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Prostaglandin Production at the Onset of Ovine Parturition Is Regulated by Both Estrogen-Independent and Estrogen-Dependent Pathways*

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ABSTRACT

A current hypothesis of ovine parturition proposes that fetal adrenal cortisol induces placental E_2 production, which, in turn, triggers intrauterine PG production. However, recent evidence suggests that cortisol may directly increase PG production in trophoblast-derived tissues. To separate cortisol-dependent and estrogen-dependent PG production in sheep intrauterine tissues, we infused singleton, chronically catheterized fetuses beginning on day 125 of gestation (term, 147–150 days) with 1) cortisol (1.35 mg/h; n = 5); 2) cortisol and 4-hydroxyandrostendione, a $P450_{aromatase}$ inhibitor (4-OHA: 1.44 mg/h; n = 5); 3) saline (n = 5); or 4) saline and 4-OHA (n = 5). Fetal and maternal arterial blood samples were collected at 12-h intervals starting 24 h before infusion and continuing during treatment for 80 h or until active labor. Uterine contractility was measured by electromyogram recording of myometrial activity. Plasma E_2, progesterone (P_4), PGE_2, and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ were quantified by RIA. PGHS-II messenger RNA (mRNA) and protein expression were determined by *in situ* hybridization and Western blot analysis, respectively. Data were analyzed by ANOVA ($P \leq 0.05$). Labor-type uterine contractions were present after 68 h of cortisol infusion and had increased significantly by 80 h. Labor-type uterine contractions were induced after 68 h of cortisol plus 4-OHA infusion, but the contraction frequency remained less than that in the cortisol-treated animals. Fetal cortisol infusion increased fetal and maternal plasma E_2 concentrations and decreased the maternal plasma P_4 concentration significantly; concurrent 4-OHA infusion attenuated the increase in fetal and maternal plasma E_2, but not the decrease in maternal plasma P_4. The fetal plasma PGE_2 concentration increased after both cortisol and cortisol plus 4-OHA infusion. The maternal plasma 13,14-dihydro-15-keto-$PGF_{2\alpha}$ concentration rose after fetal cortisol infusion, but not after cortisol plus 4-OHA infusion. Placental trophoblast PGHS-II mRNA and protein expression were increased significantly after both cortisol and cortisol plus 4-OHA infusion. Endometrial PGHS-II mRNA and protein expression increased after cortisol infusion, but not after cortisol plus 4-OHA infusion. Plasma steroid and PG concentrations, uterine activity pattern, and intrauterine PGHS-II expression were not altered in either control group. We conclude that these data suggest distinct pathways of intrauterine PG synthesis: a cortisol-dependent/E_2-independent mechanism within trophoblast tissue leading to elevations in fetal plasma PGE_2, and an E_2-dependent mechanism within maternal endometrium that leads to increased maternal plasma $PGF_{2\alpha}$ and appears necessary for uterine activity and parturition. (*Endocrinology* **141:** 3783–3791, 2000)

AN ACCEPTED CONCEPT of ovine parturition suggests that the surge in fetal adrenal cortisol production toward the end of gestation alters placental steroidogenesis, causing a decline in progesterone output and an increase in estradiol (E_2) production through the induction of the placental $P450_{C17hydroxylase}$ enzyme (1). Estrogen, in turn, stimulates intrauterine PG production, in particular PGE_2 and $PGF_{2\alpha}$, as well as triggers the expression of a specific cassette of contraction-associated proteins (CAPs) within the myometrium (2). Consequently, myometrial contractility is stimulated, and labor and delivery of the fetus ensue. However, recent evidence has led us to question this concept.

Studies examining the natural ontogeny of intrauterine PG production during late gestation and the onset of labor have found that the rise in fetal plasma PGE_2 concentration occurred with a time course similar to that of the rise in fetal plasma cortisol and preceded the rise in both fetal and maternal plasma E_2 concentrations (3). The expression of $P450_{C17hydroxylase}$, the rate-limiting enzyme of E_2 synthesis from C_{21} precursors in the ovine placenta, did not increase until the onset of early labor, well after the rise in fetal plasma PGE_2 concentration and the increase in placental PGH synthase II (PGHS-II) expression. In addition, intrafetal E_2 infusion failed to increase the expression of PGHS-II messenger RNA (mRNA) in the sheep placenta, although intrafetal administration of cortisol did increase placental PGHS-II expression and plasma PG levels (4, 5). These observations suggested that cortisol, but not E_2, might increase placental trophoblast PGHS-II expression and activity to produce PGE_2.

In related studies we also found that changes in maternal plasma concentrations of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) were correlated with increased endometrial PGHS-II expression, maternal plasma E_2 levels, and uterine activity (3, 6). Recently, E_2 has been shown to increase PGHS-II expression significantly in nonpregnant ovine myometrium and in nonpregnant ovine endometrium after progesterone priming (7). These observations suggested that placental E_2 may stimulate nontrophoblast intrauterine tissue PGHS-II expression/activity

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to produce $PGF_{2\alpha}$, which, in turn, may contribute to uterine activity.

Therefore, we hypothesized that there might be two separate pathways of intrauterine PG production: a cortisol-dependent/ E_2-independent pathway within fetal placental trophoblast tissue, and an E_2-dependent pathway within maternal intrauterine tissues. To test this hypothesis, we infused late gestation sheep fetuses with cortisol in the presence and absence of the aromatase inhibitor 4-hydroxyandrostendione (4-OHA) and determined changes in placental and uterine PGHS-II expression and PG output.

Materials and Methods

Animal preparation

Pregnant singleton ewes of mixed breeds and known gestational ages were used. Gestational age was calculated from the date of insemination, and the number of fetuses was confirmed by ultrasonography. Surgery was performed under general anesthesia as previously described (8). Briefly, polyvinyl catheters were inserted into the maternal femoral artery and vein, fetal carotid artery, and fetal jugular vein on days 120–123 of gestation (term gestation, 147–150 days). Stainless steel electrodes (electromyogram) were implanted into the myometrium to monitor uterine muscle activity. After 5 days of postoperative recovery, animals entered the experimental protocol.

Experimental protocol

Beginning on days 125–128 of gestation, fetuses received a continuous infusion of saline (3 ml/h; n = 10) or cortisol (1.35 mg/h, in the same volume of infusate; n = 10; Steraloids, Wilton, NH). After 24 h of infusion, five animals in each group received an additional intrafetal infusion of Lentaron (1.44 mg/h 4-OHA, a competitive, suicide inhibitor of the $P450_{aromatase}$ enzyme; Ciba-Geigy; Basel, Switzerland) (9). Fetal and maternal arterial blood samples were collected at 12-h intervals beginning 24 h before the start of the infusion protocol and continued through the infusion period. Blood to be used for the determination of E_2 and progesterone (P_4) was collected into syringes rinsed previously with heparinized saline; blood to be used for the determination of PGE_2 and 13,14-dihydro15-keto-$PGF_{2\alpha}$ (PGFM) was collected in heparinized syringes and then transferred to vials containing indomethacin (200 μl 1 mg/ml INDOCID; Merck Sharp and Dohme, Kirkland, Canada). Plasma was separated from blood cells by centrifugation at 1500 $\times$ *g* for 10 min at 4 C. Plasma samples were frozen at −20 C for subsequent assay.

Uterine muscle electromyogram activity was processed by a Grass wide-band AC preamplifier (model 7P511J) and was recorded using a Grass 78D EEG and polygraph data recording system (Grass Instruments, Quincy, MA). Uterine activity monitoring began 24 h before the start of the infusion protocol and continued for the infusion period. Uterine contractures were defined as a low amplitude uterine activity pattern (duration, 5–8 min; frequency, ~2–3/h); uterine contractions were defined as a high amplitude activity pattern (duration, <1 min; frequency, ≥30/h) (3).

From preliminary studies (data not shown) it was determined that an intrafetal cortisol infusion (1.35 mg/h) for a period of about 80 h was sufficient to induce a uterine contraction pattern consistent with labor. After completion of an 80-h infusion period, a terminal plasma sample was taken, then each animal was killed with an overdose of Euthanyl (sodium pentobarbital, MTC Pharmaceuticals, Cambridge, Canada), and intrauterine tissues were collected. Placental cotyledons were dissected from the uterine muscle and fetal membranes; a single piece of intercotyledonary endometrium was bluntly peeled from the myometrium at the level of the miduterine horn. An adjacent cross-sectional piece of uterine tissue, including myometrium, endometrium, chorion, and amnion, was cut, rolled, and collected. Tissues were snap-frozen in liquid nitrogen for subsequent Western blot analysis and slow frozen over dry ice for *in situ* hybridization studies.

RIA

The extraction of plasma samples and the RIA for fetal plasma PGE_2 and maternal plasma PGFM and P_4 were conducted as previously described and validated (10–12). The intraassay coefficients of variation were 8%, 4%, and 10%, respectively. The RIA for fetal and maternal plasma E_2 was performed using a commercially available ^{125}I RIA kit (ImmuChem Double Antibody 17βEstradiol ^{125}I RIA Kit, ICN Biomedicals, Inc., Costa Mesa, CA) validated for use with ovine plasma. The intraassay coefficient of variation was 3%. Fetal plasma androstenedione concentrations were measured using a commercially available ^{125}I RIA kit (ImmuChem Double Coated Tube Androstenedione ^{125}I RIA Kit, ICN Biomedicals, Inc.). The sensitivity of the assay was 0.12 pg/ml. The specificity was provided by the manufacturer; the main cross-reacting steroids were dehydroepiandrosterone, androsterone, testosterone, and estrone, with 2.08%, 1.96%, 0.83%, and 0.2% cross-reactivities, respectively. The intraassay coefficient of variation was 12%.

In situ hybridization

Sense and antisense probes based on the structure of the human PGHS-II gene were synthesized by the University of Ottawa Molecular Biology Department (Ottawa, Canada) using an Oligo1000 DNA synthesizer (Beckman Coulter, Inc., Mississauga, Canada). The oligonucleotide sequence for the PGHS-II probe was GGG ACA GCC CTT CAC GTT ATT GCA GAT GAG AGA CTG AAT TGA GGC AGT GT, corresponding to nucleotides 1734–1783 of the human PGHS-II. Northern analysis was used to confirm that the probe recognized the 4.5-kb transcript of the PGHS-II. The probe was labeled with terminal deoxynucleotidyl transferase (Life Technologies, Inc., Burlington, Canada) and ^{33}P-labeled deoxy-α-thio-ATP (1300 Ci/mmol; NEN Life Science Products, DuPont Canada, Inc., Mississauga, Canada). The probe was used at a concentration of approximately 600 cpm/μl.

The method used for *in situ* hybridization has been described previously (13). Briefly, tissue sections (10 μm) were mounted on Fisher SuperFrost glass slides (Fisher Scientific, Nepean, Canada), fixed with 4% paraformaldehyde, dehydrated through graded ethanol, and stored in 95% ethanol at 4 C. Slides were removed from ethanol and allowed to air-dry at room temperature. Tissues were incubated overnight in a moist incubation chamber at 42 C with the radiolabeled oligonucleotide PGHS-II probe diluted in hybridization buffer. Hybridization buffer was composed of 4 $\times$ SSC (single strength 1 $\times$ SSC: 150 mM sodium chloride, 15 mM sodium citrate; Sigma; St. Louis, MO), 50% deionized formamide (Life Technologies, Inc., Burlington, Canada), 0.02% BSA (Roche Molecular Biochemicals, Dorval, Canada), 10% dextran sulfate (Pharmacia Biotech, Baie d'Urfe, Canada), 200 μg hydrolyzed salmon sperm DNA/ml, 0.02% polyvinylpyrrolidone, 40 mM dithiothreitol, and 50 mM sodium phosphate (pH 7.0; Sigma). After incubation, slides were washed with 1 $\times$ SSC at room temperature for 30 min and then with 1 $\times$ SSC at 45 C for 45 min. Slides were washed with decreasing strength SSC, dehydrated in ethanol, air-dried, and exposed to x-ray film (Eastman Kodak Co., Rochester, NY). Placental tissue was exposed for 38 h, and myometrium/fetal membranes were exposed for 270 h. The autoradiographic films were developed using standard procedure. The linearity of the mRNA signals was established by simultaneous exposure of the samples with ^{14}C-labeled standards in the appropriate range (RPA504, Amersham Pharmacia Biotech, Aylesbury, UK), and the OD of PGHS-II mRNA expression was determined relative to a curve established by these ^{14}C-labeled standards. Nonspecific binding was established using a 45-mer nonsensical sequence oligonucleotide probe, and the signal was subtracted from the antisense PGHS-II mRNA signal; the specificity of the antisense PGHS-II probe was established by incubation with positive tissue controls (term ovine placentome and cultured human amnion cells, both previously shown to express PGHS-II mRNA) (3, 4). The autoradiograms were then analyzed using computerized image analysis software (Image Research, Inc., St. Catherines, Canada; Laser Scanner, Molecular Dynamics, Inc., Sunnyvale, CA; ImageQuant software, Becton Dickinson and Co., Mountain View, CA). The relative OD of placental and endometrial PGHS-II expression was assessed using nine tissue sections per animal.

Western blot analysis

Frozen tissue samples were homogenized on ice for 1 min in RIPA lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% (wt/vol) sodium deoxycholate, 0.1% sodium lauryl sulfate (SDS), 100 μM sodium orthovanadate (Sigma), 1% (vol/vol) Triton X-100 (Fisher Chemicals, Fairlawn, NJ), and Complete MiniEDTA-free protease inhibitors (Roche Molecular Biochemicals; Dorval, Canada)]. Homogenates were centrifuged at 4 C at 15,000 $\times$ *g* for 15 min, and supernatants were collected. Protein concentrations were determined by the Bradford assay (14) using BSA (Bio-Rad Laboratories, Inc., Richmond, CA) as the standard and protein absorbance at 595 nm.

Protein samples (25–100 μg) were separated by polyacrylamide gel (4–10% gradient) electrophoresis as described by Laemmli (15). Proteins were electrophoretically transferred to a 0.45-μm pure nitrocellulose membrane (Bio-Rad Laboratories, Inc.); transfer was confirmed by protein visualization with Ponceau S solution (Sigma). Blots were washed with PBS-T (150 mM NaCl, 10 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, and 0.1% Tween-20, pH 7.5; Sigma) and incubated overnight with blocking solution (5% skim milk powder in PBS-T). Blots were incubated with primary antibody for PGHS-II (1:500 dilution in blocking solution; PG27, Oxford Biomedical Research, Inc., Oxford, MA) or PGHS-I (1:4000 dilution in blocking solution; PG19, Oxford Biomedical Research Instruments, Inc.). Blots were then rinsed five times for 5 min each time with PBS-T and incubated with secondary antiserum conjugated with horseradish peroxidase for 1 h (1:1000 dilution in blocking serum; Amersham Pharmacia Biotech). Blots were washed six times for 5 min each time, and the antibody-antigen complex was detected using the Amersham Pharmacia Biotech ECL detection system (Amersham Pharmacia Biotech). Blots were exposed to x-ray film (Eastman Kodak Co.) for serial exposure times to determine the appropriate concentration of protein loading and exposure time to ensure that the protein signal was within the linear response range. The intensity of the protein signal was quantified using computerized image analysis software (Image Research, Inc.; laser scanner from Molecular Dynamics, Inc.; ImageQuant software).

Statistical analysis

Uterine contractility data were analyzed by two-way ANOVA (repeated measures) followed by *post-hoc* Tukey's test and Student's *t* test. Plasma data were analyzed by two-way ANOVA (repeated measures), followed by *post-hoc* Tukey's test. PGHS-II mRNA and protein data were analyzed by one-way ANOVA followed by *post-hoc* Tukey's test. Significance was set at $P \leq 0.05$. Data are presented as the mean $\pm$ SEM for n = 4 or 5/group.

Results

Uterine contractility

In pregnant sheep whose fetuses were infused with cortisol alone, labor-type uterine contractions were present by 68 h (12 h before death) and had increased significantly by the time of death (80 h; Fig. 1). Labor-type uterine contractions were present after 68 h of cortisol plus 4OHA infusion and had increased significantly by the time of death (80 h; Fig. 1). However, during the last 2 h before death, the cortisol-treated animals had a significantly greater contraction frequency (56.6 $\pm$ 6.7 contractions/2 h) compared with the animals treated with cortisol and 4-OHA (26.1 $\pm$ 9.0 contractions/2 h). The uterine contracture pattern was not altered at any time during the infusion in either control group (Fig. 1).

Maternal and fetal plasma steroid levels

Mean fetal and maternal plasma E_2 concentrations were increased significantly during 80 h of cortisol infusion. The fetal plasma E_2 concentration increased from 21 $\pm$ 8 to 259 $\pm$ 33 pg/ml at the time of death (Fig. 2); the maternal plasma E_2 concentration was increased from 15 $\pm$ 2 to 217 $\pm$ 59 pg/ml (Fig. 3). The increases in both fetal and maternal

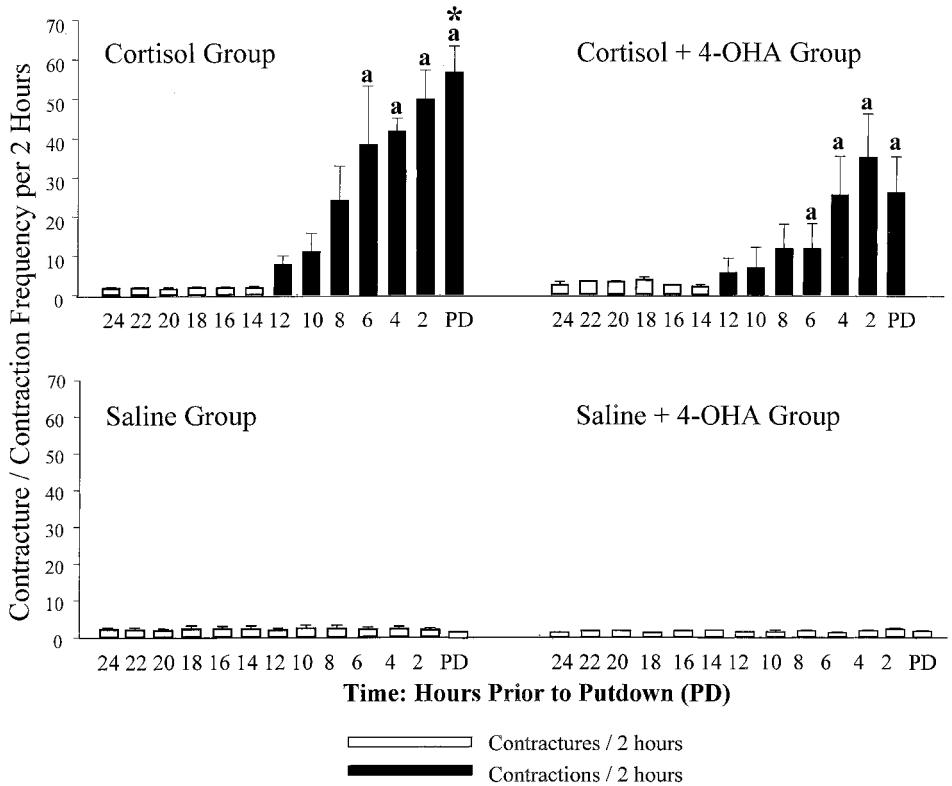

FIG. 1. Uterine activity patterns. Uterine activity, measured as the number of contractures or contractions per 2-h interval. Values are presented as the mean $\pm$ SEM (n = 5/group). Statistical analysis of the contraction frequency was performed using two-way ANOVA followed by *post-hoc* Tukey's test and Student's *t* test; significance was set at $P \leq 0.05$. Uterine contractions were present in both the cortisol- and cortisol- plus 4-OHA-treated animals after 68 h of infusion; this contraction frequency was increased significantly in the final 8 h of the infusion period (a, $P \leq 0.05$). However, in the cortisol-treated animals, contraction frequency during the final 2 h before death was significantly greater than that in cortisol- plus 4-OHA-treated animals (*, $P \leq 0.05$). There was no change in the uterine contracture pattern in either control group. PD, Putdown (80 h).

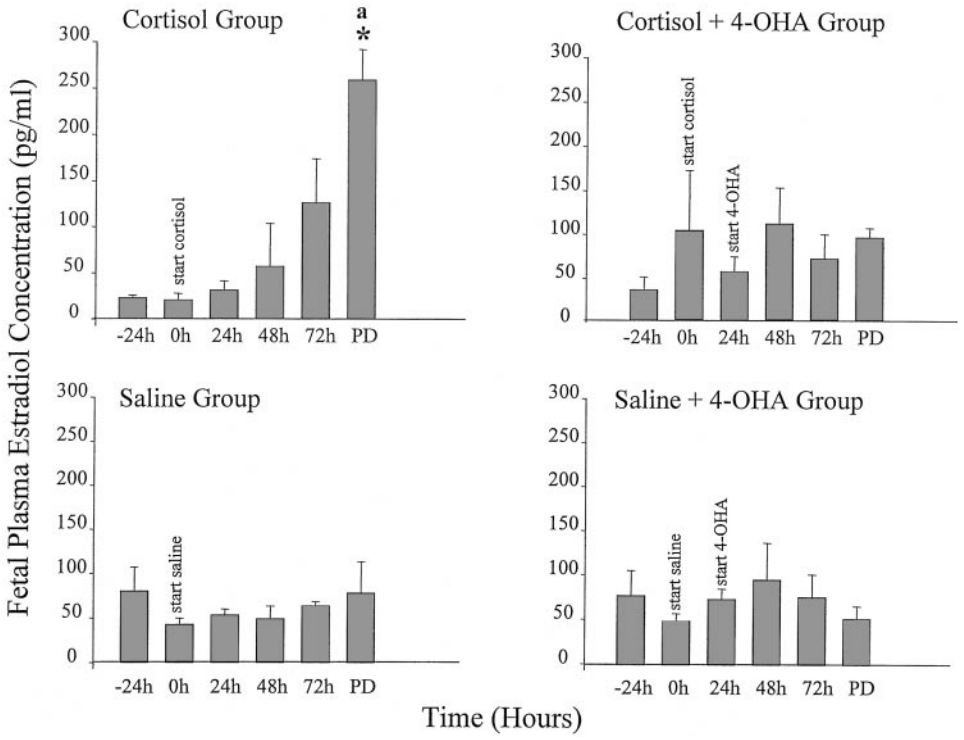

FIG. 2. Fetal plasma E_2 levels. Values are presented as the mean ± SEM over a 24-h period for five animals in each group. Statistical analysis was performed using two-way ANOVA (repeated measures) followed by *post-hoc* Tukey's test; significance was set at $P \leq 0.05$. The fetal plasma E_2 concentration in the cortisol-treated animals at the end of the infusion period (80 h; PD, putdown) was increased significantly compared with the basal level (0 h; *, $P \leq 0.05$) and to the final (80 h) fetal plasma E_2 concentration of the cortisol-plus 4OHA-treated animals (a, $P \leq 0.05$). Fetal plasma concentrations in the cortisol plus 4OHA and control groups did not change over the infusion period.

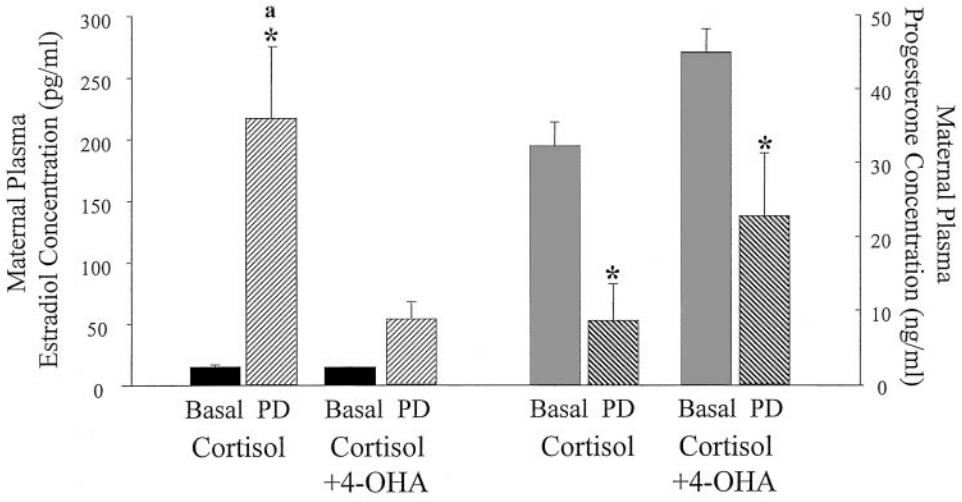

FIG. 3. Maternal plasma E_2 and P_4 levels. Values are presented as the mean ± SEM over the time period for five animals in each group. Statistical analysis was performed using two-way ANOVA (repeated measures) followed by *post-hoc* Tukey's test; significance was set at $P \leq 0.05$. The mean maternal plasma progesterone (P_4) was significantly decreased compared with the basal level (0 h) at the end of the infusion period (PD, putdown; 80 h) in the cortisol- and cortisol- plus 4OHA-treated animals. The mean maternal plasma E_2 concentration in the cortisol-treated animals was increased significantly at the end of the infusion period (PD; 80 h) compared with the basal level (0 h; *, $P \leq 0.05$) and to the final (80 h) fetal plasma E_2 concentration of the cortisol- plus 4OHA-treated animals (a, $P \leq 0.05$); plasma E_2 was not increased during the infusion period in the cortisol- plus 4-OHA-treated animals.

plasma E_2 concentrations were attenuated significantly by coinfusion with 4-OHA (Figs. 2 and 3). At the time of death, the fetal plasma E_2 concentration was 64% less, and the maternal plasma E_2 concentration was 54% less than those in the cortisol infusion group. Fetal plasma E_2 (Fig. 3) and maternal plasma E_2 were not altered at any time during the infusion period in either control group. The androstenedione concentration in fetal plasma was 342 ± 41 pg/ml before the start of the infusion. The concentration of androstenedione rose to 5340 ± 1427 pg/ml in the animals treated with cortisol and 4-OHA, but did not change significantly in any of the other animal groups (data not shown). The mean maternal P_4 concentration was decreased significantly from 33 ± 3 to 9 ± 3 ng/ml at the time of death in the cortisol-treated animals. Maternal plasma P_4 also decreased significantly in the cortisol- plus 4-OHA-treated animals (Fig. 3). The ratio of E_2/P_4 in maternal plasma was increased from 0.46 to 23.4 by cortisol infusion. The E_2/P_4 ratio change in the animals coin-

fused with 4-OHA was attenuated and rose from 0.32 to only 2.3. There was no significant change in maternal plasma P_4 before and at the end of the infusion period in either control group of animals (data not shown).

Maternal and fetal plasma PG levels

The basal fetal plasma PGE_2 values in all four groups of animals were not statistically different. Mean concentrations of PGE_2 in fetal plasma increased significantly during 80 h of either cortisol or cortisol plus 4-OHA infusion (Fig. 4) to 1618 ± 471 and 1241 ± 91 pg/ml, respectively; these values were not statistically different from each other. The mean maternal plasma PGFM concentration increased significantly from 83 ± 40 to 423 ± 85 pg/ml at the time of death in the cortisol-infused animals. However, in the cortisol plus 4-OHA-treated animals, the basal PGFM concentration was not increased significantly at the end of the infusion period (basal PGFM concentration, 149 ± 22 pg/ml; terminal PGFM concentration, 157 ± 49 pg/ml; Fig. 5). The plasma PGE_2 and PGFM concentrations in the control animals did not change throughout the infusion period (Figs. 4 and 5).

Intrauterine PGHS-II mRNA and protein expression

Placental trophoblast PGHS-II mRNA expression was increased significantly by both cortisol and cortisol plus 4OHA infusion (Fig. 6); there was a corresponding increase in placental PGHS-II immunoreactive protein expression in both of these groups of animals (Fig. 7). PGHS-II mRNA expression in the intercotyledonary endometrium was increased significantly after cortisol infusion, but not after cortsiol plus 4OHA infusion. Similarly, endometrial immunoreactive PGHS-II protein expression was increased after cortisol, but not after cortisol plus 4-OHA, infusion (Fig. 7). Placental and endometrial immunoreactive PGHS-II protein migrated an equal distance under electrophoresis (data not shown). Levels of immunoreactive PGHS-I protein expression in placenta and endometrium were not significantly different among the four groups of animals (Fig. 8).

Discussion

In this study we have shown that intrafetal cortisol infusion increased fetal trophoblast PGHS-II mRNA and protein expression, fetal plasma PGE_2 concentration, endometrial PGHS-II mRNA and protein expression, and maternal plasma PGFM concentration. In the absence of placental E_2 production, intrafetal cortisol infusion did not lead to an increase in maternal endometrial PGHS-II expression and maternal plasma PGFM concentration. Uterine activity was attenuated when placental E_2 synthesis was inhibited. Therefore, we suggest that within the ovine intrauterine environment, the increased placental PGHS-II expression and PGE_2 production associated with elevated fetal cortisol concentrations are independent of placental E_2 synthesis, but endometrial PGHS-II expression and $PGF_{2\alpha}$ production are dependent upon increased placental E_2 synthesis. Estrogen synthesis is also required for the development of a full labor-like pattern of uterine contractility.

We used Lentaron (4-OHA) to inhibit placental aromatase activity. 4-OHA is a competitive inhibitor that irreversibly binds to the active site of the aromatase enzyme (9). Nathanielsz *et al.* (16) showed that maternal iv 4-OHA infusion inhibited androgen-induced E_2 synthesis in pregnant rhesus monkeys, suggesting that 4-OHA could inhibit placental aromatase activity. France *et al.* (17) showed that 4-OHA could inhibit ovine placental aromatase activity *in vitro* with a K_i of 0.05 μM; we presume that this is the mech-

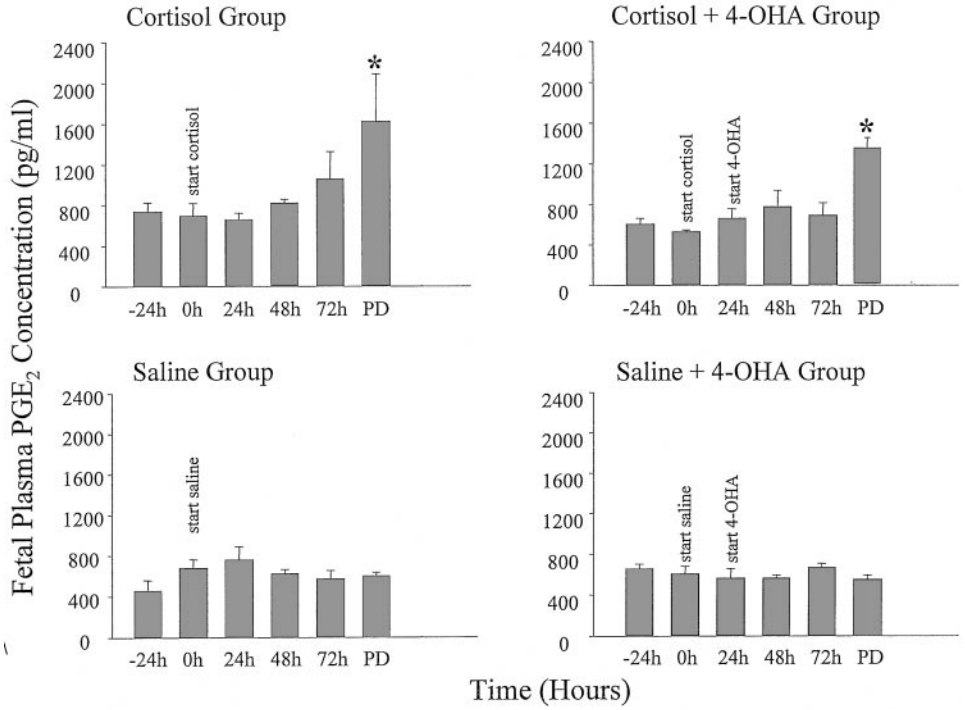

FIG. 4. Fetal plasma PGE_2 concentration. Values are presented as the mean ± SEM over the time period for five animals in each group. Statistical analysis was performed using two way ANOVA (repeated measures) followed by *post-hoc* Tukey's test; significance was set at $P \leq 0.05$. The fetal plasma PGE_2 concentration was increased significantly at the end of the infusion period (PD, putdown; 80 h) in both the cortisol- and cortisol- plus 4OHA-treated animals. There was no change in plasma PGE_2 concentration in the two control groups.

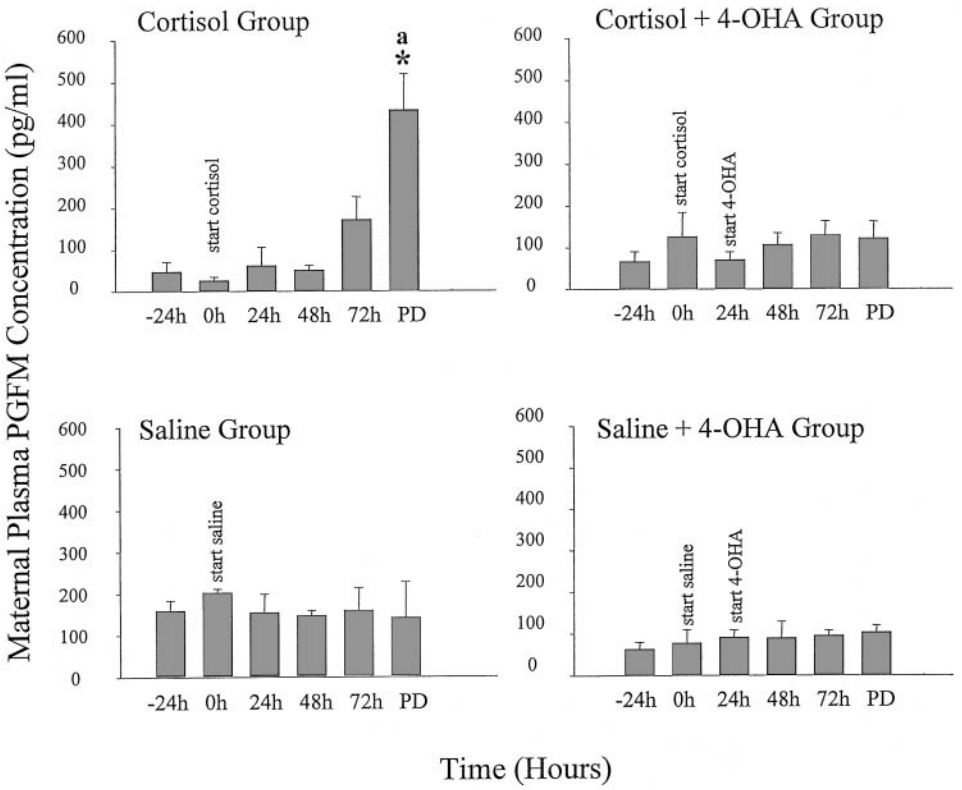

FIG. 5. Maternal plasma PGFM concentration. Values are presented as the mean ± SEM over the time period for n = 5 animals in each group. Statistical analysis was performed using two-way ANOVA (repeated measures) followed by *post-hoc* Tukey's test; significance was set at $P \leq 0.05$. Maternal plasma PGFM was increased significantly at the end of the infusion period (PD, putdown; 80 h) in the cortisol-treated animals (*, $P \leq 0.05$). Maternal plasma PGFM in the cortisol- plus 4OHA-treated animals did not change over the infusion period and was significantly less at the end of the infusion period compared with that in the cortisol-treated animals (a, $P \leq 0.05$). There was no change in plasma PGFM concentrations in the two control groups.

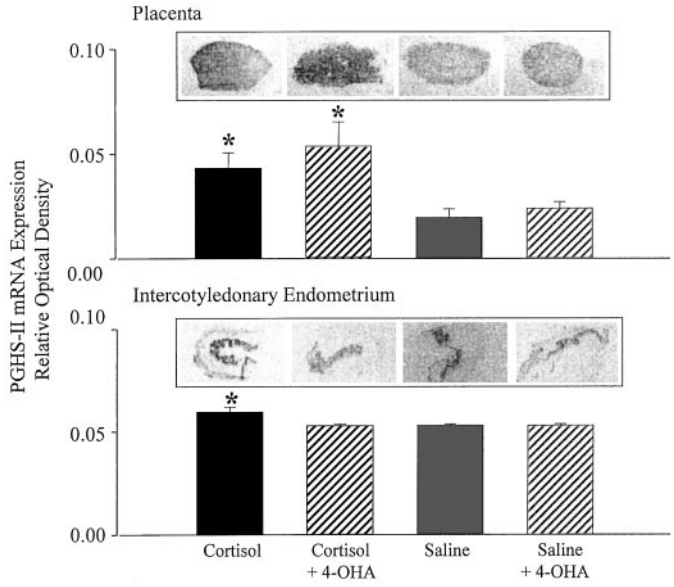

FIG. 6. Placental and intercotyledonary endometrial PGHS-II mRNA expression. Values are presented as the mean ± SEM over the time period for four animals in each group. Statistical analysis was performed using one-way ANOVA followed by *post-hoc* Tukey's test; significance was set at $P \leq 0.05$. Placental tissue had a 38-h exposure time; endometrial tissue had a 270-h exposure time. Placental PGHS-II mRNA expression was increased significantly by both cortisol and cortisol plus 4-OHA infusion. Endometrial PGHS-II mRNA expression was increased significantly by cortisol infusion, but not by cortisol plus 4-OHA infusion.

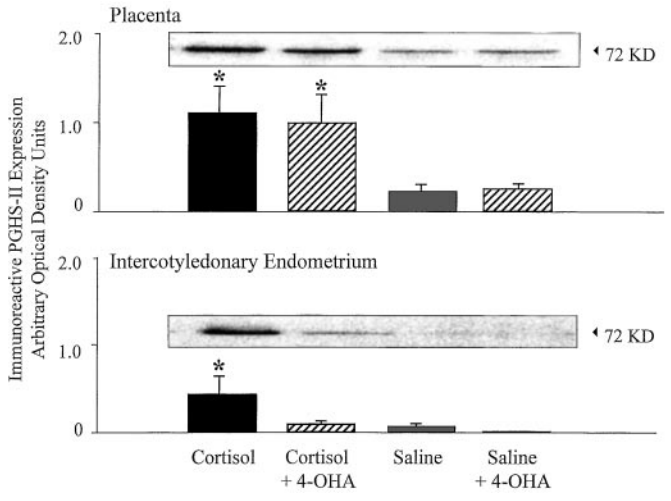

FIG. 7. Placental and intercotyledonary endometrial immunoreactive PGHS-II expression. Values are presented as the mean ± SEM over the time period for four animals in each group. Statistical analysis was performed using one-way ANOVA followed by *post-hoc* Tukey's test; significance was set at $P \leq 0.05$. Placental immunoreactive PGHS-II expression was significantly increased by cortisol and cortisol plus 4-OHA infusions. Endometrial immunoreactive PGHS-II was increased significantly by cortisol infusion, but not by cortisol plus 4-OHA infusion.

anism of the 4-OHA effect in the present study. There was no effect on basal steroid or PG concentrations in the animals receiving 4-OHA but not cortisol. The androstenedione concentration was increased in the plasma of fetuses treated with cortisol and 4-OHA. This increase was not observed in the other treatment groups, consistent with precursor build-up after cortisol infusion in the presence of 4-OHA and effective inhibition of placental aromatase activity. Using this protocol we were able to block the cortisol-induced increase in pla-

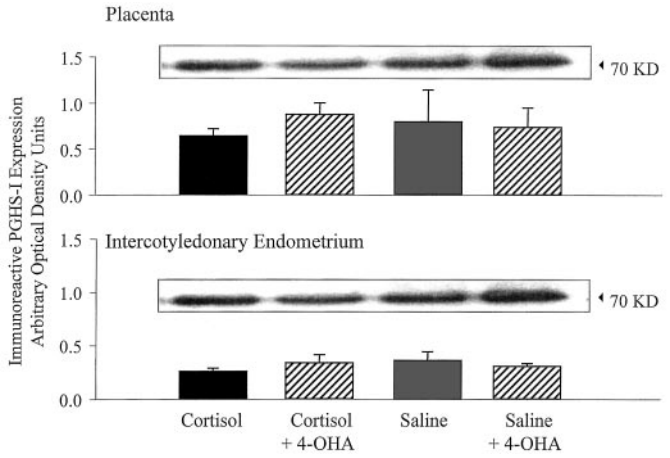

FIG. 8. Placental and intercotyledonary endometrial immunoreactive PGHS-I expression. Values are presented as the mean ± SEM over the time period for four animals in each group. Statistical analysis was performed using one-way ANOVA; significance was set at $P \leq 0.05$. Placental and intercotyledonary endometrial immunoreactive PGHS-I expression remained unchanged and was not significantly different among the four groups of animals.

cental E_2 production, and thereby we were able to determine the effects of intrafetal cortisol on intrauterine PGHS-II expression and PG output in the presence and absence of increased placental E_2 production. However, we cannot exclude the effects of basal placental E_2 production, nor can we exclude the possibility that the increase in androstenedione production and/or 4-OHA may have influenced endometrial PGHS-II expression and $PGF_{2\alpha}$ output in the animals treated with cortisol plus 4-OHA.

Recently, glucocorticoids (GC) have been found to up-regulate PGHS-II expression and activity in some trophoblast-derived cells (18–21), although inhibition of PGHS-II in amnion WISH cells treated with GC has also been reported (22). The effects of cortisol on ovine placental PGHS-II expression and PGE_2 output were independent of an increase in placental E_2 production. Ovine placental PGHS-II mRNA and protein expression have been localized previously to the mononuclear trophoblast cells (23), which also express the glucocorticoid receptor (24). The postreceptor mechanism by which GCs could regulate PGHS-II is not well understood; a specific glucocorticoid response element within the 5′-promoter region of the PGHS-II gene has been reported by two groups of investigators (25, 26). The PGHS-II promoter also contains other transcription factor-binding sites including two nuclear factor-κB (NFκB) sites (25–27). Although GCs have been shown to have a suppressor effect at the NFκB site, recent evidence has suggested that GCs may interact with the NFκB site to induce expression of acute phase hepatic reactant genes (28). A similar interaction could occur with PGHS-II. Alternatively, GCs could increase PGHS-II expression through interference with repressor transcription factors, by stimulation of promotional transcription factors, or by affecting an increase in the stability of PGHS-II mRNA (20, 21, 28, 29). Our present findings mimic the effects of GCs observed in human amnion epithelial cells (18) and amnion fibroblast cells (19). Mixed cultures of human amnion cells increased PGE_2 output in response to cortisol and dexamethasone stimulation (20, 21, 29). This stimulatory effect was receptor dependent and involved an increase in PGHS-II expression. Thus, we suggest that the prepartum increase in fetal adrenal cortisol output increases the expression of PGHS-II within fetal ovine placental trophoblast cells, leading to PGE_2 production; this PG synthesis occurs independently of an increase in placental E_2 output.

In addition, we found that endometrial PGHS-II expression and intrauterine $PGF_{2\alpha}$ output, reflected in maternal PGFM concentrations, were dependent upon placental E_2 synthesis. Previously, we were unable to demonstrate an increase in sheep placental PGHS-II expression with intrafetal E_2 infusion (4). In addition, PGHS-II expression and activity could not be stimulated by E_2 in cultured human trophoblast-derived cells (30). However, PGHS-II expression can be stimulated by E_2 in other tissues, including human monocytes, bovine oviduct, nonpregnant ovine endometrium, and myometrium (7, 31, 32). These data support a role for E_2 in the regulation of PG synthesis by intrauterine, nontrophoblast tissue, while arguing against a role for E_2 in the regulation of trophoblast PGHS-II. Recent studies have reported the presence of the estrogen receptor (ER) within nontrophoblast intrauterine tissues, including maternal placental villi, endometrium, and myometrium of the sheep in late gestation. The ER was absent from placental trophoblast cells (33). These data further preclude an E_2 effect on PGHS-II within the fetal trophoblast tissue and are consistent with ER-mediated E_2 regulation of PGHS-II within the endometrium of pregnant sheep. The PGHS-II promoter does not contain an estrogen response element, but does contain the transcription factor-binding site AP-1 (25–27). E_2 has been shown to interact with the AP-1 site to induce gene expression (28). Thus, we suggest that placental E_2 up-regulates maternal endometrial PGHS-II expression and $PGF_{2\alpha}$ output at the onset of ovine parturition; this effect may be direct, mediated by the ER. In addition, early studies using nonpregnant sheep showed that P_4 treatment increased intrauterine PG synthetic activity and was a prerequisite for the additional effects of E_2 (7, 34). Therefore, we must not exclude the role P_4 plays in the regulation of PG production.

In addition to the observed changes in PGHS-II expression within the fetal trophoblast and maternal endometrial tissue, we cannot exclude possible changes in the expression and activity of other key enzymes in the PG biosynthetic pathway, including PGE isomerase, PGF synthase, and PG dehydrogenase (PGDH). To date, little information is available regarding the expression and activity of these enzymes within ovine intrauterine tissues. PGF synthase mRNA has recently been identified in the ovine maternal placenta, endometrium, and myometrium (35). The expression of this enzyme decreased only within the endometrium during betamethasone-induced preterm labor and remained unchanged in all three tissues with spontaneous term labor (35). These data suggest that the increase in PGHS-II expression may be more important than PGF synthase in the regulation of $PGF_{2\alpha}$ production at the onset of labor. PGDH has been identified within the ovine fetal trophoblast and maternal endometrium during pregnancy, and its activity increases within the placenta at the time of active labor (36, 37). In contrast, human chorionic and placental PGDH expression

and activity have been found to decrease with the onset of labor (38). Using cultured chorionic and placental trophoblast cells, P_4 has been found to maintain PGDH expression/activity, and cortisol has been shown to decrease PGDH expression/activity (38). Evidence has suggested that at term increasing cortisol concentrations compete with P_4 in the regulation of PGDH; the resultant effect is a net decrease in PGDH expression, leading to an overall increase in intrauterine PG production (39). In our animals plasma fetal cortisol increased, and maternal plasma progesterone decreased, suggesting that PGDH expression and activity within the intrauterine tissues may decrease and contribute to the rise of PGs. Given that maternal plasma PGFM levels did not increase in the cortisol- plus 4-OHA-treated animals, increased cortisol and decreased P_4 may not be sufficient to suppress endometrial PGDH expression/activity and lead to increased $PGF_{2\alpha}$ output. In addition, E_2 may play a role in the regulation of ovine intrauterine PGDH expression/activity. The regulation of these key enzymes requires further investigation.

Placental PGE_2 production might play an important role in mediating fetal hypothalamic-pituitary-adrenal (HPA) axis activation and placental steroidogenesis at the onset of labor. Fetal plasma cortisol and PGE_2 concentrations increase with a similar time course over the last 20 days of gestation and are associated in a positive feedback manner (40). Intrafetal PGE_2 infusion increased fetal plasma cortisol and ACTH hormone concentrations in late gestation (41, 42). Recently, we have shown that specific inhibition of PGHS-II blocked the increase in fetal plasma cortisol and ACTH concentrations induced by RU486 administration in late gestation sheep (43). These data suggest that placental PGE_2 may be important for sustaining activation of the HPA axis at the end of gestation and the onset of labor. In sheep, placental E_2 production is mediated by the rate-limiting action of $P450_{C17\ hydroxylase}$, which catalyzes the conversion of C_{21} steroids to the C_{19} steroid precursors that will be aromatized to form estrogen (44, 45). Expression of placental $P450_{C17\ hydroxylase}$ at the onset of labor occurs well after the increases in placental PGHS-II expression and PGE_2 production (3, 6, 46). We propose that toward the end of gestation, fetal adrenal cortisol induces placental PGHS-II expression and PGE_2 production; in turn, PGE_2 may direct placental estrogen synthesis and act in a positive feedback loop to maintain fetal HPA activation through the onset of labor.

The frequency of uterine contractions was attenuated in the absence of placental E_2 production, although we did not find a delay in the activation of uterine contractility from contractures to contractions. Myometrial contractility is associated with induction of a specific cassette of CAPs, including connexin 43, oxytocin receptor, ion channels, and PG receptors (2); these proteins are responsible for the evolution of uterine activity from quiescence to contractility. We suggest that endometrial PGHS-II may also be considered a CAP, contributing to this evolution of uterine activity. Once CAP expression has been initiated, the uterus can be stimulated to contract by a variety of uterotonins, in particular oxytocin and PGs (1, 2, 4). CAP expression appears to be regulated by a ratio of E_2/P_4 in late gestation (2). In the present study the E_2/P_4 ratio in the cortisol-treated animals increased 24-fold at the end of the infusion period, whereas the E_2/P_4 ratio of the cortisol- plus 4OHA-treated animals increased only 2-fold. Thus, the lack of change in the E_2/P_4 ratio and/or the lack of increase in intrauterine $PGF_{2\alpha}$ production (7) observed in the cortisol- plus 4-OHA-treated animals may have failed to induce CAP expression, thereby attenuating uterine activity. Alternatively, CAPs may have been induced but uterine contractility not initiated because the production of $PGF_{2\alpha}$ did not increase. These possibilities remain to be evaluated.

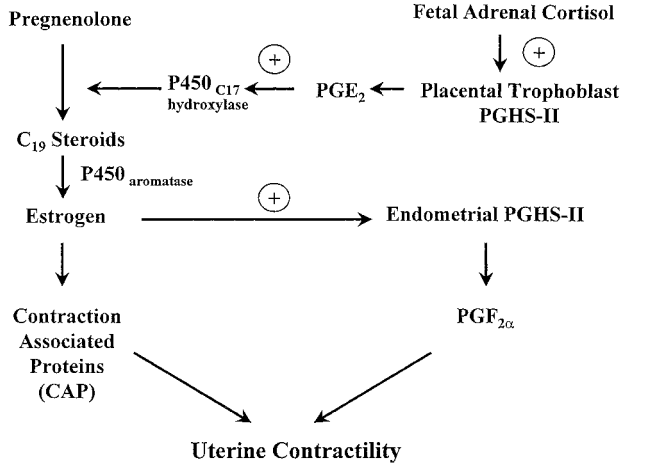

FIG. 9. Proposed hypothesis of events at ovine parturition.

Based on the observations of the present study we propose a new model for the onset of parturition in sheep (Fig. 9). We suggest that toward the end of gestation there is a gradual and sustained increase in the placental trophoblast expression of PGHS-II expression and PGE_2 production under the regulation of fetal cortisol produced from the maturation of the fetal HPA axis. Placental PGE_2, in turn, mediates an autocrine/paracrine increase in placental $P450_{C17hydroxylase}$ expression/activity to promote placental estrogen production and also acts to sustain fetal HPA axis activation. Estrogen up-regulates the expression of maternal endometrial PGHS-II and $PGF_{2\alpha}$ output as well as induces the expression of CAPs. Consequently, myometrial contractility is stimulated, and labor ensues. This hypothesis follows a tissue-specific progression of parturition events from a fetal signal to a maternal labor response.

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