pestka, 2007). One classical ISG with reported biological effects on trophoblast 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). Future research should determine the biological roles of other classical ISG in conceptus elongation and implantation in ruminants, particularly because IFNT has distinct embryotrophic effects mediated by the endometrium (Dorniak et al., 2013b; Brooks and Spencer, 2015).

Nonclassical 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 many different mammals (Hansen et al., 1999; Bazer et al., 2009). A series of global and candidate gene studies found that IFNT stimulates expression of several elongation- and implantation-related genes in the ovine uterus that are initially induced by P4 (*CST3*, *CST6*, *CTSL*, *GRP*, *HSD11B1*, *IGFBP1*, *LGALS15*, *SLC2A1*, *SLC2A5*, *SLC5A11*, and *SLC7A2*) in the endometrial epithelia (Spencer et al., 2007a, 2008; Figure 1 and Table 1). None of those genes are classical ISG, and thus are referred to as nonclassical ISG. Indeed, IFNT stimulation of many nonclassical ISG requires induction by P4 and loss of PGR in the epithelia. Importantly, many of the nonclassical ISG identified to date encode factors that have actions on the trophoblast (proliferation, migration, attachment and adhesion, nutrient transport) important for conceptus elongation (Table 1). For

example, knockdown of an AA transporter (*SLC7A1*) in the conceptus trophoblast (Wang et al., 2014) and inhibition of *HSD11B1* activity in utero or in the conceptus trophoblast compromised conceptus elongation in sheep (Dorniak et al., 2013b; Brooks et al., 2015a). The effects of IFNT in the bovine endometrium are not as well understood in terms of nonclassical ISG, but recent studies have started to identify them in cattle (Forde et al., 2011b, 2012a; Bauersachs et al., 2012; Lonergan and Forde, 2014; Bauersachs and Wolf, 2015).

PG REGULATION OF ENDOMETRIAL FUNCTION AND CONCEPTUS ELONGATION

Prostaglandins regulate expression of elongation- and implantation-related genes in the endometrial epithelia of ruminants during early pregnancy and are involved in conceptus survival and elongation (Simmons et al., 2009, 2010; Dorniak et al., 2011; Spencer et al., 2013; Brooks et al., 2015b; Figure 1 and Table 1). Several decades ago, studies found that the conceptus and endometria synthesize a variety of PG (PGE_2 , $\text{PGF}_{2\alpha}$, PGI_2 , PGD_2) during early pregnancy in both sheep and cattle (Lewis et al., 1982; Lewis and Waterman, 1983, 1985; Lewis, 1989; Charpigny et al., 1997a,b). The endometrium produces and the uterine lumen contains substantially more PG during early pregnancy than during the estrous cycle (Ellinwood et al., 1979; Marcus, 1981; Ulbrich et al., 2009). The dominant cyclooxygenase expressed in both the endometrium and trophoblast of the elongating conceptus is PTGS2 (Charpigny et al., 1997a,b). Although the antiluteolytic effects of IFNT are to inhibit expression of the *OXTR* in the endometrial LE and sGE of early pregnant ewes, IFNT does not impede upregulation of PTGS2, a rate-limiting enzyme in PG synthesis (Charpigny et al., 1997b; Kim et al., 2003). As illustrated in Figure 1, *PTGS2* expression appears between d 10 and 12 post-estrus or postmating in the endometrial LE and sGE in response to P4 (Charpigny et al., 1997b; Simmons et al., 2010). 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 (Dorniak et al., 2011, 2012). Similarly, PTGS2 is also not downregulated in endometria of early pregnant cattle, but rather is upregulated by IFNT (Arosh et al., 2004; Emond et al., 2004). Thus, IFNT can stimulate PGE_2 production in the ruminant endometrium.

Prostaglandins have a biological role in the uterus during early pregnancy, because intrauterine infusion of meloxicam, a selective PTGS2 inhibitor, into the uterus of early pregnant sheep prevented conceptus elongation

(Simmons et al., 2010; Dorniak et al., 2011). Similarly, pregnancy rates were substantially reduced in heifers that received meloxicam on d 15 after insemination (Erdem and Guzeloglu, 2010). Of note, expression of *PTGS2* in biopsies of d-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). Given that membrane and nuclear receptors for PG are present in all cell types of the ovine endometrium and conceptus during early pregnancy (Cammis et al., 2006; Dorniak et al., 2011), *PTGS2*-derived PG from the conceptus are likely have paracrine, autocrine, and, perhaps, intracrine effects on endometrial function and conceptus development during early pregnancy (Dorniak et al., 2013a; Figure 1 and Table 1).

In addition to effects via membrane receptors, both PGI_2 and PGJ_2 can activate nuclear peroxisome proliferator-activating receptors (**PPAR**; Desvergne and Wahli, 1999); PGI_2 is a ligand for PPAR delta (PPARD), and PGD_2 spontaneously forms 15-deoxy- $\Delta^{12,14}$ - PGJ_2 within cells that is a ligand for PPAR gamma (PPARG). A recent in utero study found that knockdown of PPARG in the trophectoderm compromised conceptus elongation and caused apoptosis in the trophectoderm (Brooks et al., 2015b). In other cell types and the human placenta, PPAR alter the transport, cellular uptake, storage, and use of lipids and their derivatives (Desvergne and Wahli, 1999). The PPARG-regulated genes include *FABP* and FA transport proteins [FATP or SLC27A] required for lipid uptake and triacylglycerol synthesis, which is undoubtedly important in the rapidly proliferating and growing ruminant conceptus that is also secreting large amounts of PG. In cattle, the preimplantation conceptus influences endometrial FA concentrations (Meier et al., 2011), and strategic feeding of specific FA can benefit fertility and milk production in lactating dairy cows (Thatcher et al., 2011). Future research should focus on the biological roles of lipids in the uterine environment during pregnancy in ruminants.

CONCLUSIONS

Our knowledge of the complex biological and genetic mechanisms governing conceptus elongation and implantation remains limited in domestic ruminants but is essential to improve fertility and reproductive efficiency in ruminant livestock. Individual, additive, and synergistic actions of P4, IFNT, and PG regulate expression of elongation- and implantation-related genes in the endometrial epithelia of the uterus in ruminants. The outcome of the carefully orchestrated changes in gene expression is secretion or transport of substances (e.g., glucose, AA, proteins) from the endometrium into

the uterine lumen that are embryotrophic and drive conceptus elongation via effects on trophectoderm proliferation, migration, attachment, and adhesion. Although not well understood, conceptus elongation is also likely governed by transcription factors and other factors other than IFNT and PG (Mamo et al., 2012; Forde et al., 2015). A systems biology approach is necessary to fully understand conceptus elongation and the multifactorial phenomenon of early pregnancy loss. The advent of new gene editing technologies for livestock, such as transcription activator-like effector nuclease and clustered regularly interspaced short palindromic repeats/Cas9-stimulated homology-directed repair (Tan et al., 2013), are expected to accelerate our understanding of early pregnancy biology and genetic improvements in reproductive efficiency.

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