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Mechanisms of reduced luteal sensitivity to prostaglandin $F_2\alpha$ during maternal recognition of pregnancy in ewes☆

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Abstract

During maternal recognition of pregnancy, the conceptus stimulates endometrial secretion of $PGF_2\alpha$ and PGE_2. However, $PGF_2\alpha$ is less effective in causing luteal regression in pregnant than in non-pregnant ewes. Experiments were conducted to elucidate mechanisms for reduced luteal sensitivity to $PGF_2\alpha$ during maternal recognition of pregnancy. Corpora lutea (CL) were collected from pregnant and non-pregnant ewes 0, 4, or 12 h following treatment with $PGF_2\alpha$ on day 12 after estrus. Luteal *PTGHS2* mRNA did not differ due to $PGF_2\alpha$ or pregnancy status. Luteal *PTGES* mRNA was reduced in both pregnant and non-pregnant ewes after $PGF_2\alpha$ treatment; while, luteal *PTGFS* mRNA was reduced 4 h after $PGF_2\alpha$ in pregnant, but not non-pregnant ewes. The result was a greater ratio of *PTGES* to *PTGFS* mRNA in pregnant ewes. Luteal mRNA for *HPGD* did not differ between pregnant and non-pregnant ewes on day 12. Luteal *END1* mRNA was reduced in pregnant as compared to non-pregnant ewes prior to $PGF_2\alpha$ challenge. Luteal *END1* mRNA was increased after $PGF_2\alpha$ in pregnant and non-pregnant ewes; however, *ECE1* mRNA was reduced 4 h after $PGF_2\alpha$ in pregnant, but not non-pregnant ewes. The in vitro conversion of $PGF_2\alpha$ to PGFM was greater in CL of pregnant

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than non-pregnant ewes at day 14. Luteal conversion of $PGF_2\alpha$ to PGFM appears to be regulated post-transcriptionally. During maternal recognition of pregnancy, mechanisms of reduced luteal sensitivity to $PGF_2\alpha$ may include a shift in prostaglandin production to the luteotropin PGE_2, a reduction of *ECE1* mRNA, and increased catabolism of $PGF_2\alpha$.
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Keywords: Prostaglandin; Maternal recognition of pregnancy; Endothelin; PGE_2; $PGF_2\alpha$

1. Introduction

In the ewe, at day 12 after estrus, the presence of a conceptus is required for prevention of luteal regression and establishment of pregnancy [1]. Maintenance of the corpus luteum (CL) is not due to a reduction of uterine production of the luteolysin $PGF_2\alpha$. Prostaglandins are synthesized from arachidonic acid (Fig. 1) which is first converted into PGH_2 by cyclooxygenases 1 or 2 (PTGHS1 and PTGHS2). The various prostaglandins are then produced by specific synthases that utilize PGH_2 or by the interconversion of prostaglandins. Uterine venous concentrations of $PGF_2\alpha$ [2] and jugular venous concentrations of PGFM [3] are greater in pregnant compared to non-pregnant ewes on days 13 and 14. Endometrial production of the luteotropin PGE_2 is increased during the maternal recognition of pregnancy beginning on day 13, thus increasing the ratio of PGE_2 to $PGF_2\alpha$ secreted by the endometrium [4,5].

Pregnant ewes not only have increased uterine secretion of $PGF_2\alpha$, but also have CL with reduced sensitivity to $PGF_2\alpha$. On days 12 and 13 after estrus, dosages of $PGF_2\alpha$ that initiate luteal regression and return to estrus in non-pregnant ewes fail to cause luteal regression in pregnant ewes [6–8]. Therefore, the presence of the conceptus results in a CL that is less sensitive to $PGF_2\alpha$. Few studies have compared the CL of pregnant and non-pregnant ewes, however Wiepz et al. [9] showed that lowered sensitivity to $PGF_2\alpha$ during pregnancy was not due to a reduced number of luteal receptors for $PGF_2\alpha$.

During luteal regression, CL synthesize $PGF_2\alpha$ in response to $PGF_2\alpha$ [10]. Prostaglandin $F_2\alpha$ stimulated luteal endothelial cells to increase production of endothelin-1 (EDN1), which bound to steroidogenic luteal cells via the type A endothelin receptor (EDNRA) and down-regulated progesterone synthetic enzymes [11–13]. Dosages of $PGF_2\alpha$ that consistently regressed CL at later stages of the cycle were not effective when given before day 5 [14]. In bovine CL, reduced sensitivity to $PGF_2\alpha$ early in the luteal phase appeared to be due, at least in part, to failure of upregulation of *EDN1* and *EDNRA* mRNA [15,16].

Amounts of mRNA encoding EDN1, endothelin converting enzyme-1 (ECE1) and EDNRA and EDN1 receptor type B (EDNRB) increased in bovine CL with stage of the estrous cycle, independent of $PGF_2\alpha$ [17]. After day 10, mRNA for EDN1 and EDNRA were increased by $PGF_2\alpha$. Perfusion of ovine CL in vivo on days 9–10 with $PGF_2\alpha$ increased secretion of EDN1 [18]. The presence of endothelial cells in the culture depressed secretion of progesterone from the steroidogenic cells of bovine CL collected on day 4 or 10 in comparison to enriched steroidogenic cells [19]. Secretion of progesterone was reduced by addition of $PGF_2\alpha$ only on day 10, but by EDN1 on both days. These authors concluded that EDN1 might function as a tonic inhibitor of progesterone secretion.

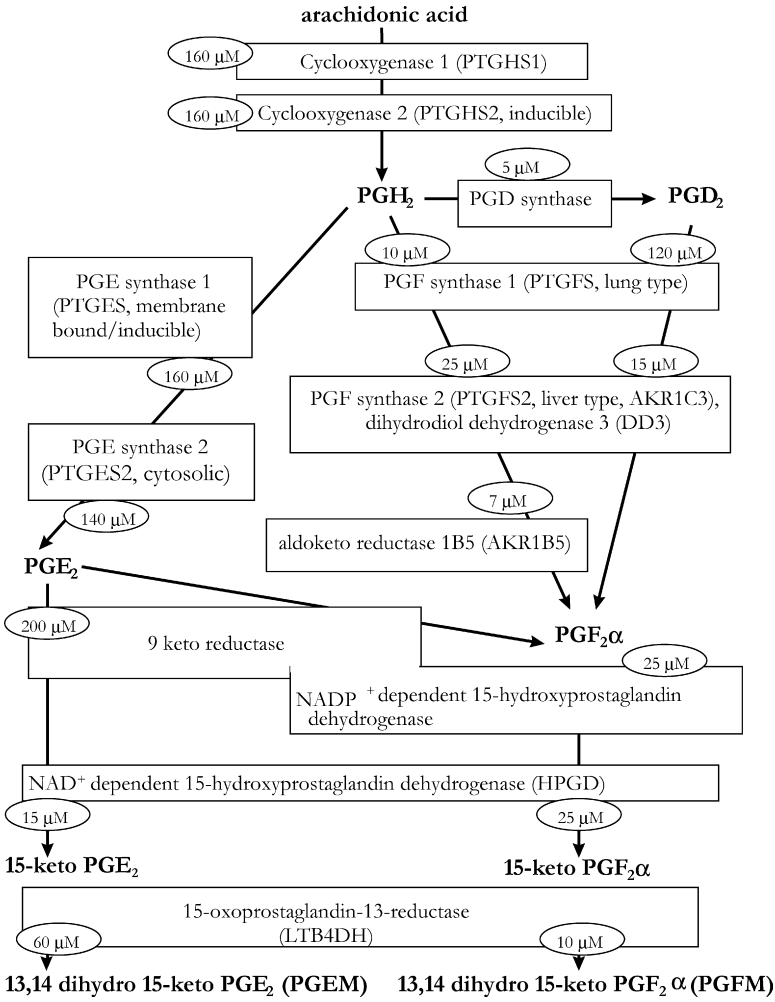

Fig. 1. Potential anabolic and catabolic pathways for PGE_2 and $PGF_2\alpha$. Lines depict conversion of substrate into product. Multiple enzymes listed on the same line demonstrate the variety of enzymes that may act on the substrate, not successive conversion steps. Enzymes named within same box have not been proven to be different enzymes. Enzymes in parentheses are acronyms for the described enzyme or another name for the same enzyme. The K_m of an enzyme for a substrate is shown within a circle. Multiple isoforms for PGE synthases 1 and 2 have not been listed. The K_m's can be obtained at http://www.brenda.uni-koeln.de.

Luteal production of luteotropins might reduce sensitivity to $PGF_2\alpha$ early in the luteal phase [20]. Both mRNA and protein of membrane bound/inducible PGE synthase (PTGES), the enzyme that converts PGH_2 to PGE_2, were present in greater quantities in CL during the first half of the luteal phase [21]. The CL metabolizes $PGF_2\alpha$ to the inactive metabolite PGFM by the sequential actions of NAD^+-dependent hydroxyprostaglandin dehydrogenase (HPGD) and 15-oxoprostaglandin-13-reductase (LTB4DH). Luteal catabolism of $PGF_2\alpha$ to

PGFM was greater on day 4 compared to day 13 of the estrous cycle in ewes [22]. Whether these mechanisms might also reduce sensitivity to $PGF_2\alpha$ during maternal recognition of pregnancy has been examined only for HPGD, which was more active in pregnant than in non-pregnant ewes on day 13 after estrus [22].

The objective of experiment 1 was to validate that 5 mg of $PGF_2\alpha$ i.m. would result in a reduction in the circulating concentrations of progesterone in non-pregnant but not pregnant ewes. The objective of experiment 2 was to determine if one mechanism by which pregnant ewes have reduced sensitivity to $PGF_2\alpha$ on day 12 involves a shift in luteal abundance of mRNA for synthetic enzymes toward luteotropic instead of luteolytic prostaglandins, an increase in the abundance of *HPGD* or *LTB4DH* mRNA, and/or altered amounts of mRNA for components of the endothelin system. The objective of experiment 3 was to determine if the presence of a conceptus results in a CL with an inherently greater capacity for catabolism of $PGF_2\alpha$ compared to CL of non-pregnant ewes on day 14 after estrus.

2. Materials and methods

2.1. General procedures

Mature ewes of primarily Suffolk and Dorset breeding were observed for estrus every 12 h with the aid of a vasectomized ram. Ewes that exhibited a normal length estrous cycle were mated to either a fertile or a vasectomized ram. At surgery, ewes were anesthetized with sodium pentobarbital (Sigma, St. Louis, MO; 2 mg/kg i.v.), and a mid-ventral laparotomy was performed to expose the reproductive tract. Individual uterine horns were flushed by placing a glass cannula into the tip of the uterine horn and infusing 10 mL of 10 mM phosphate buffered, 150 mM saline (pH 7.4) into the base of the horn. Uterine luminal contents were massaged toward the tip of the uterine horn and out through the glass cannula into a Petri dish. Flushings were examined for presence of an embryo. Ewes mated to fertile rams that did not have an embryo in the flushings were removed from the experiment. Unless otherwise stated, chemicals were purchased from VWR International, Inc. (West Chester, PA). All procedures were approved by the Animal Care and Use Committee of West Virginia University (ACUC # 01-0809).

2.2. Experiment 1

Ewes ($n = 5$ ewes/treatment/time; $N = 60$) were assigned randomly to a 2×6 factorial arrangement of treatment groups with pregnancy status and time after injection of $PGF_2\alpha$ as factors. At day 12 after estrus, all ewes were injected with 5 mg $PGF_2\alpha$ i.m. (Lutalyse, Pharmacia & Upjohn Company, Kalamazoo, MI), such that the treatment groups were ewes that were or were not pregnant. At 0, 6, 12, 18, 24, or 32 h after injection of $PGF_2\alpha$ (five pregnant and five non-pregnant ewes/time), blood was collected via the jugular vein and surgery was performed as described above. Serum concentrations of progesterone from pregnant ($n = 30$) and non-pregnant ewes ($n = 30$) were measured by a single previously validated RIA [23]. The intra-assay coefficient of variation was 11%, and sensitivity was 100 pg/mL.

2.3. Experiment 2

Ewes ($n = 5$ ewes/treatment; $N = 30$) were assigned randomly to a 2 × 3 factorial arrangement of treatment groups with pregnancy status and time after an injection of $PGF_2\alpha$ as the factors. At day 12 after estrus, all ewes were injected with 5 mg $PGF_2\alpha$ i.m. (Lutalyse). At 0, 4, or 12 h after injection of $PGF_2\alpha$, surgery was performed, and CL were collected and immediately snap frozen in liquid nitrogen and stored at −80 °C.

Luteal RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA) according to manufacturer's instructions and back extracted twice with 2 mL acid-phenol: chloroform (5:1, pH 4.5) then back extracted twice with 2 mL phenol: chloroform: isoamyl alcohol (25:24:1, pH 6.6; Ambion, Austin, TX). RNA quality was determined by separation and visualization on a 1% agarose gel stained with ethidium bromide. RNA was quantified spectrophotometrically at 260 nm. For each sample, 10 μg of RNA was reverse transcribed using random hexamers and Avian Myeloblastosis Virus reverse transcriptase (Promega, Madison, WI) for 60 min at 37 °C. Complementary DNA was quantified by real time PCR (iCycler, Bio-Rad Laboratories, Hercules, CA) using SYBR green. Primers were designed using Beacon Designer 2.1 (Premier Biosoft International, Palo Alto, CA), synthesized (Invitrogen), and optimized, utilizing a pool of RNA representing CL of all treatment groups, for β-actin (*ACTB*), cyclooxygenase-2 (*PTGS2*; EC 1.14.99.1), lung type prostaglandin F synthase (*PTGFS*; EC 1.1.1.188), aldo-ketoreductase 1B5 (*AKR1B5*; EC 1.1.1.188, EC 1.1.1.149), *PTGES* (EC 5.3.99.3), *HPGD* (EC 1.1.1.141), *LTB4DH* (EC 1.3.1.48), *EDN1*, *ECE1* (EC 3.4.24.71), and *EDNRA* (Fig. 1). Expected products were confirmed by sequencing (Retrogen, San Diego, CA). Due to very high sequence identity, primers designed for PTGFS would be expected to amplify liver prostaglandin F synthase (*PTGFS2*), 9-keto reductase like 2, dihydrodiol dehydrogenase 1, and dihydrodiol dehydrogenase 3, if present in the CL (Fig. 1), all of which convert PGH_2 and PGD_2 to $PGF_2\alpha$. Each reaction contained 25 μL iQ SYBR Green Supermix (Bio-Rad), 2 μL cDNA diluted 1:10, 300 μM (600 μM for EDNRA and PTGFS) forward and reverse primer, and water to a final volume of 50 μL. Complementary DNAs were amplified after denaturation at 95 °C followed by annealing (Table 1), and extension at 72 °C for 40 cycles. In order to generate a single amplification product, the extension step was eliminated when amplifying cDNA for *PTGES* and *HPGD* and the annealing step for *PTGHS2* started at 63 °C and decreased by 1 °C per cycle for 10 cycles, followed by 30 cycles at an annealing temperature of 53 °C. After each reaction reached completion, melting curves were determined for products. Products were separated and visualized on a 2% agarose gel stained with ethidium bromide during optimization of primers. The efficiency of each primer set was determined on cDNA that was diluted serially to 1000-fold, in duplicate, by plotting the log of cDNA versus the C_T. Efficiency was calculated by raising 10 to the power of −1 divided by the slope of the line [24]. For each sample, cDNA was amplified in duplicate with primers for the gene of interest (target gene) and *ACTB* (control gene [16]), both on the same plate. A reference pool of CL cDNA was co-amplified on each plate. Abundance of cDNA was expressed as the proportion of the reference pool, defined as a variation of the $2^{-\Delta\Delta C_T}$ method (presented by Perkin-Elmer Applied Biosystems, Foster City, CA) taking into account the actual efficiency of each primer pair, instead of assuming an efficiency of 2 [25]. Abundance of mRNA is expressed as the efficiency of the target gene, raised to the power of the difference of the C_T of sample

Table 1
Characteristics of primer sequences and PCR conditions

Gene	Primer sequence	Accession number	Product size (bp)	Annealing temperature (°C)	Intra-assay CV[a]
β-Actin	Forward 5′ ATGAGCTGCCCGATGGTC 3′, reverse 5′ GGATGTCCACGTCACACTTC 3′	gi:2182268	100	[b]	10.3[c]
Cyclooxygenase-2	Forward 5′ ATGTATCCTCCCACTGTCAAAG 3′, reverse 5′ TGTTCCCGCAGCCAAATG 3′	gi:1703495	146	53	14.0
PGE synthase 1	Forward 5′ CAACTGAGGCTGCGGAAG 3′, reverse 5′ CCAGGAACAGGAAGGGGTAG 3′	gi:31341986	150	62	12.7
PGF synthase 1	Forward 5′ TTGACTCGGTGGATCTCTG 3′, reverse 5′ TGCTTGTGGTTGAAGTTGG 3′	gi:163511	100	63	11.8
Aldoketoreductase 1B5	Forward 5′ AAGTGGTGAAGCCTGAGG3′, reverse 5′ CAGTGGATGAGGTAGAGGTC 3′	gi:265403	138	57	11.9
Hydroxyprostaglandin dehydrogenase	Forward 5′ ACCTACCTGGGCTTGGATTAC 3′, reverse 5′ CTGCCGAGCGTGTGAATC 3′	gi:4033852	150	64	15.4
15-Oxoprostaglandin-13-reductase	Forward 5′ AGCTTGAAGGAGGGTGACATG 3′, reverse 5′ ATGCGTTGTCCAGCCAGAAG3′	gi:28293974	116	56	10.4
Preproendothelin-1	Forward 5′ ATCATCTGGGTCAACACTCC 3′, reverse 5′ TAGCACACTGGCATCTCTTC 3′	gi:31341389	129	60	7.6
Endothelin converting enzyme-1	Forward 5′ GCATCCAATACCAGACAAGAAC 3′, reverse 5′ ACAGGCATAGGTGAAGAAGTC 3′	gi:31341344	127	57	8.5
Endothelin receptor type A	Forward 5′ TGTCCTTCTGGGTGGCTCTG 3′, reverse 5′ TTCGTGGGTTGATGTGTGGTG 3′	gi:27805816	144	57	11

[a] CVs were determined following linearization of C_T values and were calculated for each sample.
[b] Actin was amplified under same conditions as gene of interest.
[c] Value represents the average of actin CV for all samples.

and the C_T of the reference pool for the target gene, divided by the efficiency of the control gene (*ACTB*) raised to the power of the difference of the of the C_T of sample and the C_T of the reference pool for the control gene [25]

$$\text{mRNA abundance as a proportion of reference pool} = \frac{\text{efficiency of target}^{C_T\ \text{reference pool} - C_T\ \text{sample}}}{\text{efficiency of control gene}^{C_T\ \text{reference pool} - C_T\ \text{sample}}}$$

The coefficient of variation for each sample assayed in duplicate was determined following linearization of the C_T [26]. Samples that had a CV above 24% were re-assayed. Average intra-assay coefficients of variation are listed in Table 1.

2.4. Experiment 3

At day 14 after estrus, surgery was performed as described above, and CL were collected and immediately snap frozen in liquid nitrogen and stored at −80 °C. To determine the in vitro luteal catabolism of $PGF_2\alpha$ in CL from pregnant ($n = 8$) and non-pregnant ewes ($n = 8$), the protocol reported by Silva et al. [22] was followed. This assay was designed to determine the activity of HPGD. However, the catabolism of $PGF_2\alpha$ to PGFM occurs in two steps, thus the combined activity of HPGD and LTB4DH was measured. An aliquot of 100 mg of CL was homogenized in 1 mL of cold HPGD assay buffer (100 mM PO_4, 2 mM NAD^+, pH 7.4) and centrifuged (5000 × *g*) for 5 min at 4 °C. From the supernatant, 300 μL was incubated in duplicate at 37 °C with 50 μL of 140 nM $PGF_2\alpha$ and 650 μL of HPGD assay buffer, which resulted in a total reaction volume of 1 mL. After incubation, the reaction was stopped by adding 100 μL of the incubation reaction into tubes containing 300 μL of 200 mM formic acid. For measurement of PGFM, the pH of the reaction products was increased from 2.5 to 4.5 with the addition of 1 mL of 10 mM PBS (pH 7.4). To assess linearity of the conversion of $PGF_2\alpha$ to PGFM over time, incubations were stopped at 0, 5, 10, 15, 30, 45, 60, 90 and 180 min. After conversion of $PGF_2\alpha$ to PGFM was demonstrated to be linear over the time span examined, sample reactions were stopped at 45 min. For each sample, PGFM was measured at 0 min of incubation in duplicate in one-tenth of the incubation volume by adding 30 μL of CL homogenate, 5 μL of 140 nM $PGF_2\alpha$, and 65 μL of HPGD assay buffer to 300 μL of 200 mM formic acid to generate an acid-inactivated blank. Samples were extracted twice with fresh diethyl ether, dried under a stream of air, and reconstituted with 1 mL 10 mM PBS. Antibody for PGFM was provided by Dr. W.J. Silvia, University of Kentucky. Concentrations of PGFM were measured by a single previously-validated RIA [27]. The intra-assay coefficient of variation was 13.3%. Protein was measured in each homogenate by the Coomassie Plus Protein Assay Kit (Pierce Chemical, Rockford, IL).

2.5. Statistical analysis

In experiment 1, the effects of hour, pregnancy status, and their interaction on circulating concentrations of progesterone were tested by ANOVA utilizing the GLM procedure of SAS (version 8.0, Cary, NC), and means were separated using the LSMEANS procedure of SAS. In experiment 2, the effects of hour, pregnancy status, and their interaction on the amount of

luteal mRNA as a proportion of the reference pool for *PTGHS2*, *PTGFS*, *PTGES*, *HPGD*, *LTB4DH*, *EDN1*, *EDNRA*, and *ECE1* were tested by ANOVA utilizing the GLM procedure of SAS, and means were separated using the LSMEANS procedure of SAS. In experiment 3, the effect of pregnancy status on HPGD/LTB4DH activity was tested by ANOVA utilizing the GLM procedure of SAS.

3. Results

Concentrations of progesterone in non-pregnant ewes were reduced ($P < 0.05$) by 12 h after a single intramuscular injection of 5 mg $PGF_2\alpha$, and they remained low throughout the sampling period (Fig. 2) indicating that functional luteal regression was likely complete, although we did not monitor animals to determine whether they returned to estrus following treatment. However, in pregnant ewes, an injection of 5 mg $PGF_2\alpha$ tended to reduce ($P = 0.07$) concentrations of progesterone 6 h after the injection, but concentrations of progesterone did not differ from initial concentrations for the remainder of the sampling period (Fig. 2). Concentrations of progesterone after a challenge with $PGF_2\alpha$ were greater ($P < 0.05$) in pregnant ewes than in non-pregnant ewes sampled beginning at 18 h after the injection through the last time at 36 h (Fig. 2).

Luteal *PTGHS2* mRNA did not differ due to pregnancy status or injection of $PGF_2\alpha$ (Fig. 3A); there was less ($P < 0.05$) *PTGFS* mRNA in CL 4 h after injection of $PGF_2\alpha$ to pregnant compared to non-pregnant ewes (Fig. 3B). Luteal abundance of *PTGES* mRNA declined with time ($P < 0.05$) after injection of $PGF_2\alpha$ in both pregnant and non-pregnant ewes (Fig. 3C). The ratio of the abundance of *PTGES* to *PTGFS* mRNA was greater ($P < 0.05$) 4 h after injection of $PGF_2\alpha$ in pregnant compared to non-pregnant ewes (Fig. 3D). Pregnant ewes had more luteal *AKR1B5* mRNA than non-pregnant ewes (0.70 ± 0.07 versus

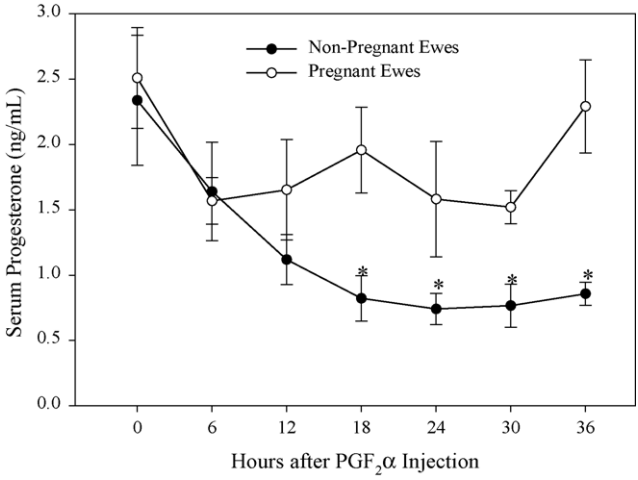

Fig. 2. Serum concentrations of progesterone in pregnant or non-pregnant ewes following injection of $PGF_2\alpha$ (5 mg). Means ± S.E.M. differed ($^*P < 0.05$) between pregnant and non-pregnant ewes at that hour.

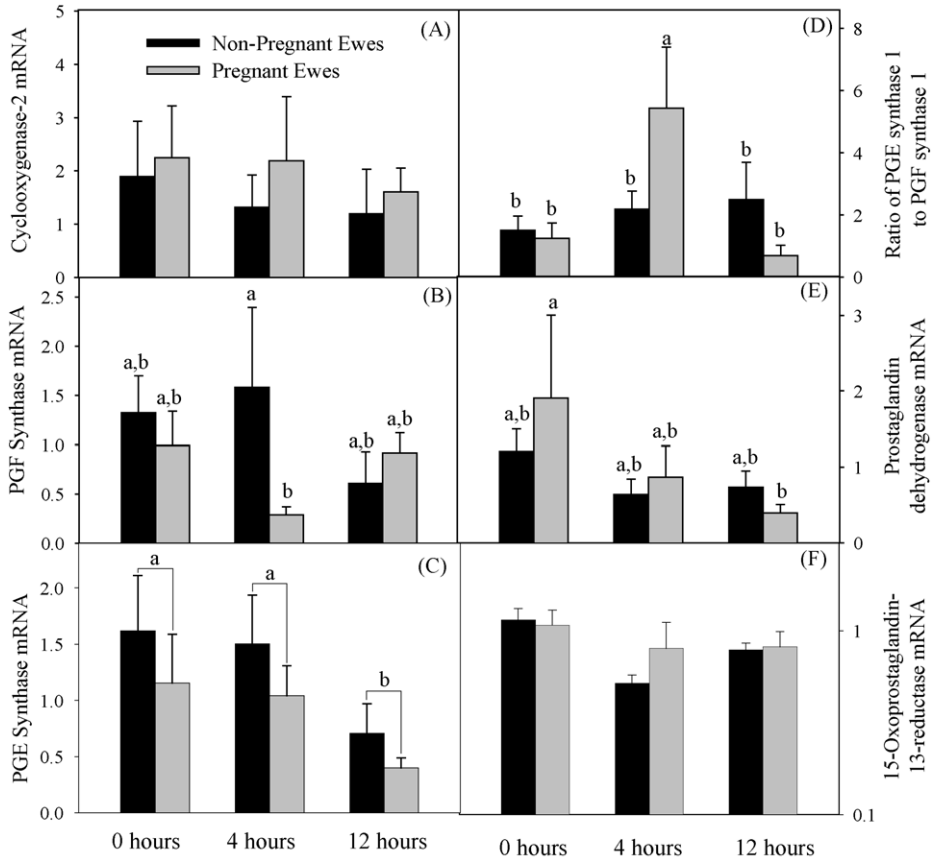

Fig. 3. Luteal abundance of mRNA for cyclooxygenase-2 (A), PGF synthase 1 (B), PGE synthase 1 (C), PGE synthase 1:PGF synthase 1 mRNA ratio (D), prostaglandin dehydrogenase (E), 15-oxoprostaglandin-13-reductase (F) 0, 4, or 12 h after an injection of $PGF_2\alpha$ (5 mg) in pregnant and non-pregnant ewes. The data are expressed as abundance of the gene of interest per actin as proportion of that in the reference pool. Means ± S.E.M. with different letters (a and b) differ ($P < 0.05$) within each panel.

0.55 ± 0.06; $P < 0.05$). There was no interaction of pregnancy status and time after injection (data not shown).

The abundance of *HPGD* mRNA did not differ ($P > 0.05$) due to pregnancy status, but there was an interaction of pregnancy status by time manifested by a lower ($P < 0.05$) amount of *HPGD* mRNA at 12 than at 0 h after injection of $PGF_2\alpha$ in pregnant ewes, while there was no change in non-pregnant ewes (Fig. 3E). There were no effects of pregnancy or treatment with $PGF_2\alpha$ on the amount of luteal *LTB4DH* mRNA (Fig. 3F). Corpora lutea collected from pregnant ewes on day 14 (experiment 3) had a greater ability to catabolize $PGF_2\alpha$ to inactive PGFM compared to CL of non-pregnant ewes (188 ± 31 ng/min/mg protein versus 48 ± 10 ng/min/mg protein, $P < 0.05$).

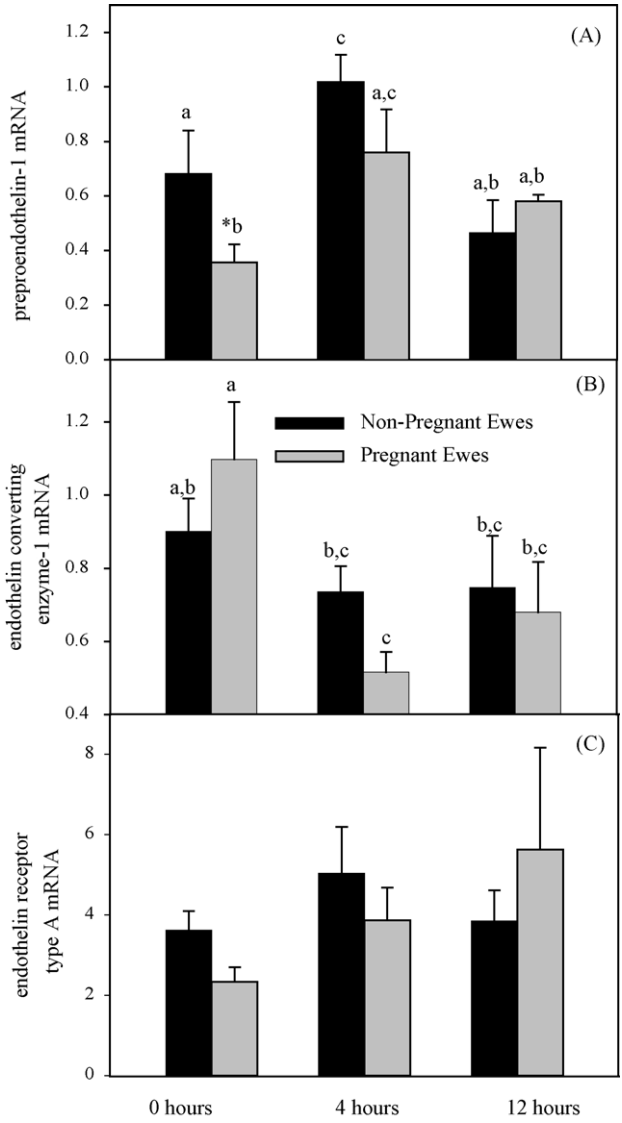

Fig. 4. Luteal abundance of mRNA for preproendothelin-1 (A), endothelin converting enzyme-1 (B), endothelin receptor type A (C) 0, 4, or 12 h after an injection of $PGF_2\alpha$ (5 mg) in pregnant and non-pregnant ewes. The data are expressed as the abundance of the gene of interest per actin as proportion of that in the reference pool. Means with different letters (a, b, and c) differ ($P < 0.05$) within each gene. Means ± S.E.M. with different letters (a and *b) tended to differ ($P = 0.08$).

Before the injection of $PGF_2\alpha$, *EDN1* mRNA tended to be lower ($P = 0.08$) in CL of pregnant than non-pregnant ewes, but increased ($P < 0.05$) at 4 h after an injection of $PGF_2\alpha$ regardless of pregnancy status (Fig. 4A). Amount of *ECE1* mRNA was altered due to pregnancy status following an injection of $PGF_2\alpha$. Abundance of *ECE1* mRNA was lower

($P < 0.05$) in pregnant, but not in non-pregnant ewes 4 h ($P > 0.05$) after injection of $PGF_2\alpha$ and remained lower at 12 h ($P < 0.05$; Fig. 4B). There was no effect of pregnancy status or time after injection of $PGF_2\alpha$ on the abundance of *EDNRA* mRNA (Fig. 4C).

4. Discussion

4.1. Prostaglandin metabolism

Corpora lutea were collected 0, 4 and 12 h following injection of $PGF_2\alpha$ to determine if alterations in the abundance of mRNA encoding enzymes involved in luteal function were different in pregnant compared to non-pregnant ewes, with the idea that these differences contribute to the divergence in progesterone concentrations depicted in Fig. 2 and observed previously [6–8]. The abundance of mRNA for *PTGFS* differed in response to $PGF_2\alpha$ in pregnant compared to non-pregnant ewes. In response to $PGF_2\alpha$, the CL produced more $PGF_2\alpha$, which was hypothesized to potentiate the effects of uterine $PGF_2\alpha$ [28]. Bovine CL may have a great potential to produce prostaglandins because luteal tissue contains five times more arachidonic acid per gram of tissue than endometrium [29].

Luteal content of *PTGHS2* mRNA did not change following an injection of $PGF_2\alpha$, and did not differ according to pregnancy status. These results were surprising considering the evidence that *PTGHS2* mRNA increased 4 h after an injection of $PGF_2\alpha$ in mid-luteal phase ovine CL, but not in CL early in the luteal phase [15,30,31]. This may be because *PTGHS2* mRNA could have increased briefly before the first CL collection at 4 h [31]. Alternatively, the dosage of $PGF_2\alpha$ in the current work was selected to result in luteal regression in non-pregnant but not in pregnant ewes, whereas others have utilized a dosage of $PGF_2\alpha$ that should cause luteal regression in both groups. The lack of difference in *PTGHS2* mRNA according to pregnancy status is consistent with experiments that compared *PTGHS2* mRNA in CL of pregnant and non-pregnant ewes [22] or in CL of cows infused with interferon-tau to non-infused controls [32]. Cyclooxygenase-2 is unlikely to be a primary point of regulation in the prostaglandin anabolic pathways in CL, considering that the product of PTGHS2, PGH_2, can be converted to either PGE_2 or $PGF_2\alpha$, prostaglandins with opposing effects.

The abundance of *PTGFS* mRNA was dramatically lower in pregnant compared to non-pregnant ewes 4 h following $PGF_2\alpha$ injection. The amount of *PTGES* mRNA, the enzyme that converts PGH_2 to the luteotropin PGE_2, did not differ between pregnant and non-pregnant ewes. However, 4 h following an injection of $PGF_2\alpha$, pregnant ewes had a greater ratio of *PTGES* to *PTGFS* mRNA, which presumably translates into a greater abundance of PGE compared to PGF synthase protein and increases the production of PGE_2 compared to $PGF_2\alpha$. Exposure of the CL to a different ratio produced by the endometrium during maternal recognition of pregnancy [5] could alter the PGE_2:$PGF_2\alpha$ ratio produced by the CL in response to a challenge of $PGF_2\alpha$. In the cow, intrauterine infusion of the conceptus-secreted protein interferon-tau increased the ratio of *PTGES* to *PTGFS* mRNA in endometrium, by reduction in *PTGFS* mRNA [32]. In contrast, the increased ratio of *PTGES* to *PTGFS* mRNA in CL occurred by increased *PTGES* mRNA. In the present study, the greater ratio of *PTGES* to *PTGFS* mRNA in the CL after a challenge with $PGF_2\alpha$ was due to a reduction

of *PTGFS* mRNA. This may indicate a species difference as ovine and bovine CL appear to produce different PGF synthase mRNA's or may reflect a difference between the effect of pregnancy and the effect of interferon-tau infusion.

In contrast to bovine CL, which appeared to express mRNA for only one PGF synthase, *AKR1B5* [21,33], ovine CL contained mRNA for both *PTGFS* and *AKR1B5*. The PTGFS we measured likely includes both *PTGFS* and *PTGFS2*. The amount of *AKR1B5* mRNA did not change with pregnancy status or in response to a challenge with $PGF_2\alpha$. Aldoketoreductase 1B5 has been hypothesized to be the major PGF synthase in bovine endometrium [34], and has the capacity to metabolize progesterone, similar to other PGF synthases such as aldoketoreductase 1C3 (also known as PGF synthase 2, which is most likely the same as dihydrodiol dehydrogenase 3 [35]), and 9-keto reductase (Fig. 1). Further studies must be performed to determine which PGF synthases are most important in the production of $PGF_2\alpha$ in the CL. Although prostaglandin synthetic enzymes that may be present in the CL (PTGFS2, AKR1B5, and 9-keto-reductase) are proposed to have 20α-hydroxysteroid dehydrogenase activity [36], the K_m's of these PGF synthases for progesterone are not known in any tissue. In addition, the actual contribution to catabolism of progesterone by PGF synthases in the CL is not known, unlike the well-characterized role of 20α-hydroxysteroid dehydrogenase that is regulated by prolactin and $PGF_2\alpha$ in the rat CL [37,38]. Although the abundance of mRNA may indicate mechanisms for reduced luteal sensitivity to $PGF_2\alpha$ during maternal recognition of pregnancy, abundance of mRNA for enzymes that metabolize prostaglandins does not necessarily reveal flux through the pathway.

Corpora lutea from pregnant ewes on day 14 had a greater capacity to catabolize $PGF_2\alpha$ into PGFM compared to CL from non-pregnant ewes, which is similar to that observed on day 13 [22]. However, the amount of *HPGD* mRNA did not differ in CL of pregnant and non-pregnant ewes before the injection of $PGF_2\alpha$ on day 12. Similarly, differences in *HPGD* mRNA were not detected after an infusion of interferon-tau [32]. In contrast, Silva et al. [22] found greater *HPGD* mRNA in CL from pregnant compared to non-pregnant ewes on day 13. These differences could indicate that the catabolic potential for $PGF_2\alpha$ in CL of pregnant ewes changes from day 12 to days 13 and 14 of pregnancy.

During early pregnancy, the endometrium increases secretion of PGE_2 [4] and $PGF_2\alpha$ [2] between days 12 and 13, which might contribute to the high variation among pregnant ewes in the amount of *HPGD* mRNA on day 12 before the injection of $PGF_2\alpha$. In addition, catabolism of $PGF_2\alpha$ to PGFM did not appear to be regulated by the amount of transcript for the second step of catabolism (i.e., *LTB4DH*). Catabolism of $PGF_2\alpha$ is likely regulated post-transcriptionally at the level of HPGD and/or LTB4DH. Although the K_m of LTB4DH for 15-keto $PGF_2\alpha$ is not known in ovine tissue, it is 0.2 μM in rat liver [39] and 10 μM in human placenta [40]. Therefore, LTB4DH activity may be rate limiting in catabolism of $PGF_2\alpha$ to PGFM in CL. Future experiments should explore possible post-transcriptional mechanisms of regulation for both HPGD and LTB4DH.

4.2. Endothelin system

One of the more noteworthy findings in the current work was the trend for a reduction in *EDN1* transcript abundance in pregnant as compared to non-pregnant ewes prior to the challenge with $PGF_2\alpha$. Whether this reduction in *EDN1* mRNA in pregnant ewes as

compared to non-pregnant ewes contributes to the differential response to a low dosage of $PGF_2\alpha$ is not known, but could be a very fruitful avenue to pursue in regard to understanding the differential regulation of luteal function at the time of maternal recognition of pregnancy. The relative abundance of luteal *EDN1* mRNA increased similarly in pregnant and non-pregnant ewes 4 h after an injection of $PGF_2\alpha$ indicating that regulation of *EDN1* mRNA did not confer reduced luteal sensitivity to $PGF_2\alpha$. Therefore, mechanisms responsible for reduced sensitivity to $PGF_2\alpha$ in CL of pregnant ewes appear to differ from those in the early luteal phase bovine CL (increased mRNA for *EDN1* and *EDNRA* [18,19]). The increase in *EDN1* mRNA after injection of $PGF_2\alpha$ was more transient than the time course reported in both ovine [13] and bovine [15] CL. This difference in the time course of EDN1 stimulation following challenge with $PGF_2\alpha$ may simply represent a dosage effect. The current work used 5 mg of $PGF_2\alpha$ which would be luteolytic only in the non-pregnant ewes, whereas the previous work used 10 mg, a dosage that might have been luteolytic in both pregnant and non-pregnant ewes.

Preproendothelin is converted to big endothelin by a variety of furin or furin-like enzymes within the cell in which it is made. Big endothelin is converted to active EDN1 by ECE1 (a zinc dependant metalloproteinase), which is present within the endothelial cell and the extracellular matrix of the CL [41,42]. Abundance of *ECE1* mRNA was lower in CL from pregnant ewes 4 h after $PGF_2\alpha$, but did not change in non-pregnant ewes. It is surprising that *ECE1* was reduced by $PGF_2\alpha$ only in the CL of pregnant ewes, because *ECE1* mRNA was decreased by EDN1 in bovine CL [42], and *EDN1* mRNA was increased in response to $PGF_2\alpha$ in both pregnant and non-pregnant ewes. Similarly, the amount of *ECE1* mRNA was lower in day-5 short-lived CL than in either day-5 normal or day-16 CL in postpartum cows [33]. In ewes, $PGF_2\alpha$ increased luteal EDN1 and reduced mRNA encoding progesterone synthetic enzymes [13]. A reduction in *ECE1* mRNA may blunt the response of the CL to $PGF_2\alpha$ by reducing the conversion of big endothelin to active EDN1 in pregnant ewes. There was no effect of $PGF_2\alpha$ or pregnancy on the abundance of *EDNRA* mRNA at 4 or 12 h after the injection, which is consistent with the 24 h previously shown to be required for upregulation of *EDNRA* mRNA in bovine CL [15]. Gelatinase A (also known as matrix metalloproteinase 2) can also act on big endothelin; however, only six amino acids are removed generating a variant of EDN1 known as EDN1[1-32] [43]. Towle et al. [44] have demonstrated that matrix metalloproteinase 2 is increased by 8 h following infusion of $PGF_2\alpha$ in sheep. It follows that some of the big endothelin produced by the CL could be converted into EDN1[1-32]. Indeed, in the bovine CL, some of the big endothelin conversion into EDN1 is not phosphoramidon (an inhibitor of ECE1) sensitive, indicating that an enzyme other than ECE1 is responsible for at least part of the measured conversion of big endothelin to the EDN1 [17].

In conclusion, day-12 CL of pregnant ewes had less *PTGFS* mRNA than CL of non-pregnant ewes 4 h after injection of $PGF_2\alpha$, resulting in a greater ratio of *PTGES* to *PTGFS* mRNA. An increased *PTGES* to *PTGFS* ratio may result in a greater luteal conversion of PGH_2 to PGE_2, acting as a luteotropic instead of luteolytic response to uterine $PGF_2\alpha$. In addition, CL of pregnant ewes had an inherently greater capacity to inactivate $PGF_2\alpha$ at day 14, and a reduced abundance of *ECE1* mRNA 4 h after an injection of $PGF_2\alpha$ on day 12. Greater ratios of PGE_2 to $PGF_2\alpha$ produced by the uterus under the influence of interferon-tau produced by the conceptus might arrive at the CL through the same route

as the uterine luteolysin and cause a similar shift in the ratio of PGE_2 to $PGF_2\alpha$ produced by the CL, which would reduce sensitivity to $PGF_2\alpha$ during the maternal recognition of pregnancy.

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