Remarkable surveillance and response systems have evolved and are conserved so that many species from the desert dwelling Western Spadefoot tadpole to the human fetus can detect threats to survival and adjust their developmental trajectory [3,40]. Rapidly evaporating pools of desert water result in elevation of a stress hormone, corticotrophic-releasing hormone (CRH), in the pathway between the brain and the pituitary gland (median eminence) of the tadpole, precipitating metamorphic climax to escape imminent peril [7,8]. If the CRH response is blocked during environmental desiccation, then the rate of development is arrested and the tadpole’s survival is compromised.

The human fetus has evolved similar mechanisms to acquire information about the environment that guide its development. The human placenta is both a sensory and effector organ that incorporates and transduces information...
from its maternal host environment into the fetal developmental program. It has receptors and expresses the genes for major stress systems, including the endocrine system and specifically CRH [10,11,20,23,28,34,35,48]. Placental CRH production increases dramatically over the course of normal human gestation [29] reaching levels at term observed only in the hypothalamic portal system (median eminence) during physiological stress [25]. Abnormally accelerating rates or excessive levels of placental CRH are significant risk factors for an earlier onset of spontaneous birth [17,18,29,30,32,45,46]. Because of this, CRH is proposed to regulate a placental clock that controls a cascade of physiological events leading to parturition [42]. Despite general agreement concerning the significance of CRH for the timing of spontaneous birth [14], there is uncertainty about when CRH exerts its effects on human parturition [9,18,29] and what regulates the CRH surge [4].

CRH, a 41-amino acid neuropeptide, is synthesized primarily in the paraventricular nucleus of the hypothalamus and has a major role in regulating pituitary-adrenal function and the physiological response to stress [5,43]. During pregnancy, CRH also is synthesized by the placenta. Placental CRH is identical to hypothalamic CRH in structure, immunoreactivity and bioactivity [36,39]. In contrast, however, to the inhibitory influence on the promoter region of the CRH gene in the hypothalamus, maternal stress signals (glucocorticoids) from the adrenal glands activate the promoter region in the placenta and stimulate its synthesis [21,41]. This positive feedback system contains both a signal to the fetus (elevated glucocorticoids) that the host environment (the mother) is threatened [44], potentially compromising fetal survival, and a response from the fetus (increased placental CRH production) that shortens gestation. The purposes of our study are to determine the critical intervals during which CRH influences the length of human gestation and the critical periods during gestation when the placenta is most vulnerable to the biological signals of threat.

1. Methods

1.1. Participants

The sample was comprised of 203 English-speaking adult women (over 18 years age, mean age = 29.9 years) consecutively recruited from two University prenatal care programs between the 6th and 10th weeks of gestation. As presented in Table 1, the majority of the sample was married and high school educated. The sample was racially/ethnically diverse with a small majority that is white/non-hispanic. All eligible subjects presented with a singleton intrauterine pregnancy, a normal uterus and cervix. For the majority the current pregnancy was their first. A comprehensive, structured medical interview and thorough chart review was conducted to exclude subjects if they presented with prior or present obstetric risk conditions including systemic maternal disease (cancer, cardiac disease, seizure history, autoimmune diseases and blood disorders), placental or cord abnormalities, uterine anomalies, infection, congenital malformations or chromosomal abnormalities determined in the first trimester.

Women also were excluded if they presented with any condition that could disr regulate neuroendocrine function, such as endocrine, hepatic or renal disorders or the use of corticosteroid medications. Interviews assessed health behaviors to exclude women who smoked or consumed alcohol or drugs of abuse 6 months before and during the index pregnancy.

1.2. Procedures

All methods and procedures in this report were approved by the Institutional Review Board of the participating institutions. Women provided informed consent to be evaluated at four intervals during gestation; 13.5–16.6 (mean = 15.3), 17.8–20.5 (mean = 19.2), 23.7–26.5 (mean = 24.9) and 29.9–32.3 (mean = 30.9) weeks gestation. A clinical ultrasound performed at the first and second intervals confirmed gestational age. Blood was collected at each interval for assessment of neuroendocrine profiles. Women were followed to term and birth outcome information was abstracted from medical charts.

The HPA and placental stress axis was evaluated by assessing levels of B-endorphin (BE), ACTH, cortisol and CRH. To control possible circadian influences, subjects were evaluated each session between 14:00 and 16:00. The times for blood draws across sessions were not significantly different (F(3,200) = 1.14, p = .34) and mean times ranged from 14:17 to 14:32. A 25 ml blood sample was withdrawn through ante-cubital venipuncture (within 20 s of venipuncture). Blood was deposited into siliconized and chilled EDTA (purple top) vacutainers, centrifuged at 2000 x g (15 min) and the plasma was decanted into polypropylene tubes containing 500 KIU/ml aprotinin (to arrest enzymatic degradation; Sigma Chemical) and stored at –70 °C until assayed.

CRH concentrations (pg/ml) were determined by radioimmunoassay (RIA; Bachem Peninsula Laboratories, SanCarlos, CA). Plasma samples (1–2 ml) were extracted with three volumes of ice-cold methanol, mixed, allowed to stand for 10 min at 4 °C, and then centrifuged at 1700 x g for 20 min at 4 °C by the modified method of Linton et al. [24]. The pellets were washed with 0.5 ml methanol, and the combined supernatants dried down (Savant SpeedVac concentrator).

| Table 1 – Subject characteristics (N = 203) |
|---------------------------------------------|
| Age (years) | 29.9 ± 2.55 |
| Marital status (%) | Married: 78 |
| Education (%) | High school: 99.5 |
| Race/ethnicity (%) | White/non-Hispanic: 54.4 |
| Parity (%) | Primiparous: 58.7 |
| Gestational intervals (weeks) | Time 1: 15.3 ± 1.02 |
| | Time 2: 19.2 ± 0.75 |
| | Time 3: 24.9 ± 0.88 |
| | Time 4: 30.9 ± 0.97 |
| Gestational age at term (weeks) | 39.1 ± 1.71 |
| Infant birth weight (g) | 3446.24 ± 565.14 |

Data are presented as mean ± standard deviation.
Reconstituted samples in assay buffer were incubated with anti-CRH serum (human) for 48 h at 4 °C followed by a 24 h incubation with 125I-CRH. Both labeled and unlabeled CRH were collected by immunoprecipitation with goat anti-rabbit IgG serum and normal rabbit serum after 90 min of incubation at room temperature. Samples were centrifuged at 1700 × g (20 min) at 4 °C and the aspirated pellets were quantified with a gamma scintillation counter. The CRH assay had less than 0.01% cross-reactivity with ovine CRH, 36% cross-reactivity with bovine CRH and non-detectable reactivity with human ACTH. The intra- and inter-assay coefficient of variance ranged from 5 to 15%, respectively.

Plasma levels of adrenocorticotropic hormone (ACTH) were measured by RIA (Nichols Institute Diagnostics). The antiserum employed was <0.001% cross-reactive with betalendorphin and ACTH fragments. Duplicate samples (200 μl/assay tube) were incubated with ACTH 125I-antibody solution and an avidin coated bead overnight at room temperature. After washing, the bead with bound radiolabeled antibody complex was quantified using an ICN Biomedical Isoflex Gamma Counter. The ACTH assay has a minimal detectable dose (MDD) of 1.0 pg/ml (95% confidence) with intra-assay CV of 3.0% at 35 pg/ml and CV = 7.8% (inter-assay) at 36 pg/ml.

Plasma levels of BE were determined by a solid phase two-site immunoradiometric assay (IRMA; Nichols Institute Diagnostics). The antiserum was 1.6% cross-reactive with betalipotropin at 500 pg/ml and was <0.01% cross-reactive with related opiates at 5 μg/ml. The BE immunoassay system has a MDD = 14 pg/ml (95% confidence limit) with a CV = 4.1% (intra-assay) and CV = 9.0% (inter-assay) at the highest concentrations measured in the present study.

Plasma cortisol levels were determined by immunofluorescence using an automated procedure on an Abbott TDx Analyzer (Abbott Laboratories). The assay was less than 5% cross-reactive with 11-deoxycortisol, corticosterone, and less than 1% cross-reactive with ten other naturally occurring steroids. The inter- and intra-assay CVs were less than 9% with a minimum detectable level (95% confidence) of 0.45 μg/dl.

Data reduction for the RIA and IRMA assays were accomplished with a computer assisted, four-parameter logistics program [38]. Differences and rates of change in endocrine profiles between women delivering term and preterm were assessed with analysis of variance with fixed (Groups [term-preterm]) and repeated (assessment intervals-time) factors. Post hoc comparisons were computed using the Tukey method. Analysis of covariance was used to control for potentially confounding variables. Greenhouse-Geisser corrections were applied when appropriate and only corrected results are reported. Logistical multivariate regression was used to predict the dichotomous variable of preterm birth. General linear regression models were used to predict the separate continuous outcomes of gestational length and CRH levels at 31 weeks. Similar analytical strategies are used for both dichotomous and continuous variables. Initially, predictors (Table 1) were entered into the regression equation one variable at a time in no preset order but with the restriction that each entry increased the ability (the variance accounted for) to predict the separate outcomes of interest (conditional forward logical regression for preterm birth, and forward stepwise regression for gestational length and CRH levels at 31 weeks). This approach was appropriate because the variables selected as predictors for this analysis were: (i) a cohesive collection of endocrine measures related to the stress axis and (ii) occurred in time before the outcomes of interest [16]. The stepwise solution was followed and confirmed by simultaneous regression, which considered concurrently all predictor variables, and then by hierarchical regression in which the order of predictor variables to enter the equation was selected by the investigator to control the influence of competing variables. The purpose of hierarchical regression was to determine causal priorities and to reduce confounding associations [6].

2. Results

2.1. Prediction of preterm birth

Placental CRH increased significantly (F₃,₂₁₀ = 106.48, p < .0001, Greenhouse-Geisser correction) during pregnancy with rapidly accelerating levels after 25 weeks gestation (Fig. 1, all pairwise comparisons p < .0001). Consistent with previous studies [29,45,46], placental CRH levels in women destined to deliver preterm (before 37 weeks) had faster rates of increase (F₃,₆₀₃ = 5.73, p < .001 [group × weeks gestation]) and significantly higher levels of CRH confined to the beginning of the early third trimester (F₁,₂₀₁ = 5.53, p = .02 [post hoc comparison at 31 weeks]) than women who subsequently delivered at term (Fig. 2).

Maternal levels of cortisol, ACTH and BE also increased significantly with advancing gestation (all p’s < .0001). The two-fold increases in maternal ACTH and BE and the three-fold increase in maternal cortisol were considerably less than the 25-fold increases in placental CRH through 31 weeks of gestation (Table 2). Of these maternal measures, only cortisol distinguished women delivering term and preterm. This is the first evidence that levels of cortisol are higher as early as 15

Fig. 1 – During the course of human pregnancy, levels of CRH increase as gestation progresses. All comparisons of CRH levels between time intervals are statistically significant.
weeks gestation \( F_{1,201} = 4.45, p = .03 \) with a similar trend at 19 weeks gestation \( F_{1,201} = 3.43, p = .065 \) in women who subsequently delivered preterm compared with women delivering after 37 weeks (Fig. 3).

Because levels of CRH late in pregnancy and concentrations of cortisol early in pregnancy both were associated with preterm birth, the association between CRH and birth outcome was re-evaluated after controlling for the level of cortisol. The rate of change of CRH during gestation \( F_{3,597} = 1.54, p = .19 \) and the relation between CRH and preterm birth \( F_{3,597} = 2.39, p = .07 \) were not significant when the statistical influence of cortisol at 15 weeks was controlled by analysis of covariance. However, when the independent contribution to the prediction of preterm birth was assessed using all of the endocrine variables (Table 2), only CRH at 31 weeks was selected by conditional logistic regression \( \chi^2_{df=1} = 3.62, p = .05 \).

### 2.2. Prediction of gestational length

Similarly, when all variables were entered into the equation simultaneously, only placental CRH at 31 weeks significantly and independently predicted gestational length \( R = .20, p = .02 \). The crucial role of CRH at 31 weeks for predicting gestational age at term was confirmed with Stepwise Regression. Elevated CRH at 31 weeks significantly (Step 1, \( R = .20, F_{1,199} = 8.36, p = .004 \)) predicted gestational length. The prediction of gestational length was improved by including in the model elevated cortisol at 15 weeks (Step 2, \( R = .26, F_{1,197} = 5.38, p = .02 \)), and finally elevated cortisol at 19 weeks (Step 3, \( R = .29, F_{1,196} = 4.08, p = .04 \)). Because levels of cortisol early in pregnancy, and levels of CRH late in pregnancy both resulted in the best prediction of gestational age at term, a final hierarchical model was constructed to control the early influence of cortisol in the equation. After cortisol levels at 15 and 19 weeks are entered in the equation (together at Step 1), elevation of CRH at 31 weeks still significantly predicted gestational age at term (Step 2, \( R = .21, F_{1,197} = 5.06, p = .02 \)).

### 2.3. Association between cortisol and CRH

The association between levels of cortisol and CRH across all time intervals is presented in Table 3. The purpose of this analysis was to examine all possible lagged and concurrent relations of these two hormones. There were no concurrent relations between cortisol and CRH for any time interval. Only the lagged associations of cortisol at 15 weeks \( R = .49, p < .001 \) and 19 weeks \( R = .28, p < .01 \) with CRH at 31 weeks were statistically significant.

### 2.4. Prediction of third trimester CRH Levels

Because CRH level at 31 weeks was the best predictor both of preterm birth and gestational length, a model to predict its
precipitous rise during gestation was constructed using all endocrine stress markers collected from the beginning of the second trimester (15 weeks) through week 26 of gestation (Table 2). The prediction of CRH levels at 31 weeks using all the endocrine markers was highly significant ($r = 0.58$, d.f. = 12,188, $F_{\text{change}} = 8.04$, $p < .0001$). Stepwise regression revealed that the single best and highly significant ($r = 0.49$, d.f. = 1,199, $F_{\text{change}} = 61.78$, $p < .0001$) independent predictor of third trimester CRH was cortisol at 15 weeks gestation. Every unit of change ($\mu g/dl$) in cortisol at 15 weeks was associated with a change in 34 units (pg/ml) of CRH at 31 weeks. The significant association between early levels of cortisol and concentrations of CRH at 31 weeks remained unchanged when analyzed by methods that controlled for the statistical influence of all other endocrine variables measured.

A final stepwise model was constructed with three variables that accounted for the most variance in the prediction of third trimester CRH (Table 4). In addition to elevated maternal cortisol at 15 weeks (Step 1), the model included elevated CRH at 26 weeks (Step 2) and elevated maternal cortisol at 19 weeks (Step 3). After the initial contribution of cortisol at 15 weeks, each of the two additional variables resulted in a progressive and significant increase in the ability to predict CRH at 31 weeks; however, the magnitude of these subsequent contributions was very small, ranging between 2 and 5% of the variance (Table 4).

3. Discussion

Our longitudinal study of human pregnancy provides convincing evidence that the earliest, and perhaps critical, period for the effects of CRH on gestational length is restricted to the interval between weeks 26 and 31. The rate of increase during this interval is faster and the level of CRH at 31 weeks is higher in women destined to deliver preterm. Serial sampling of maternal plasma provides no support for the possibility that CRH earlier in pregnancy influenced gestational length. These results confirm that elevated CRH after 26 weeks, but not before, constitute a significant risk for preterm birth [18]. New findings from this study indicate that a plausible stress-related endocrine signal, elevated cortisol [22,37] from the mother very early in pregnancy predicts the precocious rise in CRH leading to an abbreviated gestational period.

Previous research indicated that exposure to stress, especially early in pregnancy, may accelerate the rise in placental CRH and shorten the length of gestation [13]. The pattern of findings in the current study supports the argument that the effect of elevated cortisol early in pregnancy on gestational length reflects a priming or programming [2] effect on the eventual fetal/placental CRH response. First, elevation in maternal cortisol, a primary endocrine response to stress [22,37] during the first 15 weeks of gestation significantly predicts the length of gestation. This finding is consistent with the recent report of decreased gestational length among women administered corticosteroids during their first trimester [15]. Second, the relation between early elevations of cortisol and length of gestation is associated with increased levels of CRH later in pregnancy. Third, there is no effect on gestation of other maternal stress signals (ACTH or BE) at any time interval. Cortisol early in gestation is the only stress-related signal that predicts both CRH levels at 31 weeks and gestational length. Fourth, our findings in vivo of a positive association between cortisol and placental CRH are consistent with in vitro studies describing activation by glucocorticoids of placental CRH synthesis [21,41]. Furthermore, administration of synthetic glucocorticoid (betamethasone or dexamethasone) to pregnant women at risk for preterm delivery (to mature the fetal lungs) is associated with significant increases in circulating maternal CRH [28]. These findings unequivocally support the ability of cortisol to stimulate in vivo placental CRH; however, the stimulation of CRH only was seen in women after 30 weeks gestation and within 3 h of administration of the synthetic compound.

The time course for the effect of synthetic glucocorticoid on placental CRH is not consistent with the early (15 weeks gestation) and lagged (16 weeks later) association between maternal cortisol and CRH elevation observed in the present study. There are at least two explanations for these differences. First, synthetic glucocorticoid is administered in doses that profoundly alter the HPA axis [28] and reflect pharmacological rather than physiological effects. Second, an enzyme in the placenta, 11B-HSD2, oxidizes physiological (maternal) but not synthetic glucocorticoids into cortisone [19,21]. This protects the fetus from the direct and sometimes harmful effects of exposure to maternal cortisol during critical periods of development [47]. The levels of placental 11B-HSD2 rise as gestation progresses before abruptly falling near term ensuring fetal organ maturation in full term births [26,31]. It is possible that an immature placental defense system early in gestation is not capable of converting excessive levels of maternal cortisol. Early exposure to

| Table 3 – Correlations between cortisol and CRH at four gestational intervals (N = 203) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| $T_1$ CORT | $T_2$ CORT | $T_3$ CORT | $T_4$ CORT |
| CRH | .041 | | | |
| CRH | .062 | .098 | | |
| CRH | .058 | .073 | -.010 | |
| CRH | .487 | .277 | .078 | -.026 |
| $T_1$ = 15 weeks, $T_2$ = 19 weeks, $T_3$ = 25 weeks and $T_4$ = 31 weeks. $^*$ p < .01.

| Table 4 – Final model from stepwise multiple regression for the prediction of third trimester CRH |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Steps | Variable (weeks) | Standardized beta | Unstandardized beta | $R$ | $R^2$ change | $F_{\text{change}}$ | $P$-value |
|--------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Step 1 | Cortisol (15 weeks) | .49 | 33.5 | .49 | .24 | 61.70 | .000 |
| Step 2 | CRH (25 weeks) | .23 | 1.4 | -.54 | .05 | 14.61 | .000 |
| Step 3 | Cortisol (19 weeks) | .16 | 7.7 | -.56 | .03 | 6.4 | .012 |
cortisol may prime the placental clock [30,42] and accelerate placental synthesis and release of CRH [21,41]. By the same reasoning, the effects of elevated maternal cortisol at 31 weeks does not result in a simultaneous increase of maternal CRH because the activity of placental 11B-HSD2 is sufficient to convert the cortisol to cortisone. Interestingly, and in agreement with an earlier study [27], cortisol and CRH are significantly and concurrently associated only at 37 weeks in a subset of women (N = 164) in our sample consistent with the timing of the abrupt fall in 11B-HSD2. The findings suggest that a profile of endocrine markers, led primarily by biological evidence of maternal stress early in pregnancy, program a subsequent fetal/placental response that results in foreshortened gestation.

Early detection by the fetal/placental unit of stress signals from the maternal environment “informs” the fetus that there is a threat to survival. This information primes or advances the placental clock by activating the promoter region of the CRH gene and increases the synthesis of the gene product. The rapid increase in CRH begins the cascade of events resulting in myometrial activation and fetal escape from a malignant environment. Early departure from the inhospitable host environment may be essential for survival but it does have grave consequences for the tadpole and the human fetus. The immature tadpole is at a disadvantage competing with normally developing tadpoles foraging for food and reproducing [12]. Human infants born early suffer a similar fate that includes a panoply of motor, sensory and neurological impairments that persist for a lifetime [1,33].

Acknowledgements

This study was supported by US PHS (NIH) research awards from the National Institute of Child Health and Human Development (HD28413) and National Institute of Neurological Diseases and Stroke (NS41298).

REFERENCES

[1] Anderson P, Doyle LW. Victorian Infant Collaborative Study Group. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. JAMA 2003;289:3264–72.

[2] Barker DJP. Mothers babies and health in later life, 2nd ed., Edinburgh: Churchill Livingstone, 1998.

[3] Boorse GC, Denver RJ. Acceleration of ambystoma tigrinum metamorphosis by corticotropin-releasing hormone. J Exp Zool 2002;293:94–8.

[4] Challis JR. Evolution of the corticotropin-releasing hormone signaling system and its role in stress-induced phenotypic plasticity. In: Sandman CA, Strand FL, Beckwith B, Chronwall BM, Flynn FW, Nachman RJ, editors. Neuropeptides: structure and function in biology and behavior. New York: The New York Academy of Sciences; 1999. p. 46–53.

[5] Chrousos GP. Regulation and dysregulation of the hypothalamic-pituitary-adrenal axis. The corticotropin-releasing hormone perspective. Endocrinol Metab Clin North Am 1992;21:833–58.

[6] Cohen J, Cohen P, West SG, Aiken LS. Applied multiple regression/correlation analysis for the behavioral sciences. New Jersey: Lawrence Erlbaum Associates, 2003.

[7] Denver RJ. Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. Horm Behav 1997;31:169–79.

[8] Denver RJ. Evolution of the corticotropin-releasing hormone signaling system and its role in stress-induced phenotypic plasticity. In: Sandman CA, Strand FL, Beckwith B, Chronwall BM, Flynn FW, Nachman RJ, editors. Neuropeptides: structure and function in biology and behavior. New York: The New York Academy of Sciences; 1999. p. 46–53.

[9] Erickson K, Thorsen P, Chrousos G, Grigoriadis DE, Khongalsy O, McGregor J, et al. Preterm birth: associated neuroendocrine, medical, and behavioral risk factors. J Clin Endocrinol Metab 2001;86:2544–52.

[10] Florio P, Franchini A, Reis FM, Pezzani I, Ottaviani E, Petraglia F. Human placenta, chorion amnion and decidua express different variants of corticotropin-releasing hormone receptor messenger RNA. Placenta 2000;21:32–7.

[11] Frim DM, Emanuel RL, Robinson BG, Smas CM, Adler GK, Majzoub JA. Characterization and gestational regulation of corticotropin-releasing hormone messenger RNA in human placenta. J Clin Invest 1988;82:287–92.

[12] Glennemeier KA, Denver RJ. Small changes in whole-body corticosterone content affect larval Rana pипis fitness components. Gen Comp Endocrinol 2002;127:16–25.

[13] Glynn LM, Wadhwa PD, Dunkel-Schetter C, Chicz-DeMet A, Sandman CA. When stress happens matters: effects of earthquake timing on stress responsivity in pregnancy. Am J Obstet Gynecol 2001;184:637–42.

[14] Grammatopoulos DK, Hillhouse EW. Role of corticotropin-releasing hormone in onset of labour. Lancet 1999;354:1546–9.

[15] Gur C, Diav-Citrin O, Shechtman S, Arnon J, Ornoy A. Pregnancy outcome after first trimester exposure to corticosteroids: a prospective controlled study. Reprod Toxicol 2004;18:93–101.

[16] Hays WL, Statistics, 5th ed., Texas: Harcourt Brace College Publishers, 1994.

[17] Hobel C, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CP. Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks’ gestation in pregnancies ending in preterm delivery. Am J Obstet Gynecol 1999;180:5257–63.

[18] Holzman C, Jetton J, Siler-Khodr T, Fisher R, Rip T. Second trimester corticotropin-releasing hormone levels in relation to preterm delivery and ethnicity. Obstet Gynecol 2001;97:657–63.

[19] Kajantie E, Dunkel L, Turpeinen U, Stenman UH, Uitto K, Nuutila M, et al. Placental 11 beta-hydroxysteroid dehydrogenase-2 and fetal cortisol/cortisone shuttle in small preterm infants. J Clin Endocrinol Metab 2003;88:493–500.

[20] Karteris E, Grammatopoulos D, Dai Y, Olah KB, Ghobara TB, Easton A, et al. The human placenta and fetal membranes express the corticotropin-releasing hormone receptor 1 alpha (CRH-1 alpha) and the CRH-C variant receptor. J Clin Endocrinol Metab 1998;83:1376–9.

[21] King SR, Smith R, Nicholson RC. The regulation of human corticotrophin-releasing hormone gene expression in the placenta. Peptides 2001;22:1941–7.

[22] Kofman O. The role of prenatal stress in the etiology of developmental behavioural disorders. Neurosci Biobehav Rev 2002;26:457–70.

[23] Korebri C, Yu DH, Ramirez MM, Marinione E, Bocking AD, Challis JR. Antenatal glucocorticoid administration increases corticotrophin-releasing hormone in maternal plasma. Br J Obstet Gynecol 1998;105:556–61.
Linton EA, Perkins AV, Hagan P, Poole S, Bristow AF, Tilders F, et al. Corticotrophin-releasing hormone (CRH)-binding protein interference with CRH antibody binding: implications for direct CRH immunoassay. J Endocrinol 1995;146:45–53.

Lowry PJ. Corticotropin-releasing factor and its binding protein in human plasma. Ciba Found Symp 1993;172:108–15.

Ma XH, Wu WX, Nathanielsz PW. Gestation-related and betamethasone-induced changes in 11beta-hydroxysteroid dehydrogenase types 1 and 2 in the baboon placenta. Am J Obstet Gynecol 2003;188:13–21.

Magiakou M, Mastorakos G, Rabin D, Margioris AN, Dubbert B, Calogero AE, et al. The maternal hypothalamic-pituitary-adrenal axis in the third trimester of human pregnancy. Clin Endocrinol 1996;44:419–28.

Marinoni E, Korebrits C, Di Iorio R, Cosmi EV, Challis JR. Effect of betamethasone in vivo on placental corticotropin-releasing hormone in human pregnancy. Am J Obstet Gynecol 1998;178:770–8.

McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. Nat Med 1995;1:460–3.

McLean M, Smith R. Corticotrophin-releasing hormone and human parturition. Reproduction 2001;121:493–501.

Murphy VE, Clifton VL. Alterations in human placental 11 beta-hydroxysteroid dehydrogenase type 1 and 2 with gestational age and labour. Placenta 2003;24:739–44.

Ng PC, Lam CW, Lee CH, Ma KC, Fok TF, Chan IH, et al. Reference ranges and factors affecting the human corticotropin-releasing hormone test in preterm, very low birth weight infants. J Clin Endocrinol Metab 2002;87:4621–8.

Petraglia F, Florio P, Nappi C, Genazzani AR. Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. Endocr Rev 1996;17:156–86.

Petraglia F, Sawchenko PE, Rivier J, Vale W. Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. Nature 1987;328:717–9.

Petraglia F, Sutton S, Vale W. Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells. Am J Obstet Gynecol 1989;160:247–51.

Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiatry 2003;160:1554–65.

Rodbard D, Munson P, De Lean A. Improved curve fitting, parallelism testing, characterization of sensitivity and specificity, validation, and optimization for radioligand assays. RIA Rel Proc Med 1978;1:469–504.

Sasaki A, Tempst P, Liotta AS, Margioris AN, Hood LE, Kent SB, et al. Isolation and characterization of a corticotropin-releasing hormone-like peptide from human placenta. J Clin Endocrinol Metab 1988;67:768–73.

Seasholtz AF, Valverde RA, Denver RJ. Corticotropin-releasing hormone-binding protein: biochemistry and function from fishes to mammals. J Endocrinol 2002;175:89–97.

Scatena CD, Adler S. Characterization of a human-specific regulator of placental corticotropin-releasing hormone. Mol Endocrinol 1998;12:1228–40.

Smith R, Mesiano S, McGrath S. Hormone trajectories leading to human birth. Regul Pept 2002;108:159–64.

Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 1981;213:1394–7.

Wadhwa P, Dunkel-Schetter C, Chicz-DeMet A, Porto M, Sandman CA. Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. Psychosom Med 1996;58:432–46.

Wadhwa PD, Garite TJ, Porto M, Lynn L, Chicz-DeMet A, Dunkel-Schetter C, et al. Placental corticotropin-releasing hormone (CRH), spontaneous preterm birth, and fetal growth restriction: a prospective investigation. Am J Obstet Gynecol 2004;191:1063–9.

Wadhwa PD, Porto M, Garite TJ, Chicz-DeMet A, Sandman CA. Maternal corticotropin-releasing hormone levels in the early third trimester predict length of gestation in human pregnancy. Am J Obstet Gynecol 1998;179:1079–85.

Welberg LA, Seckl JR. Prenatal stress, glucocorticoids and the programming of the brain. J Neuroendocrinol 2001;13:113–28.

Wetzka B, Sehringer B, Schafer WR, Biller S, Hor C, Benedek E, et al. Expression patterns of CRH, CRH receptors, and CRH binding protein in human gestational tissue at term. Exp Clin Endocrinol Diab 2005;111:154–61.