ABSTRACT: This review integrates established and new information on the biological role of ovarian progesterone (P4) and interferon tau (IFNT), as well as other conceptus- and endometrial-derived factors, PG and cortisol, in endometrial function and conceptus elongation during the periimplantation period of pregnancy in ruminants. Interferon tau is the maternal recognition of pregnancy signal that inhibits production of luteolytic pulses of PGF2α by the endometrium to maintain corpora lutea and their production of P4, the unequivocal hormone of pregnancy. Conceptus–endometrial interactions in ruminants are complex and involve carefully orchestrated temporal and spatial alterations in endometrial gene expression during pregnancy. Available results from studies in sheep support the idea that the individual, interactive, and coordinated actions of P4, interferon tau, PG, and cortisol regulate expression of elongation- and implantation-related genes in the endometrial epithelia and that P4 and PG are essential regulators of conceptus elongation. The outcome of these gene expression changes is alterations in endometrial secretions that govern conceptus elongation via effects on trophectoderm proliferation, migration, attachment, and adhesion. An increased knowledge of conceptus-endometrial interactions during early pregnancy in ruminants is necessary to understand and elucidate the causes of recurrent pregnancy loss and to provide a basis for new strategies to improve pregnancy outcome and reproductive efficiency.

Key words: conceptus, endometrium, interferon, pregnancy, prostaglandin, ruminant

INTRODUCTION

This review integrates established and new information on the biological role of ovarian progesterone (P4) and interferon tau (IFNT), as well as other conceptus- and endometrial-derived factors, PG and cortisol, in endometrial function and conceptus elongation during the periimplantation period of pregnancy in ruminants. This area of reproduction is particularly important due to relatively high levels of pregnancy loss during that period. 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 wk after insemination (Diskin et al., 2006). Early pregnancy loss is even greater in high-yielding dairy cows, and is a major impediment to milk production efficiency and profitability (Evans and Walsh, 2011; Thatcher et al., 2011).

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., 2004a, 2007a, 2008). 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 blastocoel or central cavity surrounded by a monolayer of trophectoderm. After hatching from the zona pellucida (d 8 to 9), the
blastocyst slowly grows into a tubular or ovoid form and is then termed a conceptus (embryo-fetus and associated extraembryonic membranes). The ovoid conceptus begins to elongate on d 12 in sheep or d 15 in cattle, 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). Conceptus elongation involves exponential increases in length and weight of the trophoderm (Wales and Cuneo, 1989) and onset of extraembryonic membrane differentiation, including gastrulation of the embryo and formation of the yolk sac and allantois (Guillomot, 1995) that are vital for embryonic survival and formation of a functional placenta.

Progesterone from the ovarian CL acts on the uterus to stimulate preimplantation blastocyst growth and conceptus elongation in ruminants (Spencer et al., 2004b; Lonergan, 2011). Interferon tau is the signal for maternal recognition of pregnancy in ruminants and is secreted predominantly during conceptus elongation. Indeed, elongation of the ovoid conceptus is critical for developmentally-regulated production of IFNT as well as PG by the trophoderm. As a pregnancy recognition signal, IFNT acts in a paracrine manner on the endometrium to inhibit development of the endometrial luteolytic mechanism required for pulsatile release of PGF$_{2\alpha}$, thereby ensuring continued production of P4 by the ovarian 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 that are implicated in establishment of uterine receptivity and conceptus elongation and implantation (Hansen et al., 1999; Spencer et al., 2007a). Moreover, the endometrium itself, as well as the ovoid and elongating conceptuses, produce PG (Marcus, 1981; Lewis, 1989) and cortisol that have biological roles in endometrial function and conceptus elongation (Dorniak et al., 2011, 2012b).

Although blastocysts can develop entirely in vitro, the overall success of this process and quality of the blastocysts is markedly less than for those developed in vivo (Hasler et al., 1995), and they must be transferred into a receptive uterus for growth and development into an elongated, filamentous conceptus (Flechon et al., 1986). The endometrium of the uterus secretes substances, collectively termed histotroph, that govern elongation of the conceptus via effects on trophoderm 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, AA, sugars, and ions (Bazer, 1975; Gray et al., 2001a). 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, PG, and cortisol) as well as the endometrium itself (e.g., PG, cortisol). The genes and functions regulated by these hormones and factors in the endometrial epithelia elicit specific changes in the intrauterine milieu that support conceptus elongation (Spencer et al., 2007b, 2008; Bazer et al., 2010).

**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 (Lonergan, 2011; Mann and Lamming, 2001). In both lactating dairy cows and heifers, there is a strong positive association between the postovulatory 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), whereas 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., 2009). Indeed, 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).

Actions of ovarian P4 on the uterus are essential for conceptus survival and growth in sheep (Satterfield et al., 2006). Between d 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 in the endometrial LE and superficial GE (sGE) that encode secreted attachment and migration factors [galectin-15 (LGALS15), IGFBP1], intracellular enzymes [PG G/H synthase and cyclooxygenase 2 (PTGS2) and hydroxysteroid (11β) 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 AA (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 1 or SPP1), a regulator of calcium/phosphate homeostasis (stanniocalcin 1 or STC1), and an immunomodulatory factor (SERPINA14, also known as uterine milk proteins or uterine serpins). The initiation of expression of those genes requires P4 action and is temporally associated with the loss of progesterone receptors (PR) between d 10 and 12 in the endometrial LE and between d 12 and 14 to 16 in the GE after onset of estrus. The loss of PR in uterine epithelia immediately before implantation is a common feature across mammals (Carson et al., 2000); however, PR expression is not lost in the stroma or myometrium in the ovine uterus (Spencer and Bazer, 2002). Similar to the human (Giudice and Ferenczy, 1996; Kao et al., 2002), endometria of both cyclic and pregnant ewes 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 AA, 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).

### Table 1. Effects of ovarian progesterone and intrauterine infusions of interferon tau (IFNT), PG, and cortisol on elongation- and implantation-related genes expressed in the endometrial epithelia of the ovine uterus

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Progesterone</th>
<th>IFNT</th>
<th>PG&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Cortisol</th>
</tr>
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<tbody>
<tr>
<td><strong>Transport of glucose</strong></td>
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<tr>
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<tr>
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<td>↑</td>
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<td>++</td>
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<td>SPP1</td>
<td>↑</td>
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<td><strong>Enzymes</strong></td>
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<td>HSD11B1</td>
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<td>PTGS2</td>
<td>↑</td>
<td>+</td>
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<tr>
<td><strong>Transcription factors</strong></td>
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<tr>
<td>HIF1A</td>
<td>↑</td>
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<td>ne or +</td>
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<tr>
<td>HIF2A</td>
<td>↑</td>
<td>+</td>
<td>ne or +</td>
<td>ne</td>
</tr>
</tbody>
</table>

<sup>1</sup>Effect of hormone or factor denoted as induction (↑), stimulation (+), no effect (ne), decrease (−) or not determined (nd).

<sup>2</sup>Summary data for infusion of PGE<sub>2</sub>, PGF<sub>2α</sub> or PGI<sub>2</sub> (Dorniak et al., 2012a,b).

### 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 α (ESR1) gene in sheep and oxytocin receptor (OXTR) gene in both sheep and cattle, specifically in the endometrial LE and sGE. The absence of OXTR in the endometrium prevents the release of luteolytic pulses of PGF<sub>2α</sub>, 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 PG 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 genes, termed 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., 2009a).

### Classical Type I IFN-Stimulated Genes in the Endometrium

A common feature of the periimplantation period of pregnancy in domestic animals, rodents, and primates is the production of Type I and/or Type II IFN by trophectoderm, which induce and/or stimulate expression of classical ISG in the uterus (Hansen et al., 1999; Bazer et al., 2009a; Johnson et al., 2009). Because expression of ISG increases in a stage-specific manner within endometria of diverse species, including domestic
animals, laboratory rodents, primates, and humans, they may be universally important in establishment of uterine receptivity and conceptus implantation (Bazer et al., 2009a). 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).

Bovine endometrial, ovine endometrial, and human 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 IFN (Stark et al., 1998). Numerous classical ISG are induced or stimulated in the endometrium during conceptus elongation in both cattle and sheep. For instance, 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 are undetectable in LE by d 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 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 trophectoderm 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 classical ISG (B2M, GBP2, IFI27, IFIT1, ISG15, IRF9, MIC, OAS, RSAD2, STAT1, and STAT2) 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). This finding was initially surprising, because all endometrial cell types express IFNAR1 [interferon (α, β, and omega) receptor 1] and IFNAR2 subunits of the common Type I IFN receptor (Rosenfeld et al., 2002). 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 ISG (B2M, GBP2, IFI27, IFIT1, ISG15, MIC, OAS)

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 to 10 d downregulate expression of the progesterone receptor (PR). The loss of PR is correlated with the induction of many genes in the endometrial luminal epithelium (LE) and superficial glandular epithelia (sGE), including PG G/H synthase and cyclooxygenase 2 (PTGS2) and hydroxysteroid (11-β) dehydrogenase 1 (HSD11B1) that produce PG and cortisol, in both cyclic and pregnant ewes. If the ewe is pregnant, the trophectoderm synthesizes and secretes PG, interferon tau (IFNT), and cortisol that act on the endometrium in a cell-specific manner to upregulate the expression of many P4-induced genes that govern endometrial functions and/or elongation of the conceptus. GE, glandular epithelia; GR, glucocorticoid receptor. See online version to view figure in color.
contain 1 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 (Fig. 2A). The silencing of MIC 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). 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 (Fig. 2B). 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 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 is to determine which of the large number of classical ISG have a biological role in conceptus-endometrial interactions given that they have traditionally been associated with cellular antiviral responses, because the main function of Type I IFN is to inhibit viral infection (Pestka, 2007).

One classical ISG with reported biological effects on trophoderm growth and adhesion in ruminants is CXCL10 [chemokine (C-X-C motif) ligand 10; alias IP-10], a member of the C-X-C chemokine family that indicates that PTGS2-derived PG and HSD11B1-derived cortisol are part of the noncanonical pathway of IFNT action on the endometrium (Dorniak et al., 2011, 2012b).

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 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 nonclassical ISG in the endometrial LE during pregnancy such as WNT7A (wingless-type MMTV integration site family, member 7A), LGALS15, CTSL, CTS3, 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 (CTST, 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, 2009b). None of those genes are classical Type I ISG, and they are referred to as nonclassical or novel ISG. Indeed, IFNT stimulation of those nonclassical ISG requires induction by P4 and loss of PR in the epithelia. Importantly, all of the nonclassical ISG encode factors that have actions on the trophoderm (proliferation, migration, attachment and/or adhesion, nutrient transport) important for conceptus elongation (Table 1).

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 use a noncanonical, STAT1-independent signaling pathway to regulate expression of genes in endometrial LE and sGE and the ovine uterus (Fig. 2A). The noncanonical pathway mediating IFNT stimulation of genes in the endometrial LE and sGE has not been entirely elucidated, but other Type I IFN use 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 PGE2 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 PG and HSD11B1-derived cortisol are part of the noncanonical pathway of IFNT action on the endometrium (Dorniak et al., 2011, 2012b).

Prostaglandins and Cortisol: Potential Mediators of Progesterone and Interferon Tau Action in the Endometrium of the Ovine Uterus

Prostaglandins Regulate Endometrial Function and Conceptus Elongation. Results of recent studies in sheep clearly support the concept that PG 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). The conceptus and endometria synthesize a variety of PG during early pregnancy in both sheep and cattle (Lewis et al., 1982; Lewis and Waterman, 1983, 1985; Lewis, 1989; Charpigny et al., 1997a, 1997b). 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 (PG G/H synthase and cyclooxygenase) or PTGS2 is the dominant cyclooxygenase expressed in both the endometrium and
Figure 2. Schematic illustrating the current working hypothesis on interferon tau (IFNT) signaling in endometrial epithelia and stroma of the ovine uterus. Interferon tau, produced in large amounts by the developing conceptus, binds to the Type I interferon tau (IFN) receptor (IFNAR) present on cells of the ovine endometrium. In the cells of the endometrial luminal epithelium (LE) and superficial glands (sGE), IFNT is prevented from activating IFN-stimulated genes (ISG) by IRF2 (IFN regulatory factor two; Panel A). The potent and stable repressor IRF2, present in the nucleus, increases during early pregnancy in LE and sGE. The continual presence of IRF2 inhibits ISRE-containing target genes through direct ISRE binding and coactivator repulsion. However, IFNT can stimulate the transcription of a number of nonclassical ISG as well as increase the activity of certain intracellular enzymes (PTGS2 and HSD11B1). However, the noncanonical signaling pathways mediating these effects of IFNT in the LE/sGE are largely unknown. In cells of the stroma and middle to deep glands (Panel B), IFNT-mediated association of the IFNAR subunits facilitates the cross-phosphorylation and activation of 2 Janus kinases, Tyk2 and JAK1, which in turn phosphorylate the receptor and creates a docking site for STAT2. STAT2 is then phosphorylated, thus creating a docking site for STAT1 that is then phosphorylated. STAT1 and STAT2 are then released from the receptor and can form 2 transcription factor complexes. The ISGF3 complex, formed by association of the STAT1-2 heterodimer with interferon regulatory factor (IRF) 9 in the cytoplasm, translocates to the nucleus, and transactivates genes containing an ISRE, such as STAT1, STAT2, and IRF9. GAF is formed by binding of STAT1 homodimers, which translocates to the nucleus and transactivate genes containing a GAS element(s), such as IRF1. Interferon regulatory factor 1 can also bind and transactivate IFN-stimulated response element (ISRE)-containing genes. The simultaneous induction of STAT2 and IRF9 gene expression by IFNT appears to shift transcription factor formation from GAF toward predominantly ISGF3. Therefore, IFNT activation of the JAK-STAT signal transduction pathway allows for constant formation of ISGF3 and GAF transcription factor complexes and hyperactivation of ISG expression. IRF1, IRF-binding element; IRF2, interferon regulatory factor two; ISRE, interferon-stimulated response element; GAF, gamma activated factor. See online version to view figure in color.
trophoderm of the elongating conceptus (Charpigny et al., 1997a). 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 upregulation 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 d 10 and 12 postestrus 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 downregulated in endometria of early pregnant cattle, but rather is upregulated by IFNT (Arosh et al., 2004; Emond et al., 2004); indeed, IFNT acts as a molecular switch that stimulates PGE2 production in the bovine endometrium (Krishnaswamy et al., 2009). Further, Type I IFN were found to stimulate phospholipase A2 (PLA2) and synthesis of PGE2 and PGF2α in several different cell types over 25 yr 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, 2012b; Table 1 and Fig. 1). 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, 2012b). 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 PG in the ovine placenta and human uterine decidua, respectively (Strakova et al., 2000; Michael et al., 2003; Michael and Papageorghiou, 2008).

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 concentrations are much greater in the uterine lumen of pregnant 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 d 16 conceptuses produce substantially more of those PG than d 14 conceptuses (Lewis and Waterman, 1985). Given that PG receptors are present in all cell types of the endometrium and conceptus during early pregnancy (Cammas et al., 2006; Dorniak et al., 2011), PTGS2-derived PG 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 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). Recently, Dorniak et al. (2012a) infused PGE2, PGF2α, PGI2, or IFNT at the levels produced by the d 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 AA transporter SLC1A5, but only IFNT increased SLC7A2. In the uterine lumen, only IFNT increased glucose concentrations, and only PGE2 and PGF2α increased total AA (Dorniak et al., 2012a). Thus, available results support the idea that PG and IFNT from the conceptus coordinate to regulate endometrial functions important for growth and development of the conceptus during the periimplantation period of pregnancy. In fact, pregnancy rates were substantially reduced in heifers that received meloxicam, a partially selective inhibitor of PTGS2, on d 15 after insemination (Erdem and Guzeloglu, 2010). Thus, PG are critical regulators of conceptus elongation and implantation, 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).

Cortisol Regulates Endometrial Function

Initially identified as a candidate P4-regulated gene in the endometrium that potentially governed conceptus elongation (Satterfield et al., 2009), HSD11B1 was found to be expressed specifically in the endometrial LE and superficial GE of the ovine uterus, and was induced by P4 and stimulated by IFNT (Simmons et al., 2010; Table 1). Expression of HSD11B1 is also upregulated in the endometrium of cattle between d 7 and 13 of pregnancy (Forde et al., 2009). One of 2 isoforms of hydroxysteroid (11-β) dehydrogenases that regulate intracellular levels of bioactive glucocorticoids within key target tissues (Michael et al., 2003), HSD11B1 is a low-affinity NADP(H)-dependent bidirectional dehydrogenase and/or reductase for glucocorticoids, and the direction of HSD11B1 activity is determined by the relative abundance of NADP+ and NADPH cofactors. The endometrium of the ovine uterus, as well as the conceptus, generates active cortisol from inactive cortisone (Dorniak et al., 2012b). Cortisol regulates gene expression via the
glucocorticoid receptor (GR), a transcriptional regulator that modulates expression of primary target genes that either directly affect cellular physiology or alter the expression of other secondary target genes, which then confer hormonal responses (Yamamoto, 1985; Wang et al., 2004). Very few GR targets have been identified in the uterus or placenta, but GR targets hundreds of genes, including those involved in lipid metabolism and triglyceride homeostasis, in other organs and cell types (Wang et al., 2004; Yu et al., 2010).

Recent findings support the idea that PG mediate, in part, P4 induction and IFNT stimulation of HSD11B1 expression in the ovine endometrium (Dorniak et al., 2011, 2012b). Similarly, PG regulate activity of HSD11B1 in bovine endometria (Lee et al., 2009), and PGF2α stimulates the activity of HSD11B1 in human fetal membranes (Alfaidy et al., 2001, 2003). Whereas PG stimulate HSD11B1 activity, glucocorticoids enhance PG synthesis by upregulating expression and activity of PLA2 and PTGS2 in the ovine placenta, thereby establishing a positive feed-forward loop implicated in the timing of parturition (Challis et al., 2000). This tissue-specific stimulatory role of glucocorticoids on PG synthesis contradicts the classical concept that glucocorticoids exert antiinflammatory effects on immune cells (Goppelt-Struebe, 1997).

Recently, the elongating sheep conceptus was found to generate cortisol from cortisone via HSD11B1, and cortisol was found in the uterine lumen of early pregnant sheep (Dorniak et al., 2012b). Given that GR are present in all endometrial cells of ovine uterus during the estrous cycle and pregnancy and in the conceptus trophectoderm (Simmons et al., 2010; Dorniak et al., 2011), cortisol may have paracrine and autocrine effects on the endometrium and conceptus trophectoderm during early pregnancy (Fig. 1). Overall, relatively little is known regarding the biological activities of glucocorticoids in ruminants during early pregnancy. As summarized in Table 1, intrauterine infusions of cortisol at early pregnancy levels into the uterus of cyclic ewes from d 10 to 14 postestrus increased the expression of several elongation- and implantation-related genes expressed in the endometrial epithelia of the ovine uterus (Dorniak and Spencer, unpublished results).

Available results support the idea that cortisol from the endometrium as well as conceptus regulates endometrial functions important for conceptus elongation during early pregnancy. In humans, glucocorticoids can have positive as well as negative effects during pregnancy (Michael and Papageorghiou, 2008). Administration of synthetic glucocorticoids to women during pregnancy can alter normal development of the fetus and compromise pregnancy success by inhibiting cytokine-PG signaling, restricting trophoblast invasion, and inducing apoptosis in placenta. Similarly, administration of synthetic glucocorticoids to pregnant ewes reduced placental growth and development, numbers of trophoblast giant binucleate cells in the placenta, and circulating concentrations of placental lactogen (Braun et al., 2007). On the other hand, natural glucocorticoids are hypothesized to have positive effects during early pregnancy (Michael and Papageorghiou, 2008). In humans, the proposed positive roles of HSD11B1-generated cortisol at the conceptus-maternal interface includes stimulation of chorionic gonadotropin secretion by the trophoblast, promotion of trophoblast growth and invasion, and stimulation of placental transport of glucose, lactate, and AA. Interestingly, administration of glucocorticoids increased pregnancy rates in women undergoing assisted reproductive technologies and pregnancy outcomes in women with a history of recurrent miscarriage (Quenby et al., 2005; Boomsma et al., 2007).

**SUMMARY AND CONCLUSIONS**

Available information from the studies of sheep support the idea that the individual, additive, and synergistic actions of P4, IFNT, PG, and cortisol regulate expression of elongation- and implantation-related genes in the endometrial epithelia, and that P4 and PG are essential regulators of conceptus elongation. The outcome of these 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 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 that regulate conceptus elongation in cattle are conserved between cattle and sheep (Bauersachs et al., 2008; Forde et al., 2011b; Forde and Lonergan, 2012). Given that the endometrial LE and GE are required for conceptus elongation in sheep (Gray et al., 2002), most of the research in conceptus-endometrial interactions has been focused on those cell types; however, receptors for P4, IFNT, PG, and cortisol are present in all other uterine cell types, as well as many immune cells. In conclusion, opportunities for future research include understanding effects of PG and cortisol on other endometrial cell types, including immune cells and their potential roles in the immunobiology of early pregnancy; identifying genes regulated by PG and cortisol in the endometrium of the ruminant uterus; determining the noncanonical cell signaling pathways in the uterus used by P4 and IFNT to regulate expression of genes in the endometrial epithelia; and understanding the biological roles of ISG in endometrial function,
conceptus elongation and implantation, and function of the CL and circulating immune cells. An increased knowledge of conceptus–endometrial interactions during early pregnancy in ruminants is necessary to understand the multifactorial phenomenon of recurrent pregnancy loss and provide a basis for new strategies to improve pregnancy outcome and reproductive efficiency.

LITERATURE CITED


