Original Article

Abnormal Progesterone and Corticotropin Releasing Hormone Levels are Associated with Preterm Labour

Foteini Stamatelou,[1] *MD, SpR*, Efthimios Deligeoroglou,[2] *MD, PhD*, Georgios Farmakides,[1] *MD, PhD*, Georgios Creatsas,[2] *FACS, FRCOG, FACOG*

Abstract

Introduction: This study examined whether maternal plasma progesterone and corticotropin releasing hormone (CRH) concentrations can predict the likelihood of preterm labour. Materials and Methods: Maternal plasma progesterone and CRH concentrations were examined in a total of 51 women. The subject cohort included 20 women who were followed from the beginning of the third trimester (28 to 34 weeks gestation), half of whom delivered early preterm and half of whom were not in labour and subsequently delivered at full term (n = 10 per group). In a follow-up experiment, 31 women who were admitted during labour for delivery were examined, 15 of whom delivered preterm and 16 of whom delivered at full term. Comparisons between women who delivered preterm and those who delivered at full term were made by t-tests. Results: Mean progesterone concentration was approximately 30% lower at 28 to 34 weeks gestation in women who delivered prematurely than in women who delivered at term (*P* <0.001). Meanwhile, mean CRH concentration was 6-fold higher at 28 to 34 weeks gestation in women who experienced spontaneous preterm labour than in those who went into labour at term (*P* <0.001). Preterm mothers had lower progesterone (*P* <0.05) and CRH (*P* <0.01) levels during active labour than full-term mothers. Progesterone levels normalised within 24 hours of delivery in preterm mothers, while CRH levels remained slightly elevated (*P* <0.01). Conclusions: Maternal progesterone and CRH measurements taken early in the third trimester may be of use as biochemical markers of pregnancies at high risk of premature labour.

Ann Acad Med Singapore 2009;38:1011-6

Key words: Biochemical markers, Gestation, Hormones, Prematurity

Introduction

Preterm labour is a serious obstetrical problem. Indeed, approximately 10% of all pregnancies are affected by preterm birth and 70% of neonatal morbidity and mortality can be attributed to preterm labour.[1-3] Tocolytic and corticosteroid treatments have been used to attempt to inhibit preterm labour and ultimately to reduce the consequences of prematurity on neonates. However, these treatments have failed to reduce the rate of preterm birth.[2,4,5] Progesterone is a vital gestational-support steroid hormone produced in the adrenal glands, corpus luteum, brain and placenta. Exogenous progesterone has been used to support assisted reproduction protocols, such as in vitro fertilisation,[6] while progesterone receptor antagonists, such as mifepristone, have contraceptive and abortifacient effects.[7,8] Moreover, progesterone supplementation has been reported to reduce the incidence of spontaneous preterm delivery in women at risk for premature labour.[9]

Human parturition is characterised by a complex interplay of autocrine and paracrine signalling pathways. A key molecule in this cross-talk is corticotrophin releasing hormone (CRH), a 41 amino acid peptide that is found in the hypothalamus, pituitary and placenta.[3] As a component of the hypothalamic-pituitary-adrenal axis (HPA), CRH is the principal neuroregulator of basal and stress-induced secretion of adrenocorticotropic hormone (ACTH), b-endorphin, and propiomelanocortin-related peptides from the anterior pituitary.

The present study examined the hypothesis that abnormal CRH and progesterone levels are related to preterm onset of labour. CRH and progesterone levels were measured early in the third trimester of pregnancy (28 to 34 weeks gestation)

[1] Elena Venizelou Maternity Hospital, 6th Department of Obstetrics and Gynaecology, Athens, Greece
[2] Aretaieion University Hospital, 2nd Department of Obstetrics and Gynaecology, University of Athens, Athens, Greece
Address for Correspondence: Dr Foteini Stamatelou, Elena Venizelou Maternity Hospital, 6th Department of Obstetrics and Gynaecology, Athens, Kapandriti attikis 19014, Greece.
Email: fstamatelou@ yahoo.com

in a prospective cohort study to examine whether the levels of these hormones near the commencement of the third trimester can predict gestational length in human pregnancy. Elucidating the physiological relationships between these hormones and labour onset may have therapeutic use in preventing and/or postponing premature labour onset.

Materials and Methods

Patients

A total of 51 pregnant women aged between 26 and 34 years were included in this 1-year prospective, observational cohort study. Twenty were included in Experiment 1 and 31 were included in Experiment 2. A summary of the study cohort's clinical parameters is shown in Table 1.

The study was carried out at Helena Venizelou Maternity Hospital and Aretaieion Hospital 2nd Department of Obstetrics and Gynaecology, Athens University. All of the subjects were followed in our antenatal clinic until delivery. All of the women who ultimately delivered preterm had a medical history of preterm birth.

In order to ensure adequate statistical power for Experiment 1, venous blood was collected from 35 women between 28 and 34 weeks of pregnancy, not in labour at the time of collection. The first 10 of the 35 who went into preterm labour formed the early preterm labour group for Experiment 1; 10 of the 35 who delivered at term made up the group of women not in labour who subsequently delivered at term, for Experiment 1. Among the 10 women in the early preterm labour group, signs of preterm labour became evident 36 hours after blood collection in 1 woman, 36 to 48 hours in 6 women and 48 to 72 hours in 3 women. As all 10 women in the early preterm labour group delivered within 72 hours of having their blood samples collected, it can be considered in retrospect that they were likely in an early, though pre-symptomatic phase of labour at the time their blood was drawn. Since the tests were done in very early preterm labour, we can still hypothesise that they may represent the levels prior to the onset of labour. Importantly, the 10 controls were selected to be gestationally matched to the 10 preterm individuals at the time of blood collection.

In Experiment 2, we similarly collected venous blood samples from 31 women who were admitted to the delivery suite while in labour. Among these 31 women, 15 were experiencing spontaneous preterm labour at 28 to 34 weeks gestation (7 women were also included in Experiment 1) and they formed the preterm labour group; 16 were experiencing full-term labour (8 transferred from Experiment 1) and these formed the full-term control group.

Gestational age was determined by the date of the last menstrual period and a first trimester clinical examination. In most cases, gestational age was confirmed by ultrasonographic examination during the first trimester. All patients gave informed consent to have their blood drawn and to participate in the study. The Institutional Review Board of the hospital approved the study. None of the women were taking any medications, except ferrous sulfate and prenatal vitamins, and none received epidural anaesthesia during labour.

All pregnancies were singleton. Women with pregnancies complicated by the presence of multiple fetuses, fetal anomalies, diabetes mellitus, placental abruption, preeclampsia, intrauterine growth restriction or clinical signs of infection were excluded from the study. Caesarean section was not considered an exclusionary factor. Preterm birth was defined as delivery before completion of the 37th week of gestation with regular uterine contractions occurring at a frequency of at least 2 in every 10 min accompanied by cervical change (dilatation and/or effacement of the cervix) and/or ruptured membranes.

Blood Withdrawal and Preparation of Plasma for Hormone Measurement

A 10 mL sample of venous blood was collected from each woman by venepuncture of the antecubital vein. These blood samples were collected in a chilled syringe and transferred immediately into polypropylene tubes containing EDTA (10 mg) and Aprotinin (5000 KIU) which were held at 0°C. After centrifugation at 1600 x g for 15 minutes at 0°C, plasma was collected in duplicate aliquots, frozen and stored at -80°C. Plasma aliquots were thawed once on the day assays were performed.

Table 1. Clinical Parameters and Demographics of Study Participants

	Experiment 1		**Experiment 2**	
	Preterm mothers (n = 10)	**Term mothers** (n = 10)	**Preterm mothers** (n = 15)	**Term mothers** (n = 16)
Maternal age (y)	30.6 ± 2.4	30.2 ± 2.6	29.8 ± 1.8	29.9 ± 2.2
Gestational age (wk)	30.8 ± 1.6	30.8 ± 1.6	31.9 ± 2.2	38.7 ± 0.8
No. multiparious	10	4	11	9
No. previous preterm birth	10	3	8	4
Miscarriage history (≥2)	3	2	5	6

Plasma was extracted using a radioimmunoassay (RIA) kit according to the manufacturer's instructions (Peninsula Laboratories Inc., Bachem, UK protocol). CRH was extracted from 3 mL samples of plasma with Sep-Pak C-18 cartridges (Cat No RIK-SEPCOL1) and eluted with Buffer B (60% acetonitrile, 1% TFA, and 39% distilled water). The extracts were evaporated, reconstituted in assay buffer, and assayed for CRH immunoreactivity (Serum Biomedical Institute). The RIA kit that was used had a range 0.1-64 pg/tube. CRH was measured relative to a human CRH standard (Peninsula Laboratories Inc., Bachem, UK). A rabbit antiserum that was specific for the peptide was used. CRH iodinated with I^{125} was employed as the tracer. Bound and free I^{125}-CRH were separated by precipitation with goat anti-rabbit IgG serum and normal rabbit serum. Progesterone was similarly extracted and assayed by RIA.

Statistics

Data were presented as mean ± standard deviation. P <0.05 was considered statistically significant in all cases. Mean plasma progesterone and CRH concentrations measured between gestational weeks 28 and 34 were compared between women not in labour and who subsequently delivered at term (n = 10) and women who delivered early preterm (n = 10) by a *t*-test. Mean maternal plasma progesterone and CRH concentrations during delivery (active phase) and 24 hours after delivery were compared between a group of women with spontaneous preterm labour (n = 15) and a group of women who delivered at term (n = 16) by a *t*-test.

Results

Experiment 1: Progesterone and CRH Levels at 28 to 34 Weeks Gestation

As shown in Figure 1, a *t*-test revealed that the mean plasma progesterone concentration at 28 to 34 weeks gestation was reduced by 30% (73.0 ± 5.77 ng/mL) in the early preterm labour group than in the group of women not in labour who subsequently delivered at term (105.0 ± 10.62 ng/mL; *t*-test 8368 t, P <0.001). Also shown in Figure 1, a *t*-test revealed that the mean plasma CRH concentration at 28 to 34 weeks gestation was more than 6-fold greater in women who ultimately delivered preterm (462.0 ± 121.91 pg/mL) relative to women who delivered at term (70.8 ± 29.93 pg/mL; *t*-test 9,855 w, P <0.0001).

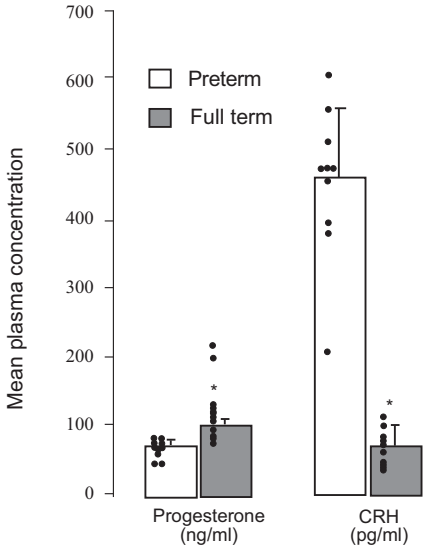

Fig. 1. At 28 to 34 weeks of pregnancy, plasma progesterone levels (ng/mL) were lower and plasma corticotropin releasing hormone levels (pg/mL) were higher in early preterm labour group women than in women not in labour who subsequently delivered at term (*P <0.001 vs full term). The individual levels of each subject are indicated with filled circles.

Experiment 2: Progesterone and CRH Levels during Active Labour and 24 Hours Postpartum

The progesterone level data are presented in Tables 2, 3 and Figure 2. Progesterone levels were markedly lower 24 hours after delivery relative to during active labour in women who delivered preterm as well as in women who delivered at term (P <0.001). However, the decline in progesterone levels was more pronounced in women who delivered at full term (10:1 labouring vs. post-partum levels) than in the women who delivered preterm (6:1 labouring vs. post-partum levels). Importantly, the relative subtlety of the progesterone decline following delivery in the preterm group (P <0.05 vs. full term, Table 3) could be attributed entirely to the 16% lower progesterone levels during active delivery in the preterm mothers relative to full-term mothers (P <0.05), as the postpartum progesterone levels were similar between the 2 groups (P >0.10).

The CRH level data are presented in Tables 4, 5 and Figure 3. The term labour patients in experiment 2 had nearly double the CRH levels when in labour (1044 pg/mL) compared to their preterm counterparts (604 pg/mL).

Table 2. Comparison of Progesterone Levels (ng/mL) During the Active Phase of Labour Relative to Levels at 24 hours Post-delivery in Women who Delivered Preterm and Women who Delivered at Full term

	Active labour	**Postpartum**	***t*-test**	***P***
Term deliveries (n = 16)	119.4 ± 15.30	10.9 ± 7.00	23.87	<0.001
Preterm deliveries (n = 15)	103.3 ± 24.75	16.9 ± 19.73	11.77	<0.001

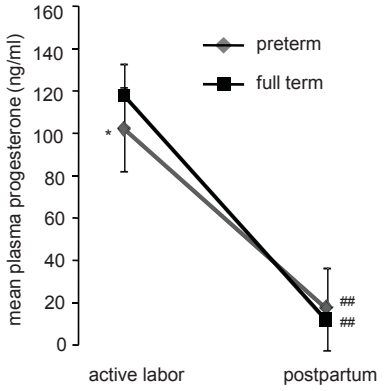

Fig. 2. Plasma progesterone levels (ng/mL) during the active phase of labour were lower in women who delivered preterm than in women who delivered at term (*P <0.05 vs full term). Progesterone levels had declined by 24 hours postpartum in both groups ($^{\#\#}P$ <0.01 vs active labour within group).

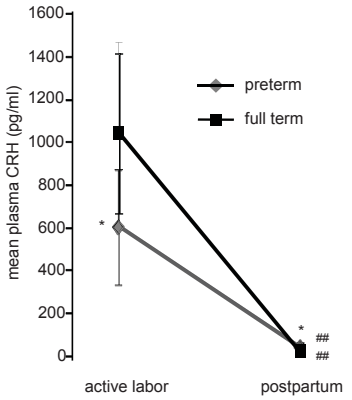

Fig. 3. In women who delivered preterm, plasma corticotropin releasing hormone levels (pg/mL) were lower during the active phase of labour but higher 24 hours postpartum relative to women who delivered at term (*P <0.01). Corticotropin releasing hormone levels had declined by 24 postpartum in both groups ($^{\#\#}P$ <0.01 vs active labour within group).

Similar to progesterone, CRH levels were lower 24 hours after delivery than during active labour in both preterm and full-term mothers (P <0.001). The decline in CRH was strikingly more pronounced in full-term mothers (44:1 labouring vs. postpartum levels) than in preterm mothers (14:1 labouring vs. postpartum levels). The relatively small active labour versus postpartum CRH differential in the preterm group (P <0.05 vs. full term, Table 5) was derived both from lower CRH levels during active labour and higher CRH levels postpartum (P <0.01 vs. respective full-term mean value).

Conclusion

The first experiment in this study revealed an association between low plasma progesterone and elevated plasma CRH levels early in the third trimester (28 to 34 weeks gestation) in women who subsequently experienced preterm labour relative to gestationally matched women whose pregnancies went to term. The key finding from the first experiment was that these abnormal levels of progesterone and CRH existed before the clinical diagnosis of preterm labour. Indeed, Experiment 1 findings showed that CRH levels were sufficiently different as to suggest that a CRH test may have good sensitivity for detecting who is at greatest risk for imminent preterm labour.

The second experiment further showed that plasma progesterone levels were low in women experiencing preterm labour during active labour relative to women experiencing labour at term. However, unlike in the experiment 1 data which were collected earlier in pregnancy, experiment 2 revealed lower plasma CRH levels during active labour in women experiencing preterm labour relative to women experiencing labour at term. Hence, while progesterone levels were consistently lower in the preterm delivery mothers relative to full-term mothers, CRH levels were elevated early in the third trimester, but relatively low during active labour, in preterm delivery mothers.

Table 3. Comparisons of Progesterone Levels (ng/mL) During the Active Phase of Labour and 24 hours Post-delivery and the Labour vs Postpartum Differential Between Women who Delivered Preterm and Women who Delivered at Full Term

	Term deliveries (n = 16)	**Preterm deliveries (n = 15)**	***t*-test**	***P***
Active labour	119.4 ± 15.30	103.3 ± 24.75	2.15	<0.05
Postpartum	10.9 ± 7.00	16.9 ± 19.73	1.11	>0.1 NS
Active labour-postpartum	108.5 ± 18.18	86.4 ± 28.43	2.59	<0.05

Table 4. Comparisons of CRH Levels (pg/mL) During the Active Phase of Labour Relative to at 24 hours Post-delivery in Women who Delivered Preterm and Women who Delivered at Full Term

	Active labour	**Postpartum**	***t*-test**	***P***
Term deliveries (n = 16)	1044 ± 419.4	24 ± 16.2	9.89	<0.001
Preterm deliveries (n = 14)	604 ± 271.9	44 ± 22.5	8.18	<0.001

Table 5. Comparisons of CRH Levels (pg/mL) During the Active Phase of Labour and 24 hours Post-delivery and the Labour vs Postpartum Differential Between Women who Delivered Preterm and Women who Delivered at Full Term

	Term deliveries (n = 16)	**Preterm deliveries (n = 14)**	***t*-test**	***P***
Active labour	1044 ± 419.4	604 ± 271.9	3.35	<0.01
Postpartum	23.6 ± 16.19	44.0 ± 22.48	2.88	<0.01
Active labour-Postpartum	1020 ± 412.8	559 ± 256.0	3.61	<0.01

Postpartum progesterone levels in preterm mothers were similar to that in full-term mothers. Although CRH levels were relatively low in preterm mothers during active labour, postpartum CRH levels were higher in preterm mothers than in full-term mothers.

CRH acts on both the fetal pituitary-adrenal axis and the uterus. It has been suggested that these actions may feed into a positive feedback loop between the fetal pituitary-adrenal axis and the placenta, which produces an upregulation of fetal secretion of cortisol and dehydroepiandrosterone-sulfate (DHEA-S). Fetal cortisol, which is important for the maturation of the fetal lungs, may also contribute to stimulating placental CRH release. Meanwhile, DHEA-S stimulates placental oestrogen production, which is thought to play a major role in the initiation of parturition. CRH receptor expression is widespread, including the myometrium, fetal membranes and placenta. Placental and fetal membrane secretion of prostaglandins E2 and F2a are upregulated in response to CRH. CRH-mediated potentiation of oxytocin actions may be important in the triggering of labour onset. The CRH binding protein, which is thought to prevent untimely CRH pituitary-adrenal stimulation, falls rapidly about 20 days before spontaneous labour while placental CRH secretion continues to rise exponentially as delivery approaches.

CRH may be regulated by an interaction between progesterone and cortisol. Specifically, progesterone has an inhibitory effect on placental CRH secretion, presumably by attenuating a positive feedback loop between CRH, adrenocorticotropic hormone and cortisol.[10] Karalis and Majzoub[11] have suggested that the inhibitory effect of progesterone is exerted through binding to glucocorticoid receptors (GRs) in trophoblast cells. At term, increased levels of cortisol may displace GR-bound progesterone as reflected by an increase in CRH output.

Our CRH findings from experiment 1 corroborate prior work in which it was found that third trimester CRH levels are elevated in women who later experience preterm labour.[6] Although we did not directly compare CRH levels between experiments 1 and 2, as they were different groups of women, it is worth noting that the hormone levels in women in established preterm labour (Experiment 2) differed substantially from that in women not yet in labour and who later delivered prematurely. Interestingly, relative to the full-term mothers, preterm mothers had elevated CRH levels early in the third trimester (Experiment 1) but low CRH levels during labour (Experiment 2). This reversal is especially noteworthy given that plasma CRH levels normally increase steadily as gestation progresses.[12] Furthermore, although preterm mothers had low CRH levels during labour, they did not experience as precipitous of a drop in CRH levels following delivery, such that their CRH levels were greater than that in full-term mothers 24 hours after delivery. These findings indicate that CRH levels in women who ultimately deliver preterm do not appear to follow the normal pattern of a steady increase throughout gestation followed by a quick decline upon delivery. Instead, our findings indicate that preterm mothers may experience an early surge in CRH levels, which subsequently declines before delivery and then continues to decline relatively gradually after delivery.

At present, it seems unlikely that measurement of maternal plasma CRH alone, or any single factor, provides an adequate means of predicting precisely which women are at risk of delivering preterm. A combination of measures, including measurements of CRH, progesterone and cervical length, may provide sufficient sensitivity and specificity for clinical use. Early identification of pregnancies at high-risk of preterm labour, such as in the mid-trimester, would make early referrals to tertiary centres possible.

It has been suggested that CRH blockade may delay labour in women at risk for premature delivery.[3] Indeed CRH1 receptor antagonism has been shown to delay the onset of parturition in sheep.[13] Progesterone and its 17aOH metabolite have been shown significantly to reduce (by almost half) the incidence of preterm birth, increase the incidence of term deliveries, and as a consequence, dramatically improve neonatal outcome. Moreover, recent clinical trials have shown that prophylactic administration of progesterone can decrease the incidence of preterm birth.[9,14,15] However, the incidence of preterm labour itself was not decreased by progesterone treatment.[9] Combinatorial therapies may provide better protection against preterm labour than any single treatment.

The present study has 2 weaknesses: a small cohort size and a range of gestational times at which blood was drawn. However, it is important to emphasise that the 2 groups in Experiment 1 were gestationally matched. Even with these limitations, this study demonstrates a close link between CRH, progesterone and the timing of human parturition. More research is needed to confirm these trends before they can be applied clinically.

Further studies on the multifactorial aspects of the initiation of preterm labour and its progression, as well as genomic approaches such as suggested by Gibb et al,[10] may allow the major obstetrical problem of premature delivery to be solved.

REFERENCES

1. Challis JR. CRH a placental clock and preterm labor. Nat Med 1995;1:416.
2. Leviton LC, Goldenburgh RL, Baker CS, Swartz RM, Freda MC, Fish LJ, et al. Methods to encourage the use of antenatal corticosteroid therapy for fetal maturation: a randomized controlled trial. JAMA 1999;281:46-52.
3. Keller PA, Kirkwood K, Morgan J, Westcott S, McCluskey A. The prevention of preterm labour-corticotrophin releasing hormone type 1 receptors as a target for drug design and development. Mini reviews in Medicinal Chemistry 2003;3:295-303.
4. Iams J. Prevention of preterm labor. N Engl J Med 1998;338:54-6.
5. Caritis SN. Treatment of preterm labour. A review of the therapeutic options. Drugs 1983;26:243-61.
6. Zarutskie PW and Phillips JA. Reanalysis of vaginal progesterone as luteal phase support in assisted reproduction cycles. Fertil Steril 2007;88:S113.
7. Loose DS and Stancel GM. Estrogens and Progestins. In: Brunton LL. Lazo JS, Parker KL, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill, 2006.
8. Fiala C, Gemzel-Danielsson K. Review of medical abortion using mifepristone in combination with a prostaglandin analogue. Contraception 2006;74:66-86.
9. Da Fonseca EB, Bittar RE, Carvalho MH, Zugaib M. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: A randomized placebo-controlled double-blind study. Am J Obstet Gynecol 2003;188:419-24.
10. Gibb W, Challis JR. Mechanisms of term and preterm birth. J Obstet Gynaecol Can 2002;24:874-83.
11. Karalis K, Majzoub JA. Regulation of placental corticotrophin releasing hormone by steroids: possible implication in labour initiation. Ann NY Acad Sci 1995;771:551-5.
12. Darne J, McGarrigle HH, Lachelin GC. Increased saliva oestriol to progesterone ratio before preterm delivery: a possible predictor for preterm labour? Br Med J (Clin Res Ed) 1987;294:270-2.
13. Mesiano S. Myometrial progesterone responsiveness and the control of human parturition. J Soc Gynecol Investig 2004;11:193-202.
14. Meis PJ, Klebanoff M, Thom E Dombroniski MP, Sibai B, Mouwad AH, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med 2003;348:2379-85.
15. Mackenzie R, Walker M, Armson A, Hannah ME. Progesterone for the prevention of preterm birth among women at increased risk: A systematic review and meta-analysis of randomized controlled trials. Am J Obstet Gynecol 2006;194:1234-42.