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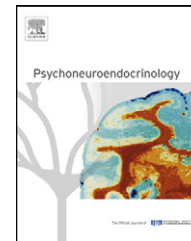
# Influence of prenatal maternal stress, maternal plasma cortisol and cortisol in the amniotic fluid on birth outcomes and child temperament at 3 months

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**Summary** This prospective, longitudinal study aimed to investigate relationships between indicators of maternal prenatal stress, infant birth outcomes and early temperament. We examined the pattern of associations and postulated pathways between physiological (cortisol plasma concentrations) and self-report indices (stress, anxiety) of maternal prenatal stress, cortisol in the amniotic fluid, birth outcomes and infant temperament at 3 months. The sample consisted of 158 women undergoing amniocentesis in the 2nd trimester of pregnancy. Questionnaire measures of maternal stress and anxiety were found to be unrelated to cortisol in plasma or amniotic fluid. Maternal cortisol was related to amniotic cortisol, which in turn was associated with lower birth weight. Birth weight predicted infant fear and distress to limitation at 3 months old. We found trend-like indirect effects of amniotic fluid on infant distress to limitation and fear via birth weight. This is one of the few studies to simultaneously assess the role of maternal and amniotic fluid cortisol on birth outcomes and infant emotional development. The results suggest that foetal cortisol may be an important predictor of infant outcomes and shed light on the mechanisms through which prenatal maternal stress affects infant psychological health.

## 1. Introduction

An increasing amount of research suggests that the prenatal environment can have a profound effect on child outcomes. Animal studies provide good evidence that prenatal stress can have long-lasting effects on the offspring (e.g. Weinstock, 1997; 2005). There is accumulating evidence that in

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humans, prenatal stress and anxiety can influence infant birth outcomes and the development of temperament and cognition (e.g. Huizink et al., 2004; Talge et al., 2007).

Various studies report a link between prenatal stress and child outcomes, with higher levels of stress having a negative impact on temperament, cognitive and motor development of the child. For example, prenatal stress and feelings of general anxiety have been linked to lower birth weight (Lobel et al., 1992; Wadhwa et al., 1993), difficult temperament and behavioural problems (Huizink et al., 2002; Buitelaar et al., 2003; O'Connor et al., 2003; Gutteling et al., 2005; Rothenberger et al., 2011). It has also been proposed that pregnancy-related anxiety specifically may be more closely linked to child outcomes than general feelings of anxiety (Huizink et al., 2004). Indeed, Buitelaar et al. (2003) found pregnancy-related anxiety (fear of having a handicapped child and fear of giving birth) to be uniquely associated with various infant temperament outcomes at age 8 months. More recently, Blair et al. (2011) found pregnancy-specific anxiety between 13 and 17 weeks of gestation to be a unique predictor of negative child temperament at 2 years.

However, the mechanisms through which prenatal maternal stress and anxiety influence subsequent child outcomes are not fully understood, with few studies investigating the possible mediating factors that might be important in linking prenatal stress and child postnatal outcome. Some of the effects of prenatal stress on child emotional development may be due to genetic inheritance. Rice et al. (2010) disentangled the influence of genetic factors and prenatal environment by examining the influence of prenatal stress in women pregnant with a genetically related child and women pregnant with a genetically unrelated child (either through egg or embryo donation through in vitro fertilization). The association between prenatal stress and child outcome (i.e., gestational age, anxiety and antisocial behaviour) was confirmed in both genetically related and unrelated mother—child pairs. This study provides evidence that maternal stress does not influence the child only through genetic factors, but also through other factors, one of which could be maternal stress hormonal activity.

The hypothalamic-pituitary-adrenal (HPA) axis is one of the major systems involved in stress response and its regulation. The HPA system is activated during stress and threat (Weinstock, 2005), and studies have looked at the concentrations of its end product, cortisol, as an endocrinological marker of stress and anxiety (Weinstock, 2008). It has been suggested that the activation of the HPA axis is one of the main biological mechanisms underlying the effects of prenatal stress (Huizink et al., 2004; Talge et al., 2007). Various studies have included measures of maternal cortisol concentrations during pregnancy in addition to, or instead of, psychosocial assessments of stress and anxiety (e.g. Buitelaar et al., 2003; Bergman et al., 2010a,b; Davis and Sandman, 2010; Rothenberger et al., 2011).

The mechanisms through which maternal HPA axis activity can influence foetal development in humans are not yet fully understood (Talge et al., 2007). A study by Radke et al. (2011) showed that prenatal stress linked to domestic violence in pregnancy was associated with glucocorticoid receptor (GR) gene methylation in children in their early teens. Increased GR methylation is associated with stronger cortisol responses

to stress. These results suggest that prenatal stress can adversely influence gene expression in the HPA-axis.

It has also been proposed that maternal cortisol can pass through the placenta and affect foetal cortisol concentration and HPA axis development. The activity of placental enzyme 11 β-hydroxysteroid-dehydrogenase type 2 protects the foetus from maternal cortisol (e.g. Benediktsson and Seckl, 1998) by converting it into inactive cortisone. However, evidence from animal studies suggests that prenatal stress can affect the function of the placenta and the expression of 11 β-hydroxysteroid-dehydrogenase enzyme (Welberg et al., 2005). Furthermore, Gitau et al. (1998) compared maternal and foetal cortisol concentrations in women undergoing clinically-indicated foetal testing, and found them to be linearly related ($r = .62$). They also found that maternal cortisol accounted for about 40% of the variance in amniotic cortisol concentrations in high stress conditions. Glover et al. (2009) looked at maternal and amniotic fluid cortisol in a sample of women undergoing amniocentesis. They also found that maternal and amniotic cortisol concentrations were significantly correlated ($r = .32$), and that in the sub-sample of more anxious women the correlation was even higher ($r = .59$). These studies support the claim that elevations in maternal cortisol can have an impact on concentrations of cortisol in the amniotic fluid. It should be noted however, that there are other possible sources of amniotic cortisol apart from maternal cortisol, such as cortisol from the fetal adrenal and the fetal membrane. In addition, Sarkar et al. (2001) have shown that 11 β-hydroxysteroid-dehydrogenase can be downregulated by norepinephrine and epinephrine. Hence, the relationship between maternal and amniotic fluid cortisol may partly be dependent on norepinephrine and epinephrine.

The question of whether amniotic fluid cortisol could explain variation in child temperament arises because concentrations of stress hormones in the amniotic fluid could affect foetal brain development. Salaria et al. (2006) found that increased prenatal cortisol exposure influenced the expression of over a thousand genes in foetal brain cells. A study by Bergman et al. (2010a) was the first to investigate the influence of amniotic fluid cortisol on child outcome. It was found that higher levels of amniotic cortisol were associated with lower cognitive scores at 17 months, but in a different paper Bergman et al. (2010b) reported no relationship with child temperament (i.e., fear reactivity at 17 months). These findings call for more research to be carried out to investigate the association between amniotic fluid cortisol and a wide range of outcomes, such as birth outcomes and temperament. Specifically, cortisol concentration in the amniotic fluid could function as a mediator of the relationship between maternal cortisol and child outcomes.

The current study combined psychosocial and hormonal assessments of maternal prenatal stress together with amniotic cortisol levels in a normal sample of healthy women undergoing amniocentesis early in pregnancy to better capture prenatal stress experience to investigate how different indices of prenatal stress are associated with birth outcomes (i.e. gestational age and birth weight) and early infant temperament. Foetal gestational age and infant birth weight are important markers of subsequent infant development. Low birth weight has been linked to lower IQ scores (Breslau, 1995) and to hyperactivity and inattention (Breslau et al.,

1996). Rice et al. (2007) found birth weight and gestational age to be associated with increased emotional problems. Gestational age and infant birth weight were therefore included as markers of infant health at delivery. We were interested in examining the effects of maternal and amniotic fluid cortisol on these early markers of infant health and well being.

We had three hypotheses. First, we hypothesized a positive association between maternal self-reports of stress and anxiety and her cortisol concentration. Second, we hypothesized that maternal plasma cortisol and amniotic fluid cortisol would be positively associated and that cortisol concentration in the amniotic fluid would be associated with infant birth outcomes (weight; gestational age). Our third hypothesis was that maternal cortisol would be indirectly associated with infant birth weight and temperament at 3 months old via its relationship with amniotic cortisol.

## 2. Method

### 2.1. Subjects and sampling

All participants in the study took part in a prospective longitudinal project into the effects of prenatal hormones on development in children. Participants were recruited from a consecutive series of referrals, between January 1999 and August 2000, to the Department of Obstetrics at the University Medical Centre in Utrecht (UMCU), the Netherlands, to undergo an amniocentesis because of prenatal diagnostic screening. All possible candidates were approached by written information and 185 women declared a willingness to participate. Only data from continued healthy singleton pregnancies were used. Abnormalities of the thyroid gland, if well treated, were allowed ($n$ = 3). Cases of twin pregnancies ($n$ = 4), diabetes ($n$ = 2), chronic use of steroid ointment ($n$ = 3), and the intake of hormonal medication because of asthma ($n$ = 6) were excluded. Two women who turned out to carry a female foetus with a sex chromosome deviation (triple-x) were excluded, as well as one woman who had general anaesthesia during her pregnancy. Four pregnancies were terminated because of an induced abortion and one because of a miscarriage. Four women were excluded because no postnatal data were collected from them. The final sample comprised 158 pregnant women (consisting of 78 male, and 80 female foetuses). Each patient gave informed consent to the procedure and the UMCU Medical Ethical Committee approved of the study. The majority (96%) was referred because of their age (36 or older); others had an amniocentesis because of a deviate serum screening (0.7%) or their medical history (3.3%). Maternal age ranged from 28 to 45 years (with a mean age of 37.6 years although our sample was older than other samples examining the relation between maternal stress and prenatal cortisol, self-reported stress levels in these older women did not seem to differ from those collected in younger women not referred for amniocentesis. For example, the mean score for fear of giving birth in our study was 5.52 (SD = 2.41), whereas that in the Huizink et al. (2003) study, using a younger Dutch sample of 170 primiparous women from the same Medical Centre, was 5.9 (SD = 2.7).

Similarly, the mean perceived stress score (PSS) in our sample was 26.04 (SD = 5.27), whereas the levels in the Huizink et al. (2002) study were 28.1 (SD = 5.5). For each parent, educational level was scored on a 7-point scale, with a score of 1 indicating "no formal qualifications" to 7 representing "a university degree." The scores of both parents were combined. The mean parental education score was 9.8 (SD = 3.1, possible range 2—14). Eighty-eight women (55.7%) had a higher education qualification; there were no differences in maternal cortisol levels, any questionnaire measures or any infant outcome measures between mothers who had a higher education qualification and those who had not. For 53 women (33.5%) this was the first child, while 102 (64.6%) had children previously, with data missing on 3 women (1.9%). There were no differences between primi- and multiparous women in perceived stress, nor on any of the cortisol or child outcomes measures. However, primiparous women scored higher on Fear of giving birth scale ($t$ (2, 153) = 2.92, $p < .05$). Blood plasma cortisol was provided by 135 women. Amniotic fluid samples were provided by 153 participants, and collected between morning and midday (9.00—12.00) in weeks 15.3—18.2 of pregnancy (see for further details, Van de Beek et al., 2009). The length of gestation was determined by the last menstrual period or ultrasonic measurement of crown-rump length (CRL) (Daya, 1993). Maternal serum was collected immediately following the amniocentesis. Serum and amniotic fluids were stored at −30 °C until assayed.

### 2.2. Measures

#### 2.2.1. Maternal questionnaires
Maternal perceived stress was assessed with a Dutch translation of the 14-item perceived stress scale (PSS; Cohen and Williamson, 1987). The scale measures perceived stress over a 4-week period, covering the 2 weeks before the puncture until the day of the result, on a 4-point scale ranging from 'never' to 'always'. Cronbach's alpha was .92. Pregnancy anxiety was assessed by means of the pregnancy related anxieties questionnaire-revised (PRAQ-R; Huizink, 2000). This questionnaire was developed by confirmatory factor analysis from the PRAQ of Van den Bergh (Van den Bergh, 1990) and consisted of 10 items that fitted to a three-factor model: fear of giving birth (3 items; scores ranging from 3 to 15), Fear of bearing a physically or mentally handicapped child (4 items; scores ranging from 4 to 20), and Concern about one's own appearance (3 items; scores ranging from 3 to 15; Cronbach's alphas for the scales were all >76. Pregnancy anxiety rather than general anxiety is related to birth outcome and activation of the neuroendocrine axis in pregnancy (Wadhwa et al., 1993; Killingsworth Rini et al., 1999). For the purposes of present study, only the 'Fear of giving birth' scale was used due to past findings of its particular relevance to infant outcomes (Huizink et al., 2003).

Fifteen minutes prior to amniocentesis, women were given the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983). This 20-item questionnaire assesses levels of anxiety experienced at the time (state) on a 4-point Likert scale ranging from 1 (not at all) to 4 (very much). For this scale the scores can range from a minimum of 10 to a

maximum of 40. The STAI state scale has been demonstrated to have a good internal consistency, with coefficients for the scale ranging from .86 to .95 (Spielberger et al., 1983).

### 2.2.2. Hormone assays

Cortisol in amniotic fluid was determined by radioimmunoassay after heat denaturing of the binding protein CBG. A polyclonal cortisol antibody was used and [1,2-$^3$H(N)]-Hydrocortisone (NEN - DUPONT, Dreiech, Germany) as a tracer. The lower limit of detection was 0.5 nmol/L and interassay variation was on average 6%. Maternal cortisol samples were collected immediately following the amniocentesis. Cortisol in serum was measured using a competitive technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 0.01 μmol/L and within run precision was 4% at 0.55 μmol/L. All samples were analyzed in one batch.

Cortisol binding globulin was measured using the CBG RIA kit (BioSource Europe S.A., Nivelles, Belgium). The lower limit of detection was 10 mg/L for serum and 1.6 mg/L for amnion fluid. Interassay variation was 4% on average.

*Perinatal outcome*. Infant gestational age (in weeks) and infant birth weight (in grams) were used as perinatal outcomes.

*Infant temperament*. Temperament was assessed at 3 months. Women in the Netherlands go back to work 3 months after having birth and we theorised that the time after 3 months is a period of change for mother and child. Moreover, by 3 months mothers are able to rate their child's temperament more reliably than at earlier time points. Mothers filled in the Dutch translation of the infant behaviour questionnaire-revised (IBQ-R, Garstein and Rothbart, 2003). The IBQ-R assesses 14 domains of infant temperament and the current study used the two subscales, which have been used in research on infant temperament and cortisol: 'distress to limitations' and 'fear' (Gunnar et al., 1992). Infant 'distress to limitations' refers to baby's fussing and crying in confining positions, during caretaking activities and when unable to perform a desired action. 'Fear' refers to baby's startle or distress to changes in stimulation, novel physical objects and social stimuli, and inhibited approach to novelty. The 'fear' and 'distress to limitations' subscales each consist of 16 items ($\alpha$ = .87 and .82, respectively).

### 2.2.3. Missing data

The following variables had missing values: maternal cortisol levels (14%), amnion cortisol levels (6.35%), infant birth weight (11%), maternal concerns about giving birth (7%), maternal perceived stress (12.7%), infant distress to limitation (11.4%) infant fear at (11.4%). The pattern of missingness was tested with Little's MCAR test, which assesses whether missing data are missing completely at random (MCAR) or missing at random (MAR) with the null hypothesis being that missing data are MCAR (Acock, 2005). The results of the test indicated that there was no systematic pattern of missing data and that missing values were randomly distributed across all observations ($\chi^2$(887) = 910.67, $p$ = .28).

### 2.2.4. Statistical analysis

ormality of distribution of the variables was checked using a histogram with a normal curve. The moderately positively skewed variable of maternal cortisol underwent natural log (ln) transformation, which is recommended for this type of skewness (Tabachnik and Fidel, 2001), after which the distribution of the variable appeared normal. The non-transformed variable was used in the descriptive statistics table and the frequency distribution, but the transformed variable was used in all further analyses. We also tested for differences by child sex and maternal stress and anxiety.

Structural equation modelling using full information maximum likelihood estimation (FIML) in LISREL 8.50 (Jöreskog and Sörbom, 2001) was used to test a model linking maternal cortisol with infant temperament at 3 months old via amniotic cortisol and birth weight. FIML estimation directly fits the model to the non-missing values for each observation, allowing the use of all cases including those with missing data (Widaman, 2006). The significance level across all analyses was set at $p$ < .05.

## 3. Results

The characteristics of the sample are presented in Table 1 and the correlations between study variables are shown in Table 2. There were no sex differences in amniotic fluid cortisol concentration ($t$ = .08, $p$ = .94), birth weight ($t$ = .52, $p$ = .64), IBQ-fear ($t$ = −.51, $p$ = .61), or IBQ-distress to limitation ($t$ = 1.84, $p$ = .08). Given that Glover et al. (2009) found that the association between maternal and amniotic fluid was moderated by maternal state anxiety, we checked for differences between high and low-anxious and stressed mothers. We found no differences in the magnitude of associations for high and low anxious women based on the STAI questionnaire, nor between high and low stressed mothers as measured by the perceived stress questionnaire (PSS). The comparisons

**Table 1** Descriptive statistics for the variables during prenatal and postnatal assessment of mothers and infants.

| Study variable | Mean | SD | Range |
|---|---|---|---|
| *Prenatal* | | | |
| Maternal cortisol (μmol/L) | 0.46 | .13 | 0.25—1.06 |
| Amniotic cortisol (nmol/L) | 42.08 | 6.84 | 27.0—63.0 |
| Time of puncture (weeks) | 16.41 | .47 | 15.30—18.20 |
| Fear of giving birth | 5.51 | 2.41 | 3.0—13.0 |
| Perceived stress (PSS) | 26.04 | 5.27 | 15.0—40.0 |
| STAI state | 43.59 | 4.71 | 30—58 |
| *Postnatal* | | | |
| Birth weight (grams) | 3483.80 | 551.75 | 1435.0—4772.0 |
| Gestational age (weeks) | 39.83 | 1.65 | 30.71—42.73 |
| IBQ-distress to limitation | 3.12 | 1.01 | 1.36—6.33 |
| IBQ-fear | 1.97 | .84 | 1.00—5.10 |

**Table 2** Inter-correlations among prenatal measures, birth outcomes and infant temperament at 3 months.

| | Study variable | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Prenatal measures* | | | | | | | | | | |
| 1 | Maternal cortisol | — | | | | | | | | |
| 2 | Amniotic cortisol | .18[*] | — | | | | | | | |
| 3 | Fear of giving birth | .15 | −.05 | — | | | | | | |
| 4 | Perceived stress (PSS) | .00 | .09 | .30[**] | — | | | | | |
| 5 | STAI state | .09 | −.03 | −.07 | −.09 | — | | | | |
| *Postnatal measures* | | | | | | | | | | |
| 6 | Birth weight | −.15 | −.25[**] | −.11 | .03 | −.06 | — | | | |
| 7 | Gestational age | −.07 | −.18[*] | .07 | .07 | −.01 | .44[**] | — | | |
| 8 | Distress to limitation (IBQ) | .02 | .06 | .11 | .20[*] | .08 | −.21[*] | −.07 | — | |
| 9 | Fear (IBQ) | .03 | −.02 | −.03 | .10 | .18 | −.19[*] | −.15 | .33[**] | — |

[*] $p < .05$.
[**] $p < .01$.

between low- vs. high-anxious and stressed women were based on a median split and were performed using Fishers r-to-z transformation.

## 3.1. Relationship between prenatal and postnatal variables

Maternal plasma and amniotic fluid cortisol concentrations were positively associated ($r = .18$, $p < .05$). The two stress questionnaire measures, fear about giving birth and perceived stress in pregnancy, were also positively correlated ($r = .30$, $p < .01$), but both prenatal stress measures were not correlated with maternal state anxiety (STAI). As shown in Table 2, maternal and foetal cortisol levels were not correlated with self-reported maternal stress or anxiety.

Birth weight and gestational age were positively associated ($r = .44$, $p < .01$). Amniotic cortisol was significantly correlated with both birth weight ($r = −.25$, $p < .01$) and gestational age ($r = −.18$, $p < .05$). Only birth weight, and not gestational age, in turn, was correlated with both IBQ-distress to limitation ($r = −.21$, $p < .05$) and IBQ-fear ($r = −.19$, $p < .05$). Perceived stress was correlated with IBQ-distress to limitation ($r = −.20$, $p < .05$). Maternal and foetal cortisol levels were not associated with infant temperament at 3 months old (Fig. 1).

## 3.2. Influence of prenatal stress and child birth and temperament outcomes

A variable can have an indirect effect on an outcome variable in the absence of correlation if the predictor variable influences a third intervening variable, which in turn, affects the outcome variable (MacKinnon et al., 2002). We tested the indirect influence of prenatal cortisol (maternal, amniotic) on temperament outcomes via birth weight. Although birth weight and gestational age were associated, the pattern of correlations clearly showed that birth weight rather than age was the best birth outcome variable. Moreover, the effects of stress (i.e., cortisol) on physical development are well known (Weinstock, 2005). We therefore chose not to include gestational age in further analysis (Fig. 2).

Maternal cortisol was related to increased amniotic cortisol ($\beta = .18$, $p < .05$) and amniotic cortisol predicted decreased birth weight ($\beta = −.19$, $p < .05$). Birth weight, in turn, was a significant predictor of both infant fear and distress to limitation at 3 months ($\beta = −.16$ and $−.18$, $p < .05$, respectively). The indirect effects of maternal cortisol on infant fear and distress to limitation via birth weight were not significant ($t = 1.35$, $b = .03$ and $t = 1.42$, $b = .03$) while the indirect effects of amniotic cortisol on infant fear and distress to limitation via birth weight resembled a trend ($t = 1.78$, $b = .05$; $t = 1.94$, $b = .05$, $p < .10$). The fit indices
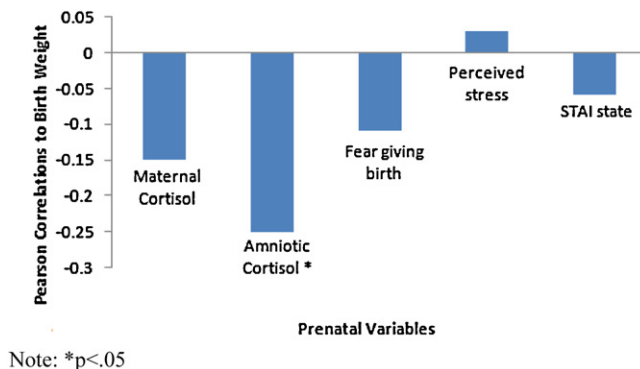


Note: *p<.05

**Figure 1** Pearson correlations between prenatal predictors and birth weight. *Note*: $*p < .05$.
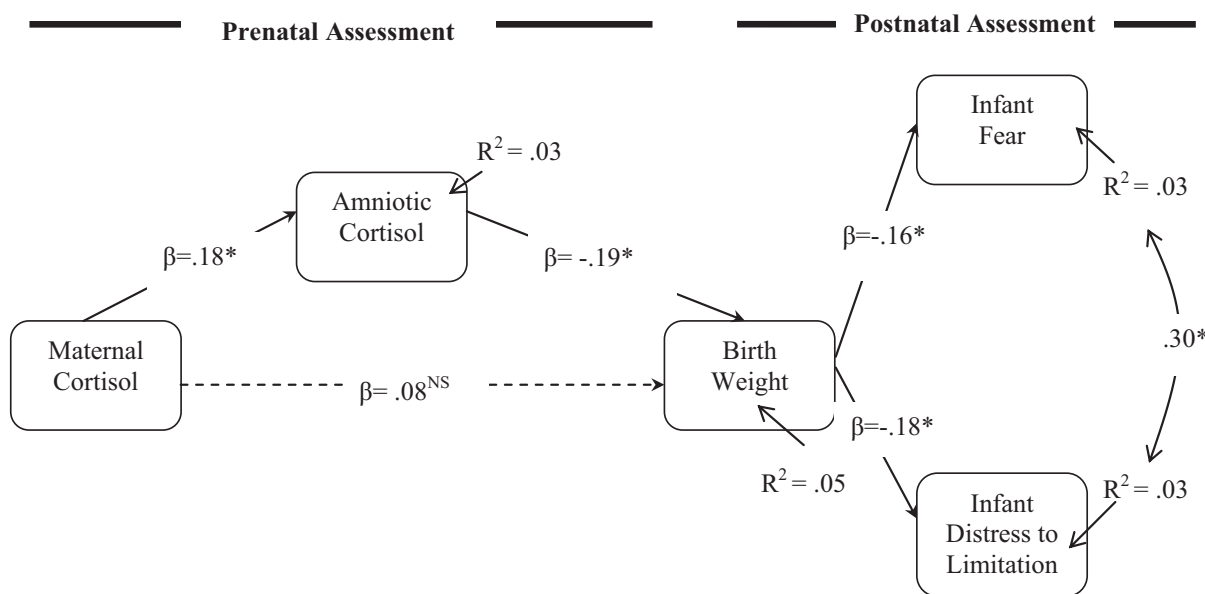
**Figure 2** Maximum likelihood estimation of relationships between prenatal cortisol, birth weight and infant temperament at three months old. $N$ = 158, *$p < .05$. Goodness of fit indices: $\chi_4^2 = .72$, $p = .95$, RMSEA = .00, CFI = 1.00, GFI = 1.00, AGFI = .99, *Note*: *$p < .01$.

indicated an acceptable fit between the model and the data ($\chi_4^2 = .72$, $p = .95$, RMSEA = .00, CFI = 1.00, GFI = 1.00, AGFI = .99, $df = 4$).

## 4. Discussion

We examined the relationships between physiological and self-rated measures of prenatal maternal stress and assessed their relationship with infant birth weight and early temperament. We used different assessments of prenatal stress (i.e., perceived stress and pregnancy-related anxiety) around amniocentesis in early pregnancy and maternal cortisol levels during this procedure. Cortisol concentrations in the amniotic fluid were used as an indicator of foetal cortisol concentration.

Contrary to our first hypothesis, maternal self-reports of perceived stress, fear of giving birth and anxiety at the time of the puncture (STAI) were not associated with either maternal or foetal cortisol levels, suggesting a discrepancy between self-reports and physiological/endocrine measures of stress. Some previous studies have also failed to establish a relation between self-reported stress or anxiety, on the one hand, and cortisol levels, on the other (e.g. Harville et al., 2009).

It is possible that the cortisol concentrations collected in this study partly reflect the stress of the amniocentesis procedure, whereas reports of perceived stress and anxiety assess more general and trait-related feelings of stress and anxiety. It might be useful in the future to obtain more than one cortisol sample during pregnancy to better capture maternal cortisol levels. The lack of association between

self-reported and physiological assessments of stress not only highlights the challenges of conducting research on stress in pregnancy but also the need to combine subjective and more objective assessments to gain a more complete understanding of the maternal stress experience.

As hypothesised, maternal cortisol and amniotic fluid cortisol were positively associated. This finding, in addition to work of Gitau et al. (1998), Sarkar et al. (2006) and Glover et al. (2009) provides further evidence that maternal cortisol is related to amniotic cortisol concentration, and supports the idea that prenatal maternal stress could impact foetal HPA-axis activity and brain development. The correlation in the present study ($r = .18$), however, was considerably lower than coefficients identified in previous work. For example, Glover et al. (2009) found a correlation of .32 in a sample of women also undergoing amniocentesis. There are two possible explanations for the weaker association, the first being that our sample of pregnant women was less anxious. Glover et al. (2009) observed a mean STAI state score of 49.7 (SD = 13.6), whereas our mean was 43.59 (SD = 4.71), and they also found a stronger association between maternal an foetal cortisol in more anxious than less anxious women. Secondly, our cortisol samples were collected early in pregnancy (weeks 15—18), whereas the age ranges in the studies by Gitau et al. (1998) and Glover et al. (2009) were much larger, being 13—35, and 15—37 weeks gestation, respectively. Diaz et al. (1998) report that the rat foetal brain is protected from some of the damaging effects of glucocorticoids by 11 β-HSD, which is highly expressed around mid-pregnancy. This expression dramatically reduces in late pregnancy, which could result in

higher levels of glucocorticoids affecting foetal brain development (Diaz et al., 1998). Huizink et al. (2003) collected maternal salivary cortisol in early, mid and late pregnancy, and found only late pregnancy cortisol to be related to infant mental and motor development. This suggests that in normal healthy samples the association between maternal and amniotic fluid cortisol may be stronger in late pregnancy, and that late pregnancy cortisol in particular might affect infant outcome. Our finding of a positive association — even in mid pregnancy — provides further support that amniotic fluid cortisol concentrations are influenced by maternal HPA-axis functioning and are not fully protected by 11 β-HSD as has been assumed by previous studies (e.g. Benediktsson and Seckl, 1998). Similarly, Mairesse et al. (2007) found that chronic stress during late pregnancy in rodents was associated with strong attenuation of placental 11 β-HSD2 expression, therefore allowing increased access of active glucocorticoids to the foetus.

An additional aim of this study was to investigate the influence of prenatal factors on birth outcome and child temperament. Perceived stress during pregnancy was found to be associated with more difficult temperament (distress to limitation) at 3 months. Our main interest, however, lay in the contribution of physiological stress assessed through maternal plasma and amniotic fluid cortisol to birth outcomes and temperament. We found no relation between maternal cortisol and any of our infant outcome measures. This is consistent with previous research. Gutteling et al. (2005), for example, found no relationship between early, mid- or late pregnancy cortisol levels and infant temperament, while Rothenberger et al. (2011) found no association between maternal salivary cortisol and infant emotional reactivity at 5 months. Davis and Sandman (2010) only found a relation between 3rd trimester maternal cortisol and infant emotional reactivity.

We did, however, observe an association between amniotic fluid cortisol and infant gestational age and birth weight whereby higher levels of cortisol in the amniotic fluid were associated with shorter gestational age and lower birth weight. We went on to assess whether maternal cortisol exerted indirect effects on birth outcomes and infant temperament via amniotic cortisol. The pattern of results showed that maternal cortisol was associated with levels of amniotic cortisol, which was associated with lower infant birth weight. Birth weight, in turn, was related to infant fear and distress to limitation assessed at three months old. It is important to note, however, that the overall indirect effect of maternal cortisol on infant temperament was non-significant and that further research, perhaps using a larger sample, is warranted.

Gestational age and birth weight have been found to be important indicators of infant health and predictors of later development (Schlotz and Phillips, 2009), with lower birth weight being associated with developmental delays in infancy and childhood (e.g. Breslau, 1995; Breslau et al., 1996; Rice et al., 2007). In our study, only birth weight was associated with more difficult temperament. These results suggest that the prenatal hormonal environment may have a direct influence on the physical development of the foetus, which is associated with a more difficult temperament in early infancy. It is important to acknowledge that other systems may be involved in influencing birth weight and subsequent temperament development. For example, whilst Field et al. (2010) demonstrated that elevations in prenatal norepinephrine were also associated with lower birth weight, other studies have found that norepinephrine is related to behavioural inhibition and approach (Rothbart et al., 2000).

The study had some limitations. First, although this study focused on the role of maternal prenatal stress on infant outcomes, it is important to note that the cortisol and subjective stress measures may also reflect maternal genetic contributions to infant phenotype (Rice et al., 2010). Second, we already mentioned the fact that one should ideally collect more than one maternal (plasma or salivary) cortisol sample at different time points in pregnancy to examine the longitudinal effects of maternal stress and to improve the reliability of the measurement during this particular period of development. A single measure of amniotic cortisol also may not adequately capture the foetal stress experience. However, taking more amniotic fluid samples for research purposes only raises ethical questions. Other issues are that although all cortisol samples were taken in the morning, we were unable to control for the exact time of day the sample was taken. This issue may have introduced some noise to the data. It is possible that increased precision in estimating the associations between key study variables would have been possible if we had been able to control for the timing of sampling.

Additionally, infant temperament was not assessed objectively (e.g. using observation). A reliance on maternal report might, at least partially, explain the association between maternally reported perceived stress and infant temperament. This was an additional reason for not including self-report data in the SEM model. Finally, we did not collect any postnatal infant cortisol samples. It would be interesting to investigate whether individual differences in foetal and infant cortisol levels are stable over time and positively associated with different measures of temperament.

We focused on the early stages of development (indexed using birth weight, temperament at three months) in an attempt to control some of the environmental moderators of prenatal effects. It has been demonstrated, however, that maternal behaviour is a potentially important moderator of the effects of prenatal factors on later outcome (e.g. Kaplan et al., 2008; Bergman et al., 2010a,b). In future research, it would be important to obtain information on postnatal maternal state and care-giving behaviours to better understand the joint effects of prenatal and postnatal maternal factors on infant outcome and later development.

The study supports earlier findings by Gitau et al. (1998) and Glover et al. (2009), that maternal HPA-axis functioning influences cortisol concentration in the amniotic fluid. Amniotic cortisol was associated with infant birth weight, with birth weight, in turn, predicting child temperament. Overall, the findings of our study indicate that relatively small variations in prenatal endocrine stress levels, in a normal, healthy sample of mothers, are associated with infant birth weight and more difficult temperament at 3 months of age, and highlight the important role of prenatal cortisol in the process of physical and emotional development.

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