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Citation for final published version:

Nazzari, S., Fearon, P., Rice, F., Ciceri, F., Molteni, M. and Frigerio, A. 2020. The biological underpinnings of perinatal depressive symptoms: a multi-systems approach. *Journal of Affective Disorders* 274 , pp. 1004-1012. 10.1016/j.jad.2020.05.023 file

Publishers page: <http://dx.doi.org/10.1016/j.jad.2020.05.023>  
< <http://dx.doi.org/10.1016/j.jad.2020.05.023> >

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The biological underpinnings of perinatal depressive symptoms: a multi-systems approach.

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## Abstract

**Background.** Well-established evidence exists of an association between depressive symptoms and alterations in the stress and inflammatory response systems; however, the picture is far less coherent during the perinatal period. This study combines the assessment of multiple stress and inflammatory biomarkers in late pregnancy and after delivery in order to investigate cross-sectional and prospective associations with perinatal depressive symptoms. **Methods.** One-hundred-ten healthy women were assessed in late pregnancy (mean gestational age=34.76; SD=1.12) and 89 were re-evaluated after delivery (mean hours after delivery=52.36; SD=19.70) for depressive and anxiety symptoms through the Edinburgh Postnatal Depression Scale and the State-Trait Anxiety Inventory. Serum Interleukin-6 (IL-6), C-Reactive Protein (CRP) and diurnal salivary cortisol levels were measured on both occasions, while diurnal salivary alpha amylase (sAA) levels were assessed in late pregnancy. **Results.** Using Hierarchical Linear Models, higher depressive symptoms were found to be associated with higher IL-6 levels, lower morning cortisol levels and a flatter cortisol diurnal slope during pregnancy, while adjusting for potential confounders. No significant associations were found after delivery or with change in biomarker levels from pre- to post-partum. Furthermore, preliminary evidence of a positive association between inflammation and stress markers in women with higher antenatal depressive symptoms was found. **Limitations:** The sample was relatively small and highly selected, thus limiting generalizability of the findings. **Conclusions.** Results emphasize the need for an integrated multi-systems approach to the understanding of the biological underpinnings of perinatal depression and suggest that the stress-immune interactions represent a promising avenue for future endeavor.

## Main text

### 1. Introduction

Perinatal depressive symptoms affect up to 12% of women in high-income countries (Woody et al., 2017), with possible negative implications for both maternal and offspring's health and well-being (Gentile, 2017). Research on the biological underpinnings of perinatal depression has mostly focused on the role of the Hypothalamic-Pituitary-Adrenal (HPA) axis and its end-product cortisol, while relatively little is known about the contributions of the Inflammatory Response System (IRS) and Sympathetic Nervous System (SNS). The current study adopts a short-term longitudinal design and combines the assessment of multiple stress and inflammatory biomarkers in late pregnancy and after delivery in order to elucidate possible associations with pre- and postnatal depressive symptoms and investigate their change across the perinatal period.

#### 1.1 Stress markers and perinatal depression

Despite extensive research investigating the associations between perinatal depression and cortisol (Orta et al., 2018; Seth et al., 2016), mood-related alterations in the diurnal cortisol pattern have been poorly investigated. Alterations in cortisol diurnal rhythm, as indicated by a lower waking level (O'Connor et al., 2014), a blunted cortisol awakening response (CAR; Osborne et al., 2018), a flatter diurnal decline (O'Connor et al., 2014), increased evening (O'Keane et al., 2011; Osborne et al., 2018) and averaged daily cortisol (O'Connor et al., 2014; Osborne et al., 2018), in clinically depressed pregnant women have been found in some studies, though not all (Shea et al., 2007; Hellgren et al., 2013). Likewise, a blunted CAR have been reported in depressed women at 6-8 weeks (Taylor et al., 2009) and 6 months (De Rezende et al., 2016) postpartum, whereas Corwin and colleagues (2015) reported higher daily cortisol in depressed women 14 days, but not 7 days or 6 months after delivery. Conversely, studies on non-clinical samples have failed to detect any associations between depressive symptoms and diurnal cortisol measures both prenatally (Rash et al., 2015; Heuvel et al., 2018) and postnatally (Cheng & Pickler, 2010, Scheyer & Urizar, 2016). Large heterogeneity in cortisol measures may play a role in inconsistent findings with

studies differing in the selection and computation of composite scores (e.g. CAR, decline, etc.). Multilevel analytical models have been recommended for analysing repeated cortisol measures (Hruschka et al., 2005; Zijlmans et al., 2015) and might help to detect subtle mood-related alterations in cortisol diurnal pattern, yet they are scarcely applied. Furthermore, most studies have been cross-sectional and it is unknown whether depressive symptoms are associated with changes in diurnal cortisol from pre- to postpartum.

Initial evidence suggests that salivary  $\alpha$ -amylase (sAA), a non-invasive marker of SNS activity (Nater & Rohleder, 2009), can be reliably assessed during pregnancy (Nierop et al., 2006) and its diurnal patterning (characterized by a rapid decrease after awakening and a gradual increase throughout the day) is largely maintained during the prenatal period (Giesbrecht et al., 2013). However, knowledge about the association between sAA diurnal levels and perinatal depression is limited. Braithwaite and colleagues (2015) found higher sAA waking levels in pregnant depressed women, while Giesbrecht and colleagues (2013) found no association between sAA diurnal trajectory and prenatal depression, although both momentary depressed and positive mood were positively associated with sAA.

### *1.2 Inflammatory markers and perinatal depression*

Mounting evidence indicates a role for inflammation in the pathophysiology of depression (Miller and Raison, 2016), with levels of inflammatory markers such as Interleukine-6 (IL-6) and C-Reactive Protein (CRP) being found consistently increased in depressed patients (e.g. Valkanova et al., 2013). However, the generalizability of these findings to the perinatal period is still debated (Osborne & Monk, 2013). Either positive (e.g. Cassidy-Bushrow et al., 2012; Christian et al., 2009; Liu et al., 2016) or null associations (e.g. Skalkidou et al., 2009; Blackmore et al., 2011; Buglione-Corbett et al., 2018) between depressive symptoms and IL-6 and/or CRP levels have been reported during pregnancy and following birth-to-6 months postpartum. To our knowledge, only Osborne and colleagues (2019) have examined whether prenatal depressive symptoms are associated with changes in cytokines levels from pre- to post-partum. The authors reported a

greater increase in pro-inflammatory cytokines levels, including IL-6, from the second to the third trimester of gestation, followed by a greater decrease at 6 weeks postpartum in a small group of women (N=12) with higher prenatal depressive symptoms, as compared to non-depressed women (N=37).

### *1.3 Stress-immune interactions*

Besides their individual effects, the stress and inflammatory response systems exhibit significant bidirectional interactions (Kuhlman et al., 2017), with a disruption of these circuits being hypothesized to increase vulnerability to several disorders (Raison and Miller, 2003), including depression (Pariante, 2017). However, studies investigating the concurrent activity of the HPA-axis and IRS during the perinatal period are scarce (Corwin et al., 2013; Shelton et al., 2015; Walsh et al., 2016) and, to our knowledge, only Corwin and colleagues (2015) examined the link between variability in stress-immune interactions and risk for depression. Results showed a positive association between inflammation and cortisol in postnatally depressed women, and no association in euthymic women.

### *1.4 Current study*

Although it is increasingly acknowledged that the coordination of the SNS, HPA-axis and IRS is essential to health and well-being (Gunnar & Quevedo, 2007; Kuhlman et al., 2017), knowledge about the stress-immune interplay during the perinatal period as well as the association with perinatal depressive symptoms remains vague. Furthermore, most studies have been cross-sectional and mood-related changes in biomarker levels over the perinatal period are largely unknown. This limited knowledge results in a lack of biomarkers for the diagnosis, prevention and treatment of perinatal depression.

The current study begins to fill these gaps by investigating the cross-sectional and prospective associations among naturally occurring variations in depressive symptoms and multiple stress (i.e. diurnal cortisol and sAA) and inflammatory (i.e. IL-6 and CRP) markers in late

pregnancy and after delivery. We hypothesized that higher depressive symptoms would be associated with heightened inflammation and an altered cortisol diurnal pattern. Conversely, no formal hypothesis was made regarding the association between depressive symptoms and diurnal sAA due to limited available literature. Additionally, supplementary analyses investigated an exploratory hypothesis and examined the robustness of the associations found for depression. Specifically, we sought to extend findings from Corwin and colleagues (2015) by exploring the association among stress and inflammatory markers in women with higher versus lower depressive symptoms pre- and post-partum. Furthermore, as perinatal depression and anxiety are strongly correlated (Falah-Hassani et al., 2017), we explored whether the observed biological alterations were specific to depression or can be replicated for anxiety.

## 2 *Material and methods*

### 2.1 *Participants and procedures*

Women at 30-33 gestational weeks were recruited in three Italian hospitals and followed longitudinally within the Effects of Depression on Infants (EDI) Study, an on-going study investigating the effects of maternal prenatal depression on infants' development (Nazzari et al., 2019). Prenatal inclusion criteria were: aged 18-45 years, normotensive, with singleton uncomplicated pregnancy, non-smoker, not afflicted by any disease, not taking any chronic medications, and with no substance/alcohol abuse problems or psychiatric disorders (except for depression and anxiety) as ascertained through the *Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I*; First, et al., 2002). One-hundred-ten women (mean age= 33.00; SD= 3.85), were included in the current sample (Table 1). Nineteen caesarean sections and 1 intrauterine death were excluded from the postnatal phase, and 1 woman withdrew due to her newborn's health problems. Thus, data from 89 women were available at the postnatal phase (see Supplemental Figure 1 for a flow diagram of the study design). Women who were excluded from the postnatal phase did not differ from participants on any demographic variables, depression or

anxiety scores.

Depressive/anxiety symptoms and biomarkers levels were evaluated at 34-36 gestational weeks (mean gestational age=34.76; SD=1.12) and soon after delivery (mean hours after delivery=52.36; SD=19.70). On both occasions, women filled out the Edinburgh Postnatal Depression Scale, the State-Trait Anxiety Inventory, a health-related information form and provided blood samples. Diurnal salivary samples were collected over the two consecutive days preceding the pre- and postnatal sessions as described below.

The Ethics Committee of University College London, of Scientific Institute Eugenio Medea and of the hospitals involved approved the study protocol, and all participants gave their written informed consent.

## 2.2 Self-report measures

*Depressive symptoms* were evaluated through the Italian version (Benvenuti et al., 1999) of the Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987), a 10-items questionnaire widely used to screen for perinatal depression. The Italian version of the EPDS shows good internal consistency (alpha of Cronbach=0.79), high sensitivity and convergent validity with other measures of depression, comparably to the original version (Benvenuti et al., 1999).

*Anxiety symptoms* experienced in the last few days were assessed through the Italian version (Pedrabissi and Santinello, 1989) of the 20-items state-anxiety subscale of the State-Trait Anxiety Inventory (STAI-S; Spielberger et al. 1970). The Italian version of the STAI-S shows high coefficients of internal consistency (alpha of Cronbach=.91-.92) and good test-retest reliability (Spearman correlation=0.49;  $p<0.01$ ) (Pedrabissi and Santinello, 1989). Furthermore, moderate concurrent validity with other measures of anxiety has been reported as well as high ability to discriminate between clinical and nonclinical Italian samples (Balsamo et al., 2013).



*Health-related information* was collected through ad-hoc forms in order to examine potential health-related confounders of the association between depressive symptoms and biological levels (further details in Supplemental Methods).

### 2.3 Biological measures

*Blood collection and assay.* Antecubital venous blood samples were collected in the morning (with the exception of 8 postnatal samples) and kept refrigerated at +4° until they reached the laboratory where serum was centrifuged, aliquoted, and stored at -80 °C. Serum CRP and IL-6 concentrations were assayed in duplicate by using Quantikine High Sensitivity ELISA kits (R&D Systems Europe, LTD). Ninety-seven women out of 110 (88%) agreed to blood sampling during pregnancy and 66 out of 89 (74%) after delivery. As IL-6 and CRP levels assessed in the afternoon (N=8) were comparable to those assessed in the morning (respectively,  $F(1,65)= 0.326$ ,  $p=0.57$  and  $F(1,65)= 1.05$ ,  $p=0.31$ ), all postnatal samples were included in the analyses.

*Saliva collection and assay.* Six unstimulated saliva samples were collected on two consecutive days upon awakening, 30 minutes post-waking and before going to bed respectively, at home, during pregnancy and, at the hospital, after delivery, according to the same protocol. All samples were stored frozen at -80° until assayed for cortisol in duplicate using a competitive high sensitivity enzyme immunoassay kit (Salimetrics, UK) and for sAA using a kinetic enzyme assay kit (Salimetrics, UK). Due to budget restrictions, sAA was examined only in pregnancy. All women provided completed saliva samples during pregnancy, 12 women had incomplete postnatal salivary data and 9 women had no postnatal salivary data. Women with complete, partial or missing postnatal data did not differ on any sociodemographic, health-related variables, depression or anxiety scores. One prenatal 30-min post-waking sample and 4 postnatal 30-min post-waking samples, collected more than one hour from awakening, were excluded from analyses (e.g. O'Connor et al. 2014). Further details are reported in Supplemental Methods.

### 2.4 Statistical analyses

Variables were first examined for outliers and skewness. The distributions of the biological markers were positively skewed even after removing samples greater than 3 SD from the mean (n=7 samples for prenatal cortisol, n=8 for postnatal cortisol, n=4 for sAA, n=3 for prenatal IL-6), thus measures were natural log transformed prior to analysis to approximate normal distributions.

Given the low-risk nature of the current sample and the focus of the study on naturally occurring variations in maternal symptoms across the whole normative range, the total score of the EPDS/STAI was employed as a continuous variable in the main analyses.

Hierarchical Linear Models (HLMs) were estimated to investigate: 1) the cross-sectional association between depressive symptoms and biological levels in pregnancy and after delivery; 2) the prospective association between prenatal depressive symptoms and the change in markers levels from pre- to post-partum. HLMs allow to partition different sources of variance and provide robust estimates despite missing values by maximizing all valid data point (Hruschka et al., 2005). Final sample size for the main analyses is provided in Supplemental Figure 1. Full model specification is provided in Supplemental Methods. Preliminary models examined demographic, health-related and situational factors, known to affect stress and immune physiology. Variables significantly associated with biological markers in these analyses were included as controls in subsequent analyses. The fixed effects of maternal depressive symptoms on the intercept and time slopes were tested sequentially. Model fit was tested with likelihood deviance difference test for nested models. Model parameters for ln-transformed cortisol values are presented in tables, while results in figures are presented using anti-logged values. A sensitivity power analyses estimated that with the current sample size and 80% power at a 0.05 level of significance, we were able to detect effects ranging from  $f^2=.11$  to  $f^2=.16$ , while we would have missed any effect smaller than this.

Supplementary analyses preliminarily explore stress-immune interactions. Daily average cortisol and sAA were computed for each day as the area under the curve according to the trapezoid method with respect to the ground (AUCg, Pruessner et al., 2003) and, as they were highly correlated ( $r(104)=.57$ ,  $p<.001$  for cortisol and  $r(106)=.75$ ,  $p<.001$  for sAA), the mean of the

2 days was used. After Corwin and colleagues (2015), graphical analyses were used to explore different patterns of correlations between sAA or cortisol AUCg and inflammatory markers between women scoring above versus under the EPDS median during pregnancy and after delivery. At time points where significant differences were identified, the relationship trends were further investigated through multiple linear regressions. In these models, levels of inflammation were the outcomes variables and the difference in the relationship patterns between stress and inflammatory markers was evaluated by testing the significance of the interaction term between EPDS and cortisol/sAA AUCg on levels of inflammation.

Lastly, given the considerable overlap between depression and anxiety ( $r=.65$  prenatally and  $r=.46$  postnatally,  $p<.001$ ), comparable HLMs examined anxiety, rather than depression, as individual-level predictor.

Statistical analyses were performed using SPSS 24 and MLWiN 3.02.

### 3. Results

Descriptive raw values for all study variables are presented in Table 2.

#### 3.1 Diurnal cortisol

##### 3.1.1 Diurnal variation in prenatal cortisol

The baseline model for prenatal cortisol (Table 3) indicated significant between-person variation in mean waking cortisol levels ( $p<.001$ ) and showed the typical cortisol diurnal pattern found in non-pregnant samples (both morning and afternoon slopes  $ps<.001$ ). Fetal sex was significantly associated with cortisol mean waking level ( $p=.005$ ), with 2.9% lower values in women carrying a female fetus as compared to male, and was thus included as a control in all subsequent models. No other significant associations between cortisol levels and demographic, pregnancy-related or situational variables were found (all  $ps>.05$ ). Higher prenatal depressive symptoms, as assessed through the EPDS, were associated with lower cortisol levels at waking and 30 minutes after waking (respectively, 2.6% and 3% lower with every 1-point EPDS increase,  $ps=.002$ ), and

with a flatter afternoon cortisol decline (2.2% smaller slope with every 1-point increase on the EPDS,  $p=.019$ ), after adjusting for covariates. While the EPDS score was modeled as a continuous variable, for illustrative purposes, prenatal maternal cortisol trajectories are displayed in Figure 1 for women with higher (+1SD) and lower (-1SD) EPDS score. Inclusion of depressive symptoms resulted in a significant improvement of model fit over the baseline model (deviance difference (3)=12.44,  $p=.01$ ).

### 3.1.2 Diurnal variation in postnatal cortisol

The expected cortisol diurnal pattern was found soon after delivery (both morning and afternoon slopes  $p<.001$ ), with significant between-day (level-2,  $\sigma^2_{u0}=0.017$ ,  $p<.001$ ) and between-person (level-3,  $\sigma^2_{v0}=0.004$ ,  $p<.001$ ) variation in mean waking cortisol levels. There was a marginally significant effect of time from delivery (estimate=-0.002, SE=0.001,  $p=.07$ ) on cortisol waking levels. Thus, time from delivery was included as a covariate in subsequent models. All other demographic and health-related variables were not significantly associated with cortisol values (all  $p>.05$ ). No significant effects of postnatal EPDS scores on mean cortisol waking levels ( $p=.15$ ), on morning ( $p=.21$ ) and afternoon ( $p=.18$ ) slopes were found.

### 3.1.3 Diurnal variation in cortisol from pregnancy to delivery

The unconditional means model for the change in diurnal cortisol from pregnancy to delivery revealed significant variability between occasions (i.e. pregnancy versus delivery; level-3  $p<.001$ ). As expected, mean cortisol waking values were 3.25% higher at delivery as compared to prenatal levels ( $p<.001$ ), while no effect of sampling occasion (i.e. pregnancy or delivery) on cortisol diurnal slopes was found ( $p>.05$ ). Both foetal sex and time from delivery were retained in the model as they were previously associated with cortisol levels. Prenatal depressive symptoms were associated with lower waking cortisol values prenatally (estimate=-0.03, SE=0.01;  $p=.01$ ), but not postnatally (estimate=0.01, SE=0.01;  $p=.31$ ). Additionally, prenatal depressive symptoms were related to a greater increase in cortisol waking levels from pregnancy to delivery (estimate=0.046,

SE=0.017;  $p=.006$ ). However, the inclusion of a three-way interaction between prenatal depressive symptoms, time of sampling and occasions did not significantly improve the model fit over the baseline model (deviance difference (6)=11.3,  $p=0.08$ ).

### 3.2 *Diurnal sAA*

The expected sAA diurnal trajectory was observed (both morning and afternoon slopes  $ps<.001$ ), with significant variability in sAA waking values between persons and days ( $ps<.001$ ). There was a significant effect of day of collection on mean sAA waking values ( $p=.002$ ), but not on diurnal slopes, with higher levels on day 2 as compared to day 1. Thus, day of collection was retained as a covariate. Demographic, pregnancy-related and situational variables were not significantly associated with diurnal sAA levels (all  $ps>.05$ ).

As shown in Table 3, there was no effect of depressive symptoms on mean sAA waking levels ( $p=.22$ ) or diurnal pattern (note that there was a non-significant tendency for depressive symptoms to be related to a flatter morning sAA decline ( $p=.07$ ), while no effects on sAA afternoon slope,  $p=.35$ ). Inclusion of depressive symptoms did not significantly improve model fit over the baseline model (deviance difference (3)=3.78,  $p=.29$ ).

### 3.3 *Inflammatory markers*

As expected, both CRP and IL-6 levels were significantly higher after delivery as compared to late pregnancy (both  $ps<.001$ , Table 4), with significant between-person variations in prenatal markers levels as well as change from pregnancy to delivery (all  $ps<.001$ ). There was a significant effect of pre-pregnancy Body Mass Index (BMI), as assessed through the health-related form, and age on CRP prenatal levels and on the time slope. Specifically, we observe 5.5% lower CRP levels per year older ( $p=.003$ ) and 8.9% higher CRP with each point on the BMI ( $p<.001$ ). Additionally, a higher BMI and a younger age were related to a flatter CRP increase from pregnancy to delivery (deviance difference (4)=30.3,  $p<.001$ ). Thus, both maternal age and BMI were included in all subsequent models. Furthermore, BMI was significantly associated with prenatal IL-6 levels

( $p=.016$ ), with 4.4% higher IL-6 concentrations with each BMI point, and was included as a covariate in subsequent analyses.

There was a significant effect of prenatal depressive symptoms on IL-6 levels during pregnancy with around 7.7% higher IL-6 concentrations with every 1-point EPDS increase ( $p=.04$ ), although no effect on IL-6 pre- to post-partum slope ( $p=.72$ ). The inclusion of depressive symptoms significantly improved the model fit over the baseline model (deviance difference (1)= 4.02,  $p=0.04$ ). In contrast, prenatal depressive symptoms were not significantly related to mean prenatal CRP levels or slope (all  $ps>.05$ ). Similarly, no effects of postnatal depressive symptoms on mean CRP or IL-6 postnatal levels were found (all  $ps>.05$ ).

### 3.4 *Supplementary Results*

#### 3.4.1 *Correlations between systems*

Table 5 shows bivariate correlations between EPDS scores and biological levels.

As shown in Supplemental Figure 2, the relationship patterns for IL-6 and both cortisol and sAA during pregnancy were quite different among women who scored above or below the EPDS median. Specifically, in women scoring above the EPDS median ( $N=66$ ), prenatal cortisol AUCg and sAA AUCg increased with increasing levels of IL-6 (respectively,  $p=.03$  and  $p=.002$ ), while this was not true in women scoring below the median (both  $ps>.05$ ). No differences in relationship trends were apparent after delivery.

To further explore these relationship patterns during pregnancy, separate regression models were fitted. There was no main effect of cortisol or sAA AUCg on mean IL-6 or CRP levels during pregnancy (all  $ps>.05$ ). However, there was a significant interaction between prenatal depressive symptoms and sAA ( $p<.01$ ) in relation to IL-6 levels during pregnancy, while the interaction between prenatal depressive symptoms and cortisol was not significant ( $p=.06$ ). Specifically, higher sAA levels were associated with higher IL-6 levels only in women with higher EPDS scores (+1SD;  $p<.05$ ), while the association was non-significant in women with lower EPDS

scores ( $p > .05$ ). The sAA model yielded a significant improvement in model fit as compared to the baseline model (deviance difference (2)=12.97,  $p < .01$ ).

### 3.4.2. Anxiety symptoms

There were no significant effects of anxiety symptoms, as assessed through the STAI-S, on cortisol, IL-6 and CRP levels both prenatally and postnatally (all  $p$ s  $> .05$ ). However, there was a significant main effect of prenatal anxiety on mean waking sAA levels (estimate=-0.663, SE=0.312;  $p = .033$ ) and on sAA morning slope (estimate=0.594, SE=0.209;  $p = .004$ ). Specifically, higher STAI scores were related to lower waking sAA concentrations and a flatter decline from waking to 30 minutes after. This model significantly improved model fit over the baseline model (deviance difference (3)=19.71,  $p < .001$ ).

## 4 Discussion

The present study is among the first to investigate the concurrent and short-term functioning of the HPA-axis, SNS and IRS from pre- to post-partum in association with perinatal depressive symptoms.

In line with our predictions, higher depressive symptoms in late gestation, as assessed through the EPDS, were associated with heightened IL-6 levels and an altered cortisol diurnal pattern, as indexed by lower morning cortisol levels (i.e. at waking and 30 minutes after) and a flatter diurnal decline. In contrast with our initial hypothesis, however, no significant associations were found in the early postpartum. Furthermore, exploratory analyses indicate that anxiety, but not depressive, symptoms were associated with an altered sAA diurnal profile and that the interplay between inflammatory and stress markers during pregnancy might be different in women with higher depressive symptoms.

Evidence exists of an altered diurnal cortisol pattern in clinically depressed (O'Connor et al., 2014) or minority high-stressed (Suglia et al., 2010) pregnant women; likewise, an association

between depressive symptoms and elevations in circulating IL-6 levels has been reported in samples of low-income high-psychosocial risk (Cassidy-Bushrow et al., 2012; Christian et al., 2009) and clinically depressed (Haeri et al., 2013; Osborne et al., 2018) pregnant women. The current findings support and extend these studies by showing that depressive symptoms, even without meeting the full criteria for a clinical diagnosis, are associated with an altered stress-related physiology in a low-risk sample of pregnant women. These results converge with evidence of depression as a dimensional “trait” in the general population, rather than a discrete entity, with a continuous measure of risk underlying the clinical phenotype (e.g. Wray et al., 2018). It is important to note that the overall diurnal cortisol output during pregnancy, as indexed by the AUCg, was not significantly associated with depressive symptoms. One methodological implication is that a detailed characterization of the diurnal pattern, both through multiple sampling and multilevel analytical techniques, is needed in order to detect reliable associations between depressive symptoms and subtle disturbances in diurnal cortisol profile that would be undetectable otherwise. Indeed, as cortisol levels increase (e.g. Allolio et al., 1990) and maternal HPA-axis becomes gradually less responsive to stress as pregnancy advances (Kammerer et al., 2002), the association between maternal distress and cortisol might become more difficult to detect. Furthermore, the current findings align with increasing evidence indicating that diurnal cortisol measures, rather than absolute levels, better allow to capture the HPA-axis individual variability and are more strongly associated with psychosocial stress measures (Pruessner et al., 2003, Harville et al., 2009). Additionally, evidence of an association between prenatal depressive symptoms and a flatter diurnal cortisol decline is in line with recent meta-analytical findings in the general population (Adam et al., 2017) and possibly indicates that the diurnal cortisol slopes represent a biological marker of emotional health that deserves further investigation.

Findings of an association between increased IL-6 levels and depressive symptoms during pregnancy converge with meta-analytical evidence in non-pregnant samples (Dowlati et al., 2010; Valkanova et al., 2013). This result in a sample of healthy pregnant women might suggest that the experience of depressive symptoms per se is associated with a higher pro-inflammatory state



during pregnancy. As initial evidence in humans indicates that higher than typical elevations in pro-inflammatory cytokines during pregnancy are associated with a range of altered neurodevelopmental outcomes in the offspring (reviewed in Nazzari & Frigerio, 2020), including birth outcomes (e.g. Ernst et al., 2011; Nazzari et al., 2019), cognitive outcomes (e.g. Nazzari et al., in press; Rasmussen et al., 2019) and structural and functional brain alterations (e.g. Graham et al., 2017; Rudolph et al., 2018), future studies should investigate possible inflammatory pathways from maternal depression to adverse offspring's development.

It is noteworthy that the current study was sufficiently powered to detect medium to large effect size, thus the fact that we did find significant associations between antenatal depressive symptoms and biological alterations is possibly suggestive of a relatively large size of the effect, although further replication is needed. The pathways underlying the observed associations are still to be understood. Depression and both inflammation and dysregulation of the HPA-axis might share a somewhat common genetic background (Spijker & van Rossum, 2009; Barnes et al., 2017; Wray et al., 2018). Additionally, as both the stress and immune response systems are extremely responsive to environmental adversities, early adverse experiences might affect their functioning and confer vulnerability for later mental health problems (Pariante et al., 2017).

However, the correlational nature of this study and the short-term longitudinal design do not allow to establish whether the observed associations reflect causal processes.

Importantly, the inflammatory and stress response systems function in a complex interdependent manner over time. Thus, abnormal inflammation or dysregulation of the HPA-axis has the potential to disrupt this circuit, carrying potentially adverse consequences for mental and physical health (Khulman et al., 2017). While a disruption of the stress-immune circuits is hypothesized to play a role in the pathophysiology of depression in the general population (Pace et al., 2007; Irwin & Cole, 2013), the interplay among stress hormones and inflammation across the perinatal period is largely unexplored. Our exploratory analyses provide evidence of a positive association between IL-6 levels and both higher diurnal sAA and, to a lesser extent, cortisol concentrations in women with higher depressive symptoms during pregnancy. It has been

hypothesized that increased levels of stress hormones in late gestation might inhibit pro-inflammatory cytokines production and potentiate production of anti-inflammatory cytokines (Elenkov & Chrousos, 2002). Initial evidence indicates an impairment of the negative feedback relationship between cortisol and pro-inflammatory cytokines in high-risk pregnant samples (Corwin et al., 2013; Walsh et al., 2016). Current results show a positive association between stress and inflammatory markers in pregnant women with higher depressive symptoms, possibly suggesting that the stress-immune interplay offers a promising avenue for future endeavor into the biological underpinnings of perinatal depression. Nevertheless, it is important to emphasize that the current findings are small-scale and exploratory and need replication in larger and clinical samples.

It is noteworthy that despite the strong association between prenatal depression and anxiety, cortisol diurnal alterations and heightened inflammation were more robustly observed for depressive symptomatology, whereas weak and non-significant associations were found for anxiety symptoms. Conversely, prenatal anxiety symptoms were significantly associated with an altered sAA diurnal pattern, as indicated by lower sAA waking levels and a flatter morning decline, in line with few reports of an alteration of sympathetic function in prenatally anxious women (e.g. Field et al., 2006; Giesbrecht et al., 2013). Future studies should directly compare these two conditions in larger samples and elucidate whether, despite being comorbid, depression and anxiety during the perinatal period differ in some respects in their underlying biology.

Some non-significant findings are worth mentioning. First, we did not report any significant association between depressive symptoms and either inflammation or cortisol after delivery. Similarly, there was no substantial evidence of an association between prenatal depressive symptoms and change in cortisol and inflammatory markers concentrations from pre- to postpartum. A lack of association among postnatal depression and levels of inflammation or cortisol in the immediate postpartum period is in line with previous evidence (e.g. Skalkidou et al., 2009; Corwin et al., 2015) and supports the hypothesis that, despite the strong continuity between pre- and postnatal depression, they might reflect somewhat different endocrine and inflammatory states

(Kammerer et al., 2006). Alternatively, methodological issues might account for these null findings. First, the lack of associations in the postnatal period might be an issue of power, as the sample size at the postnatal phase was slightly decreased. Alternatively, the association between depressive symptoms and biomarkers levels during the first week after childbirth might be masked by the strong hormonal and inflammatory response to delivery. It is possible that, as the biological response to delivery normalizes, significant associations might emerge. Indeed, significant associations between diurnal cortisol levels and depression 2 weeks, but not 1 week (Corwin et al., 2015), 7 weeks (Taylor et al., 2009) and 6 months postpartum (De Rezende et al., 2016) have been observed. Similarly, Osborne and colleagues (2019) reported a greater decrease in IL-6 levels from late pregnancy to 6 weeks postpartum among women with higher prenatal depressive symptoms. Furthermore, a follow-up evaluation of depressive symptoms could reveal significant differences in levels of cortisol and inflammation after delivery in women who later developed postpartum depression. Consistently with this, Liu and colleagues (2016) reported higher CRP and IL-6 levels after delivery in women with postpartum depression 6 months later.

Lastly, in contrast with previous studies (e.g. Scrandis et al., 2008; Azar & Mercer, 2013), CRP levels were not related to depressive symptomatology. Although CRP is considered a non-specific marker of systemic inflammation, evaluating “basal” CRP levels is challenging and it is not possible to rule out a wide range of factors influencing circulating levels (Du Clos and Mold, 2004), including acute infections (e.g. Rasmussen et al., 2018). Therefore, the lack of association between CRP and depression may be due to methodological issues. Alternatively, as null associations have been already reported (e.g. Cassidy-Bushrow et al. 2012; Catov et al., 2014), it remains plausible that IL-6, rather than CRP, is a more adequate biomarker for perinatal depression, driving the association between inflammation and depressive symptoms.

## 5 *Limitations*

Some limitations of the present study are noteworthy. First, data are based on a relatively small community sample of middle-high SES healthy women and, as expected, self-reported

depressive symptoms were relatively low, thus limiting generalizability to high psychosocial risk or clinical psychiatric populations. Second, data were collected only in late pregnancy and early puerperium. We cannot rule out that effects earlier in pregnancy or later postpartum may be different. Third, compliance with the saliva collection protocol was not objectively measured. Fourth, although several potential confounders were statistically controlled, we cannot rule out unmeasured confounds or third variables that might explain the observed associations, including genetic variants. Lastly, we did not evaluate sAA levels postpartum. While conclusions that can be drawn from the current study are to be regarded as preliminary and require replication in different and larger cohorts, the current study leads the way to future research adopting a multi-systems approach to the investigation of perinatal depression.

## 6 Conclusions

The current study adds to the growing literature suggesting a dysregulation of the HPA-axis and IRS in prenatal depression and emphasizes the need for an integrated multi-systems approach to the understanding of the biological underpinnings of perinatal depression. Confirming our findings in larger samples may help to identify early biomarkers and treatment targets for perinatal depression, which is now one of the most compelling issue in maternal-child health worldwide.

### *Acknowledgments:*

The authors wish to thank all the families and children who participated in this study. We are extremely grateful to Dr. Stefano Norchi and Dr. Daniele Merazzi from Ospedale Valduce di Como, Dr. Rinaldo Zanini from Ospedale Mandic di Merate and Dr. Alberto Zanini from Ospedale Fatebenefratelli di Erba, as well as to all the midwives and nurses from the hospitals involved for their help in recruitment and data collection. The assistance of Nadia Dottori, Enrita Pozzi, Marco Redaelli and Marta Calcinati in data collection is gratefully acknowledged.

### *Funding sources:*

This work was supported by a PhD studentship provided by the Stanley Thomas Johnson Foundation awarded to Sarah Nazzari [grant number: 4461]. Funding was also received by Fondazione Banca del Monte di Lombardia and Soroptimist Club di Lecco, Italy.

*Conflict of interest:* none

### *Ethical standards:*

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Table 1 – Description of the study population (N=110)

<b>Variables</b>	<b>N (%) or Mean (SD)</b>
Age	M=33.01, SD=3.85
Education	
< 10 years	11 (10.0%)
> 10 years	99 (90.0%)
Family Socio-Economic Status (SES)*	
Low	5 (4.5%)
Middle	46 (41.8%)
High	50 (48.2%)
Marital Status	
Married	69 (62.7%)
Cohabiting	39 (35.5%)
Divorced	1 (0.9%)
Single	1 (0.9%)
Parity	
Primiparous	99 (90.0%)
Multiparous	11 (10.0%)
Baby's gender	
Male	57 (51.8%)
Female	53 (48.2%)
Pre-pregnancy Body Mass Index (BMI)	22.00 (0.4)
Actual BMI	25.00 (0.4)

\* Percentages for Family SES do not add to 100% due to missing values.

Table 2 - Descriptive statistics for study variables during pregnancy and after delivery

	Pregnancy			Delivery		
	Mean	SD	Range	Mean	SD	Range
<i>Cortisol (µg/dl)</i>						
Waking	0.38	0.13	0.13-0.83	0.43	0.18	0.02-0.97
Waking +30'	0.50	0.15	0.10-0.91	0.57	0.25	0.03-1.60
Bedtime	0.18	0.06	0.01-0.41	0.20	0.09	0.03-0.45
<i>sAA (U/ml)</i>						
Waking	68.68	63.75	3.00-463.84	--	--	--
Waking +30'	47.60	37.97	2.80-190.10	--	--	--
Bedtime	97.17	79.58	3.28-562.71	--	--	--
<i>CRP (ng/ml)</i>	3786.74	2772.98	480.04-11244.10	11660.28	3541.09	6981.27-18156.9
<i>IL-6 (pg/ml)</i>	1.67	1.02	0.48-6.47	7.02	2.86	1.68-12.62
<i>EPDS</i>	5.37	4.41	0-19	5.53	4.77	0-28
<i>STAI-S</i>	35.14	8.87	21-71	34.49	9.64	20-64



Table 3 – Preliminary (Model 1) and full (Model 2) prediction models for prenatal diurnal cortisol and sAA

Prenatal cortisol	Model 1			Model 2			Prenatal sAA	Model 1			Model 2		
	Estimate	SE	p	Estimate	SE	p		Estimate	SE	p	Estimate	SE	p
<i>Fixed effects</i>							<i>Fixed effects</i>						
Intercept	0.329	0.008	<.001	0.327	0.008	<.001	Intercept	3.731	0.079	<.001	3.731	0.079	<.001
Morning	0.086	0.010	<.001	0.092	0.024	<.001	Morning	-0.242	0.052	<.001	-0.241	0.051	<.001
Afternoon	-0.152	0.007	<.001	-0.188	0.017	<.001	Afternoon	0.460	0.066	<.001	0.461	0.066	<.001
Fetal sex	-0.029	0.010	0.005	-0.026	0.010	0.009	Sampling day	0.140	0.042	0.002	0.140	0.042	0.002
EPDS				-0.026	0.008	0.002	EPDS				-0.126	0.104	0.223
EPDS X Morning				-0.004	0.014	0.778	EPDS X Morning				0.128	0.069	0.065
EPDS X Afternoon				0.022	0.009	0.019	EPDS X Afternoon				0.083	0.090	0.354
<i>Random effects</i>							<i>Random effects</i>						
<i>Level 3 (individual)</i>							<i>Level 3 (individual)</i>						
Intercept variance	0.002	0.001	<.001	0.002	0.001	<.001	Intercept variance	0.498	0.077	<.001	0.497	0.077	<.001
Morning slope variance	0.005	0.001	<.001	0.005	0.001	<.001	Afternoon slope variance	0.193	0.058	<.001	0.194	0.057	<.001
Intercept/morning slope covariance	0.000	0.001	0.668	0.000	0.001	0.871	Intercept/afternoon slope covariance	-0.127	0.050	0.011	-0.127	0.050	0.011
<i>Level 2 (day)</i>							<i>Level 2 (day)</i>						
Intercept variance	0.005	0.000	<.001	0.005	0.000	<.001	Intercept variance	0.287	0.020	<.001	0.285	0.019	<.001
<i>Level 1 (times)</i>							<i>Level 1 (times)</i>						
Intercept variance	0.000	0.000	0.998	0.000	0.000	0.998	Intercept variance	0.000	0.000	0.999	0.000	0.000	0.999

Table 4 – Full prediction models for inflammatory markers change from pregnancy to delivery

	IL-6			CRP		
	Estimate	SE	p	Estimate	SE	P
<i>Fixed effects</i>						
Intercept	0.935	0.029	<.001	7.968	0.070	<.001
Time	1.089	0.050	<.001	1.355	0.074	<.001
Age				-0.054	0.018	0.003
BMI	1.699	0.709	0.016	0.086	0.018	<.001
EPDS	0.077	0.039	0.045	0.074	0.094	0.436
Age x Time				0.039	0.019	0.046
BMI x Time				-0.081	0.019	<.001
EPDS X Time	-0.023	0.064	0.724	-0.155	0.098	0.114
<i>Random effects</i>						
<i>Level 2 (individual)</i>						
Intercept variance	0.081	0.012	<.001	0.473	0.068	<.001
Time variance	0.166	0.030	.001	0.485	0.075	<.001
Intercept/time covariance	-0.051	0.015	<.001	-0.445	0.068	<.001
<i>Level 1 (times)</i>						
Intercept variance	0.000	0.000	1.000	0.000	0.000	1.000

Table 5 - Bivariate correlations for primary study variables

	1	2	3	4	5	6	7	8
1. Prenatal EPDS								
2. Prenatal Cortisol AUCg	-.15							
3. Prenatal sAA AUCg	.05	<b>.25**</b>						
4. Prenatal IL-6	<b>.19*</b>	.10	.17					
5. Prenatal CRP	.11	.08	-.01	<b>.31**</b>				
6. Postnatal EPDS	<b>.44**</b>	-.13	.07	.14	.19			
7. Postnatal Cortisol AUCg	-.13	.09	-.16	-.09	-.03	-.20		
8. Postnatal IL-6	.12	.01	.06	<b>.27*</b>	.08	.005	.09	
9. Postnatal CRP	-.19	.001	-.15	<b>-.33**</b>	.22	-.02	-.09	-.18

\* p<.05; \*\*p<.01

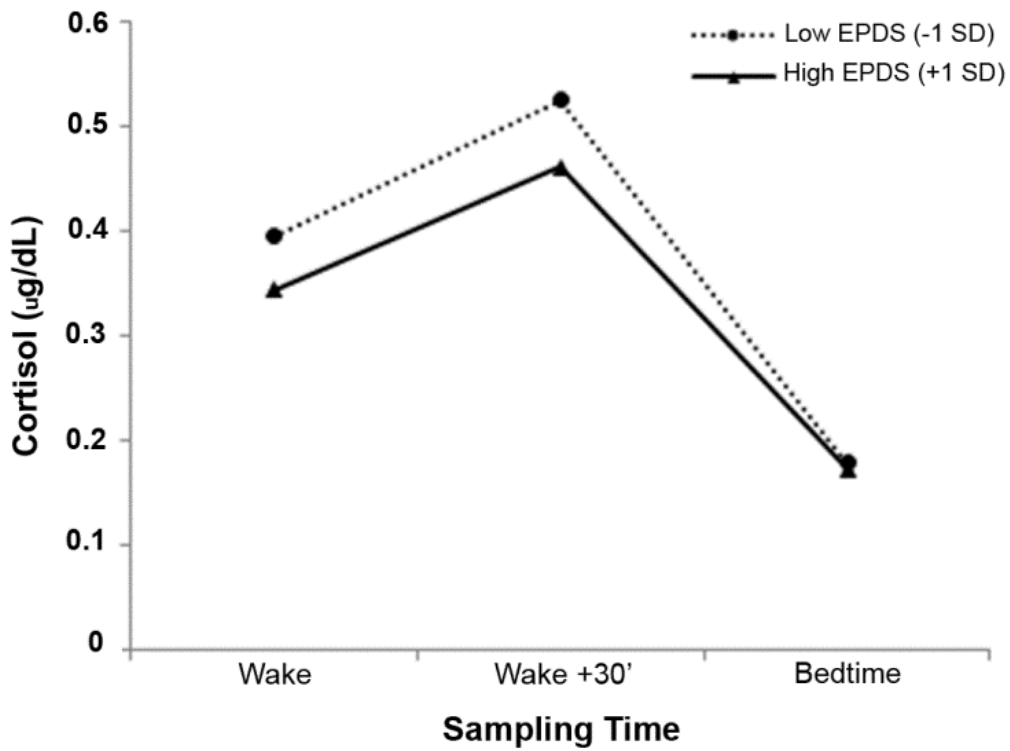


Figure 1- Graphical representation of the effect of maternal depressive symptoms on diurnal cortisol levels during pregnancy. Cortisol levels are plotted for women with higher (+1 SD) and lower (-1SD) depressive symptoms during pregnancy, after adjusting for covariates.