Effects of periconceptional undernutrition on the initiation of parturition in sheep

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Kumarasamy, Vasumathy, Murray D. Mitchell, Frank H. Bloomfield, Mark H. Oliver, Melanie E. Campbell, John R. G. Challis, and Jane E. Harding. Effects of periconceptional undernutrition on the initiation of parturition in sheep. Am J Physiol Regul Integr Comp Physiol 288: R67–R72, 2005. First published August 26, 2004; doi:10.1152/ajpregu.00357.2004.—In sheep, parturition is initiated by increased fetal hypothalamic-pituitary-adrenal axis (HPAA) activity leading to PGE2 and PGF2α production and a rise in the 17β-estradiol-progesterone (E2/P4) ratio. Uteroplacental PG production can also increase fetal HPAA activity. Periconceptional maternal undernutrition accelerates fetal HPAA maturation resulting in preterm labor. We determined whether preterm labor was preceded by an increase in PG concentrations and E2/P4 ratio and whether these increases preceded or followed the corresponding rise in cortisol concentrations. Singleton-bearing ewes were nourished ad libitum (N, n = 9) or undernourished (UN, n = 10) to reduce maternal weight by 15% from 61 days (d) to +30 d after mating with ad libitum intake thereafter. Paired maternal and fetal blood samples were collected from 126 d until delivery. Half the UN group delivered prematurely (>2 SD below mean gestation for the flock). PG and cortisol concentrations and E2/P4 ratio increased before delivery in the same way in both groups. However, the increases occurred 7–10 d earlier in UN than in N animals. In both UN and N fetuses cortisol concentrations rose before fetal and maternal PG concentrations and maternal E2/P4 ratio. Periconceptional maternal undernutrition induces preterm delivery in sheep by advancing the expected prepartum rise in cortisol and PG concentrations and E2/P4 ratio. The rise in fetal cortisol concentration precedes the rise in fetal and maternal PG concentrations and maternal E2/P4 ratio, suggesting that the underlying mechanism is likely to be acceleration of fetal HPAA maturation, resulting in initiation of the normal process of parturition.

hypothalamic-pituitary-adrenal axis; prostaglandin; cortisol; estrogen-progesterone ratio

IN MOST ANIMAL SPECIES birth is triggered through the activation of the fetal hypothalamic-pituitary-adrenal axis (HPAA), whereas preterm birth may be associated with precocious activation of this axis (5). Despite the existence of a functional negative feedback mechanism in the fetal HPAA at 121–131 days gestation, plasma adrenocorticotropic (ACTH) and cortisol concentrations rise progressively in a semilogarithmic pattern in the last 15–20 days of gestation (14, 32–34). This sustained release of glucocorticoids is essential for organ maturation and also provides the trigger for the initiation of parturition (7).

An accepted pathway for the initiation of parturition suggests that the prepartum surge of fetal cortisol leads to an increase in the estrogen-progesterone ratio via the activation of placental enzymes. Estrogen, in turn, stimulates intrauterine PG production (16, 17). It also triggers the expression of contraction-associated proteins within the myometrium, leading to labor and delivery of the fetus (19). However, recent evidence suggests two separate pathways for the production of PGs: a cortisol-dependent/estrogen-independent pathway within the fetal placental trophoblast tissue and an estrogen-dependent pathway within the maternal intrauterine tissue (31). PGE2 appears to be important for the endocrine responses of the fetus and the induction of placental P450C17 hydroxylase enzyme, whereas PGF2α is better correlated with changes in uterine activity.

The alternative hypothesis suggests that placental PGE2 production might play a key role in mediating fetal HPAA activation, as fetal PGE2 infusion increased cortisol and ACTH concentrations (18, 28), and specific inhibition of PGH synthase (PGHS)-2 blocked the increase in fetal plasma cortisol and ACTH concentrations in late-gestation sheep (20). Hence placental PGE2 may directly stimulate the fetal adrenal gland, sustaining the activation of the fetal HPAA at the end of gestation via a feed forward loop (1, 18).

Previous experiments from our group show that undernutrition from 61 days before until 30 days after mating results in an earlier rise of plasma cortisol concentrations in the fetuses of undernourished ewes. Half of the fetuses had a precocious rise and delivered preterm, suggesting an accelerated maturation of fetal HPAA (3). Fetal ACTH concentrations were higher in all undernourished fetuses regardless of the timing of birth, consistent with the hypothesis that maturation of the HPAA must reach a threshold level to initiate a feed forward loop in producing increased ACTH and cortisol concentrations concomitantly, leading to labor and delivery of the fetus (3). However, it is also possible that undernutrition might have altered placental function, thus initiating parturition via the alternative pathway where an increase in PGE2 synthesis rather than cortisol might initiate the events leading to parturition.

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We therefore undertook the present study to determine whether preterm labor and delivery induced by periconceptional maternal undernutrition was preceded by the expected increase in fetal PGE2 and maternal 13,14-dihydro-15-keto-prostaglandin F (PGFM, a stable metabolite of PGF2α) concentrations and an increase in maternal 17β-estradiol-progesterone (E2/P4) ratio, and also whether these increases preceded or followed the corresponding rise in cortisol concentrations.

METHODS

Animals and surgical procedures. Experiments were approved by the institutional Animal Ethics Committee of the University of Auckland. Animal management techniques are as described previously (3, 25). Five-year-old Romney ewes were acclimatized to indoor conditions and feeding on a concentrate diet consisting of 65% lucerne and 30% barley, with limestone, molasses, and trace element supplements. Sixty-one days before mating, ewes were weighed and then randomly assigned to maintenance feeding (N, concentrates at 3–4% of body wt/day) or low-plane feeding (UN, fasted for 2 days then fed concentrates at 1–2% of body wt/day). Ewes were weighed weekly, and individual rations for UN ewes were adjusted to maintain a 10–15% reduction in body weight. A fortnight before mating the estrous cycles of ewes were synchronized with intravaginal devices containing progesterone. The feed restriction of UN ewes continued until 30 days after mating, and thereafter all ewes were kept on the maintenance level of feeding. Ewes were ultrasound scanned 62 days after mating, and only singleton-bearing ewes were included in the experiment. At 105 days of gestation, ewes were transported to the laboratory and acclimatized for 7 days before surgery. With the sheep under general anesthesia, polyvinyl catheters were inserted into tarsal vein and artery of both fetal hindlimbs and a maternal femoral artery and vein, carotid artery, and jugular vein. Paired maternal and fetal blood samples were collected between 0800 and 1000 before feeding every second day from 126 days gestation, daily from 135 days, and then twice daily from 142 days until delivery. Blood was collected into EDTA tubes containing 30 μl of acetic acid (5 ng/ml) on ice and centrifuged for 10 min at 3,000 rpm and 4°C. Plasma was removed and stored at −20°C until analysis.

Radioimmunoassays. Reversed-phase solid-phase extraction (SPE)-C18 cartridges were obtained from Alltech Associates (Deerfield, IL). Methanol and cyclohexane were purchased from Scharlau (Barcelona, Spain), and ethyl acetate was obtained from APS (Seven Hills, NSW, Australia). PGE2 and PGFM standards were acquired through Cayman Chemical (Ann Arbor, MI). PGE2 antibody was raised in house and PGFM antibody was a gift from Professor F. Dray (Pasteur Institute, Paris, France). [5,6,8,11,12,14,15(15)-3H]-PGE2 and [5,6,8,11,12,14,15(15)-3H]-PGFM were obtained from Amersham-Pharmacia Biotech (Aylesbury, UK). Activated charcoal and dextran were purchased from Sigma (St. Louis, MO).

The extraction procedure was validated for use in sheep plasma. Reproducibility was assessed with repeated measurements from 10 plasma pool samples, and the coefficient of variation (CV) was 26% for PGE2 and 9% for PGFM. Parallelism was assessed by extracting PGE2 and PGFM from varying volumes (0.125–0.5 ml) of plasma pool in triplicate. There was a strong linear relationship between volume of plasma and extracted PGE2 (y = 101.6x, r² = 0.54, P < 0.0001) and PGFM (y = 18.44x, r² = 0.89, P < 0.0001) concentrations. Accuracy was assessed by the addition of known amounts of PGE2 (0–2,500 pg) and PGFM (0–300 pg) to fetal and maternal plasma, respectively. The measurements were corrected for endogenous PG concentrations in the plasma. There was a strong linear relationship between added and recovered PGE2 (y = 1.071x, r² = 0.95, P < 0.0001) and PGFM (y = 1.000x, r² = 0.88, P < 0.0001) concentrations.

Plasma samples were thawed on ice and centrifuged for 10 min at 3,000 g and 4°C. Based on the Cayman Chemical EIA Kit sample purification protocol, SPE-C18 cartridges were activated with methanol and milliQH2O. Acidified plasma samples and PG spiked samples were applied onto cartridges, rinsed with milliQH2O and cyclohexane, and eluted with ethyl acetate containing 1% methanol. The eluent was evaporated under a stream of nitrogen, and the dry residue was dissolved in PG buffer (8.0 g/l Na2HPO4, 2.4 g/l anhydrous NaH2PO4, 9.0 g/l NaCl, and 1.0 g/l bovine γ-globulin). Concentrations of PGE2 and PGFM were measured by specific radioimmunoassay as described previously (15, 23). Cortisol was assayed by an in-house radioimmunoassay, and these data have been reported previously (3). Estradiol was measured using a double antibody radioimmunoassay kit (Diagnostic Products, Los Angeles, CA). This kit has previously been validated in the rat (12), and we validated it in sheep plasma. Stripped ovine plasma was spiked with serial concentrations of 17β-estradiol from 5–500 pg/ml in 1% bovine serum albumin (BSA). Extractions of varying volumes of spiked plasma (50–400 μl) together with assay standards, were made using diethyl ether containing 5% vol/vol 0.1% ascorbic acid. After extraction and drying under a stream of nitrogen, samples were reconstituted with 1 ml of 1% BSA. Recovery of 17β-estradiol was 89–94%. Extracted samples were assayed in duplicate. Parallelism was observed, and the CV for the spiked plasma was 6.4%. The minimum detectable dose was 0.14 pg/ml. For the actual samples from this study, 100 μl of plasma were extracted together with assay standards, reconstituted in 1% BSA, and assayed in duplicate. The intra-assay CV for the actual samples was 9.2%. Progesterone was measured by an in-house radioimmunoassay following extraction with diethyl ether as described previously (2) (intra-assay CV 5.4%, detection limit 1.5 ng/ml).

Statistical analysis. The UN group (UN-Total) was further divided into two groups: animals that delivered at term (UN-Term) and animals that delivered preterm (UN-Preterm), defined as a gestational length >2 SD below the mean for the flock (i.e., <138 days). Data were analyzed in two ways: comparison between two groups, maintenance fed/delivered at term (N-Term) and UN-Total, and comparison between three groups, N-Term, UN-Term, and UN-Preterm. Baseline concentrations of PG, cortisol, and estradiol were defined as the mean of all results from samples taken ≥7 days before delivery. The timing of the rise in PG, cortisol, and estradiol concentrations was taken as the day on which they first exceeded 2 SD above mean baseline concentrations. Data are presented as means ± SE. Hormone levels were compared between treatment groups using one-way analysis of variance (ANOVA). The time of rise of hormones in relation to each other and to treatment group was analyzed by two-way ANOVA. (Statview 5.0.1; SAS institute, Cary, NC).

RESULTS

We have previously reported that the fetuses from UN ewes delivered earlier than N ewes across the gestational age range (Table 1) (3). All lambs were normally grown at birth, although all preterm lambs died soon after birth (3, 26). The basal concentration of fetal plasma PGE2 in our study was 0.5 ± 0.3 ng/ml and increased rapidly to peak in the range of 0.35–12.44 ng/ml. The basal concentration of maternal plasma PGFM in our study was 0.2 ± 0.1 ng/ml and increased to peak in the range of 0.23–14.97 ng/ml.

There was no difference between the N-Term and UN-Total groups in the baseline concentrations of either fetal PGE2 or maternal PGFM (Table 1). When N-Term, UN-Term, and UN-Preterm groups were compared, there was no difference among groups for fetal PGE2 concentrations, but maternal PGFM concentrations were higher in UN-Preterm than UN-Term ewes (P < 0.05, Table 1). (Fig. 1). The timing of the first
rise in fetal PGE2 and maternal PGFM concentrations occurred about 2 wk earlier in UN-Preterm than in UN-Term and N-Term groups ($P < 0.0001$, Table 1) (Fig. 1), but when analyzed in relation to time of delivery, rather than gestational age, fetal PGE2 and maternal PGFM concentrations increased before delivery in the same way in all groups (Fig. 2). There was no difference between the fetus and ewe in the timing of the rise in PG concentrations in all animals (141.1 ± 6.8 vs. 139.8 ± 5.7 days, not significant (ns)). However, in N-Term ewes, but not in any other group, maternal PGFM concentrations increased 3 days before fetal PGE2 concentrations ($P = 0.03$).

These data on changes in PG concentrations were then related to maternal $E_2/P_4$ ratio and to previously published changes in cortisol concentrations (3). There were no differences among the groups in the baseline concentrations of maternal $17\beta$-estradiol and progesterone, in $E_2/P_4$ ratio or of either fetal or maternal cortisol (Table 1). The first rise in maternal $E_2/P_4$ ratio occurred 11–13 days earlier in UN-Preterm ewes than in UN-Term or N-Term ewes ($P < 0.0001$, Table 1). Similarly, the first rise in fetal cortisol concentration occurred ~10 days earlier in UN-Preterm fetuses than in UN-Term and N-Term fetuses (Table 1). The first rise in maternal cortisol concentration also occurred 10 days earlier in UN-Preterm ewes than in N-Term and 1 day earlier than in UN-Term ewes (Table 1). However, when analyzed in relationship to time of delivery rather than gestational age, once again maternal $E_2/P_4$ ratio and fetal and maternal cortisol concentrations increased before delivery in the same way in all groups (Fig. 3). There was no significant difference between the fetus and ewe in the timing of the rise in cortisol concentrations in all animals (136.8 ± 5.3 vs. 137.7 ± 8.2 days, ns) or in each group individually.

We then compared fetal cortisol concentrations in the days leading up to delivery with fetal PGE2 and maternal PGFM concentrations and with maternal $E_2/P_4$ ratio. As described above, there were no significant differences in the concentrations of any of these hormones among groups (N-Term, UN-Term, and UN-Preterm) when analyzed in relationship to time.

### Table 1. Gestational age at delivery, baseline plasma prostaglandin, cortisol, $17\beta$-estradiol, and progesterone concentrations and time of their rise in well-nourished and undernourished groups

<table>
<thead>
<tr>
<th></th>
<th>UN-Total</th>
<th>UN-Term</th>
<th>UN-Preterm</th>
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<tbody>
<tr>
<td>N-Term</td>
<td>$n = 9$</td>
<td>$n = 10$</td>
<td>$n = 5$</td>
</tr>
<tr>
<td>Gestational age at delivery, days</td>
<td>144.7 ± 1.9</td>
<td>138.9 ± 7.0$^a$</td>
<td>145.1 ± 1.1</td>
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<td>Baseline concentration</td>
<td>PGE2 (ng/ml)</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>PGFM (ng/ml)</td>
<td>2.6 ± 0.8</td>
<td>3.4 ± 3.2</td>
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<tr>
<td></td>
<td>Fetal cortisol (ng/ml)</td>
<td>1.7 ± 0.02</td>
<td>0.16 ± 0.03</td>
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<tr>
<td></td>
<td>Maternal cortisol (ng/ml)</td>
<td>142 ± 3</td>
<td>138 ± 7</td>
</tr>
<tr>
<td></td>
<td>Maternal PGFM (ng/ml)</td>
<td>143 ± 4</td>
<td>133 ± 9$^a$</td>
</tr>
<tr>
<td></td>
<td>Maternal $E_2$ (pg/ml)</td>
<td>142 ± 1</td>
<td>137 ± 3</td>
</tr>
<tr>
<td></td>
<td>Maternal $E_2/P_4$ ratio</td>
<td>143 ± 1</td>
<td>139 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. N-Term, maintenance-fed animals delivered at term; UN-Total, low plane-fed group, delivered both term and preterm; UN-Term, low plane-fed animals delivered at term; UN-Preterm, low plane-fed animals delivered preterm; PGFM, 13, 14-dihydro-15-keto-prostaglandin F; $E_2$, $17\beta$-estradiol; $P_4$, progesterone. $^aP < 0.05$, $^bP < 0.06$, $^cP < 0.0001$, vs. N-Term group. $^dP < 0.05$, $^eP < 0.0001$ vs. UN-Term group.
of delivery, nor were there significant differences among groups in the relationship between the time of rise before delivery of cortisol and PGE2 (\(P = 0.48\)), PGFM (\(P = 0.25\)), and E2/P4 ratio (\(P = 0.12\)). For all animals, however, fetal cortisol concentrations rose significantly earlier than fetal PGE2 concentrations (\(P < 0.0001\)), maternal PGFM concentrations (\(P = 0.0005\)), and the maternal E2/P4 ratio (\(P = 0.005\), Fig. 3). The time of rise before delivery of fetal PGE2, maternal PGFM, and maternal E2/P4 ratio was not significantly different from each other (fetal PGE2 vs. maternal PGFM, \(P = 0.8\) and vs. maternal E2/P4 ratio, \(P = 0.7\); maternal PGFM vs. maternal E2/P4 ratio, \(P = 0.1\)).

**DISCUSSION**

The fetal PGE2 and maternal PGFM concentrations we report here are similar to those reported in other studies of PG secretion in sheep (8, 13, 20–22, 24, 29, 31, 32).

We have previously demonstrated that periconceptional undernutrition from 61 days before until 30 days after mating induces preterm birth in sheep (3). Preterm birth was characterized by an early fetal cortisol surge and, in the present study, by an early rise in fetal PGE2 and maternal PGFM concentrations and in maternal E2/P4 ratio. The rise in fetal cortisol concentrations preceded the rise in PG concentrations and in maternal E2/P4 ratio. No difference was seen between the fetus and the ewe in the timing of the rise in cortisol or PG concentrations. These findings suggest that moderate maternal undernutrition in the periconceptional period results in preterm labor and delivery by accelerating the development of the fetal HPAA, leading to early onset of the normal mechanisms of initiation of parturition in sheep. Although the UN group was divided into preterm and term groups for the sake of this analysis, the UN group as a whole delivered early across a wide gestational age range, suggesting that maturation of the fetal HPAA must reach a threshold level to trigger the processes leading to parturition (3). The possible mechanisms underlying this maturation are discussed elsewhere (3, 4).

We found that the prepartum rise in fetal plasma cortisol concentrations preceded the rise in fetal plasma PGE2 concentrations and in maternal E2/P4 ratio. This is in accordance with early studies in sheep and goats by Currie and colleagues.
and with the hypothesis currently favored in the literature suggesting that the surge in fetal adrenal cortisol production at the end of gestation resulting from sustained activation of the fetal HPAA leads to increased placental trophoblast PGHS-2 expression and PGE2 production (31). The alternative hypothesis, not supported by our data, suggests that placental PGE2 production might play a key role in mediating fetal HPAA activation in late-gestation sheep fetuses (18, 20, 28). This does not exclude the possibility that placental PGE2 may directly stimulate the fetal adrenal, sustaining the activation of the fetal HPAA at the end of gestation via a feed forward loop (1, 18). However, our data do not suggest that altered placental PG production is the primary event leading to preterm delivery after periconceptional undernutrition.

Both fetal PGE2 and maternal PGFM concentrations increased with gestational age in all animals. Undernutrition in the periconceptional period led to an earlier increase in PG concentrations in preterm animals. Preterm labor induced by a continuous infusion of dexamethasone to the sheep fetus, commencing at 138 days gestation, resulted in delivery within 50 h of the onset of infusion (29). Similarly, labor was induced within 100 h of pulsatile infusion of ACTH to the sheep fetus commencing at 127 days gestation (27). In the present study, however, the trigger was introduced in the periconceptional period yet led to early labor and delivery with an increase in PG concentrations some 4 mo later.

We also found that the timing of the rises in fetal PGE2 and maternal PGFM concentrations before delivery was similar in all groups and that PG concentrations did not rise significantly earlier than the maternal E2/P4 ratio. This is in contrast with a recent study, suggesting that placental PGE2 stimulates placental P450C17 hydroxylase activity and contributes to the placental estrogen production (31). Estrogen in turn upregulates maternal endometrial PGHS-2 expression and PGE2α output at the onset of parturition, suggesting that the rise in placental PGE2 synthesis should precede the rise in maternal PGFM and 17β-estradiol concentrations (31). In sheep, fetal plasma PGE2 concentrations rise progressively over the last 15–20 days of pregnancy, whereas maternal plasma PGE2α concentrations rise sharply only 12–24 h before delivery (6, 30). However, the observation from the present study could be explained by the following mechanism. In fetal sheep, the cortisol surge before parturition is known to be responsible for the activation of P450C17 enzymes in the placenta, leading to an increase in the estrogen-progesterone ratio. This increase in turn stimulates the intrauterine production and release of PGE2α (16, 17).

Therefore, PGE2α production at the onset of parturition could be regulated by an estrogen-dependent, PGE2-independent pathway, resulting in a simultaneous rise in PGs in both maternal and fetal circulation. If the action of estrogen on intrauterine production of PGs is largely paracrine or autocrine, then we would not expect to be able to detect differences in the timing of rise of estrogens and PGs in the maternal circulation.

In this study we were unable to collect samples near labor, as preterm birth in undernourished animals was not expected and the onset of labor was not monitored directly. Others have shown that PG concentrations increase significantly before delivery and continued to increase to maximum concentrations at delivery (29). Therefore, our peak PG concentrations might not be the maximal concentrations as samples were not obtained near delivery. In addition, the infrequent initial sampling regimen used in our study might have led us to miss the real time of the first rise in cortisol, PG, and 17β-estradiol concentrations, limiting our ability to detect differences in the time of rise of these hormones between groups. Clearly our findings show the need for close monitoring and frequent sampling during pregnancy and delivery in any future studies.

In summary, the present study suggests that moderate maternal undernutrition around the time of conception induces preterm delivery in sheep by advancing the expected prepartum increase in cortisol and PG concentrations and in maternal E2/P4 ratio. The prepartum rise in fetal cortisol concentration precedes the rise in fetal PGE2 and maternal PGFM concentrations and in maternal E2/P4 ratio, suggesting that the underlying mechanism is likely to be acceleration of fetal HPAA maturation resulting in initiation of the normal process of parturition, thus leading to preterm labor and delivery.

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