CLINICAL STUDY

Maternal plasma corticotropin-releasing factor (CRF) and CRF-binding protein (CRF-BP) levels in post-term pregnancy: effect of prostaglandin administration

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Abstract

Objective: Placental corticotropin-releasing factor (CRF) affects myometrial contractility and the secretion of several uterotonins such as prostaglandins (PGs); however, the activity of CRF is counteracted by CRF-binding protein (CRF-BP). At term and pre-term labor, CRF levels in maternal plasma are highest whereas those of CRF-BP are falling, and the cause of this fall is unknown. Thus, in this study, we investigated the effect of PG administration for labor induction on maternal plasma CRF and CRF-BP concentrations.

Design: Maternal plasma CRF and CRF-BP levels were assayed before and after (2 h later) induction of labor by intracervical administration of prostaglandin E₂ (PGE₂), and at delivery in a group of healthy post-term pregnancies (*n* = 18). Controls were women at term out of labor (*n* = 22), who subsequently progressed to deliver a healthy singleton baby.

Methods: CRF was measured by two-site immunoradiometric assay, and CRF-BP was assayed by radioimmunoassay.

Results: Maternal plasma CRF levels were significantly (*P* < 0.0001) lower and CRF-BP significantly (*P* < 0.0005) higher in post-term than in term pregnancies. With respect to induction of labor, 2 mg PGE₂ were sufficient to increase maternal plasma CRF levels at delivery (*P* < 0.005). While 0.5 mg PGE₂ significantly decreased maternal plasma CRF-BP levels at delivery (*P* < 0.001), 2.0 mg PGE₂ significantly reduced CRF-BP concentrations both after 2 h (*P* < 0.05) and at delivery (*P* < 0.0001).

Conclusions: In the light of the well-known stimulation of prostaglandin release by CRF, these data suggest a positive feedback effect of PGE₂ on maternal CRF release during induced labor.

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Introduction

The mechanisms underlying the onset of parturition emphasize the role played by the paracrine intrauterine interactions (1, 2). Several locally produced factors, corticotropin-releasing factor (CRF), adrenocorticotropin (ACTH), prostaglandins (PGs), and oxytocin (OT) modulate myometrial contractility (2–4).

In addition to CRF, the human placenta also expresses specific CRF receptors (5), by which CRF stimulates secretion of prostaglandin (6–8), ACTH (8, 9), and OT (10), all specific uterotonins. Moreover, as term approaches, the human myometrium expresses specific CRF receptors having increased affinity (11), by which CRF may potentiate PG-induced myometrial contractility, an effect reversed by the addition of a synthetic CRF antagonist (12).

Intrauterine tissues also express CRF-binding protein (CRF-BP) (13), a 37 kDa protein of 322 amino acids able to bind CRF with high affinity (14) and blocking the

CRF actions on intrauterine target organs (14, 15). It counteracts the local CRF-induced secretion of ACTH (13, 16) and PG (7) as well as the effect of CRF on PG-induced myometrial contractility (7). CRF is released into maternal circulation from early in gestation and its peripheral plasma concentration increases until the third trimester, when a more incisive rise associated with the onset of parturition occurs (17, 18). On the contrary, CRF-BP levels do not change until late gestation (19–22), when they fall rapidly in the last 3 weeks before delivery (19, 23, 24). Thus, reciprocal changes of CRF and CRF-BP levels occur in the biological fluids of pregnancy, with higher CRF levels in the presence of lower CRF-BP concentrations at term as well as at pre-term labor, suggesting an involvement of both CRF and CRF-BP in the events controlling the length of human pregnancy (1, 4).

The fact that both CRF and PGs interact with the myometrium, and that PGs have been reported to increase placental CRF secretion from cultured human

placental cells prompted us to examine the effect of PGE₂ on maternal plasma CRF and CRF-BP levels.

Materials and methods

Patients

A group of 40 healthy pregnant women were consecutively enrolled. Informed written consent was obtained from all patients prior to inclusion in the study for which local Human Investigation Committee approval was obtained.

In a longitudinal study, we included a group of healthy post-term pregnancies ($n=18$; age range from 27 to 39 years; gestational age: 41.5 ± 0.14 weeks (mean $\pm$ S.E.M.); Table 1), who underwent induction of labor by intracervical administration of PGE₂ (Prepidil Gel, Pharmacia & Upjohn, Sweden) 0.5 ($n=10$) and 2.0 mg ($n=8$). Blood samples were collected before and after (2 h later) PGE₂ administration, and at delivery. None of these patients received OT during the course of labor induction.

As a control group, a blood sample was collected from women at term in absence of labor ($n=22$; age range from 25 to 38 years, between 40 and 41 gestational weeks; Table 1). They subsequently (4 ± 2 days interval (mean $\pm$ S.E.M.) from blood collection) progressed to deliver a healthy singleton baby, without any obstetric complications at delivery.

Gestational age was calculated on the basis of the last menstrual period and confirmed by the use of ultrasound evaluation (Real Time Ultrasound Scan Equipment, Siemens Sonoline Elegra, Germany Millennium edition, with a probe at 2.5–5.0 MHz). The exclusion criteria were multiple pregnancies, diabetes, hypertension, fetal anomaly, maternal or fetal infection, fetal growth restriction, cardiotocographic evidence of fetal distress, and an Apgar score at 1 min of <7 .

Blood samples were drawn from the antecubital vein with a polypropylene syringe and a butterfly needle, and then transferred to chilled tubes containing ethylenediaminetetraacetic acid (10 mg/ml blood) and aprotinin (50 μ l/tube from a solution of 20 000 UI/ml; Trasylol 100 000 UIC; Bayropharm, Milan, Italy). The tubes were

immediately centrifuged at 4 °C (3000 *g* for 10 min). All plasma samples were kept at -80 °C until assay.

CRF-BP assay

CRF-BP levels were measured by RIA. Purified recombinant CRF-BP was radioiodinated by the glucose oxidase/-lactoperoxidase method and was separated on a 90×1 cm bed of Sephacryl S200 developed with 0.05 ml/l phosphate buffer (pH 7.4), containing 0.5% BSA and 0.1% sodium azide at a flow rate of 3 ml/h, collecting fractions every 20 min. Only a radiolabel constituting the peak eluting with a K_{av} of 0.46 was used as tracer for the CRF-BP RIA. Seventy-nine percent of the radioactivity from these peak fractions was precipitated by the addition of an excess of the rabbit antibody raised against recombinant CRF-BP as used in the RIA. The immunoassay was performed essentially as described previously (21). Briefly, CRF-BP stocks (3.28 mg/l) were prepared in aliquots of 0.5 ml in sheep serum and stored frozen at -20 °C. Assay standards were prepared by dilution of stock aliquots in 0.05 M phosphate buffer (pH 7.4), containing 0.5% wt/vol BSA and 0.1% wt/vol sodium azide to obtain a range of concentrations from 0.9 to 464 mg/l. To 50 ml of the above buffer were added 50 ml standard or a column fraction, 100 ml tracer containing 20 000 c.p.m. ¹²⁵I-CRF-BP, and 100 ml rabbit anti-CRF-BP antibody diluted 4000-fold in the same buffer. Standard and samples were prepared in duplicate, and the assay was incubated for 16 h at 4 °C before separation. Separation was achieved by a precipitating antibody consisting of 10% sheep anti-rabbit antiserum directed against the Fc fragment containing 0.5% vol/vol normal rabbit serum and 4% polyethylene glycol 6000. Inclusion of hCRF in standards or human plasma samples in concentrations ranging from 1.6 to 25 mg/l had no effect on CRF-BP measurements (21). Assay sensitivity was 3.125 ng/ml. Intra-assay coefficient of variation was 7%.

CRF assay

Maternal plasma samples were submitted to an extraction procedure as previously described (21). The acid extraction method we used to strip CRF from the

Table 1 Clinical characteristics of study groups. Values are expressed as means $\pm$ S.E.M.

	Controls	PGE ₂ 0.5 mg	PGE ₂ 2.0 mg	P
<i>n</i>	22	10	8	–
Parity	1.18 $\pm$ 0.18	1.28 $\pm$ 0.19	1.16 $\pm$ 0.16	NS
Maternal age (years)	30.18 $\pm$ 1.55	30.4 $\pm$ 1.5	32.5 $\pm$ 1.2	NS
Weeks at delivery	40.4 $\pm$ 0.5	41.3 $\pm$ 0.23	41.6 $\pm$ 0.14	NS
Birthweight (g)	3344 $\pm$ 66.31	3285 $\pm$ 73.5	3421 $\pm$ 52.44	NS
Apgar <7 at 1 min	0	0	0	NS
Placental weight (g)	556.6 $\pm$ 13.65	546.0 $\pm$ 28.53	550.3 $\pm$ 31.83	NS
CRF (pmol/l)	3708.31 $\pm$ 176.9	1740.51 $\pm$ 396.22	1163.32 $\pm$ 378.81	<0.0001
CRF-BP (nmol/l)	42.66 $\pm$ 3.78	74.25 $\pm$ 12.04	83.09 $\pm$ 10.78	<0.0005

NS, not significant.

CRF-BP allowed us to measure total CRF (25). Briefly, cyclohexyl columns (500 mg; Bondelut, AnalyLichem International, Arbor City, CA USA) were washed with methanol (0.5 ml) and a 2 ml mixture of formic acid plus triethylamine and 0.2% β -mercaptoethanol (pH 3) and loaded slowly into the column. The peptide was finally eluted with a mixture of 75% acetonitrile, 25% triethylamine, and 0.2% mercaptoethanol (2 ml). The final recovery of the peptide evaluated with cold (100 ng) or labeled (^{125}I) CRF was 85%. All extracted samples were then dried in a speed vacuum concentrator (Savant, Hicksville, NJ, USA). All reagents were purchased from Sigma–Aldrich Corp.

Each dried sample was redissolved in buffer (0.1% BSA, 0.05% Triton X-100, and PBS (pH 7.3)), and CRF concentrations were measured by two-site immunoradiometric assay (IRMA) as previously described (21), except that the immunoglobulin G for radioiodination was purified from a sheep antibody raised against the hCRF (sequence 36–41) conjugate, and the second epitope antibody was from a rabbit that had been immunized with the hCRF (sequence 1–20) conjugate. The assay sensitivity was 10 pg/ml. Inter-assay coefficient of variation was 6%.

Statistical analysis

Results are expressed as mean and S.E.M. Statistical significance of hormone concentrations was assessed using Mann–Whitney *U* test for comparison between term and post-term pregnancies. Friedman test followed by *post hoc* Dunn's test was applied to evaluate the statistical significance of hormone changes throughout induction of labor.

Results were considered statistically significant when $P < 0.05$.

Results

Clinical findings

Table 1 shows the clinical characteristics of all patients. There were no significant differences between maternal age, parity, and gestational age. In addition, all patients had a vaginal delivery, without difference in the Apgar score, birthweight, and placental weight.

Hormone levels

CRF and CRF-BP were measurable in all plasma samples and correlated with the duration of labor ($r = -0.58$ and $r = 0.57$ for CRF and CRF-BP respectively; $P < 0.05$).

Maternal plasma CRF levels were significantly lower ($P < 0.0001$) in post-term pregnancies than in control women (Table 1; Fig. 1A). With respect to induction of labor, whilst the administration of 0.5 mg PGE_2 failed to

Figure 1 Maternal plasma CRF (A) and CRF-BP (B) levels in term and post-term pregnancies. Mean value of each group is represented by horizontal bars. * $P < 0.0001$ for CRF levels (A); * $P < 0.0005$ for CRF-BP concentrations (B).

affect CRF levels at the prescribed time points (Fig. 2A; Table 2), the intracervical administration of 2 mg PGE_2 was associated with significantly increased ($P < 0.005$) maternal plasma CRF levels at delivery (Fig. 3A; Table 2).

Maternal plasma CRF-BP levels were significantly lower ($P < 0.0005$) in control subjects than in post-term pregnancies (Fig. 1B; Table 1). In addition, while the administration of 0.5 mg PGE_2 significantly decreased ($P < 0.001$) maternal plasma CRF-BP levels only at delivery, PGE_2 at 2.0 mg significantly reduced CRF-BP concentrations both after 2 h ($P < 0.05$) and at delivery ($P < 0.0001$; Fig. 3B; Table 2).

Discussion

The present study shows that post-term pregnancies had significantly lower CRF and higher CRF-BP levels, and primarily that the therapeutic use of PGs affects maternal CRF and CRF-BP levels.

CRF and CRF-BP are expressed by human placenta and secreted differentially during pregnancy. In fact, while CRF concentrations increase progressively until the third

Figure 2 Changes of maternal plasma CRF (A) and CRF-BP (B) levels throughout induction of labor by intracervical administration of 0.5 mg PGE₂. CRF-BP levels were significantly reduced at delivery. * $P < 0.001$ (Friedman test for repeated measures).

trimester (1, 3, 4, 18), the concentrations of CRF-BP do not change significantly during most of gestation (19–22), but they fall during the final weeks of normal pregnancy (19, 23, 24) and still further with the onset of labor (19). In pre-term labor, the highest CRF and lowest CRF-BP concentrations are measurable in maternal and fetal circulation, as well as in amniotic fluid (19, 20, 22–24, 26). During the course of spontaneous labor at term, there is no further change in maternal plasma CRF (27), and both CRF and CRF-BP return to normal non-pregnancy levels within 48 h *post partum* (28, 29). The dynamics of these proteins in maternal circulation through the various stages of spontaneous labor either pre- or post-term remains unknown.

In the present study, we found significantly lower CRF and higher CRF-BP levels in post-term pregnancies, suggesting that the lack of the reciprocal changes in CRF and CRF-BP levels may delay the onset of labor. As already mentioned, CRF-BP, because it binds CRF, has been postulated to affect levels of 'free' CRF and, thus, its bioactivity (14–16), counteracting placental CRF-induced secretion of ACTH (13, 30) and PG (7) as well as the effect of CRF on PG-induced myometrial contractility (7). Consequently, the failure of CRF levels to increase and CRF-BP levels to decrease in the last weeks of gestation may postpone the spontaneous onset of labor. Our data describing the impairment of CRF and CRF-BP secretion in post-term deliveries agree with that reported by McLean *et al.* (20), who first suggested that CRF and CRF-BP levels trigger parturition after a predetermined length of gestation.

In the present *in vivo* study, we have also found that the intracervical administration of PGE₂ increased maternal plasma CRF levels at delivery and decreased maternal plasma CRF-BP levels, extending the observation *in vitro* that PGE₂ stimulates both the placental secretion (9) and expression (31) of CRF in a dose-dependent manner. We found that PGE₂ increased CRF levels only at the highest dose (2.0 mg), while the effect on maternal plasma CRF-BP concentrations was obtained using a lower dose (0.5 mg), with an early effect (after 2 h) when the highest dosage was used. Because the average concentrations of CRF in the two treatment groups differed at baseline, it can be argued that the group treated with 0.5 mg PGE₂ might have responded similarly to the group receiving 2.0 mg PGE₂, if they had the same CRF concentrations pre-treatment. However, the increase of plasma CRF concentrations following 2.0 mg PGE₂ administration was consistently seen in all patients, covering a wide range of pre-treatment CRF concentrations, contrasting with no effect of 0.5 mg PGE₂ administration even in the women with the lowest baseline CRF levels.

In summary, CRF concentrations increased and CRF-BP concentrations decreased in maternal plasma after intracervical administration of PGE₂ for labor induction in post-term gestation. Taken together with the observation that CRF stimulates prostaglandin release, these data suggest a positive feedback effect of PGE₂ on maternal CRF release during induced labor. Thus, in addition to the

Table 2 Changes (mean $\pm$ S.E.M.) of maternal plasma corticotropin-releasing factor (CRF) and CRF-binding protein (CRF-BP) levels before and after intracervical PGE₂ administration, and at delivery.

	Pre	Post (2 h later)	Delivery	P
0.5 mg PGE ₂				
CRF (pmol/l)	1740.51 $\pm$ 396.22	1827.57 $\pm$ 417.04	1960.91 $\pm$ 429.47	NS
CRF-BP (nmol/l)	74.25 $\pm$ 12.04	73.82 $\pm$ 15.53	48.19 $\pm$ 6.99	<0.001
2.0 mg PGE ₂				
CRF (pmol/l)	1163.32 $\pm$ 378.81	1497.34 $\pm$ 478.04	1624.69 $\pm$ 511.24	<0.005
CRF-BP (nmol/l)	83.09 $\pm$ 10.78	70.02 $\pm$ 9.08	61.76 $\pm$ 8.45	<0.0001

NS, not significant.

Figure 3 Changes of maternal plasma CRF (A) and CRF-BP (B) levels throughout induction of labor by intracervical administration of 2.0 mg PGE₂. PGE₂ significantly increased CRF levels at delivery (* $P < 0.005$), also decreasing significantly CRF-BP concentrations both after 2 h (* $P < 0.05$) and at delivery (** $P < 0.0001$).

direct PG effects on cervical dilatation and myometrial contraction, the mechanism of labor induction with PGE₂ may also benefit from decreased CRF-BP and increased CRF levels in maternal circulation.

References

- Reis FM, D'Antona D & Petraglia F. Predictive value of hormone measurements in maternal and fetal complications of pregnancy. *Endocrine Reviews* 2002 **23** 230–257.
- Romero R, Espinoza J, Gotsch F, Kusanovic J, Friel L, Erez O, Mazaki-Tovi S, Than N, Hassan S & Tromp G. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. *British Journal of Obstetrics and Gynecology* 2006 **113** 118–135.
- Petraglia F, Florio P, Nappi C & Genazzani AR. Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. *Endocrine Reviews* 1996 **17** 156–186.
- Challis JRG, Matthews SG, Gibb W & Lye SJ. Endocrine and paracrine regulation of birth at term and preterm. *Endocrine Reviews* 2000 **21** 514–550.
- Florio P, Franchini A, Reis FM, Pezzani I, Ottaviani E & Petraglia F. Human placenta, chorion, amnion and decidua express different variants of corticotropin-releasing factor receptor messenger RNA. *Placenta* 2000 **21** 32–37.
- Jones SA & Challis JRG. Local stimulation of prostaglandin production by corticotrophin-releasing hormone in human fetal membranes and placenta. *Biochemical and Biophysical Research Communications* 2000 **159** 192–199.
- Petraglia F, Benedetto C, Florio P, D'Ambrogio G, Genazzani AD, Marozio L & Vale W. Effect of corticotropin-releasing factor-binding protein on prostaglandin release from cultured maternal decidua and on contractile activity of human myometrium *in vitro*. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 3073–3076.
- Petraglia F, Florio P, Benedetto C, Marzio L, Di Blasio AM, Ticconi C, Piccione E, Luisi S, Genazzani AR & Vale W. Urocortin stimulates placental adrenocorticotropin and prostaglandin release and myometrial contractility *in vitro*. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 1420–1423.
- Petraglia F, Sawchenko PE, Rivier J & Vale W. Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. *Nature* 1987 **328** 717–719.
- Florio P, Lombardo M, Gallo R, Di Carlo C, Sutton S, Genazzani AR & Petraglia F. Activin A, corticotropin-releasing factor and prostaglandin F_{2α} increase immunoreactive oxytocin release from cultured human placental cells. *Placenta* 1996 **17** 307–311.
- Grammatopoulos DK. The role of CRH receptors and their agonists in myometrial contractility and quiescence during pregnancy and labour. *Frontiers in Bioscience* 2007 **12** 561–571.
- Benedetto C, Petraglia F, Marozio L, Chiarolini L, Florio P, Genazzani AR & Massobrio M. Corticotropin-releasing hormone increases prostaglandin F_{2α} activity on human myometrium *in vitro*. *American Journal of Obstetrics and Gynecology* 1994 **171** 126–131.
- Petraglia F, Potter VA, Cameron A, Sutton S, Behan DP, Woods RJ, Sawchenko PE, Lowry PJ & Vale W. Corticotropin-releasing factor-binding protein is produced by human placenta and intrauterine tissues. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 919–924.
- Kemp CF, Woods RJ & Lowry PJ. The corticotrophin-releasing factor-binding protein: an act of several parts. *Peptides* 1998 **19** 1119–1128.
- Petraglia F, Florio P, Gallo R, Salvestrone C, Lombardo M, Genazzani AD, Di Carlo C, Stomati M, D'Ambrogio G & Artini PG. Corticotropin-releasing factor-binding protein: origins and possible functions. *Hormone Research* 1996 **45** 187–191.
- Linton EA, Wolfe CDA, Behan DP & Lowry PJ. A specific carrier substance for human CRF in late gestational maternal plasma which could mask the ACTH releasing activity. *Clinical Endocrinology* 1988 **28** 315–324.
- Florio P, Severi FM, Ciarmela P, Fiore G, Calonaci G, Merola A, De Felice C, Palumbo M & Petraglia F. Placental stress factors and maternal-fetal adaptive response: the corticotropin-releasing factor family. *Endocrine* 2002 **19** 91–102.
- Florio P, Vale W & Petraglia F. Urocortins in human reproduction. *Peptides* 2004 **25** 1751–1757.
- Perkins AV, Eben F, Wolfe CDA, Schultze HM & Linton EA. Plasma measurements of corticotrophin-releasing hormone-binding protein in normal and abnormal human pregnancy. *Journal of Endocrinology* 1993 **138** 149–157.
- McLean M, Bisits A, Davies J, Woods R, Lowry P & Smith, . A placental clock controlling the length of human pregnancy. *Nature Medicine* 1995 **1** 460–463.
- Petraglia F, Florio P, Benedetto C, Gallo C, Woods RJ, Genazzani AR & Lowry PJ. High levels of corticotropin-releasing factor (CRF) are inversely correlated with low levels of maternal CRF-binding protein in pregnant women with pregnancy-induced hypertension. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 852–856.
- Berkowitz GS, Lapinski RH, Lockwood CJ, Florio P, Blackmore-Prince C & Petraglia F. Corticotropin-releasing factor and its binding protein: maternal serum levels in term and preterm deliveries. *American Journal of Obstetrics and Gynecology* 1996 **174** 1477–1483.

- 23 Petraglia F, Florio P, Simoncini T, Woods RJ, Giuntini A, Serra GB, Genazzani AR & Lowry PJ. Cord plasma corticotropin-releasing factor-binding protein (CRF-BP) in term and preterm labor. *Placenta* 1997 **18** 115–119.
- 24 Florio P, Woods RJ, Genazzani AR, Lowry PJ & Petraglia F. Changes in amniotic fluid immunoreactive corticotropin-releasing factor (CRF) and CRF-binding protein levels in pregnant women at term and during labor. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 835–838.
- 25 Woods RJ, Grossman A, Saphier P, Kennedy K, Ur E, Behan D, Potter E, Vale W & Lowry PJ. Association of human corticotropin-releasing hormone to its binding protein in blood may trigger clearance of the complex. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 73–76.
- 26 Erickson K, Thorsen P, Chrousos G, Grigoriadis DE, Khongsaly O, McGregor J & Schulkin J. Preterm birth: associated neuroendocrine, medical, and behavioral risk factors. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 2544–2552.
- 27 Florio P, Cobellis L, Woodman J, Severi FM, Linton EA & Petraglia F. Levels of maternal plasma corticotropin-releasing factor and urocortin during labor. *Journal of the Society for Gynecologic Investigation* 2002 **9** 233–237.
- 28 Linton EA, Perkins AV, Woods RJ, Eben F, Wolfe CD, Behan DP, Potter E, Vale WW & Lowry PJ. Corticotropin releasing hormone-binding protein (CRH-BP): plasma levels decrease during the third trimester of normal human pregnancy. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 260–262.
- 29 Campbell EA, Linton EA, Wolfe CD, Scraggs PR, Jones MT & Lowry PJ. Plasma corticotropin-releasing hormone concentrations during pregnancy and parturition. *Journal of Clinical Endocrinology and Metabolism* 1987 **64** 1054–1059.
- 30 Linton EA, Behan DP, Saphier PW & Lowry PJ. Corticotropin-releasing (CRH)-binding protein: reduction in the adreno-corticotropin-releasing activity of placental but not hypothalamic CRH. *Journal of Clinical Endocrinology and Metabolism* 1990 **70** 1574–1580.
- 31 Dibbs KI, Anteby E, Mallon MA, Sadovsky Y & Adler S. Transcriptional regulation of human placental corticotropin-releasing factor by prostaglandins and estradiol. *Biology of Reproduction* 1997 **57** 1285–1292.

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