*DOI:*
10.1016/j.bbi.2021.09.020

*Document Version*
Peer reviewed version

**INCREASED MATERNAL INFLAMMATION AND POORER INFANT NEUROBEHAVIOURAL COMPETENCIES IN WOMEN WITH A HISTORY OF MAJOR DEPRESSIVE DISORDER FROM THE PSYCHIATRY RESEARCH AND MOTHERHOOD – DEPRESSION (PRAM-D) STUDY**

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**Abstract:**

**Introduction:** Stress in pregnancy is associated with adverse outcomes in offspring and developmental programming is a potential mechanism. We have previously shown that depression in pregnancy is a valid and clearly defined stress paradigm and both maternal antenatal and offspring stress-related biology is affected. This study aims to clarify whether maternal biology in pregnancy and offspring outcomes can also be influenced by a history of prior depression. Our primary hypothesis is that, similarly to women with depression in pregnancy, women with a history of depression but who are not depressed in pregnancy will have increased cortisol secretion and markers of immune system function, and that their offspring will have poorer neuro-developmental competencies and increased cortisol stress response.

**Methods:** A prospective longitudinal design was used in 59 healthy controls and 25 women with a past history of depression who were *not depressed* in pregnancy, named as 'history-only', and their offspring. Maternal antenatal stress-related biology (cortisol and markers of immune system function) and offspring outcomes (gestational age at birth, neonatal neurobehaviour (Neonatal Behavioural Assessment Scale, NBAS), cortisol stress response and basal cortisol at 2 and 12 months) and cognitive, language and motor development (Bayley Scales of Infant and Toddler Development (BSID)) were measured.

**Results:** Compared with healthy pregnant women, those with a history of MDD who remain free of MDD in pregnancy exhibit increased markers of immune system function in pregnancy: IL-8 (d=0.63, p=0.030), VEGF (d=0.40, p=0.008) and MCP-1 (d=0.61, p=0.002) and have neonates with lower neurobehavioural scores in most areas, reaching statistical significance in the social-

interactive (d=1.26, p=0.015) **cluster**. However, there were no differences in maternal or offspring HPA axis function or in infant development at 12 months.

**Conclusion:** Our study indicates that pregnant women with a history of depression have increased markers of immune system function and their offspring show behavioural alterations that may be the effects of in utero programming, epigenetic factors or genetic predisposition.

**Keywords:** depression, pregnancy, offspring, developmental programming, genetics

**Funding and disclosure**

The work was supported by the following grants: The UK National Institute for Health Research (NIHR) Biomedical Research Centre at the South London and Maudsley NHS Foundation Trust and King's College London (CMP, SO); The Lullaby Trust (formerly known as the Foundation for the Study of Infant Deaths) (263) (CMP, SC, SO); the Psychiatry Research Trust (CMP, SO); and the Brain and Behavior Research Foundation (SO, CMP); The funding sources played no role in the study design, writing, or analysis of the paper and results.

**Declaration of interest statement**

CMP has received research funding from pharmaceutical companies interested in the development of new antidepressants, such as Johnson & Johnson and Boehringer Ingelheim, but this project is unrelated to this funding; there are no further declarations of interest.

## Highlights

- Compared with healthy pregnant women, those with a history of MDD who remain free of MDD in pregnancy exhibit increased **markers of immune system function** in pregnancy
- Compared with neonates born to healthy pregnant women, those born to women with a history of MDD who remain free of MDD in pregnancy have lower neurobehavioural competencies in most areas
- These maternal and offspring differences are not simply related to depression during pregnancy and thus may be the effects of epigenetic factors or genetic predisposition.

**1. Introduction**:

In the elucidation of potential underlying mechanisms for the association of antenatal depression with suboptimal outcomes in offspring, our previously reported findings have shown that women with Major Depressive Disorder (MDD) in pregnancy differ from the psychiatrically healthy in antenatal stress-related biology (increased inflammation and evening cortisol), and furthermore their babies have a shorter length of gestation, poorer neonatal neurobehavioural competences and an enhanced cortisol response to stress at 1 year postnatal. Additionally, positive correlations between maternal antenatal inflammation and infant stress response suggest a mechanistic link (Osborne et al., 2018; Sawyer, 2019; Sawyer et al., 2019). Thus, this line of research establishes MDD as a clearly defined and clinically relevant paradigm for the much-studied 'stress in pregnancy' biological and psychological framework, and especially in the context of psychoneuroimmunology (Osborne & Munk, 2013; Sherer et al., 2018). Moreover, it offers one possible explanation for the replicated association between depression in pregnancy and increased risk of psychopathology in the offspring (Goodman et al., 2011; Sawyer et al., 2019), with a particular emphasis on the relationship between perinatal mood, maternal immune response, and childhood development (Osborne & Munk, 2013; Sherer et al., 2018). Indeed, animal models have offered extensive evidence that maternal immune activation disrupts brain molecular and cellular processes, and leads to anxiety- and depression-like behaviours, in the offspring (Gumusoglu & Stevens, 2019).

However, existing literature regarding developmental programming in humans often states that, despite demonstrating a possible developmental programming effect, such studies are not designed to test the possible pre-pregnancy basis of the differences found in prenatally

stressed infants, such as genetic influences or long-term biological, epigenetics and psychological effects of pre-existing depression bleeding into the perinatal period (O'Donnell & Meaney, 2017; Sawyer et al., 2019). Indeed, a recent investigation in a cohort of more than twenty thousand women has found that the associations between maternal prenatal depressive symptoms and offspring behavioural outcomes in early childhood are likely to be explained, at least in part, by shared genes (Hannigan et al., 2018). Other studies found that women with a history of depression are more likely to display negative affect or reduced sensitivity while interacting with their infants even if well in the perinatal period (Bind et al., 2021; Forbes, Cohn, Allen, & Lewinsohn, 2004), again indicating possible effects of previous depression on biological or psychological mechanisms even in the absence of active psychopathology in pregnancy.

To address this issue, in the current study we compare the same, previously defined group of healthy pregnant women (and their offspring) with a group of pregnant women with a history of depression but who were not actively depressed during pregnancy. This strategy enables us to elucidate further whether maternal pregnancy markers of immune system function, stress biomarkers, and offspring outcomes, are affected by a pre-existing MDD phenotype, in the absence of active depression during pregnancy. The primary hypothesis is that women with a history of depression, but who are not depressed in pregnancy, will have the same pattern of overactivity of the HPA axis and markers of immune system function as women with MDD in pregnancy, as previously described by Osborne et al., 2018. The secondary hypotheses are similarly, that there will be overactivity of the HPA axis and poorer neuro-developmental competencies in their offspring (Osborne et al., 2018).

## 2. Methodology:

**2.1. Design:** In a prospective longitudinal observational study, we compared a 'history-only' group with a DSM-IV diagnosis of MDD prior to pregnancy (and their offspring) with a control group of healthy pregnant women (and their offspring) up to one year postnatally. Maternal socio-demographics, obstetric and physical risk factors, together with clinical status and markers of immune system function, were assessed at baseline (25 weeks gestation), and HPA axis at 32 weeks of pregnancy. Gestational age at birth was recorded, and neonatal neurobehavioral function was assessed at 6-days postnatal. Infant cortisol reactivity (response to the pain stress of routine immunizations) and basal activity (morning and evening) was also assessed at 2- and 12-months postnatal. Finally, infant development was assessed at 12-months postnatal. Outcome measures were assessed blind to caseness. The study was approved by King's College Hospital Research Ethics Committee, and all participants provided written informed consent.

**2.2. Sample:** The sample comprised 84 women recruited in the late second trimester of pregnancy (gestational age, m=27.4±2.2 weeks): 25 history-only (referred to Maudsley Perinatal Psychiatry Services) and 59 healthy controls (attending routine antenatal ultrasound scan) all at King's College Hospital. Of the 25 history-only, 12 (48%) had a past history of recurrent MDD, 11 (44%) had a past history of a single episode of depression and 2 (8%) had a past history of depression not otherwise specified (NOS). None were taking antidepressant medication at the baseline assessment (when inflammatory biomarkers were assessed) but one (4%) took antidepressant medication during pregnancy but prior to baseline. Inclusion criteria were: women over 18 years of age with a singleton pregnancy;

history-only women with a DSM-IV diagnosis of MDD prior to pregnancy; and controls without any current or past DSM-IV axis I diagnosis. Exclusion criteria were: uterine anomaly, known obstetric complications in the index pregnancy, severe or relevant chronic medical conditions, such as cardiovascular disease, metabolic or endocrine disorder, for example gestational diabetes and hypertension. History-only women were excluded if presenting with any current DSM-IV diagnosis, if having a past history of psychosis or bipolar affective disorder, or if taking antidepressant medication at baseline.

As expected in a longitudinal study of an inner city group of people, subject retention reduced over time, and at 1 year postnatal only 73 mother-infant dyads (51 controls and 22 history-only) were assessed. However, there was no statistically significant difference at any time point between the proportion of cases and controls remaining in the study. Unsurprisingly, those who stayed in the study were less deprived (according to IMD score), were more likely to be of professional or managerial employment categories and married or cohobiting; otherwise there were no statistically significant differences in socio-demographic information at baseline nor in antental psychopathology as rated by BDI and STAI.

**2.3. Clinical assessment:** All subjects were assessed for current and past DSM-IV axis I disorders at baseline using the Structured Clinical Interview for DSM-IV (SCID I – CV) (First, 1996). We additionally used (at baseline and 32 weeks gestation) the Beck Depression Inventory (BDI, version IA; (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961a) and the State-Trait Anxiety Inventory (STAI; (Spielberger, 1983), two self-rated instruments measuring

intensity or frequency of, respectively, depressive and anxiety symptoms. The BDI and STAI were also administered at 6-days, 2- and 12-months postnatal.

The most relevant socio-demographic and medical factors are presented in Table 1. There were some statistically significant group differences in baseline socio-demographic and clinical characteristics in pregnancy, with the history-only group having lower levels of education and employment than controls. As in our previous paper, in order to condensed the information from these socio-demographic variables, the Index of Multiple Deprivation (IMD) score was examined (Noble M, 2004), a UK government measure of relative deprivation for small areas that covers seven aspects of deprivation; there were no statistically significant group differences in IMD score, ethnicity, smoking in pregnancy or pre-pregnancy BMI. Compared with the controls, the history-only group had higher STAIS, and BDI scores at baseline and at 32 weeks gestation, although only baseline STAIS reached statistical significance. In any case, average scores of both scales in the history-only group were below the threshold for clinically-significant symptoms, that is, BDI>14 (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961b) and STAI>40 (Bunevicius et al., 2013) (Table 1). The groups did not differ in obstetric history or obstetric risk factors at baseline, and there were no significant group differences in medical conditions, use of medication and other health indicators or health behaviors (data not shown).

**2.4. Inflammatory markers:** Blood was obtained between 12pm and 3pm at a visit early in the third trimester (median = 27.1 weeks, range 24.1 to 34.6 weeks); there was no statistically significant difference in gestational age at sample acquisition between history-only and controls (z=1.0, p=0.30). All samples were transported to the laboratory in a cooled

box and processed within 2 hours of venipuncture. Aliquots of serum were immediately frozen at -80°C pending analysis. Serum high sensitivity C-reactive protein (hsCRP) was measured using an ELISA kit supplied by PZ Cormay, Poland; the assay was analyzed in batches on the Cobas Mira (intra- and inter-assay CV were 2.96% and 3.85% respectively). Serum IL-1α, IL-1β, IL-2, IL-4, Il-6, IL-8, IL-10, TNFα, vascular endothelial growth factor (VEGF), EGF, MCP-1, and INF-γ were measured using a cytokine chip array kit supplied by Randox Laboratories, UK; the kit employs a sandwich chemiluminescent immunoassay, described in our previous work (Di Nicola et al., 2013). For IL-1α, IL-4 and INF-γ, >50% of the sample was at the lowest detectable level of the assay, so these measures were not included in the subsequent analyses.

**2.5. Salivary cortisol:** Maternal saliva samples were obtained in the third trimester (median = 32.5 weeks, range 31.4 to 37.1 weeks); there was no statistically significant difference in gestational age at sample acquisition between cases and controls (z=0.74, p=0.46). All subjects collected two samples, using Salivettes containing a polymer swab (Sarstedt, UK), at awakening and 8pm. Cortisol awakening response (CAR) was assessed in a subset (n=44) that also collected samples at +15min, +30min, and +60min after awakening. Subjects were given a practical demonstration, verbal and written instructions, a recording log and a mechanical timer for sample collection; emphasis was placed on the accuracy of timings and procedure, and subjects were instructed not to eat, drink or smoke in the first hour after awakening or in the thirty minutes before sample collection at 8pm. There were no differences between history-only and controls, in awakening time (7:31hrs ±0:54), the time of awakening sample collection (7:32hrs ±0:54), the interval between awakening and

sample acquisition (0.27mins ±1.09) and the time of evening sample collection (20:12hrs ±0:41).

Infants' saliva samples were collected by a researcher before and 20 minutes after the routine immunizations at 2 and 12 months (median age = 2.07 months, range 1.74 to 3.94, and = 12.48 months, range 11.99 to 17.31, respectively); there was no statistically significant difference in age for infants of cases or controls at both time points (z= 1.0, p=0.31, and z= 0.5, p=0.64, respectively). Infants' saliva samples were also obtained on the following day, by the mother, at morning awakening and at 8pm. Care was taken to avoid feeding for 15 minutes before a sample was taken. A Salivette and Salimetrics childrens swab (SCS) were used to collect saliva by the researcher on the immunization day; while, for ease of use, a Sorbette arrow was used for infant saliva collection the following day by the mother. As for mothers, a sample log was used to record timings and relevant information. There were no differences between infants of cases and controls, in awakening time (7:30hrs ±1:17 at 2 months and 7:18 ±1:08 at 12 months), the interval between awakening and sample acquisition (14.14 mins ±21.79 at 2 months and 15.22 ±21.75 at 12 months) and the time of evening sample collection (19:41hrs ±0:40 at 2 months and 20.15 ±0:47 at 12 months).

Salivary samples were frozen at -20°C pending analysis. Saliva cortisol was measured using a high sensitivity salivary cortisol enzyme immunoassay kit supplied by Salimetrics Europe Ltd, UK. Samples were assayed in duplicate where an adequate volume of saliva allowed. The inter-and intra-assay CV ranged from 8-11% and 6-10% respectively. The formula for the area of a trapezoid (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) was used to calculate (i) CAR area under the curve (AUCi) using the four samples acquired within the first

hour of waking, and (ii) diurnal cortisol secretion using awakening and evening cortisol values.

**2.6. Neonatal neurobehavioral function and infant development:** The Neonatal Behavioral Assessment Scale (NBAS) (Brazelton, 1995) was used to measure neurobehavioral function in term-born babies at a median age of 7.0 days (range from 4 to 27 days); there was no statistically significant difference in age at NBAS between infants of cases or controls (z=0.35, p=0.73). Twenty-eight behavioral items were rated and pooled into five clusters (autonomic stability, motor, orientation, range of state and regulation of state). The Bayley Scales of Infant and Toddler Development (Bayley-III, BSID) were used to evaluate cognitive, language and motor development using a series of developmental play tasks (Bayley, 2005). BSID was used at a median age of 13.17 months (range 12.07 to 15.90); there was no statistically significant difference in age at BSID between infants of cases or controls (z= 0.4, p=0.65).

**2.7. Data analysis:** The statistical analyses were performed in SPSS Statistics Version 26 (IBM Ltd, UK). The analysis plan comprised cross-sectional group comparisons of maternal antenatal biomarkers, birth outcomes, neonatal neurobehavior, infant HPA axis, and development in 1-year-olds, as well as the associations between infant factors and maternal biomarkers. For all statistical tests, the data were first examined to ensure that the assumptions of the General Linear Model (GLM) were met. In order to reduce bias, data were winsorized or log-transformed prior to analyses or the bootstrap method (with 1000 samples) was employed; raw data are presented in the figures and tables. Pearson's chi-square ($\chi^2$) test of the independence of variables was used for the analysis of categorical

data. Pearson's correlation ($r_p$) was used for the analysis of association between parametric continuous variables, and Spearman's correlation ($r_s$) was applied to non-parametric continuous variables. In univariate analyses, group comparisons of continuous data were made using the independent samples t-test. For non-parametric data, the Mann-Whitney test was used and the z score reported. Univariate analyses that showed significant differences between history-only and controls were repeated after adjustment for IMD, smoking in pregnancy, prepregnancy BMI and measures of maternal antenatal psychopathology if appropriate. Cohen's δ was calculated to estimate the effect size for group differences, or effect size was expressed by partial eta squared ($\eta_p^2$) where ANOVA and ANCOVA were applied. Family-wise adjustment for multiple comparisons was used to identify the strongest findings. Mean and standard error of the mean are presented in graphs.

## 3. RESULTS

### 3.1 Women with a history of MDD have increased markers of immune system function in the early 3rd trimester

Markers of immune system function were compared between the two groups of pregnant women at a single time point in the early 3rd trimester (mean weeks ± SD, 27.4±2.2). Compared with the control group, women with a history of MDD had statistically significant higher IL-8 (d=0.63), VEGF (d=0.40) and MCP-1 (d=0.61), with a trend-level statistical significance for TNFα (Table 2). It is important to highlight that only MCP-1 remained statistically significant after adjustment for multiple comparisons, and thus should be

considered the most robust finding among the inflammatory biomarkers. Neither IMD, smoking in pregnancy, pre-pregnancy BMI, nor baseline BDI or STAIS scores were correlated with these markers of immune system function, so were not included in covariate models. There were no statistically significant group differences in IL-1β, IL-2, IL-6, IL-10, EGF or hsCRP (Table 2).

**3.2 Women with a history of MDD have normal cortisol secretion in the 3rd trimester**

Basal HPA axis and CAR were compared between the control and history-only groups of pregnant women at a single time point in the early 3rd trimester (mean weeks ± SD, 32.7±1.1); there were no statistically significant group differences (Table 2). Neither IMD, smoking in pregnancy, pre-pregnancy BMI, nor baseline BDI or STAIS scores were correlated with these markers of immune system function, so were not included in covariate models.

**3.3 Women with a history of MDD do not have babies with lower gestational age at birth**

Gestational age at birth for infants of women with spontaneous onset of labour was compared between controls, there was no significant group difference (see Table 2).

**3.4 Neonates born to women with a history of MDD have poorer neurobehavioural competencies at 6 days postnatal**

The neurobehavioral assessment (NBAS) of full-term infants conducted at an average of 6 days postnatal showed that, compared with infants born to women of the control group, those born to history-only women demonstrated poorer performance on the social-orientation cluster (d=1.26). Moreover, although not reaching statistical significance, the infants of history-only women also had poorer neurobehavioural competences in most

other clusters (autonomic stability, regulation of state and motor, range of d=0.36-0.43) (Table 2). It is important to highlight that no clusters remained statistically significant after adjustment for multiple comparisons, and therefore these findings need to be considered as suggestive and in need of replication. Neither IMD, smoking in pregnancy nor gestational age at birth were correlated with NBAS social-orientation, so were not included in covariate models. However, baseline BDI and STAIS scores were correlated with NBAS social-orientation cluster (r=-.32, p=0.005, r=-.25, p=0.028, respectively), though, when included as covariates, neither measure of maternal antenatal psychopathology accounted for the group difference in NBAS score. Contemporraneous postnatal BDI and STAI scores did not correlate with the social-orientation cluster (r=-.03, p=0.82 and r=-.21, p=0.13, respectively).

### 3.5. Infants born to women with a history of MDD have normal cortisol function at 2 and 12 months of age

*3.5.1. Cortisol reactivity to stress was assessed in the two-and twelve-month-old infants by measuring cortisol before and 20 minutes after immunizations.*

For 2-month old infants, mixed design ANOVA showed a statistically significant effect of time on cortisol ($F_{(2, 64)}$=27.4, p<0.001, $\eta_p^2$=0.30); that is, cortisol increased following immunization in infants of both history-only and healthy control women. However, there was no interaction between caseness and time ($F_{(2, 64)}$=0.0, p=0.99, $\eta_p^2$=0.00) and no between-subjects effects ($F_{(2, 64)}$=0.7, p=0.42, $\eta_p^2$=0.01). Thus, the magnitude of the cortisol response did not differ between infants born to control or to history-only women (Table 3 and Figure 1, Panel A).

In contrast, for the 12-month old infants there was no statistically significant main effect of time on cortisol ($F_{(2, 59)}$=3.0, p=0.09, $\eta_p^2$=0.05); that is, cortisol did not increase following immunisation in either group of infants; moreover, as at 2 months of age, there was no interaction between caseness and time ($F_{(2, 59)}$=3.7, p=0.06, $\eta_p^2$=0.06) and no between-subjects effects ($F_{(2, 59)}$=0.5, p=0.5, $\eta_p^2$=0.01) (Table 3 and Figure 1, Panel B).

*3.5.2. Basal HPA axis activity in the two- and twelve-month-old infants was assessed the day after their immunization by measuring awakening and evening cortisol.*

Surprisingly, at 2 months, morning (but not evening) cortisol was lower in the infants of history-only women than of control women ($t_{(61)}$=2.1, p=0.040, d= 0.54 and $t_{(61)}$=1.7, p=0.10, d= 0.43 respectively). It is important to highlight that no measures remained statistically significant after adjustment for multiple comparisons. Neither IMD, smoking in pregnancy nor length of gestation were correlated with infant morning cortisol, so were not included in covariate models.

At 12 months, there were no statistically significant group differences in morning or evening cortisol ($t_{(56)}$=-0.6, p=0.52, d=0.16 and $t_{(56)}$=-0.5, p=0.59, d=0.13 respectively).

**3.6 Infants of women with a history of MDD have normal development at 12 months postnatal**

Infant development, measured by BSID, was compared between infants of healthy and history-only women; there were no statistically significant differences in cognitive (mean ±SD, 114.0 ±14.0 vs. 107.3 ±17.2, $t_{(70)}$=1.2, p=0.22, d=0.29 respectively), language (100.6

±12.4.0 vs. 99.2 ±15.8, $t_{(69)}$=0.4, p=0.69, d=0.10 respectively) or motor (101.4 ±10.3 vs. 103.4 ±17.0, $t_{(27.9)}$=-0.5, p=0.62, d=0.19 respectively) development.

### 3.7 Associations between maternal antenatal stress-related biology and infant measures

Correlations were used to examine the associations between the pregnancy and the infant measures that differed between history-only and controls, i.e., markers of immune system function and NBAS social-orientation cluster (see Table 4). There were no statistically significant correlations between NBAS social-orientation and maternal antenatal IL-8, TNFα or MCP-1, although the direction of the associations was always negative (i.e., higher inflammation associated with poorer neurobehavioral competency, range of r=-.01 to r=-.13, and that with VEGF was at a trend level of significance, r=-.20, p=0.098).

## 4. DISCUSSION

We use a prospective longitudinal design and demonstrate that, compared with healthy pregnant women, those with a history of MDD who remain free of MDD in pregnancy (history-only) exhibit increased markers of immune system function in pregnancy, and neonates with poorer neurobehavioural competences – albeit the latter findings did not survive multiple corrections. These are two features that we had described before in association with the presence of active depression in pregnancy (Osborne et al., 2018). Moreover, and, again, similarly to women with active depression in pregnancy (Osborne et al., 2018), we find some correlational evidence indicating an association between higher maternal markers of immune system function and poorer neurobehavioural competences, although these findings are at best suggestive. In contrast to results in women with active

depression in pregnancy, those with history-only do not appear to show abnormal HPA axis activity during pregnancy or shorter length of gestation, nor do their 12-month old offspring appear to show increased cortisol reactivity to stress, or basal cortisol activity.

Compared with healthy women, those with a history of depression have higher levels of 3rd trimester markers of immune system function (IL-8, VEGF and MCP-1, with trend-level significance for TNFα) but not HPA axis overactivity. This finding differs in part from our previous findings in women with depression pregnancy, mostly because the latter additionally have overactivity in the HPA axis in the evening and a blunted CAR (Osborne et al., 2018). The lack of HPA axis abnormalities in history-only women in this study could be explained by methodological factors such as sensitivity of the cortisol assay or by reduced statistical power; however, it is interesting that previous studies have shown that a blunted awakening response characterises the most severe, anhedonic forms of depression (Dedovic & Ngiam, 2015), and that both the increased awakening and diurnal cortisol levels, and the blunted cortisol awakening response, tend to normalise with successful improvement of depression (Ruhé et al., 2015), thus suggesting that HPA axis biological abnormalities are driven predominantly by 'state' depression.

In contrast, the pattern of elevation in markers of immune system function is similar across the two studies, with differences (if any) possibly related to the power of the two studies. Specifically, VEGF and TNFα are statistically elevated, and IL-8 and MCP-1 are numerically elevated, in the women with antenatal depression, while IL-8, VEGF and MCP-1 are statistically elevated, and TNFα numerically elevated, in the history-only women. Taken together with our data in depressed pregnant women, our findings are consistent with the

increased inflammation (hsCRP and high glycoprotein acetyls) found, in a recent larger study, in both depressed pregnant women and pregnant women with a history of depression (Lahti-Pulkkinen et al., 2019). However, two points from the above study by Lahti-Pulkkinen et al. are relevant to the interpretation of our findings: first, around one fifth of women with a history of depression also tended to have clinically-significant depressive symptoms, while we specifically excluded women with current depression from the history-only group; and, second, the increased inflammation was mediated by a higher early BMI in both groups of women with depression or history of depression, while in both our reports the increased inflammation is independent from BMI (Osborne et al., 2018, and the present study). Preclinical studies further confirm the importance of these findings, bringing evidence that the peripheral immune activation induced during pregnancy by models of antenatal stress are associated with changes in inflammatory signals in the brain of both mothers and offspring (Cattaneo et al., 2018; Sherer et al, 2018). Taken together, our study indicates that increased markers of immune system function in pregnant women with a history of depression is not just related to current depressive symptoms or increased BMI, and thus may represent the effects of epigenetics or genetic predisposition, as is also proposed for the long-term increased inflammation described in adult offspring of women with antenatal depression (Plant et al., 2016) and in general for inflammation in depression outside pregnancy (Barnes, Mondelli, & Pariante, 2017). It is also interesting to notice previous studies in depressed patients that the activation of the immune system may represent more of a 'trait marker for this condition, that persists after successful improvement of symptoms (Cattaneo et al., 2013) and is present before and after the onset of depression (Pitharouli et al., 2021).

Interestingly in this study, and contrary to women who were depressed in pregnancy, we found no evidence of maternal HPA axis hyperactivity or increased infant cortisol stress reactivity (if anything, infant morning cortisol is lower in infants of history-only than control women). Again, together with our data in depressed pregnant women, this evidence indicates that not all stress biomarkers in pregnancy 'are equal in their causes and consequences': maternal increased inflammation can be driven by both current depression and a previous history, and does not affect infant cortisol, while maternal HPA axis hyperactivity appears only to be driven by current depression, and may be required in order to increase infant cortisol reactivity – which is thus more likely to be the effect of 'programming in utero' (Figure 1, Panels A - D). This notion is consistent with multiple clinical and preclinical studies that have found an association between maternal antenatal cortisol levels and infants cortisol levels (Almanza-Sepulveda et al., 2020).

This however does not mean that a history of depression alone does not affect the infant at all. In fact, compared with neonates of healthy women, those born to the history-only group show poorer neurobehavioural competences at 6 days, specifically indexed by the social-orientation cluster of the NBAS examination, and with a general pattern of numerically suboptimal scores in most other clusters. Interestingly, we have also found sub-optimal mother-infant interactions in in history-only dyads at 2-months postnatal (Bind et al., 2021). Indeed, infants of both the history-only women and women with depression in pregnancy (as described in our previous paper (Osborne et al., 2018)) have very similar scores on all NBAS clusters, suggesting that neurobehavioural competences may be driven by epigenetic or genetic predisposition. The presence of suboptimal development in infants of women with a history of depression is consistent with the aforementioned study by Hannigan et al.

(Hannigan et al., 2018) indicating that the transmission of maternal depression into offspring behavioural alterations may be genetically driven. However, the elevated pregnancy proinflammatory cytokines in both the depressed (Osborne et al., 2018) and history-only women (this study), together with the consistent pattern of negative correlations between the cytokines and infant NBAS social-orientation cluster (with virtually identical correlational coefficients for VEGF in the two groups, $r=-.22$, $p\leq0.05$ and $r=-.20$, $p=0.098$, respectively) suggest that a programming effect may also be present. Indeed, a number of genes relevant to the intergenerational transmission of psychopathology have been shown to be epigenetically influenced by antenatal stress, both in clinical samples and animal models (Sawyer et al., 2019).

Interestingly, in both this study and our previous report in women depressed in pregnancy we do not find any group difference in the development of one-year-old infants. This replicates a similar study in one-year-old infants (O'Leary et al., 2019) and suggests that the early suboptimal development or behaviour captured in the NBAS does not translate into cognitive, language or motor deficits, at least not visible at 12 months of age. Of course, it is still possible that this early suboptimal development translates into later emotional and behavioural difficulties.

Our series of studies in antenatally-depressed and history-only women try to address at least some of the limitations described by Osborne & Monk (2013) in their review on the literature of the inflammatory morbidity in perinatal depression, including the use of a clearly defined antenatal sample, the use of more structured measures to define depression, a relatively narrow time-window for the blood sample collection in terms of

both gestational period and time during the day, and the concomitant assessment of hormonal status. However, we do need to emphasise the small number of women in the history-only group (and the overall sample of depressed and healthy controls), which obviously limits the impact of our findings. Furthermore, the sample of women with a history of depression is a diagnostically heterogenous sample as it includes women with recurrent MDD as well as single depressive episodes and depression not otherwise specified; as these conditions differ in various diagnostic aspects they may have differential effects on psychosocial and biological factors. Thirdly, BDI and STAI were used to assess symptoms of depression and anxiety in pregnancy, however, neither of these scales are specially designed, or validated, for use in pregnancy, unfortunately, at the time of this study, there were no such scales available; none-the-less, no women met diagnostic criteria for MDD during pregnancy. Finally, we did not collect information on antidepressant treatment before pregnancy, and on current or lifetime psychotherapeutic treatment, thus missing information that may have an effect on depressive symptoms as well as stress-related biology. Nevertheless, the consistency with the previous study in women with antenatal depression, together with the aforementioned evidence in the same sample of impaired mother-infant interaction in both women with antenatal depression and in history-only women (Bind et al., 2021), strongly suggest that our main conclusion – that a history of depression, even in the absence of current depression, increases the risk of inflammation in pregnancy and of suboptimal development in the infants – has both clinical and biological relevance.

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**Table 1: Maternal socio-demographic and clinical characteristics**

| | **Controls (n=56-59)** | **History-only (n=23-25)** | **Statistical test and significance** |
|---|---|---|---|
| **Maternal age, mean ±sd** | 32.2 ±4.3 | 33.2 ±6.3 | $t_{(33.8)}$=-0.7, p=0.51 |
| **Ethnicity, white, n (%)** | 44 (75) | 22 (88) | $X^2$=1.9, p=0.25 |
| **Education, A levels and above, n (%)** | 55 (93) | 18 (72) | **$X^2$=6.9, p=0.014** |
| **Employment outside the home, n (%)** | 48 (81) | 19 (76) | $X^2$=0.3, p=0.57 |
| **Employment, professional or managerial, n (%)** | 43 (73) | 11 (44) | **$X^2$=6.4, p=0.014** |
| **Marital status, married or cohabiting, n (%)** | 51 (86) | 20 (80) | $X^2$=0.6, p=0.52 |
| **IMD score, mean ±sd** | 28.4 (7.9) | 28.0 (7.9) | $t_{(81)}$=-0.2, p=0.84 |
| **Pre-pregnancy BMI, mean ± sd** | 23.1 (3.7) | 24.6 (7.9) | z=0.2, p=0.81 |
| **Cigarette use in index pregnancy, n (%)** | 2 (5) | 2 (8) | $X^2$=0.2, p=0.64 |
| **BDI score at baseline, mean ±sd** | 3.8 ±2.6 (n=57) | 5.1±5.1 (n=23) | z=1.4, p=0.16 |
| **STAI score at baseline, mean ±sd** | 27.2 ±6.8 (n=56) | 34.3 ±12.5 (n=23) | **z=2.3, p=0.02** |
| **BDI score at 32/40, mean ±sd** | 3.4 ±2.6 (n=31) | 5.1±3.7 (n=21) | z=1.8, p=0.07 |
| **STAI score at 32/40, mean ±sd** | 28.8 ±8.2 (n=30) | 34.4±10.1 (n=21) | z=1.8, p=0.07 |

Note BDI: Beck Depression Inventory score, STAIS: State Trait Anxiety Inventory score

**Table 2: Maternal antenatal markers of immune system function and HPA axis, and neonates' gestational age at birth and NBAS clusters**

| | **Controls mean ±SD (n=52-57)** | **History-only mean ±SD (n=20-23)** | **Statistical test and significance** | **Cases mean ±SD (n=40-41)** |
|---|---|---|---|---|
| **INFLAMMATION** | | | | |
| IL-1 β (ng/l) | 1.3 ±1.2 | 1.4 ±1.1 | $t_{(71)}$=-0.8, p=0.40 | 2.0±4.9 |
| IL-2 (ng/l) | 2.0 ±2.1 | 1.6 ±1.0 | $t_{(71)}$=0.6, p=0.46 | 1.9±2.2 |
| IL-6 (ng/l) | 0.8 ±0.5 | 0.9 ±0.4 | $t_{(70)}$=-0.7, p=0.47 | **1.6±3.1*** |
| IL-8 (ng/l) | 2.0 ±1.3 | 3.3 ±2.6 | **$t_{(71)}$=-2.2, p=0.030** | 5.0±11.3 |
| IL-10 (ng/l) | 0.7 ±0.6 | 0.7 ±0.4 | $t_{(71)}$=-1.5, p=0.14 | **1.6±5.2*** |
| TNFα (ng/l) | 1.1 ±0.9 | 1.3 ±0.9 | $t_{(71)}$=-1.7, p=0.09 | **1.6±1.0**** |
| VEGF (ng/l) | 3.2 ±3.4 | 6.1 ±9.8 | **$t_{(71)}$=-2.7 p=0.008** | **6.5±12.6**** |
| EGF (ng/l) | 16.1 ±23.8 | 23.0 ±27.1 | $t_{(71)}$=-1.3 p=0.21 | 21.1±26.8 |
| MCP-1 (ng/l) | 51.6 ±38.2 | 85.5 ±68.0 | **$t_{(71)}$=-3.5, p=0.002** | 67.7±60.3 |
| hsCRP (mg/l) | 5.31 ±6.8 | 5.10 ±6.4 | $t_{(72)}$=-0.1 p=0.99 | 5.14±6.3 |
| **HPA AXIS** | | | | (n=31) |
| Diurnal cortisol, (AUCg, nmol/m | 81.9 ±38.5 | 91.7 ±59.0 | z=0.54, p=0.59 | **123.2±104.3 **** |
| Awakening cortisol (nmol/ml) | 9.8 ±4.5 | 9.8 ±4.3 | z=0.41, p=0.68 | **11.9±5.9*** |
| Evening cortisol (nmol/ml) | 3.5 ±2.9 | 4.8 ±7.8 | z=0.59, p=0.56 | **8.1±14.6**** |
| CAR, (AUCi, nmol/ml/min) | 127.6 ±228.7, n=26 | 102.8 ±174.0, n=18 | $t_{(42)=}$0.75, p=0.46 | **22.3±156.6* n=23** |
| **Gestational age at birth** | **(n=38)** | **(n=13)** | | |
| **Mean weeks ±SD** | **40.5 ±1.1** | **40.2 ±1.0** | **$t_{(49)}$=0.80, p=0.39** | **39.2±2.6*** |
| **NBAS** | | | | |
| Autonomic stability | 6.2 ±1.1 | 5.6 ±1.5 | $t_{(78)}$=1.6, p=0.17 | **5.3±1.0***** |
| Range of state | 3.2 ±0.8 | 3.**3** ±0.7 | $t_{(78)}$=-0.**7**, p=0.**33** | **3.3±0.8** |
| Regulation of state | 6.4 ±1.3 | 5.7 ±1.4 | $t_{(78)}$=1.9, p=0.062 | **5.5±1.6**** |
| Social-orientation | 7.5 ±1.2 | 6.4 ±1.8 | **$t_{(25.8)=}$3.2, p=0.015** | **6.5±1.4**** |
| Motor | 5.6 ±0.7 | 5.3 ±0.6 | $t_{(78)}$=1.6, p=0.10 | **5.3±0.8*** |

**Note: Cases were reported in Osborne S et al., 2018. Data have been included to aid the discussion of points of interest. Difference between cases and controls *<0.05, **<0.01.**

**Table 3: Infant cortisol response to pain stress and basal cortisol at 2 and 12 months**

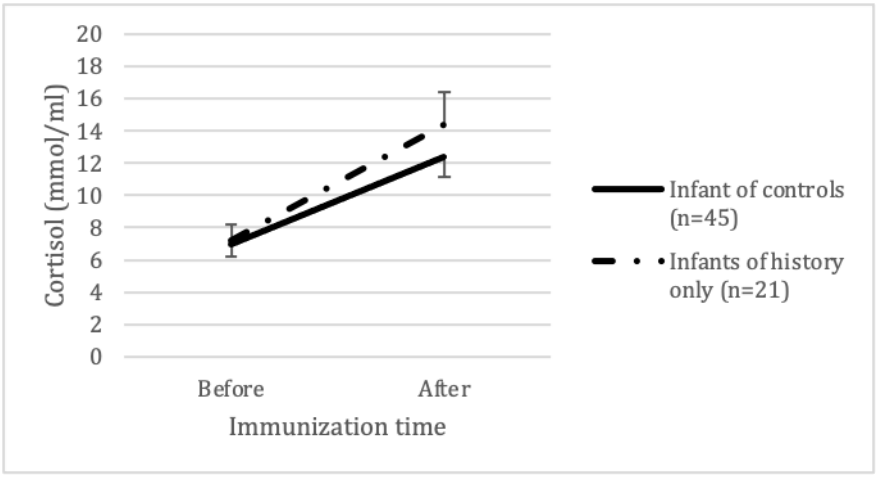
| | Infants of controls mean ±SD | Infants of history-only mean ±SD | Statistical test and significance | Infants of depressed cases mean ±SD |
|---|---|---|---|---|
| Cortisol response to stress at 2 months (nmol/ml) | n=45 | n=21 | | n=38 |
| Pre-immunisation | 6.98±5.30 | 7.22±4.45 | Mixed design ANOVA – see text | 10.98±14.78 |
| Post-immunisation | 12.36±8.28 | 14.36±9.34 | | 16.28±17.29 |
| Basal cortisol at 2 months (nmol/ml) | n=47 | n=16 | | n=34 |
| Morning | 9.84±8.66 | 7.34±13.00 | $t_{(61)}$=2.1, p=0.040* | 12.57±15.37 |
| Evening | 7.46±12.99 | 3.89±5.03 | $t_{(61)}$=1.7, p=0.10 | 6.83±14.62 |
| Cortisol response to stress at 12 months (nmol/ml) | n=45 | n=16 | | n=19 |
| Pre-immunisation | 5.94±11.98 | 6.43±11.82 | Mixed design ANOVA – see text | 11.37±20.24 |
| Post-immunisation | 5.91±11.91 | 6.14±5.30 | | **14.43±23.57*** ** |
| Basal cortisol at 12 months (nmol/ml) | n=39 | n=19 | | n=25 |
| Morning | 10.37±12.89 | 12.75±17.98 | $t_{(56)}$=-0.6, p=0.52 | 14.07±21.38 |
| Evening | 4.29±11.45 | 7.28±18.63 | $t_{(56)}$=-0.5, p=0.59 | **13.21±26.26*** |

Note: Cases were reported in Osborne S et al., 2018. Data have been included to aid the discussion of points of interest. Difference between cases and controls *<0.05, **<0.01.

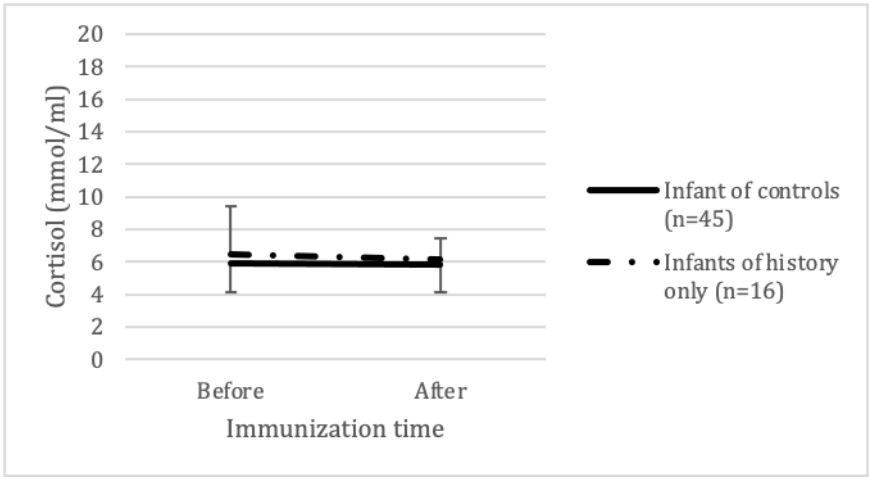
**Table 4: Correlations table (Spearman's r): maternal antenatal markers of immune system function and NBAS**

| | Autonomic stability | Range of state | Regulation of state | Social-orientation | Motor |
|---|---|---|---|---|---|
| IL-1 β | .02 | -.15 | -.04 | .06 | .13 |
| IL-2 | -.00 | -.12 | -.08 | .16 | .05 |
| IL-6 | .06 | -.04 | .02 | .06 | .07 |
| IL-8 | .03 | -.07 | -.20 | -.03 | -.04 |
| IL-10 | .00 | -.12 | -.16 | .03 | .14 |
| TNFα | -.16 | -.04 | -.04 | -.01 | -.01 |
| VEGF | -.08 | .01 | -.22* | -.20* | -.06 |
| EGF | .16 | .05 | -.06 | -.07 | .03 |
| MCP-1 | .02 | .16 | -.07 | -.13 | .06 |
| hsCRP | .00 | -.12 | -.11 | -.02 | -.03 |

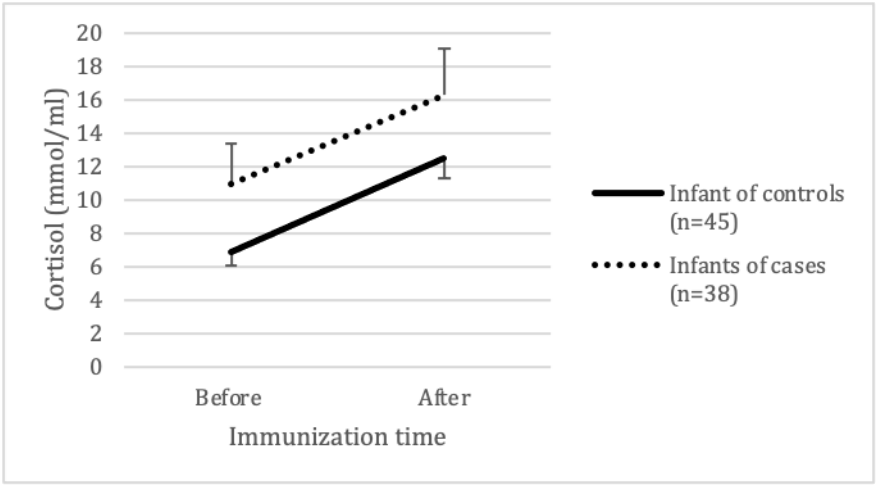
**Note. *trend level of significance**



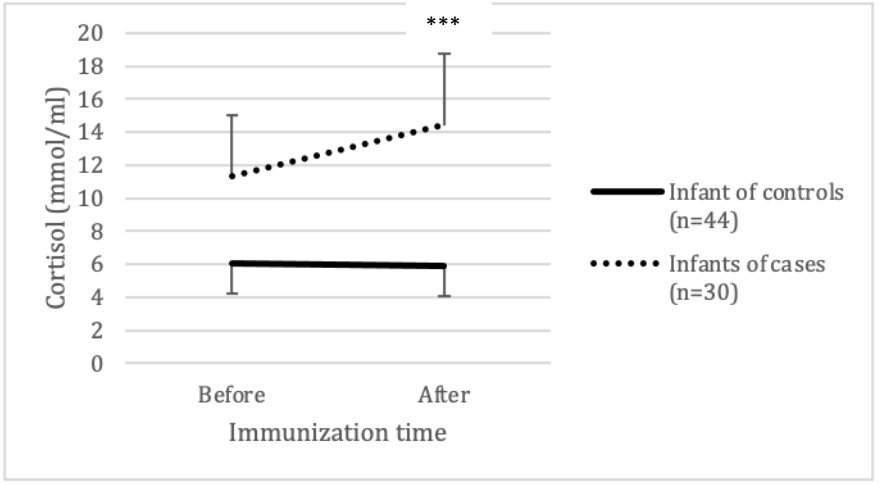

Panel A: Infants of history-only women at 2 months.
Note: within-subjects effect of time, $p < .001$




Panel B: Infants of history-only women at 12 months

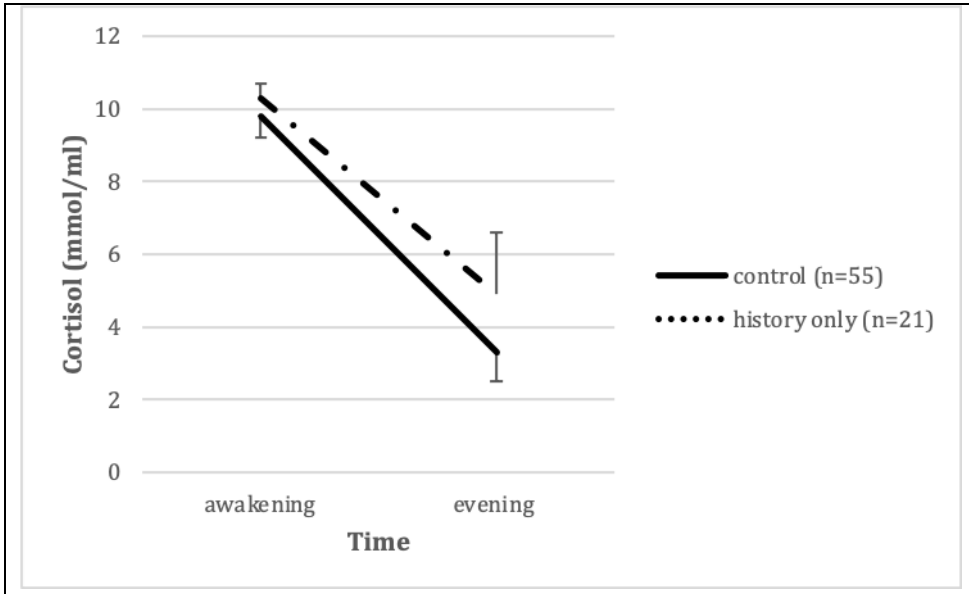



Panel C: Infants exposed to depression in pregnancy at 2 months.
Note: within-subjects effect of time, $p < .001$

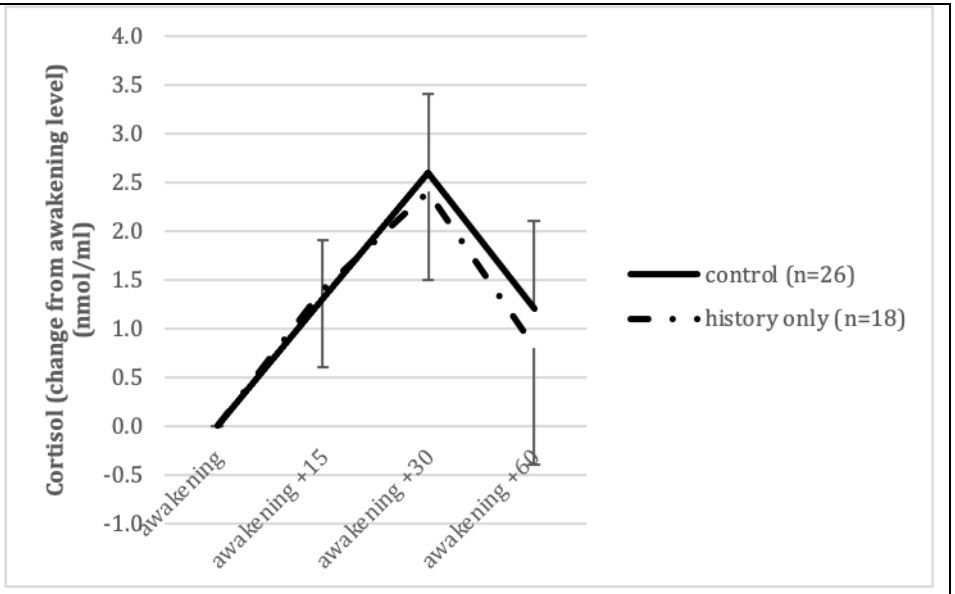



Panel D: Infants exposed to depression in pregnancy at 12 months.
Note: ***$p < .001$

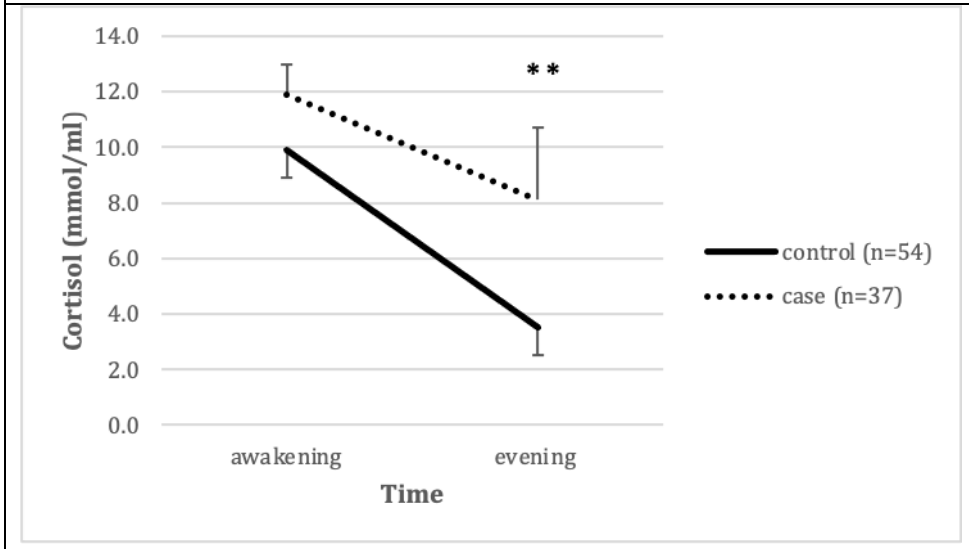
**Figure 1: Infant cortisol response to pain stress at 2 and 12 months (Panels A and B). For comparison, the corresponding figures are shown (Panels C and D) for infants exposed to MDD in pregnancy (from Osborne et al., 2018).**



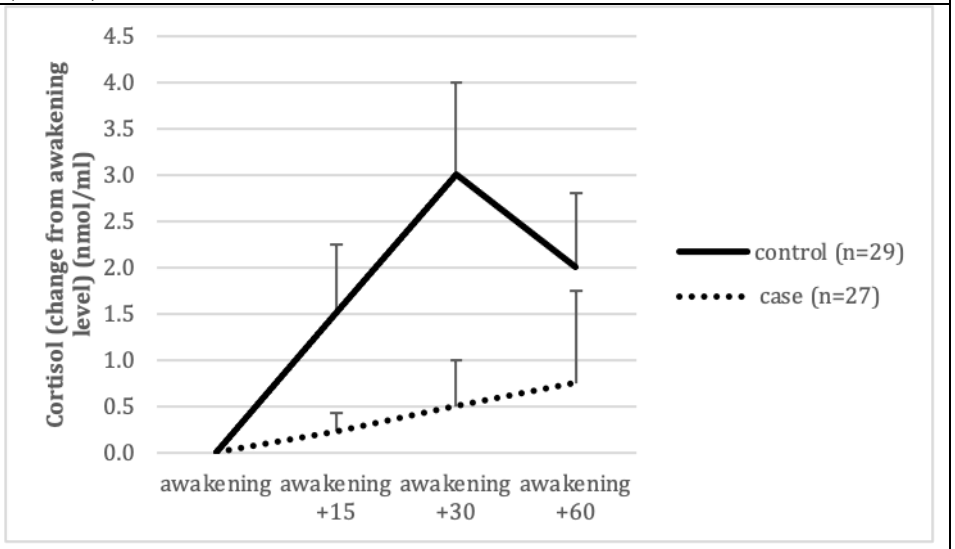

Panel A: History-only: maternal antenatal diurnal cortisol




Panel B: History-only: maternal antenatal Cortisol Awakening Response (AUCi)




Panel C: MDD: maternal antenatal diurnal cortisol ( **p=0.004)




Panel D: MDD: maternal antenatal Cortisol Awakening Response (AUCi, p=0.020)

**Figure 2: Maternal hypothalamic-pituitary axis for history-only women at 32 weeks gestation (Panels A and B). For comparison, the corresponding figures are shown (Panels C and D) for women with MDD in pregnancy (from Osborne et al., 2018).**