ABSTRACT

In ruminants, the corpus luteum (CL) of early pregnancy is resistant to luteolysis. Prostaglandin (PG)E\textsubscript{2} is considered a luteoprotective mediator. Early studies indicate that during maternal recognition of pregnancy (MRP) in ruminants, a factor(s) from the conceptus or gravid uterus reaches the ovary locally through the utero-ovarian plexus (UOP) and protects the CL from luteolysis. The local nature of the embryonic antiluteolytic or luteoprotective effect precludes any direct effect of a protein transported or acting between the gravid uterus and CL in ruminants. During MRP, interferon tau (IFNT) secreted by the trophoblast of the conceptus inhibits endometrial pulsatile release of PGF\textsubscript{2\alpha}, and increases endometrial PGE\textsubscript{2}. Our recent studies indicate that (1) luteal PG biosynthesis is selectively directed toward PGF\textsubscript{2\alpha} at the time of luteolysis and toward PGE\textsubscript{2} at the time of establishment of pregnancy (ESP); (2) the ability of the CL of early pregnancy to resist luteolysis is likely due to increased endometrial biosynthesis and signaling of PGE\textsubscript{2}; and (3) endometrial PGE\textsubscript{2} is transported from the uterus to the CL through the UOP vascular route during ESP in sheep. Intrauterine co-administration of IFNT and prostaglandin E\textsubscript{2} synthase 1 (PGES-1) inhibitor reestablishes endometrial PGF\textsubscript{2\alpha} pulses and regresses the CL. In contrast, intraovarian co-administration of IFNT and PGES-1 inhibitor along with intraovarian administration of PGE\textsubscript{2} rescues the CL. Together, the accumulating information provides compelling evidence that PGE\textsubscript{2} produced by the CL in response to endometrial PGE\textsubscript{2} induced by pregnancy may counteract the luteolytic effect of PGF\textsubscript{2\alpha} as an additional luteoprotective mechanism during MRP or ESP in ruminants. Targeting PGE\textsubscript{2} biosynthesis and signaling selectively in the endometrium or CL may provide luteoprotective therapy to improve reproductive efficiency in ruminants.

Key words: prostaglandin, corpus luteum, endometrium, establishment of pregnancy

PROSTAGLANDINS

Prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT) are classified as eicosanoids (from the Greek \textit{eicosa}, meaning 20), which describe the broad group of compounds derived from C\textsubscript{20} fatty acids. Prostaglandins are 20-carbon unsaturated hydroxyl fatty acids with a cyclopentane ring. Arachidonic acid, an essential fatty acid, is the principal precursor for PG. In mammals, PG play important roles in several physiological and pathological processes (McCracken, 2005).

Biosynthesis of PG

Phospholipase A\textsubscript{2} (PLA\textsubscript{2}) liberates arachidonic acid from membrane phospholipids. Cyclooxygenases (COX) 1 and 2 convert arachidonic acid into PGH\textsubscript{2}, the common intermediate metabolite for biosynthesis of various PG, and PGH\textsubscript{2} is then converted into selective PG including PGF\textsubscript{2\alpha}, PGE\textsubscript{2}, PGD\textsubscript{2}, PGI\textsubscript{2}, and TXA\textsubscript{2} by specific synthases (Smith and Dewitt, 1996; Kudo and Murakami, 1999; Thorén and Jakobsson, 2000; Smith and Song, 2002; Thorén et al., 2003). Prostaglandin F synthase (PGFS, such as AKR1B1, AKR1C1, AKR1C2, and AKR1C3) and prostaglandin E synthase (PGES-1, -2, and -3) convert PGH\textsubscript{2} into PGF\textsubscript{2\alpha} and PGE\textsubscript{2}, respectively. Catabolism of PG is governed by prostaglandin 15-dehydrogenase (PGDH), which catabolizes PGF\textsubscript{2\alpha} into inactive 15-keto-13,14-dihydro PGF\textsubscript{2\alpha} (PGFM), and catabolizes PGE\textsubscript{2} into inactive 13,14-dihydro-15-keto PGE\textsubscript{2} (PGEM; Tai et al., 2002). An overview on PG metabolic pathways is depicted in Figure 1.
Transport of PG

Transport of PG through plasma membranes is poorly understood, with proposed mechanisms including from simple diffusion, passive transport, active transport, counter current exchange, and carrier-mediated transport. The PG are organic anions and cross cellular membranes by simple diffusion; however, the estimated flow rate is too low and insufficient to bring forth their biological effects. In the pulmonary circulation, PGE2 and PGF2α are catabolized rapidly in one passage through the lungs. Therefore, a carrier-mediated transport mechanism is required for cellular transport of PG. Prostaglandin transporter (PGT) is a member of the 12-transmembrane solute carrier organic anion transporter (OATP) 2A1 (SLCO2A1) family (Schuster, 1998, 2002); PGT transports PGF2α, PGE2, PGD2, and TxA2, in a competitive manner, with different affinities for each PG. We and others using pharmacological and genomic approaches have shown that inhibition of PGT prevents PGT-mediated transport of PG in various cell types (Kanai et al., 1995; Chan et al., 1998; Banu et al., 2003, 2008).

Signaling of PG

Prostaglandin F2α and PGE2 elicit their autocrine, paracrine, or endocrine biological actions through FP and EP (EP1, EP2, EP3, and EP4) receptors, respectively, by activating multiple signaling cascades (Coleman et al., 1994; Narumiya et al., 1999; Narumiya and FitzGerald, 2001). The FP and EP1 receptors are coupled to the Gq protein and activate phospholipase C, which generates 2 second messengers: inositol triphosphate and diacylglycerol. Further signaling events involve the activation of protein kinase C, mitogen-activated protein kinases (ERK1/2), and transcriptional factors such as nuclear factor kappa B (NF-kB) and cyclic AMP-responsive element-binding protein (CREB) (Narumiya, 1999).

Figure 1. Overview on biosynthesis and signaling of prostaglandins, which is developed based on published information (Kanai et al., 1995; Smith and Dewitt, 1996; Kudo and Murakami, 1999; Narumiya et al., 1999; Narumiya and FitzGerald, 2001; Smith and Song, 2002; Tai et al., 2002; Thorén and Jakobsson, 2000; Thorén et al., 2003; Castellone et al., 2005; Buchanan et al., 2006; Cha and DuBois, 2007). PG = prostaglandin; PPAR = peroxisome proliferator-activated receptor; COX = cyclooxygenase; DP = prostaglandin D2 receptor; EP = prostaglandin E2 receptor; FP = PGF2α receptor; ERK1/2 = extracellular signal-regulated protein kinases 1 and 2; AKT = protein kinase B; NF-kB = nuclear factor kappa B; IP3 = inositol trisphosphate; PGDH = prostaglandin 15-dehydrogenase; PGEM = inactive 13,14-dihydro-15-keto PGE2; PGFM = inactive 15-keto-13,14-dihydro PGF2α; TX = thromboxane. Color version available online.
sphosphate (IP₃) mobilizes intracellular calcium (Ca²⁺) and diacylglycerol activates protein kinase C (PKC). The EP2 and EP4 receptors are coupled to Gs protein and activate adenyl cyclase and generate cyclic (c) AMP, which in turn activates protein kinase A (PKA). The EP3 receptor is coupled to Gq, Gs, and Gi proteins and is associated with inhibition or induction of cAMP. Activation of EP3 receptor also increases mobilization of Ca²⁺ and IP₃ (Narumiya et al., 1999). The EP (Castellone et al., 2005; Buchanan et al., 2006; Cha and DuBois, 2007; Banu et al., 2009) and FP (Sales et al., 2005, 2007; Guo et al., 2012) receptors cross-talk with multiple intracellular pathways. An overview on PG signaling pathways is depicted in Figure 1.

**Requirement for Pulsatile Release of Endometrial PGF₂α During Luteolysis in Ruminants**

Luteolysis is a neuroendocrine-mediated event. Functional luteolysis denotes the decline in progesterone (P₄) production by the corpus luteum (CL). Structural luteolysis denotes the physical involution and regression of luteal tissues. The relationship between functional and structural luteolysis has not been clearly defined; however, functional luteolysis appears to precede structural luteolysis (McCracken et al., 1999; Niswender et al., 2000). In ruminants, endometrial PGF₂α is the luteolytic hormone that causes functional and structural luteolysis through multiple mechanisms (McCracken et al., 1999; Niswender et al., 2000). At the time of luteolysis, PGF₂α is released from the endometrium in a pulsatile pattern. Development of the luteolytic mechanism is largely under the regulation of P₄, estradiol, and oxytocin (Hooper et al., 1986; McCracken et al., 1999). In sheep, continuous exposure of endometrium to P₄ for 8 to 10 d downregulates expression of nuclear P₄ receptor (PGR) in luminal epithelial (LE) cells between d 11 and 13, thereby allowing a rapid increase in expression of estrogen receptor α (ESR1) after d 13, followed by an increase in expression of oxytocin receptor (OXTR) after d 14 of the estrous cycle (McCracken et al., 1999; Spencer et al., 2004, 2007). Pulsatile release of oxytocin from the posterior pituitary after d 13 to 14 of the estrous cycle acts on endometrial OXTR and induces the release of luteolytic pulses of PGF₂α from the endometrial LE cells between d 14 and 16 of the estrous cycle. Luteal oxytocin acts as a supplemental source of oxytocin to boost the oxytocin pulse from the posterior pituitary and amplify the luteolytic pulses of PGF₂α from the endometrium during luteolysis (McCracken et al., 1995, 1999). Five endometrial PGF₂α pulses of 1-h duration over a period of 48 h at 8-h intervals are required to consistently cause complete CL regression in sheep. Reducing the duration of pulses of PGF₂α from 1 to 0.5 h failed to cause luteolysis, even after six 0.5-h pulses (McCracken et al., 2012). Endometrial PGF₂α pulses are secreted into the uterine vein, which joins the ovarian vein to form the utero-ovarian vein. These luteolytic PGF₂α pulses are transported from the utero-ovarian vein into the ovarian artery locally through a unique vascular structure called the utero-ovarian plexus (UOP; McCracken et al., 1972, 2012). Local transport of PGF₂α through the UOP is obligatory for regression of the CL in sheep, because 99% of PGF₂α secreted by the endometrium is catabolized into its inactive stable metabolite PGFM by PGDH after a single systemic passage through the lungs (Davis et al., 1980). The reviews by McCracken et al. (1999) and Niswender et al. (2000) provide more details on functional and structural luteolysis in ruminants.

**Requirement for Luteal Biosynthesis of PGF₂α During Luteolysis in Ruminants**

Administration of PGF₂α during the mid-luteal phase of the estrous cycle increases luteal PGF₂α production which is inhibited by pretreatment with indomethacin (COX-1/2 inhibitor), as measured in CL explant cultures (Rexroad and Guthrie, 1979). Systemic blockade with indomethacin inhibits endometrial and luteal PGF₂α biosynthesis. Importantly, indomethacin blockade does not inhibit the decrease in P₄ levels (functional luteolysis) in response to luteolytic PGF₂α (Guthrie, 1979). Both Rexroad and Guthrie (1979) and Guthrie (1979) suggest that luteal synthesis of PGF₂α is not required for functional luteolysis; however, the effect of indomethacin treatment on structural luteolysis (size and weight of the CL) was not measured. Administration of luteolytic PGF₂α, in vivo and treatment of luteal cells with PGF₂α, in vitro induce expression of COX-2 mRNA in luteal tissues and cells in sheep (Tsai and Wiltbank, 1997, 1998; Anderson et al., 2001). These findings strengthen the view that luteal synthesis of PGF₂α is likely to be an important part of a positive feedback loop between the uterus and the CL during the process of luteolysis. In support of this view, a recent study (Niswender et al., 2007) indicates that indomethacin, in the form of a polymerized gel implanted surgically into the ovine CL at mid cycle, does not inhibit the effects of luteolytic PGF₂α on decreasing circulating P₄ levels (functional luteolysis); however, it does inhibit the effects of luteolytic PGF₂α on size and weight of the CL and maintains the structure (structural luteolysis). These studies together suggest that endometrial PGF₂α causes functional luteolysis, whereas luteal PGF₂α may cause structural luteolysis (Sawyer et al., 1990; Juen-
gel et al., 1993; Zheng et al., 1994; Niswender et al., 2007; Sugino and Okuda, 2007). However, the absolute requirement for luteal PGF$_{2\alpha}$ in functional versus structural luteolysis has yet to be established.

**Molecular Control of Luteal P4 Biosynthesis in Ruminants**

The CL has large steroidogenic cells (LLC) and small steroidogenic cells (SLC) besides fibroblasts, immune cells, and endothelial cells. The LLC and SLC are thought to be derived from the granulosa and theca cells, respectively (McCracken et al., 1999; Niswender et al., 2000). Luteal steroidogenesis depends on transport of cholesterol. The steroidogenic acute regulatory protein (StAR) protein transports the cholesterol from the outer to the inner mitochondrial membrane. The cholesterol-side-chain cleavage enzyme (P-450scs), located on the inner membrane of mitochondria, converts cholesterol to pregnenolone. 3β-Hydroxysteroid dehydrogenase (3β-HSD) enzyme converts pregnenolone to P4 (Niswender, 2002). The StAR protein is expressed in LLC and SLC and regulated by cAMP/PKA and ERK1/2 pathways (Wiltbank et al., 1993; Manna et al., 2009). The SLC secrete basal levels of P4 but respond to LH/cAMP/PKA with an increase in P4 production (McCracken et al., 1999; Niswender et al., 2000). The LLC secrete high levels of basal P4 and do not respond to LH stimulation. The driving factor for the constitutive cAMP/PKA activation and P4 production by the LLC is largely unknown. Prostaglandin E$_2$/EP2/EP4 signaling activates cAMP/PKA pathways in most cell types under physiological conditions (Narumiya et al., 1999). It is possible that luteal PGE$_2$/EP2/EP4 signaling could drive the constitutive activation of cAMP/PKA in LLC, but this notion needs be examined in functional studies. The review by Niswender (2002) provides more details on molecular control of luteal P4 biosynthesis in ruminants.

**Maternal Recognition and Establishment of Pregnancy in Ruminants**

During the maternal recognition (MRP) and establishment of pregnancy (ESP) in ruminants, interferon tau (IFNT), a type 1 interferon, is secreted by the mononuclear cells of trophoderm of the conceptus (Imakawa et al., 1987). Interferon tau acts on LE cells in a paracrine manner and suppresses transcription of both ESR1 and OXTR genes in sheep (Spencer et al., 2004, 2007) and cows (Mann et al., 1999; Robinson et al., 2008), and thereby inhibits oxytocin-induced pulsatile release of luteolytic PGF$_{2\alpha}$ by the endometrium. Interferon tau signaling is mediated through a cell surface receptor that is composed of 2 subunits, IFNAR1 and IFNAR2. The well-characterized downstream signal transduction pathways of type 1 IFN is activation of tyrosine kinase 2 (TYK2) and Janus kinase 1 (JAK1) and signal transducer and activator of transcription (STAT), collectively known as the JAK/STAT pathways (Stewart et al., 2001; Wang and Roberts, 2004). Activation of JAK/STAT in turn regulates phosphorylation and activation of STAT and IFN-stimulated genes (ISG). Phosphorylated STAT1 and STAT2 (heterodimers) together with IFN regulatory factor-9 (IRF-9) form IFN-stimulated gene factor 3 (ISGF3) transcriptional complex that translocates to the nucleus and binds with IFN-stimulated response elements (ISRE) to initiate transcription of specific ISG. Interestingly, IFNAR1 and IFNAR2 genes are expressed (Rosenfeld et al., 2002) but STAT1, STAT2, and IRF-9 genes are not expressed in endometrial LE cells at the time of MRP in sheep (Choi et al., 2001; Spencer et al., 2007). On the other hand, IFNT-stimulated genes are expressed in LE cells (Song et al., 2006). This suggests that IFNT can activate STAT-independent cell-signaling pathways in endometrial LE cells at the time of MRP/ESP (Spencer et al., 2007).

**Novel IFNT Downstream Signaling During MRP in Ruminants**

In deciphering novel IFNT signaling, we have recently identified that IFNT activates JAK-SRC-EGFR-RAS-RAF-ERK1/2-EGR-1 signaling pathways in the ovine endometrial luminal epithelial (oLE) cells in vitro (Bannu et al., 2010). Using oLE cell cultures, immunoprecipitation, and ERK1/2 short interfering (si)RNA approaches, we have shown that IFNT phosphorylates PGT protein at tyrosine and threonine residues and concomitantly dephosphorylates PGT protein at serine residues, and thus inhibits PGT-mediated release of endometrial PGF$_{2\alpha}$ through ERK1/2 pathways. These findings indicate that interactions between ERK1/2 and PGT are the important cell-signaling cascades required to inhibit PGT-mediated pulsatile release of PGF$_{2\alpha}$ from the ovine endometrium (Bannu et al., 2010; Lee et al., 2013). Next, we analyzed expression, co-localization, and interaction between pERK1/2 and PGT proteins in endometrial LE cells in vivo. Results demonstrate that IFNT induces activation of pERK1/2 proteins and increases its interaction with PGT protein, whereas co-treatment with ERK1/2 inhibitor (U0126) and IFNT decreases interaction between pERK1/2 and PGT protein in the endometrial LE and restores 3 to 4 pulses of PGF$_{2\alpha}$ (Lee et al., 2014). These results
clearly indicate that IFNT interacts with PGT through ERK1/2 pathways and inhibits PGT-mediated release of PGF2α from endometrial LE in sheep in vivo (Lee et al., 2014). Restoration of PGF2α pulses in the IFNT and ERK1/2 inhibitor-treated group is positively associated with the expression of OXTR and ESR1 and negatively associated with the expression of p-ERK1/2 proteins in endometrial LE cells (Lee et al., 2014). These results suggest an important interplay among IFNT, ESR1, and OXTR through ERK1/2 pathways in endometrial LE cells in sheep. The underlying molecular pathways through which inhibition of ERK1/2 pathways reestablishes expression of OXTR and ESR1 in sheep are not presently known.

Earlier studies have proposed that IFNT suppresses ESR1 and OXTR through IRF2-dependent mechanisms in endometrial LE in sheep (Spencer et al., 2004, 1995c, 1998). However, the cell signaling pathways through which IFNT induces IRF-2 protein in endometrial LE are yet to be established. Our present results indicate that IFNT does not induce IRF-2 protein, and co-treatment of ERK1/2 inhibitor with IFNT does not modulate expression of IRF2 protein in endometrial LE cells on d 16 of the estrous cycle. Early pioneering work reported in one study that IFNT induced expression of IRF-2 protein in endometrial LE cells (Spencer et al., 1998); in contrast, the same researchers reported in their next study that IFNT did not induce expression of IRF-2 protein in endometrial LE in cyclic sheep (Choi et al., 2001). Taken together, our present results along with a previous study (Choi et al., 2001) suggest that IFNT suppresses ESR1 and OXTR through yet unidentified mechanisms independent of IRF-2 pathways in endometrial LE at the time of MRP.

In a later study, Bazer and coworkers reported that IFNT precludes binding of ESR1/SP1 with GC-rich regions of the OXTR promoter and thereby indirectly inhibits transcription of OXTR genes through ESR1/SP1 complex (Fleming et al., 2006). We have recently identified that IFNT activates JAK-SRC-EGFR-RAS-RAF-ERK1/2-EGR-1 novel signaling pathways in endometrial LE cells in vitro (Banu et al., 2010), and that IFNT increases pERK1/2 and EGR-1 proteins in endometrial LE in vivo in sheep. Both EGR-1 and SP1 are competing for the same GC-rich element, and EGR-1 acts as a tissue-specific transcriptional suppressor or activator of various genes under different physiological and pathological conditions (Raychowdhury et al., 2002; Al-Sarraj et al., 2005; Fernandez-Alvarez et al., 2008). It is possible that IFNT suppresses ESR-1 and OXTR though EGR-1 at the time of the MRP in ruminants; further functional studies are required for confirmation. By contrast, in cows, ESR1 is expressed in LE cells on d 12 to 14 of pregnancy. Whether suppression of ESR1 gene is required for IFNT to suppress OXTR gene has yet to be established in cows (Mann et al., 1999).

**Transport of IFNT from the Uterus to Ovary Through UOP at MRP in Ruminants**

Intrauterine infusions of IFNT in cyclic sheep and cows suppress endometrial pulses of PGF2α, and prevent luteolysis (Spencer et al., 1995a,b,c). Recent studies reported that infusion of IFNT (200 μg/d) into the uterine vein maintained functional CL in 80% of sheep up to 32 d through yet unidentified physiological mechanisms (Oliveira et al., 2008; Bott et al., 2010). As suggested by those authors, this could be due to increased intraluteal PGE2 biosynthesis or luteal immune cell activation but this end-point was not measured. In another study (Antoniazzi et al., 2013), the authors showed that infusion of 20 or 200 μg of IFNT into the uterine vein or 200 μg into the jugular vein protects the CL from the luteolytic action of PGF2α, irrespective of dose or route of administration in sheep. Further, that group of investigators concluded that IFNT is released from the uterus into the uterine vein and acts through an endocrine mechanism to induce ISG in the CL and thus delays luteolysis (Oliveira et al., 2008; Bott et al., 2010). However, pioneering work by Roberts and colleagues (Godkin et al., 1984) indicated that intrauterine infusion of 125I-labeled ovine trophoblast protein-1 (oTP-1, later named IFNT) into d-12 nonpregnant ewes is retained within the uterus. Only very small amounts of subunits of 125I oTP-1 but not intact 125I oTP-1 protein appeared to enter the maternal vasculature. We examined the transport of IFNT from the uterus to the ovary through the uterine vein-UOP-ovarian artery vascular route on d 12 to 16 of the estrous cycle and pregnancy in sheep. Western blot and ELISA (sensitivity of the assay was 1 ng/mL, IFNT antibody, a generous gift from Fuller W. Bazer, Texas A&M University) analyses indicate that IFNT protein is abundantly present in uterine flushing but it is not detectable in the utero-ovarian vein, ovarian artery, and CL on d 12 to 16 of pregnancy.

Hansen and colleagues, in their first report, used an antiviral assay that was not specific for IFNT (Oliveira et al., 2008). In their second report (Bott et al., 2010), they used rolIFNT antibody to preabsorb d 15 uterine venous blood samples but in their ELISA, they used type 1 IFN instead of rolIFNT as their antigen and standard. Thus, in these 2 studies, they measured type 1 IFN but not IFNT per se in their samples. In their third study (Romero et al., 2015), they used rolIFNT antibody and RIA, and detected IFNT in uterine venous...
blood of approximately 250 to 275 pg/mL (sensitivity of the assay was 148 pg/mL). The data sets on the presence of IFNT in uterine venous blood in sheep at the time of MRP from these research groups (Godkin et al., 1984; Oliveira et al., 2008; Bott et al., 2010; Lee et al., 2012b; Romero et al., 2015) do not agree with each other. The reasons for this discrepancy are most likely model, approach, methodology, and sensitivity of the assays used. It is possible that very small amount (0.2–0.3 ng/mL) of IFNT escapes from the gravid uterus into uterine venous blood at the time of MRP or ESP in sheep. It is estimated that daily IFNT production by a d-14 ovine conceptus is about 600 ng/l or 14.4 μg/d (Ashworth and Bazer, 1989). Compared with large amounts of IFNT retained inside the uterus, the physiological importance of a small amount of IFNT (0.2–0.3 ng/mL) released into the uterine venous blood during MRP or ESP still needs be established.

Early experiments involving anastomosis of the uterine vein to the ovarian artery from the pregnant to the nonpregnant uterine horn indicate that both luteolytic and luteoprotective mediators need to be transported from the uterine vein to the ovarian artery via the UOP in sheep and cattle (Mapletoft and Ginther, 1975; Mapletoft et al., 1975, 1976a,b; Ginther, 1981). Furthermore, embryo/conceptus transfer and hysterectomy experiments indicate that the luteolytic and luteoprotective mechanisms are locally mediated between uterus and CL of the ipsilateral ovary and do not act systemically in sheep (Moor and Rowson, 1964, 1966a,b; Rowson and Moor, 1967; Moor et al., 1969, 1970). Interferon tau is a relatively large protein (19 kDa) and it could not be transferred locally from the uterus to the ovary via the UOP. Hansen and colleagues failed to show the presence of IFNT protein in the ovarian arterial blood or corpus luteum per se or to confirm the transport of IFNT through uterine vein-UOP-ovarian artery vascular route in their series of studies (Oliveira et al., 2008; Bott et al., 2010; Romero et al., 2015). The local action of the embryonic antiluteolytic or luteoprotective effect does call into question the physiological relevance of any protein that is not locally transported from the gravid uterus to the CL on luteal maintenance in ruminants (Silvia and Niswender, 1986).

Infusions of roIFNT into uterine vein increased levels of ISG15 mRNA in the CL (Oliveira et al., 2008; Bott et al., 2010), suggesting that high levels of IFNT in the circulating blood may cause such an increase either directly or indirectly by downstream mediators. Previous studies from 2 different laboratories showed that subcutaneous (Spencer et al., 1999) and intramuscular (Chen et al., 2006) injections of 2 mg of roIFNT from d 11 to 17 of the estrous cycle increased expression of ISG15 mRNA in the CL in sheep, which supports the systemic action of IFNT on peripheral tissues. However, none of the systemic administration of roIFNT delivery induced an extended delay in return to estrus in sheep (Spencer et al., 1999; Chen et al., 2006), suggesting that expression of ISG15 is not associated with antiluteolytic action of IFNT.

In contrast, Hansen and colleagues recently reported (Antoniazzi et al., 2013) that infusion of IFNT (20 or 200 μg) into the uterine vein or administration of IFNT (200 μg) into the jugular vein, regardless of route or dose, effectively protects CL from the luteolytic actions of PGF2α by mechanisms that may involve luteal ISG and stabilization of cell survival genes. Further functional studies are required to resolve these conflicting findings on the role of ISG in luteal maintenance in sheep or cows. On the other hand, a recent study by Spencer and colleagues (Dorniak et al., 2011) indicated that IFNT increased ISG15 mRNA in the endometrium on d 10 to 14 of the estrous cycle and this effect was abolished by co-infusion of the COX-2 inhibitor meloxicam in sheep. These data suggest that IFNT increases expression of ISG15 mRNA in the endometrium indirectly through COX-2–derived PG, presumably PGE2. A recent study by Spencer et al. (2013) indicated that PG, including PGE2, directly increased ISG15 mRNA in the endometrium in cows and sheep. Thus, it is possible that uterine venous or systemic administrations of roIFNT may reach the ovary through the systemic circulation and interact with type 1 IFN receptors and either directly increase ISG15 mRNA or indirectly increase it by stimulating intraluteal PGE2 production, which was not measured by Hansen and colleagues in their studies (Oliveira et al., 2008; Bott et al., 2010; Antoniazzi et al., 2013). Thus, further work will be required to determine the physiological significance of uterine venous IFNT in sheep and mechanisms by which uterine venous administrations of IFNT maintain luteal structure and function.

**Source and Origin of a Luteoprotective Mediator During MRP in Ruminants**

In ruminants, the CL of early pregnancy is more resistant to the luteolytic action of PGF2α (Inskeep et al., 1975; Pratt et al., 1977; Nancarrow et al., 1982; Silvia and Niswender, 1984, 1986) on d 12 to 16, and the resistance is even greater when multiple embryos are present (Nancarrow et al., 1982). Injection of PGF2α into the ovarian artery or follicles of early pregnant sheep causes luteolysis in 28 or 17% of animals compared with 78 or 83% in nonpregnant sheep, respectively (Inskeep et al., 1975; Pratt et al., 1977). Exogenous estradiol at doses causing premature luteolysis in cyclic sheep is less effective in pregnant sheep (Kittok and Britt, 1977).
In hysterectomy (Anderson et al., 1969; Ginther, 1981; McCracken et al., 1999; Moor et al., 1970) and ovarian transplant models (McCracken et al., 1971, 1973, 1999) in sheep, the CL is maintained for >100 d, which is due to the absence of endometrial PGF2α pulses in the hysterectomy model and the failure of endometrial luteolytic PGF2α pulses to reach the ovary locally via UOP in the ovarian transplant model (Anderson et al., 1969; Moor et al., 1970; McCracken et al., 1971, 1973, 1999; Ginther, 1981). Although the CL is maintained in both of these models, it regresses readily to exogenous PGF2α (McCracken et al., 1971, 1973, 1999). In contrast, the CL in early pregnancy is resistant to exogenous PGF2α and the pregnancy is maintained (Inskeep et al., 1975; Mapleton and Ginther, 1975, 1976a; Silvia and Niswender, 1986). In sheep, secretion of PGE2 from the uterus into the uterine vein increases during the period of luteal resistance in early pregnancy on d 12 to 16 (Lewis et al., 1978, Ellinwood et al., 1979, Silvia et al., 1984; Silvia and Niswender, 1984, 1986; Rawlings and Hyland, 1985; Vincent and Inskeep, 1986; Vincent et al., 1986). Intraovarian administration of PGE2 at certain doses counteracts the luteolytic actions of PGF2α (Henderson et al., 1977). Prostaglandin E2 is secreted by both conceptus (Hyland et al., 1982; Lacroix and Kann, 1982; Charpigny et al., 1985) and endometrium in vitro (Ellinwood et al., 1979; Marcus, 1981; Lacroix and Kann, 1982) from pregnant ewes. Intrauterine or intraovarian infusions of PGE2 in nonpregnant ewes extend the interestrus interval and reduce luteal sensitivity to both endogenously secreted and exogenously administered PGF2α (Henderson et al., 1977, 1978, 1979; Magness et al., 1981; Reynolds et al., 1981). Similar effects of uterine infusion of PGE2 have been observed in cows (Reynolds et al., 1983). Prostaglandin E2 is a lipid-soluble mediator with a low molecular weight of 0.35 kDa and is structurally similar to PGF2α, which most likely can be transported locally from the uterus to the ovary (Silvia and Niswender, 1986) through PGT-mediated mechanisms in the UOP (Banu et al., 2003; Lee et al., 2010). As a stimulator of cAMP and a vasodilator, PGE2 has properties that are opposite to that of PGF2α (McCracken et al., 1999). Thus, PGE2 may be one of the factors that protect the CL from luteolysis during MRP or ESP in ruminants. The review by Weems et al. (2006) provides more detail on the role for PGE2 on luteal protection in ruminants.

**Selective and Preferential Transport of PGF2α and PGE2 from the Uterus to the Ovary Through the UOP During Luteolysis and MRP in Ruminants**

Endometrial production of PGF2α peaks on d 14 to 15 after estrus in both pregnant and nonpregnant sheep (Hooper et al., 1986; Zarco et al., 1988). Nonpregnant sheep display pulsatile release of PGF2α superimposed on a constant baseline, whereas pregnant sheep show increased continuous basal release of PGF2α (Zarco et al., 1988). During the period of MRP, one of the fascinating aspects is that pulsatile release of PGF2α is inhibited, whereas the basal concentration of PGF2α is increased in pregnant compared with nonpregnant sheep (Hooper et al., 1986; Zarco et al., 1988). We have shown that the pulsatile release of PGF2α is regulated by central oxytocin release and PGT-mediated transport, whereas the basal release of PGF2α is controlled by simple diffusion in sheep (Banu et al., 2008; Lee et al., 2013).

Concentrations of PGE2 and PGF2α are increased in uterine venous plasma at the time of ESP in sheep (Lewis et al., 1978; Ellinwood et al., 1979; Silvia et al., 1984; Silvia and Niswender, 1984, 1986; Rawlings and Hyland, 1985; Vincent and Inskeep, 1986). We determined the transport of PGE2 and PGF2α from the uterus to the ovary through the uterine vein-UOP-ovarian artery vascular route on d 12 to 16 of the estrous cycle and pregnancy in sheep (Lee et al., 2012b). The intrauterine concentration of PGF2α is significantly higher compared with that of PGE2 on d 16 of the estrous cycle and pregnancy. Importantly, ~85% of PGF2α is transported from the uterus to the uterine vein at the time of luteolysis, whereas only ~35% of PGF2α is transported from the uterus to the uterine vein at the time of MRP or ESP. These results indicate that PGF2α is secreted and transported from the endometrium to the uterine vein (endocrine secretion) at the time of luteolysis and from the endometrium to the uterine lumen (exocrine secretion) at the time of MRP or ESP in ruminants, as has been proposed in pigs (Bazer and Thatcher, 1977). It is possible that PGT (Banu et al., 2003, 2008, 2010), either individually or in coordination with other transporters such as MRP4 (Reid et al., 2003), could regulate this endocrine versus exocrine transport of PGF2α in ruminant endometrium. Functional studies are needed to understand this important mechanism.

Prostaglandin E2 is preferentially transported from the uterus to the ovary at the time of ESP. The concentration of PGE2 is higher in the uterine vein (~30 vs. ~0.5 ng/mL) and in the ovarian artery (~4 vs. ~0.5 ng/mL) on d 16 of pregnancy compared with that of the estrous cycle, respectively (Lee et al., 2012b). Our pres-
ent data are in agreement with several previous findings that secretion of PGE2 from the uterus into the uterine vein increases during the period of luteal resistance in early pregnancy in sheep (Lewis et al., 1978; Ellinwood et al., 1979; Silvia et al., 1984; Silvia and Niswender, 1984, 1986; Rawlings and Hyland, 1985; Vincent and Inskeep, 1986; Vincent et al., 1986). Transport of PGE2 from the uterus to the uterine vein and from the uterine vein to the ovarian artery appears to depend on its net concentration present in the uterine lumen and uterine vein, respectively. These results suggest that PGE2 is transported from the uterus to the ovary selectively or preferentially and not regulated by endocrine-exocrine secretory mechanisms at the time of luteolysis versus MRP or ESP in sheep.

Selective Luteal PGF2α and PGE2 Biosynthesis and Signaling During Luteolysis and MRP in Ruminants

We determined the regulation of PGF2α and PGE2 biosynthesis and signaling components in the CL on d 12 to 16 of the estrous cycle and pregnancy (Lee et al., 2012b). Our results indicate that the ratio between AKR1B1-PGFS and PGES-1 or PGES-3 is higher on d 14 to 16 of the estrous cycle. In contrast, the ratio between PGES-1 or PGES-3 and AKR1B1-PGFS is higher on d 14 to 16 of pregnancy (Lee et al., 2012b). To evaluate the functional aspects of these enzymatic changes, we measured luteal production and secretion of PGF2α and PGE2. The ratio of PGF2α to PGE2 in the ovarian venous plasma is higher on d 14 and 16 of the estrous cycle, whereas the ratio of PGE2 to PGF2α is increased on d 14 and 16 of pregnancy (Lee et al., 2012b). Analyses of luteal lysates indicate that intraluteal production of PGF2α is higher on d 16 of the estrous cycle, whereas intraluteal PGE2 production is increased on d 16 of pregnancy (Lee et al., 2012b). Data from the CL explant tissue cultures indicate that PGH2 is selectively converted to PGF2α on d 16 of the estrous cycle, whereas PGE2 is selectively converted to PGE2 on d 16 of pregnancy (Lee et al., 2012b). These results together indicate that luteal PG biosynthesis is selectively directed toward PGF2α at the time of luteolysis and toward PGE2 at the time of ESP. Our present results support previous reports that PGES-1 protein is highly expressed in the CL at the time of ESP in cows (Arosh et al., 2003), and that exogenous administration of PGF2α increases the ratio of PGES-1 to PGFS-AKR1B1 mRNA in luteal tissue on d 12 in pregnant compared with cyclic sheep (Costine et al., 2007).

Earlier studies reported that administration of PGF2α on d 11 or 12 of the estrous cycle induced expression of COX-2 mRNA in the luteal tissues at 1 to 4 h but it returned to basal level at 12 and 24 h in sheep (Tsai and Wiltbank, 1997, 1998). Our recent results indicate that COX-2 protein is constantly expressed in the CL on d 12 to 16 of the estrous cycle and pregnancy. Expression of COX-2 is required for both PGF2α and PGE2 biosynthesis. Whereas PGES-1 is functionally coupled to COX-2 and controls >90% of total PGE2 production, PGES-2 is preferentially coupled to constitutive COX-1, and PGES-3 is coupled to both COX-1 and COX-2 (Kudo and Murakami, 1999; Thorén and Jakobsson, 2000) depending on the need. Our results indicate that the COX-2-AKR1B1-PGFS pathway is involved in luteal PGF2α biosynthesis at the time of luteolysis; in contrast, the COX-2-PGES-1-PGES-3 pathway is involved in luteal PGE2 biosynthesis during the ESP (Lee et al., 2012b).

The net luteal production of PGF2α versus PGE2 is regulated not only by biosynthetic enzymes but also by the catabolic enzyme PGDH. Our results (Lee et al., 2012b) indicate that PGDH protein is highly expressed on d 12 to 16 of pregnancy but not on d 12 to 16 of the estrous cycle in sheep. To determine the functional aspects of luteal PGDH in vivo, we measured the concentrations of PGFM and PGEM in the ovarian venous plasma. The concentration of PGFM is higher on d 14 and 16 of pregnancy compared with that of the estrous cycle. Surprisingly, the concentration of PGEM is very low and does not appear to be regulated (Lee et al., 2012b). Three isoforms of PGDH are identified and these isoforms are differentially expressed in human placental tissues (McCracken et al., 2012) and their functions are not completely understood. The action of PGDH on PGF2α catabolism is well supported in the ovine CL (Silva et al., 2000); however, the action of PGDH on PGE2 catabolism is largely unknown. Our result suggests that the PGDH protein detected at 29 kDa in the ovine CL catalyzes only PGF2α into PGFM but not PGE2 into PGEM during early pregnancy. We believe that other PGDH isoforms yet to be characterized or discovered may control the catabolism of PGE2 to PGEM in the ovine CL. Our recent results support previous findings that PGDH mRNA expression is increased in the CL on d 13 to 14 of pregnancy compared with that of the estrous cycle in sheep, and this increase is critical for catabolism of PGF2α by the CL of early pregnancy (Silva et al., 2000). These results together indicate that intraluteal PGF2α production at the time of luteolysis is governed by increased ratios of AKR1B1-PGFS to PGES-1 and PGES-3, whereas the increased intraluteal PGE2 production at the time of ESP is governed by increased ratios of PGES-1 and PGES-3 to AKR1B1-PGFS and increased PGF2α catabolism by PGDH (Lee et al., 2012b).
Figure 2. Caption on facing page.
Figure 2. Working model on novel concepts on action of prostaglandins (PG) on luteal maintenance and maternal recognition of pregnancy (MRP) and establishment of pregnancy (ESP) in ruminants: (I) Luteolysis (panel A): (1) Oxytocin (OT) acts on its receptor OXTR, (2) activates Ca²⁺ and protein kinase C (PKC) pathways, (3) which in turn activates the endometrial PGF₂α, biosynthetic machinery, and (4) induces endometrial PGF₂α production. Luteolytic PGF₂α pulses are transported through (5) prostaglandin transport (PST)–dependent mechanisms and basal release of PGF₂α is mediated through (6) PGT–independent mechanism or simple diffusion from the endometrial epithelial cells to the corpus luteum (CL) through the utero-ovarian plexus (UOP). (Panel B) Endometrial luteolytic PGF₂α pulses (7) act on the PGF₂α receptor (FP) in large luteal cells, (8) activate Ca²⁺ and PKC pathways, (9) which in turn activates the luteal PGF₂α, biosynthetic machinery, and (10) induces intraluteal PGF₂α production, and (11) further auto-amplifies luteal PGF₂α production by autocrine and paracrine mechanisms. In addition, PGF₂α–FP signaling (12) suppresses survival pathways and (13) activates apoptotic pathways. (14) Importantly, endometrial PGF₂α production is suppressed at the time of luteolysis, which leads to (15) repressed PGF₂α–EP2/EP4 (PGE₂ receptor) signaling in large luteal cells, thus expediting (16) suppression of survival and (17) activation of apoptotic pathways in large luteal cells. Together, activation of FP and suppression of EP2/EP4 signaling (18) decrease progesterone (P₄) biosynthesis and secretion, (19) induce apoptosis of large luteal cells, which eventually culminates in (20) functional and structural luteolysis. II. MRP/ESP: (panel C) (21) Interferon-tau (IFNT) acts on its receptors IFNR1 and IFNR2 and (22) suppresses estrogen receptor α (ESR-1) and oxytocin receptor (OXTR) through (23) IFN regulatory factor-2 (IRF-2) or (24) specificity protein 1 (SP1) (sequential signaling cascades from IFNR to IRF2 or SP1 is yet to be confirmed). In parallel, IFNT activates (25) the IFNR-JAK-SRC-EGFR-RAS-RAF-ERK1/2-EGR1 module. (26) Early growth response protein 1 (EGR1) and SPI are competing for the same GC-rich elements, and EGR1 may compete or replace binding with SPI and suppresses OXTR (this mechanism yet to be confirmed). (27) ERK1/2 and EGR1 inhibits PGT function and (28) suppresses PGT-mediated transport of pulsatile release of PGF₂α from the endometrium to the CL through the UOP. Interestingly, (29) IFNT does not inhibit basal endometrial production of PGF₂α, which is transported by PGT-independent mechanism or simple diffusion from the endometrium to the UOP. It supports the increased basal concentration of PGF₂α (30) found in the UOP at the time of ESP. (31) PGF₂α acts on the large luteal cells but the CL of early pregnancy is resistant to both basal and luteolytic PGF₂α, activity. Concurrently, IFNT acts on (32) the endometrial epithelial cells and (33) stromal cells through multiple mechanisms, (34) increases net endometrial PGE₂ production, (35) which further auto-amplifies endometrial PGF₂α production through autocrine-paracrine EP2/EP4 signaling. (36) PGE₂ is transported from the endometrium to the CL through the UOP via PGT- or MRP4-mediated mechanisms. (Panel D) (37) Endometrial PGE₂ acts on the EP2/EP4 in the large luteal cells, activates cAMP/PKA and SRC pathways, and in turn auto-amplifies (38) intraluteal PGE₂ production via (39) autocrine and paracrine EP2/EP4 signaling. In parallel, EP2/EP4 signaling activates (40) antiapoptotic or survival pathways, (41) suppresses proapoptotic pathways, thus protecting the CL from structural luteolysis. PGE₂ through (42) cAMP/protein kinase A (PKA) and (43) SRC-ERK1/2 or SRC-AKT pathways may drive the (44) constitutive production of progesterone by the large luteal cells and thus protects the CL from functional luteolysis. Collectively, (45) IFNT or pregnancy-induced endometrial PGE₂ and luteal PGE₂ promote resistance of the CL against PGF₂α through multiple intracellular mechanisms and protect the CL from regression during MRP or ESP. Note: Considering the focus of this review, we are not able to include other important signaling pathways such as nitric oxide, endothelin, cytokines, and antioxidants in the large luteal cells. Given the complexity of the signaling network, we limited our model to large luteal cells for clarity and readability. Color version available online.

Biosynthesized luteal PGF₂α and PGE₂ need to be transported out of luteal cells and act through their specific receptors FP or EP, respectively, to produce their physiological effects. Prostaglandin transporter competitively transports PGF₂α and PGE₂ with equal affinity (Banu et al., 2003). Our results indicate that expression of PGT protein in the CL is not regulated by the estrous cycle or pregnancy on d 12 to 16. This constant expression of PGT suggests that the amount of PGT protein available to transport PGF₂α or PGE₂ from luteal cells may not differ between luteolysis and ESP.

Our results (Lee et al., 2012b) further indicate that expression of FP protein in the CL on d 12 to 16 is also not regulated by the estrous cycle or pregnancy. Activation of FP in turn activates PKC, IP₃, and Ca²⁺ cell signaling pathways (Narumiya et al., 1999) and interacts with multiple intracellular cell signaling pathways in the luteal cells in sheep and cows (Wiltbank et al., 1995; Anderson et al., 2001; Davis and Rueda, 2002). Our present results support previous findings that FP numbers or FP mRNA in the CL are similar in cyclic and early pregnant sheep and that the resistance of the CL of early pregnancy to exogenous PGF₂α is apparently not due to a change in FP receptor concentration (Wiepz et al., 1992).

In addition, our results indicate that luteal EP2- and EP4-mediated PGE₂ signaling is activated at the time of ESP, whereas it is suppressed or inhibited at the time of luteolysis (Lee et al., 2012b). Both EP2 and EP4 activate cAMP and PKA pathways. Expression of EP3 protein is marginally increased on d 12 and 14 of pregnancy compared with that of the estrous cycle. The 4 EP3 isoforms, EP3A, EP3B, EP3C, and EP3D, are produced by alternative splicing of the C-terminal, and show different efficiency in activation of the Gq, Gs, and Gi proteins and inhibition or stimulation of adenylate cyclase and cAMP (Narumiya et al., 1999). Activation of EP3A decreases cAMP, EP3B and EP3C increase cAMP, and EP3D decreases cAMP and increases IP₃ (Narumiya et al., 1999). The commercially available EP3 antibody used in our study recognized N-terminal but not C-terminal splice variants and thus specific EP3 isoforms were not detectable. However, a recent study in cows showed that expression of EP3B mRNA increased in the CL in response to PGE₂ and was associated with maintenance of the CL (Weems et al., 2012). Further, our results show that expression of the
EP1 protein in the CL is decreased on d 16 of pregnancy. Activation of EP1 activates PKC, IP3, and Ca\textsuperscript{2+} cell signaling pathways (Narumiya et al., 1999). The results suggest that luteal PGE\textsubscript{2} signaling is specifically directed toward cAMP-PKA pathways through EP2, EP4, and EP3B subtypes during the ESP in ruminants (Lee et al., 2012b; Weems et al., 2012). It is possible that EP2, EP4, and EP3B could activate or share the common cAMP-PKA intracellular pathways in the ovine CL. However, this is yet to be determined by receptor-specific functional studies.

**Selective Cell Survival and Apoptosis Signaling in the CL During Luteolysis and MRP in Ruminants**

We determined the regulation of important components of survival and apoptotic pathway networks in the ovine CL (Lee et al., 2015). We found that p-ERK1/2, p-AKT, β-catenin, p-NFκB, p-Src416, p-β-arrestin, and p-GSK3β signaling proteins are temporally suppressed in the CL from d 14 to 16 of the estrous cycle. By contrast at time of ESP, expression or activation of p-ERK1/2, p-AKT, β-catenin, p-NFκB, p-Src416, p-β-arrestin, and p-GSK3β proteins in the CL is sustained or increased on d 12 to 16 to maintain its function and structure. Our results further demonstrate that expression of Bcl-XL, Bcl2, Bad, and Bax proteins are not regulated in the CL on d 12 to 16 of the estrous cycle. In contrast, expression of cleaved (active) caspase-3 and apoptosis-induction factor (AIF) proteins are increased and x-linked inhibitor of apoptosis protein (XIAP) protein is decreased in the CL on d 16 of the estrous cycle. By contrast, expression of Bcl2, Bcl-XL, and XIAP proteins are sustained or increased, and activation of caspase-3 protein is suppressed in the CL on d 12 to 16 of pregnancy (Lee et al., 2015). These results together indicate that programmed suppression of cell survival pathways and programmed activation of caspase-3 dependent and independent intrinsic apoptotic pathways are required for luteolysis. Whereas, these multiple and redundant cell survival and antiapoptotic pathways need to be sustained and proapoptotic proteins need to be suppressed to rescue the CL from functional as well as structural luteolysis at the time of MRP or ESP (Lee et al., 2015). Prostaglandin E\textsubscript{2} acts as a mitogenic, antiapoptotic, angiogenic, steroidogenic, and immunomodulatory mediator in diverse systems (Cleveland, 2006; Wang and Dubois, 2006; Cha and Dubois, 2007; Eisinger et al., 2007). In addition, our results indicate that luteal EP2- and EP4-mediated PGE\textsubscript{2} signaling is activated at the time of ESP and suppressed or inhibited at the time of luteolysis (Lee et al., 2012b). Prostaglandin E\textsubscript{2} activates ERK1/2, AKT, β-catenin, and NFκB pathways in tumor cells (Castellone et al., 2005; Buchanan et al., 2006; Cha and DuBois, 2007) and various other cell types (Bamu et al., 2009). Thus, activation of EP2 and EP4 may be one of the critical mechanisms to protect the CL of early pregnancy from luteolytic challenges in ruminants. Our ongoing studies will identify interactions between PGE\textsubscript{2}, EP2/EP4 signaling and cell survival and apoptosis protein machinery in the CL during MRP or ESP in ruminants.

**Novel Concepts on PGE\textsubscript{2} Signaling on Luteal Maintenance During MRP in Ruminants**

In our ongoing studies, we find that IFNT increases expression of PGES-1 in endometrial epithelium in sheep. Intrauterine co-administration of IFNT and PGES-1 inhibitor restores expression of ESR-1 and OXTR, reestablishes endometrial PGF\textsubscript{2α} pulses, and causes luteolysis. By contrast, intrauterine co-administration of IFNT and PGES-1 inhibitor along with intraovarian administration of PGE\textsubscript{2} rescues the CL. These results suggest that, in addition to suppression of endometrial PGF\textsubscript{2α} pulses, IFNT may prolong the lifespan of the CL by increasing endometrial secretion of PGE\textsubscript{2}, which in turn increases luteal PGE\textsubscript{2} biosynthesis and signaling and thus promotes luteal resistance. We have reported that IFNT or early pregnancy increases expression of EP2 and EP4 in the endometrial LE on d 12 to 16 in sheep (Lee et al., 2012a). Interferon tau increases the concentration of PGE\textsubscript{2} in uterine flushing and utero-ovarian vein in sheep (Arosh et al., 2011). Intrauterine infusion of EP2 and EP4 inhibitors decreases IFNT production and constrains the elongation of the conceptus on d 16 of pregnancy in sheep (Nithy et al., 2011). These emerging studies together suggest possible interactions between IFNT and EP2/EP4 signaling in the endometrium in ruminants.

**CONCLUDING REMARKS AND IMPLICATIONS**

Accumulating information provides compelling evidence that PGE\textsubscript{2} produced by the CL in response to endometrial PGE\textsubscript{2} induced by IFNT or pregnancy may act as a luteoprotective mechanism and thus prolong the lifespan of the CL during MRP and ESP in ruminants. The potential mechanisms are depicted in Figure 2. Further functional studies are required to uncover these underlying molecular and cellular mechanisms. It is evident that antiluteolytic and luteoprotective mechanisms are complementary, and both mechanisms are required to rescue and protect the CL at the time of MRP and ESP. Deregression of either one of the mechanisms may cause premature functional as well as structural luteolysis and thus lead to a compromised pregnancy in ruminants. Targeting PGE\textsubscript{2} biosynthesis
and signaling selectively in the endometrium or CL is expected to provide luteoprotective therapy to improve reproductive efficiency in ruminants.

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