Cortisol and Interferon Tau Regulation of Endometrial Function and Conceptus Development in Female Sheep

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During early pregnancy in sheep, the elongating conceptus secretes interferon-τ (IFNT) and the conceptus as well as endometrial epithelia produce prostaglandins (PG) via PG synthase 2 (PTGS2) and cortisol via hydroxysteroid (11-β) dehydrogenase 1 (HSD11B1). Ovarian progesterone induces and PG and IFNT stimulates endometrial *HSD11B1* expression and keto-reductase activity as well as many epithelial genes that govern trophectoderm proliferation, migration, and attachment during elongation. The primary aim of these studies was to test the hypothesis that HSD11B1-derived cortisol has a biological role in endometrial function and conceptus development during early pregnancy in sheep. In study 1, cyclic ewes received vehicle, cortisol, PF 915275 (PF; a selective inhibitor of HSD11B1), cortisol and PF, meloxicam (a selective inhibitor of PTGS2), cortisol and meloxicam, recombinant ovine IFNT, or IFNT and PF into the uterus from day 10 to day14 after estrus. Cortisol and IFNT stimulated endometrial *HSD11B1* expression and activity, increased endometrial PTGS2 activity and the amount of PG in the uterine lumen, and up-regulated many conceptus elongation-related genes in the endometrium. Some effects of cortisol and IFNT were mediated by PTGS2-derived PG. In study 2, bred ewes received PF 915275 or recombinant ovine IFNT and into the uterus from day 10 to day 14 after mating. Inhibition of HSD11B1 activity in utero prevented conceptus elongation, whereas IFNT rescued conceptus elongation in PF-infused ewes. These results suggest that HSD11B1-derived cortisol mediates, in part, actions of ovarian progesterone and the conceptus on endometrial function and support the hypothesis that IFNT, PG, and cortisol coordinately regulate endometrial functions important for conceptus elongation and implantation during early pregnancy in sheep. **(*Endocrinology* 154: 931–941, 2013)**

Maternal support of blastocyst growth and development into an elongated conceptus (embryo/fetus and associated membranes) is critical for pregnancy recognition signaling and implantation in ruminants (1, 2). After hatching from the zona pellucida on day 8, the blastocyst develops into an ovoid or tubular form by days 11-12 and is then termed a conceptus (1, 3). The conceptus begins to elongate on day 12 and reaches up to 14 cm or more in length by day 16. Conceptus elongation requires secretions of the endometria produced by the luminal (LE) and glandular epithelia (GE) (4, 5). During early pregnancy in ruminants, endometrial epithelial gene expression and function are regulated primarily by progesterone from the ovarian corpus luteum and secreted factors from the elongating conceptus including interferon-τ (IFNT) and prostaglandins (PGs) (1, 6-10). IFNT exerts an antiluteolytic effect on the endometrium that maintains function of the corpus luteum for continued production of progesterone, the unequivocal hormone of pregnancy necessary for conceptus survival and growth (8).

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Abbreviations: CORT, cortisol; CST3, cystatin C; Ct, threshold cycle; CTSL, cathepsin L; CX, control; EIA, enzyme immunoassay; GE, glandular epithelia; GR, glucocorticoid receptor; GRP, gastrin-releasing peptide; HIF1A, hypoxia inducible factor-1α; HSD11B1, hydroxysteroid (11β) dehydrogenase 1; IFNT, interferon-τ; IGFBP1, IGF binding protein 1; LE, luminal epithelia; LGALS15, galectin-15; LSM, least squares means; MEL, meloxicam; PF, PF915275; PG, prostaglandin; PR, progesterone receptor; PTGS2, PG synthase 2; SLC, solute carrier; SPP1, secreted phosphoprotein 1.

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The combinatorial effects of ovarian progesterone, PGs, and IFNT on the endometrium result in specific changes in gene expression and thus the intrauterine milieu that govern conceptus elongation (for review see Refs 2, 7, and 11). Progesterone induces the expression of many elongation- and implantation-related genes in the endometrial LE and superficial GE between days 10 and 12 after estrus or mating in both pregnant and nonpregnant ewes that encode intracellular enzymes such as prostaglandin G/H synthase and cyclooxygenase 2 [(PG synthase 2 (PTGS2)] and hydroxysteroid (11β) dehydrogenase 1 (HSD11B1), transcription factors [hypoxia inducible factor-1α (HIF1A), HIF2A], migration, and attachment proteins [galectin-15 (LGALS15)], IGF binding protein 1 (IGFBP1), a protease [cathepsin L (CTSL)], a protease inhibitor [cystatin C (CST3)], a cell proliferation factor [gastrin releasing peptide (GRP)], glucose transporters [solute carrier (SLC) family 2 member A1, SLC5A1], and amino acid transporters (SLC1A5, SLC7A2). In the endometrial GE, progesterone induces expression of several genes between days 12 and days 14-16, including a cell proliferation factor (*GRP*), adhesion protein [secreted phosphoprotein 1 (*SPP1*)], and a glucose transporter (*SLC5A11*). The timing of their expression and biological effects of those progesterone-induced genes implicate them as regulators of conceptus elongation and implantation (11), and they are also increased by IFNT (2, 7, 12, 13) as well as PTGS2-derived PGs (9, 14). Indeed, inhibition of PTGS2 activity in utero by meloxicam prevents conceptus elongation during early pregnancy in sheep (9).

In the ovine uterus, HSD11B1 acts predominantly as a keto-reductase-generating biologically active cortisol from the biologically inactive cortisone (14). In sheep, PG stimulate endometrial *HSD11B1* expression and keto-reductase activity, which increases cortisol in the uterine lumen, and the elongating conceptus also regenerates cortisol from cortisone during early pregnancy (14). Prostaglandins also regulate activity of HSD11B1 in the endometrium of cattle (15). In the ovine placenta, PGs stimulate HSD11B1 activity and glucocorticoids enhance PG synthesis by up-regulating expression and activity of 2 important enzymes, phospholipase A2 and PTGS2, thereby establishing a positive feed-forward loop implicated in the timing of parturition (16). Endometrial *HSD11B1* expression is markedly increased by endometrial PGs and IFNT and PGs from the conceptus during early pregnancy (14, 17), and the glucocorticoid receptor (GR) is present in cells of the uterus and conceptus (14, 17). The primary aim of these studies was to test the hypothesis that HSD11B1-derived cortisol has a biological role in endometrial function and conceptus development during early pregnancy in sheep.

Materials and Methods

Experimental design

Mature Rambouillet female ewes (*Ovis aries*) were checked daily for onset of estrus (designated as day 0 of the estrous cycle or pregnancy) using a vasectomized ram. All experimental and surgical procedures were approved by the Institutional Animal Care and Use Committee of Washington State University.

Study 1

Ewes (n = 40) were detected for estrus using a vasectomized ram. Using a surgical approach described previously (9, 14), ewes on day 10 after estrus were subjected to a midventral laparotomy, and the lumen of each uterine horn received a vinyl catheter (0007760; Durect Corp, Cupertino, California) connected to an Alzet 2ML1 osmotic pump (Durect Corp) secured within the infundibulum of the oviduct and mesovarium. Ewes (n = 5 per treatment) received pumps containing the following: 1) 2 mL of vehicle as a control [CX; 2% ethanol (vol/vol) in saline]; 2) 156 ng cortisol (CORT) in 2 mL of vehicle; 3) 840 ng PF915275 (PF) (Tocris Bioscience, Bristol, United Kingdom) in 2 mL of vehicle; 4) CORT+PF; 5) 1225 ng meloxicam (MEL) (Boehringer Ingelheim Pharamaceuticals, Ridgefield, Connecticut); 6) CORT+MEL; 7) 101 μg recombinant ovine IFNT (10^7 antiviral units); or 8) IFNT+PF. The Alzet 2ML1 osmotic pump has a reservoir volume of 2 mL and a pumping rate of 10 μL/h for 7 days. The amount of cortisol pumped into the uterine lumen on a daily basis (23 ng) mimics cortisol production by an elongating day 14 ovine conceptus (14). PF is a potent and selective HSD11B1 inhibitor that displays little activity for HSD11B2 (18, 19). Meloxicam is a partially selective PTGS2 inhibitor (20) that is 13 times more inhibitory for PTGS2 than PTGS1 and effective in sheep (21-26).

Recombinant ovine IFNT was prepared as described previously (27), and the amount of recombinant IFNT pumped into the uterine lumen on a daily basis (14.4 μg) mimics IFNT production by an elongating day 14 ovine conceptus, which is 600 ng/h (28). Our previous studies found that infusion of that amount of IFNT in the uterine lumen each day mimics effects of the conceptus on endometrial expression of hormone receptors and IFNT-stimulated genes during early pregnancy in ewes (9, 29). At necropsy on day 14 after estrus, the female reproductive tract was excised, and the uterine lumen carefully flushed with 20 mL of 10 mM Tris (pH 7.2). The volume of the uterine flushing recovered from the lumen was measured and recorded, and then the flush was clarified by centrifugation (3000 $\times$ *g* at 4°C for 15 minutes). The supernatant was carefully removed with a pipette, aliquoted, frozen in liquid nitrogen, and stored at −80°C. Several sections (~0.5 cm) from the midportion of each uterine horn were fixed in fresh 4% paraformaldehyde in PBS (pH 7.2). After 24 hours, fixed tissues were changed to 70% ethanol for 24 hours and then dehydrated and embedded in Paraplast-Plus (Oxford Labware, St Louis, Missouri). The remaining endometrium was physically dissected from myometrium, frozen in liquid nitrogen, and stored at −80°C for subsequent RNA extraction.

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Study 2

Ewes (n = 15) were mated at estrus (day 0) to rams of proven fertility. On day 10 after mating, ewes were subjected to a midventral laparotomy, and the lumen of each uterine horn received a vinyl catheter connected to an Alzet 2ML1 osmotic pump that was implanted as described in study 1. Ewes (n = 5 per treatment) received pumps containing PF (Tocris Bioscience) in vehicle [840 ng of PF in 2 mL of 2% ethanol (vol/vol) in saline] or recombinant ovine IFNT (101 μg) and PF (IFNT+PF) in 2 mL of vehicle. The remaining ewes (n = 5) were not implanted with osmotic pumps to provide tissues from an untreated pregnant uterus. All the ewes were necropsied on day 14 after mating to obtain the uterine flushings, and the uteri were processed as described for study 1.

Total RNA isolation and real-time PCR analysis

Using methods described previously (9), total RNA was isolated from endometrial samples and reverse transcribed and analyzed by real-time PCR using an ABI Prism 7900HT system (Applied Biosystems, Foster, California) with Power SYBR Green PCR master mix (Applied Biosystems). Specific oligonucleotide primers were designed by the Oligo 7 program (Molecular Biology Insights, Inc, Cascade, Colorado) (14) (see Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Primer specificity and efficiency (−3.6 > slope > −3.1) were confirmed using a test amplification run. Each individual sample was run in triplicate under the following conditions: 50°C for 2 minutes; 95°C for 10 minutes; 95°C for 15 seconds; and 60°C for 1 minute for 40 cycles. A dissociation curve was generated at the end of amplification to ensure that a single product was amplified. PCR without template or template substituted with total RNA was used as a negative control to verify experimental results. The threshold line was set in the linear region of the plots above the baseline noise, and threshold cycle (Ct) values were determined as the cycle number at which the threshold line crossed the amplification curve. Ovine *GAPDH* was used as the reference gene.

Bioassay of HSD11B1 activity in the endometrium

Endometrial HSD11B1 keto-reductase activity was quantified by measuring the net rate of conversion of cortisone to cortisol using methods described previously (14). Briefly, pieces of endometrium (150 mg) were placed in a 10-cm culture dish with 6 mL MEM (Sigma-Aldrich Co, St Louis, Missouri) containing [0.1% (wt/vol)] BSA (Sigma), penicillin (100 U/mL), and streptomycin (100 μg/mL). Endometrial explants were treated with vehicle as a control or cortisone (3 μM final). To examine nonspecific interconversion, cortisone (3 μM final) was added to the medium in the absence of tissue (blank). Endometrial explants and blanks, each in triplicate, were placed on the orbital shaker within the incubator at 37°C in a humidified atmosphere of 5% carbon dioxide in air for 4 hours. The medium was then collected and stored at −20°C until assayed for cortisol using a single antibody-charcoal separation assay described previously (14, 30). Counts per minute were obtained from a liquid scintillation spectrophotometric β-counter (LS 6500; Beckman Coulter, Indianapolis, Indiana). Radioimmunoassay data were processed using Assay Zap software (Biosoft, Cambridge, United Kingdom). The detection limit for cortisol was 15.6 pg/mL, and the intra- and interassay coefficients of variation were 4.1% and 7.0%, respectively. Rabbit anticortisol antiserum (Pantex, Santa Monica, California) was used with a cross-reactivity of 60%, 48%, 0.01%, and 0.01% with corticosterone, deoxycorticosterone, progesterone, and estradiol, respectively. The rate of conversion of cortisone to cortisol is expressed as picogram cortisol per milligram of tissue per 4 hours and represents HSD11B1 activity in the endometrium.

Enzyme immunoassay of cortisol in the uterine flush

A sensitive enzyme immunoassay (EIA) kit from Cayman Chemical (500360; Ann Arbor, Michigan) was used according to the manufacturer's recommendations to determine the amount of cortisol in uterine flushings. Assay sensitivity for cortisol was 6.6 pg/ml and the intra- and interassay coefficients of variation were 3.2% and 6.7%, respectively. The data are expressed as total amount of cortisol in the uterine lumen determined by multiplying assay results by the recovered volume of the uterine flushing.

Enzyme immunoassay of prostaglandins in the uterine flush

An EIA kit (catalog no. 514012; Cayman Chemical) was used to measure PGs in the uterine flush and measured according to the manufacturer's recommendations. This assay determines the relative amount of total PG in samples using an antiserum that exhibits high cross-reactivity for most PGs, which allows quantification of all the PGs in a given sample with a single assay. Assay sensitivity was 15.6 pg/mL, and the intra- and interassay coefficients of variation were 2.8% and 5.4%, respectively. The data are expressed as total amount of PG in the uterine lumen, which was determined by multiplying assay results by the recovered volume of uterine flushing.

Assay of PTGS2 activity in the endometrium

Endometrial tissue (1 g) was placed in ice-cold buffer containing 100 mM Tris-HCl (pH 7.5) and cOmplete Protease Inhibitor Cocktail (catalog no. 05892791001; Roche Applied Science, Indianapolis, Indiana) and then homogenized for 2 minutes using a tissue homogenizer. Homogenates were then clarified by centrifugation (10,000 × *g* for 15 minutes at 4°C). The protein content in the supernatant was determined using a Bradford protein assay (Bio-Rad Laboratories, Burlingame, California). The supernatant was aliquoted and then stored at −80°C. Endometrial PTGS2 activity was measured using a cyclooxygenase colorimetric activity assay (catalog no. 760151; Cayman Chemical) performed according to the manufacturer's recommendation. The intra- and interassay coefficients of variation were 4.9% and 7.3%, respectively. The data are expressed as PTGS2 activity in nanomoles per minute.

Statistical analysis

All quantitative assay data were subjected to least-squares ANOVA using the general linear models procedures of the Statistical Analysis System (SAS Institute Inc, Cary, North Carolina). For analysis of the real-time PCR data, the Ct values of the target mRNA was analyzed for the effects of treatment with the *GAPDH* reference gene values used as a covariate. In study 1,

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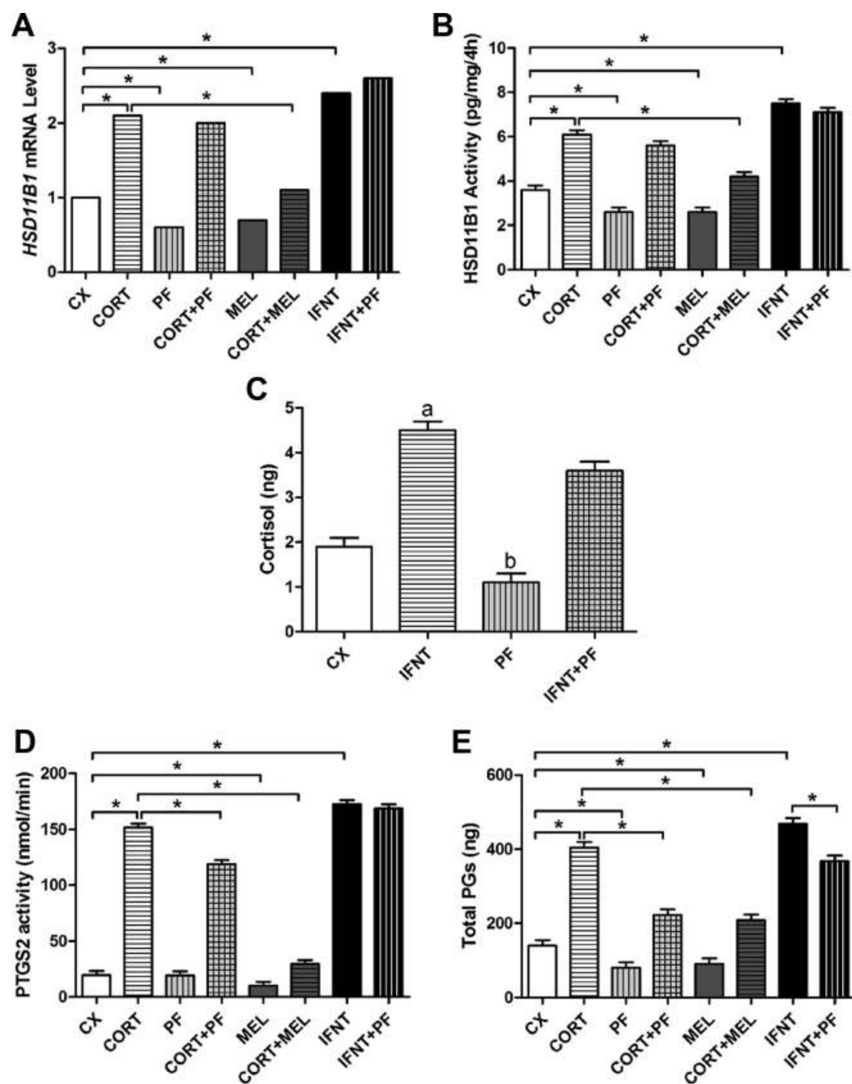

Figure 1. Effects of intrauterine treatments on endometrial HSD11B1 mRNA abundance, HSD11B1 keto-reductase activity, and PTGS2 activity and the amount of cortisol and prostaglandins in the uterine lumen. Cyclic ewes received intrauterine infusions of vehicle as a CX, CORT, PF (HSD11B1 inhibitor), CORT and PF, MEL (PTGS2 inhibitor), CORT and MEL, recombinant ovine IFNT, or IFNT and PF from day 10 to day 14 after estrus. A, Endometrial *HSD11B1* mRNA was measured by real-time PCR and expressed as fold change relative to CX ewes. B, Endometrial HSD11B1 keto-reductase activity was measured by determining the ability of explants to generate cortisol from cortisone. C, The quantity of cortisol in the uterine lumen was measured using an enzyme immunoassay. Data are presented as total amount (nanograms) in the uterine lumen. D, Endometrial PTGS2 activity was measured using a bioactivity assay of extracts. E, The quantity of total PGs in the uterine lumen was measured using an EIA. Data are presented as total amount (nanograms) in the uterine lumen. Differences are denoted as follows: *, $P < .01$.

orthogonal contrasts were used to determine effects of treatment (CORT vs CX, PF vs CX, CORT+PF vs CORT, MEL vs CX, CORT+MEL vs CORT, IFNT vs CX, and IFNT+PF vs IFNT). In all analyses, error terms used in the tests of significance were identified according to the expectation of the mean squares for error. Significance ($P < .10$) was determined by the probability differences of the least squares means (LSM). With the exception of real-time PCR, data are presented as LSM with SE. Real-time PCR data are presented as fold change relative to the mRNA levels in the CX samples calculated using the LSM Ct values from the statistical analysis.

Results

Cortisol increases endometrial *HSD11B1* expression and HSD11B1 and PTGS2 activity and amount of PG in the uterine lumen (study 1)

Study 1 was conducted to determine the effects of cortisol and IFNT on endometrial function and determine the extent to which the actions of cortisol were mediated by HSD11B1 or PTGS2 activity and those of IFNT by HSD11B1 activity. Infusion of cortisol into the uterus of cyclic ewes increased ($P < .01$, CX vs CORT) *HSD11B1* mRNA by 2.1-fold (Figure 1A) and HSD11B1 keto-reductase activity by 1.7-fold (Figure 1B) in the endometrium. Infusion of PF 915275, a selective HSD11B1 inhibitor, decreased ($P < .05$, CX vs PF) *HSD11B1* mRNA and HSD11B1 activity in the endometrium as well as cortisol in the uterine lumen (Figure 1C). However, neither endometrial *HSD11B1* mRNA nor HSD11B1 activity was different in ewes treated with CORT and PF as compared with those receiving CORT alone.

Infusion of cortisol into the uterus substantially increased ($P < .01$, CX vs CORT) endometrial PTGS2 activity and PG in the uterine lumen by 7.7- and 2.9-fold, respectively (Figure 2A). Intrauterine infusion of PF 915275 alone did not affect endometrial PTGS2 activity but decreased ($P < .05$) total PG in the uterine lumen. Furthermore, infusion of the PF 915275 along with CORT diminished ($P < .01$, CORT vs CORT+PF) the effects of cortisol on endometrial PTGS2 activity, and ewes infused with CORT and PF had less ($P < .01$) total PG in the uterine lumen compared with ewes infused with CORT alone (Figure 2, D and E).

Intrauterine infusion of MEL, a selective PTGS2 inhibitor, reduced ($P < .01$, CX vs MEL) PTGS2 activity in the endometrium as well as total PG in the uterine lumen (Figure 2, D and E). Intrauterine infusion of MEL decreased ($P < .05$, CX vs MEL) endometrial *HSD11B1*

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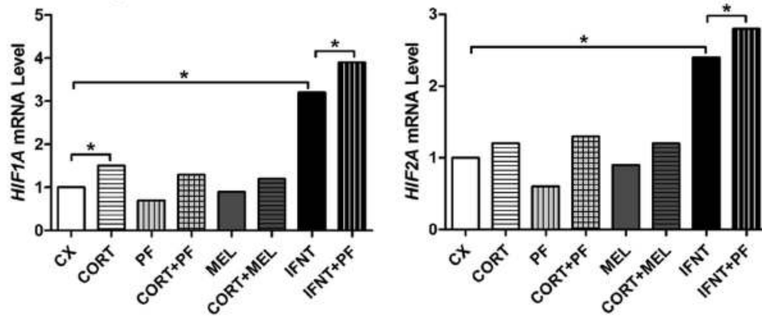
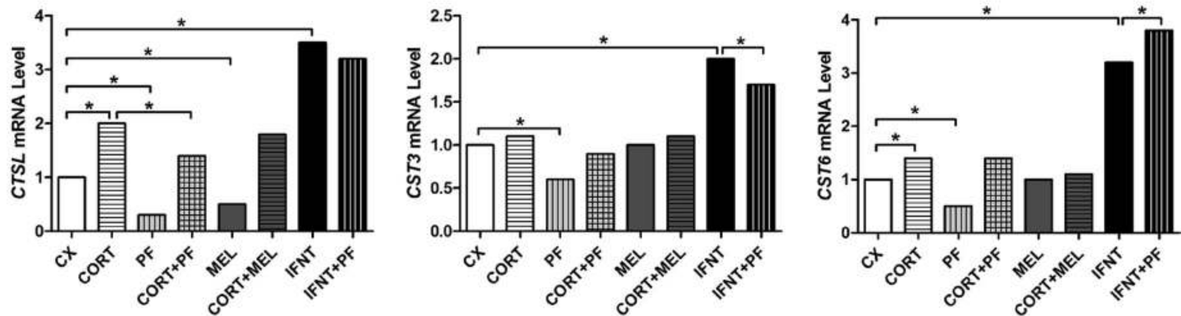
Transcription factors



Extracellular protease and protease inhibitor



Secreted cell proliferation, migration and attachment factors

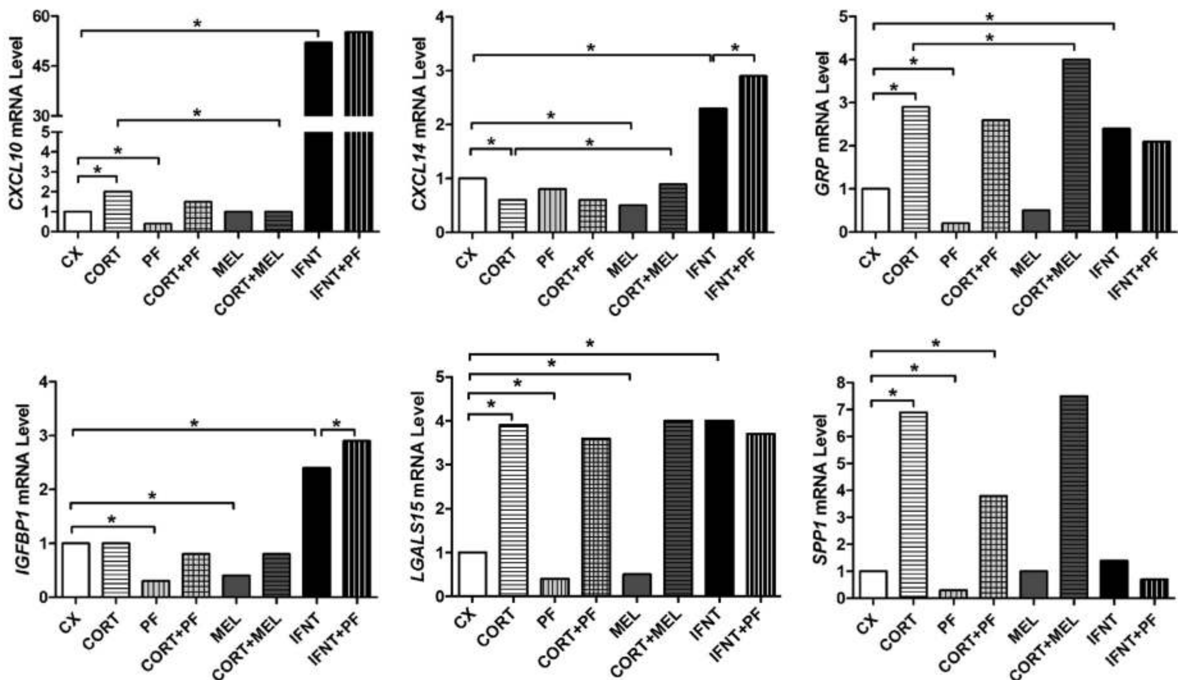


Figure 2. Effects of intrauterine treatment on expression of transcription factors, extracellular proteases and their inhibitor, and mediators of cell proliferation, migration, and attachment in the endometrium of cyclic sheep. Cyclic ewes received intrauterine infusions of vehicle as a CX, CORT, PF (HSD11B1 inhibitor), CORT and PF, MEL (PTGS2 inhibitor), CORT and MEL, recombinant ovine IFNT, or IFNT and PF from day 10 to day 14 after estrus. Endometrial mRNA abundance was measured by real-time PCR and expressed as fold change relative to CX ewes. Differences ($P < .05$) are denoted with an asterisk (*) for the different treatments determined by orthogonal contrast.

mRNA by 1.4-fold (Figure 1A) and HSD11B1 activity by 20% (Figure 1B). Infusion of MEL diminished ($P < .01$, CORT vs CORT+MEL) the stimulatory effects of cortisol on *HSD11B1* mRNA and HSD11B1 activity (Figure 1, A and B). Furthermore, infusion of MEL diminished ($P < .01$, CORT vs CORT+MEL) the effects

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of cortisol on endometrial PTGS2 activity and the amount of PG in the uterine lumen (Figure 1, D and E). Collectively, these results support the ideas that cortisol increases total PGs in the uterine lumen via effects on both PTGS2 as well as HSD11B1 in the endometrium.

IFNT regulates HSD11B1 expression and activity in the endometrium (study 1)

Infusion of IFNT into the uterus increased ($P < .01$, CX vs IFNT) *HSD11B1* mRNA (Figure 1A) and HSD11B1 activity (Figure 1B) in the endometrium as well as cortisol in the uterine lumen (Figure 1C). However, infusion of PF 915275, the HSD11B1 inhibitor, had no effect on IFNT stimulation of endometrial *HSD11B1* expression or HSD11B1 activity or cortisol in the uterine lumen (Figure 1, A–C). Infusion of IFNT into the uterus substantially increased ($P < .01$, CX vs IFNT) PTGS2 activity in the endometrium and the amount of PG in the uterine lumen (Figure 1, D and E). Although endometrial PTGS2 activity was not different in the endometrium of IFNT- and IFNT+PF-infused ewes (Figure 1D), total PGs in the uterine lumen were about 22% lower ($P < .05$) in IFNT- and PF-infused ewes as compared with ewes infused with IFNT alone (Figure 1E).

Cortisol and IFNT effects on expression of elongation- and implantation-related genes in the endometrium (study 1)

As compared with the endometrium of CX ewes, intrauterine infusion of cortisol increased ($P < .05$) the expression of *HIF1A*, *CTSL*, *CST6*, *CXCL10*, *GRP*, *LGALS15*, *SPP1*, *SLC2A1*, *SLC2A5*, *SLC2A12*, and *SLC1A5* (Figs. 2 and 3). Intrauterine infusion of PF 915275 decreased ($P < .05$) expression of *HIF2A*, *CTSL*, *CST3*, *CST6*, *CXCL10*, *GRP*, *IGFBP1*, *LGALS15*, *SPP1*, *SLC2A12*, and *SLC1A5*. However, coinfusion of the PF 915275 inhibitor along with cortisol decreased cortisol stimulation of only *CTSL*, *LGALS15*, and *SPP1* ($P < .05$,

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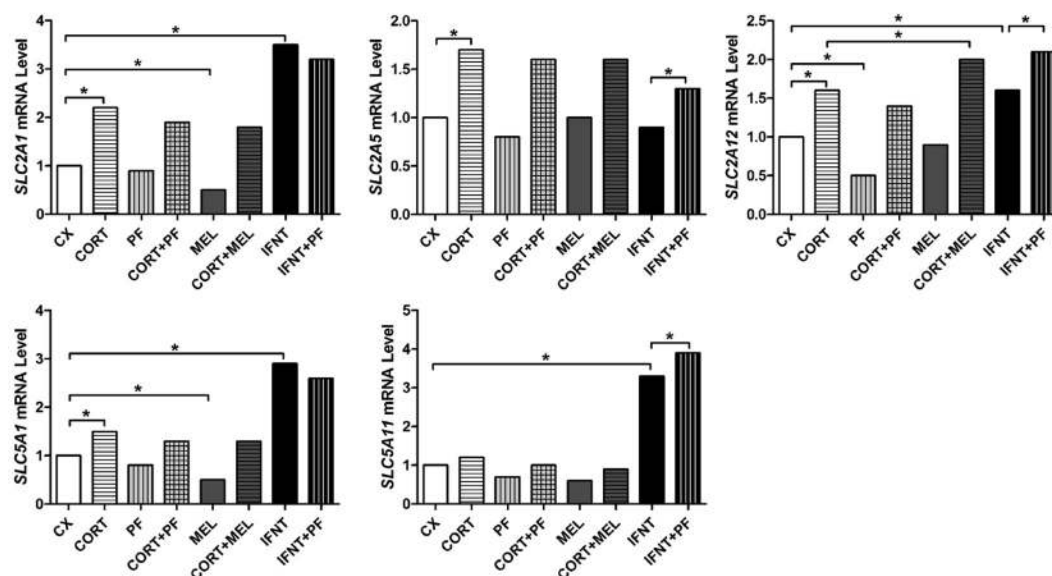

Figure 3. Effects of intrauterine treatment on expression of glucose and amino acid transporters in the endometrium of cyclic sheep. Cyclic ewes received intrauterine infusions of vehicle as a CX, CORT, PF (HSD11B1 inhibitor), CORT and PF, MEL (PTGS2 inhibitor), CORT and MEL, recombinant ovine IFNT, or IFNT and PF from day 10 to day 14 after estrus. Endometrial mRNA abundance was measured by real-time PCR and expressed as fold change relative to CX ewes. Differences ($P < .05$) are denoted with an asterisk (*) for the different treatments determined by orthogonal contrast.

CORT+PF vs PF alone). Intrauterine infusion of the PTGS2 inhibitor, MEL, decreased ($P < .05$, CX vs MEL) the expression of *CTSL*, *CXCL14*, *GRP*, *IGFBP1*, *LGALS15*, *SLC2A1*, *SLC5A1*, *SLC5A11*, and *SLC7A2* in the endometrium. However, coinfusion of MEL along with cortisol decreased ($P < .05$, CORT vs CORT+MEL) only cortisol stimulation of *CXCL10*.

Intrauterine infusion of IFNT increased ($P < .05$, CX vs IFNT) expression of *HIF1A*, *HIF2A*, *CTSL*, *CST3*, *CST6*, *CXCL10*, *CXCL14*, *GRP*, *IGFBP1*, *LGALS15*, *SLC1A5*, *SLC2A1*, *SLC2A12, SLC5A1, SLC5A11,* and *SLC7A2* in the endometrium (Figs. 2 and 3). Coinfusion of the PF 915275 inhibitor somewhat decreased ($P < .05$, IFNT+PF vs IFNT) IFNT stimulation of *CST3* and somewhat increased IFNT stimulation of *HIF1A*, *HIF2A*, *CST6*, *CXCL14*, *IGFBP1*, *SLC2A5*, *SLC2A12*, *SLC5A11*, *SLC1A5*, and *SLC7A2*.

Intrauterine infusion of a HSD11B1 inhibitor prevents conceptus elongation, reduces endometrial HSD11B1 and PTGS2 activity, and reduces cortisol and PG in the uterine lumen (study 2)

This study tested the hypothesis that HSD11B1-derived cortisol from the conceptus and/or endometrium regulates conceptus elongation during early pregnancy in sheep. Ewes were infused with PF 915275, a selective inhibitor of HSD11B1, or PF 915275 and recombinant ovine IFNT from day 10 to day 14 of early pregnancy. Ovoid conceptuses of 0.5 cm (n = 4) or 1 cm (n = 1) in length were found in uteri of ewes infused with PF 915275, a selective inhibitor of HSD11B1 (Figure 4). In contrast, fully elongated conceptuses of 12-14 cm in length (n = 5) were recovered from ewes coinfused with PF and pregnancy levels of IFNT (Figure 4) as well as from untreated pregnant control ewes (n = 5) (data not shown).

As summarized in Table 1, activities of endometrial HSD11B1 and PTGS2 enzymes were substantially lower ($P < .05$), respectively, in ewes receiving intrauterine infusion of PF as compared with ewes infused with PF+IFNT or day 14 pregnant control ewes (PX). Furthermore, cortisol and total PG were lower ($P < .05$) in the uterine lumen of PF-treated ewes as compared with values for the IFNT+PF-infused ewes or day 14 pregnant control ewes. In contrast, coinfusion of PF along with IFNT had no effect on either endometrial HSD11B1 and PTGS2 activities or amounts of cortisol and PG in the uterine lumen when compared with day 14 pregnant control ewes.

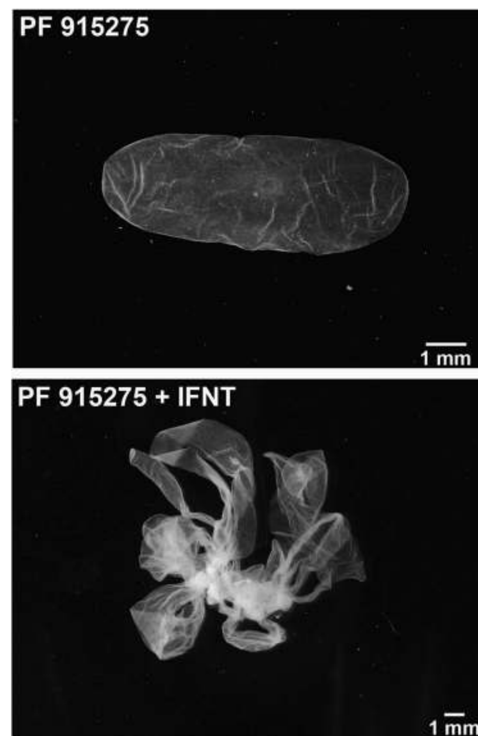

Figure 4. Inhibition of HSD11B1 activity in utero inhibits conceptus development. Ewes (n = 5 ewes per treatment) received intrauterine infusions of PF, a selective HSD11B1 inhibitor, or recombinant ovine IFNT and PF (IFNT+PF) from day 10 to day 14 after mating. Ovoid conceptuses of 0.5 cm (n = 4) or 1 cm (n = 1) in length were found in uteri of ewes infused with PF, a selective inhibitor of HSD11B1. In contrast, fully elongated conceptuses of 12-14 cm in length (n = 5) were recovered from ewes coinfused with PF and IFNT.

Discussion

Results of these studies support our central hypothesis that ovarian progesterone and factors from the endometrium (PG and cortisol) and conceptus (IFNT and PG) coordinately regulate conceptus elongation during early pregnancy in ewes. Most importantly, the present studies provide novel insights into the biological roles of HSD11B1 and cortisol during early pregnancy. We first identified HSD11B1 as a progesterone-regulated gene in the endometrium that was implicated in conceptus elongation (31). Ovarian progesterone is critical for conceptus survival and growth in sheep because conceptus survival is compromised in early pregnant ewes treated with mifepristone (RU486), a progesterone receptor (PR) and GR antagonist, from day 8 to day 12 after mating (32). Indeed, increasing the circulating levels of progesterone immediately after ovulation and mating accelerates conceptus elongation in sheep and cattle via effects on the endometrium (32-34). Progesterone induces the expression of many genes in the endometrial LE/superficial GE of the ovine uterus between day 10 and day 12 in both cyclic and pregnant ewes (for review see Refs 2, 7, 11, and 12), including *HSD11B1* and *PTGS2* (17, 31, 35).

Results of study 1 suggest that HSD11B1-derived cortisol, a biologically active glucocorticoid, mediates, in part, actions of ovarian progesterone on the endometrium.

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TABLE 1. Effects of PF and IFNT infusion on HSD11B1 and PTGS2 enzyme activities in the endometrium and amount of cortisol and prostaglandin in the uterine lumen (study 2)

	Endometrial Enzyme Activity		Uterine Lumen	
Treatment	HSD11B1 (pg/mg per 4 h)	PTGS2 (nmol/min)	Cortisol (ng)	Prostaglandin (ng)
PF	3.3[a]	257.1[a]	1.6[a]	369.3[a]
IFNT+PF	10.6	470.6	9.1	668.7
Day 14 PX[b]	11.3	521.1	8.5	643.3
SE	0.5	5.4	0.4	55.6

[b] PF vs IFNT+PF and PF vs day 14 PX ($P < .05$).
[a] Endometria from day 14 pregnant (PX) ewes.

Glucocorticoid receptors are abundant in all endometrial cell types during the estrous cycle and early pregnancy as well as the conceptus trophectoderm in sheep (17). Inhibition of HSD11B1 activity in the endometria of cyclic ewes reduced expression of many progesterone-induced epithelial genes, including *CTSL*, *CST3*, *CST6*, *GRP*, *HSD11B1*, *IGFBP1*, *LGALS15*, *SLC1A5*, *SLC2A12*, and *SPP1* that are implicated in governing conceptus elongation. In study 1, intrauterine infusion of cortisol stimulated expression of a number of those genes, including *HSD11B1*, even when coinfused with the PF 915275 HSD11B1 inhibitor; thus, these genes are likely directly regulated by cortisol via the GR. Indeed, the promoter region of the human *HSD11B1* gene contains glucocorticoid response elements (36). In addition to being an antiprogestin, RU486 is a high-affinity antagonist of both PR and GR (37), and loss of expression of GR as well as *HSD11B1* mRNA occurred in endometria of ewes treated with RU486 (17). The present study investigated a targeted set of known genes expressed specifically in the endometrial epithelia during the cycle and early pregnancy, but comprehensive transcriptome studies are needed to identify novel glucocorticoid-regulated genes, given that GR is expressed in all other cell types of the endometrium, including resident immune cells, as well as the conceptus trophectoderm (17).

Results of study 1 found that endometrial PTGS2 activity and PG production are regulated by cortisol in the ovine uterus. This tissue-specific stimulatory action of cortisol on PG synthesis is contrary to the classical concept that glucocorticoids exert antiinflammatory effects on immune cells in inflamed and wounded tissues by suppressing PG production (37). However, in the ovine placenta, glucocorticoids increase PTGS2 activity and decrease expression of hydroxyprostaglandin dehydrogenase 15-(nicotinamide adenine dinucleotide) or hydroxyprostaglandin dehydrogenase that inactivates PG, thereby increasing PG synthesis and secretion (36). We reported recently that PTGS2-derived PG from the endometrium mediate, in part, effects of progesterone on *HSD11B1* expression and that intrauterine infusion of PG, including PGE2, PGF2α, and PGI2, increase endometrial *HSD11B1* expression and keto-reductase activity, resulting in higher levels of cortisol in the lumen of the ovine uterus (11). Similarly, PG stimulate HSD11B1 activity in bovine endometria (15), and PGF2α stimulates HSD11B1 activity in human fetal membranes (38). In study 1, only 1 of the genes stimulated by cortisol in the endometrium was affected by infusion of the PTGS2 inhibitor, meloxicam, suggesting that the effects of cortisol are direct and not indirectly mediated by PG. As illustrated in Figure 5, available results support the idea that PG enhance cortisol generation by up-regulating *HSD11B1* expression and keto-reductase activity and that cortisol stimulates PTGS2-mediated PG production in the endometrial epithelia, which establishes a positive feed-forward stimulation of progesterone-induced genes in the LE that govern survival and elongation of the conceptus during early pregnancy. Indeed, our recent study in sheep revealed that in utero inhibition of PTGS2 activity by infusion of meloxicam beginning on day 10 of pregnancy prevented conceptus elongation as assessed on day 14 (9), indicating a vital role for PG in endometrial function and conceptus elongation during early pregnancy in sheep.

A novel finding of the present study 2 was that inhibition of HSD11B1 activity in utero, by infusion of a selective HSD11B1 inhibitor (PF 915275) beginning on day 10 of pregnancy, also prevented elongation of the ovoid conceptus as assessed on day 14. Relatively little is known regarding the biological activities of glucocorticoids during early pregnancy in any species. Of note, pregnancy rates were reported to be greater in women undergoing standard in vitro fertilization who receive synthetic glucocorticoids prior to or immediately after embryo transfer (39), although this study may need to be confirmed (40). A recent study found that glucocorticoid and eicosanoid pathways are hyperactivated in eutopic endometria of patients with ovarian endometriosis (41). The uterus of PF 915275-infused ewes contained ovoid conceptuses and had substantially lower amounts of cortisol as well as PG

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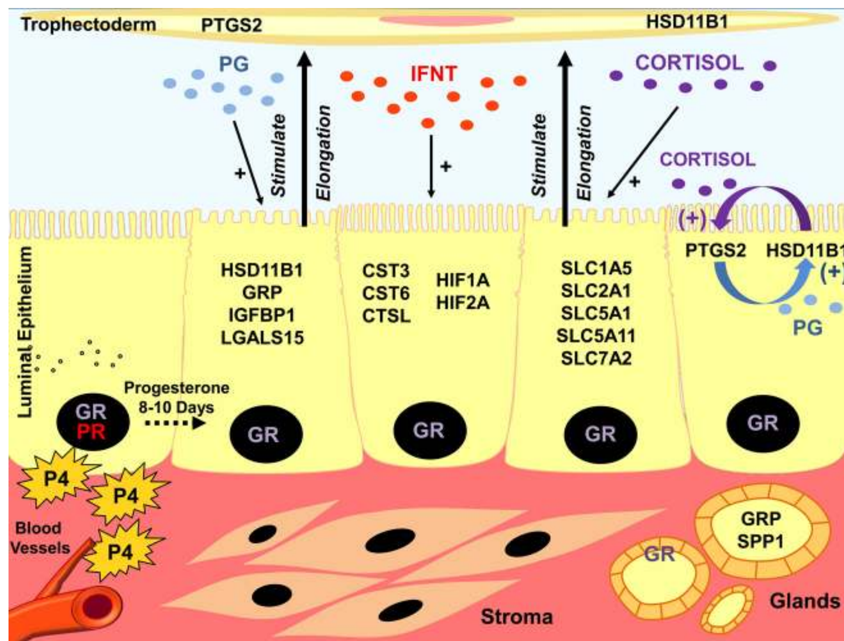

Figure 5. Schematic illustrating the effects of ovarian progesterone and factors from the endometrium and conceptus trophectoderm on expression of elongation- and implantation-related genes in the endometrial epithelia of the ovine uterus during early pregnancy. Ovarian progesterone induces a number of elongation-regulating genes (*PTGS2, HSD11B1*, *GRP*, *IGFBP1*, *LGALS15*, *CST3*, *CST6*, *CTSL*, *SPP1*, *SLC transporters*) in the endometrial luminal epithelium and/or glands after 8-10 days of action (between day 10 and day 12 after estrus/mating), and their induction is associated with a loss of PR in the luminal epithelium. The HSD11B1 and PTGS2 generate cortisol and prostaglandins, respectively, which act via receptors in the endometrium to stimulate expression of elongation-regulating genes as well as the expression and/or activity of HSD11B1 and PTGS2 in a feed-forward mechanism. The trophectoderm of the elongating conceptus also synthesizes and secretes cortisol and PG as well as IFNT, which act on the endometrium to stimulate elongation-regulating genes. It is likely that IFNT, PG, and cortisol regulate genes in other endometrial cell types (stroma and immune cells) as well as perhaps the conceptus trophectoderm, given the distribution of their receptors. P4, progesterone.

in the uterine lumen as compared with day 14 pregnant ewes that contained elongating filamentous conceptuses. These results strongly support the idea that cortisol generated by HSD11B1 the endometrium and/or conceptus trophectoderm is important for conceptus elongation during early pregnancy in sheep as well as in cattle (42).

Results of the present studies also solidify the role of IFNT as a key regulator of endometrial function governing conceptus elongation in ruminants because intrauterine infusion of IFNT rescued PF 915275 inhibition of conceptus elongation. During early pregnancy in ewes, elongating conceptuses produce and secrete IFNT that acts in a paracrine manner on the endometrium to stimulate endometrial functions hypothesized to govern conceptus elongation and implantation (see Refs 2, 7, and 11-13 for review and also Figs. 1-3). The IFNT-stimulated genes include transporters of glucose (*SLC2A1*, *SLC2A12*, *SLC5A1*) and amino acids (*SLC1A5* and *SLC7A2*) that are important for trophectoderm proliferation and/or migration (43) and other factors (*CXCL10*, *GRP*, *IGFBP1*, *LGALS15*) secreted into the uterine lumen that mediate trophectoderm cell proliferation, migration, and/or attachment (12, 44-46). As found previously (9, 10, 14), intrauterine infusion of IFNT into cyclic ewes in study 1 increased the expression of progesterone-induced genes that are implicated in conceptus elongation and also increased the activity of HSD11B1 and PTGS2 in the endometrium, resulting in higher levels of cortisol and PG in the uterine lumen. Of note, infusion PF 915275 did not diminish the effects of IFNT to stimulate endometrial gene expression and activity of HSD11B1 and PTGS2 in study 1, although infusion of the PTGS2 inhibitor meloxicam revealed that effects of IFNT on the endometrium are mediated, in part, by PTGS2-derived PG (9). In study 2, the uterus of IFNT and PF 915275 infused ewes contained elongated filamentous conceptuses as compared with PF 915275-infused ewes that contained ovoid conceptuses. Indeed, the levels of HSD11B1 and PTGS2 activity in the endometrium and cortisol and PG in the uterine lumen were substantially higher in IFNT and PF-infused as compared with PF-infused ewes and not different from day 14 pregnant ewes. The higher levels of cortisol and PG in the uterine lumen are likely the result of increased HSD11B1 and PTGS2 activity in the endometrium as well as increased amount of trophectoderm cells that also synthesize and secrete cortisol and PGs (14). The precise mechanism of how PF 915275 inhibits HSD11B1 enzymatic activity is not understood, although it is specific for HSD11B1 and has little effect on HSD11B2 (18, 19).

Another plausible hypothesis is that the actions of PG increased via IFNT effects on PTGS2, via their receptors increases HSD11B1 activity at a posttranscriptional level involving the availability of nicotinamide adenine dinucleotide phosphate via hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase) or perhaps glucose-6-phosphate via glucose (47). Interferon-τ increases expression of EP2 and EP4 prostaglandin receptors in the LE and increases the amount of glucose in the uterine lumen via increased glucose transporter expression in the early pregnant ovine uterus (11, 48-50). Collectively, available results from published and the present studies

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support the idea that IFNT rescues conceptus elongation by increasing the activity of HSD11B1 and PTGS2 and expression of IFNT-stimulated genes in the endometrial epithelium, which results in higher levels of cortisol, PG, and elongation and implantation-related genes that govern growth and development of the trophectoderm. Another possibility is that IFNT has direct effects on trophoblast gene expression and functions, but autocrine effects of IFNT have not been reported, even though it expresses type I interferon receptors (51).

As summarized in Figure 5, results of the present studies support the following ideas: 1) cortisol stimulates *HSD11B1* expression and HSD11B1 keto-reductase activity in the endometrium; 2) cortisol and PG modulate, in part, actions of ovarian progesterone on endometrial gene expression; 3) cortisol stimulates PG synthesis and PG stimulate cortisol generation establishing a positive feedforward mechanism in the endometrium during early pregnancy; 4) IFNT stimulates HSD11B1 activity and endometrial generation of cortisol; 5) HSD11B1-derived cortisol regulates endometrial function important for conceptus elongation; and 6) IFNT is a fundamental regulator of conceptus elongation via effects on expression of elongation- and implantation-related epithelial genes as well as PTGS2 and HSD11B1 activity.

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