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# Prenatal hair cortisol concentrations during the COVID-19 outbreak: Associations with maternal psychological stress and infant temperament

Nina Bruinhof[a,*], Stefania V. Vacaru[a], Marion I. van den Heuvel[b], Carolina de Weerth[a], Roseriet Beijers[a,c]

[a] Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, P.O. Box 9010, 6500 GL Nijmegen, the Netherlands
[b] Department of Cognitive Neuropsychology, Tilburg University, 5037 AB Tilburg, the Netherlands
[c] Department of Social Development, Behavioural Science Institute, Radboud University, P.O. Box 9104, 6500 HE Nijmegen, the Netherlands

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ABSTRACT

*Background:* Maternal psychological stress during pregnancy, including stress resulting from disasters and trauma, has been linked to temperamental difficulties in offspring. Although heightened cortisol concentrations are often hypothesized as an underlying mechanism, evidence supporting this mechanism is not consistent, potentially because of methodological issues and low stress in the population.
*Aim:* To address these issues, this preregistered study investigated the following associations between: 1) prenatal psychological stress and hair cortisol, as a biomarker for chronic stress, during the COVID-19 outbreak (i.e., as a major worldwide psychological stressor), and 2) maternal hair cortisol during the COVID-19 outbreak and later infant temperamental negative affectivity and orienting/regulation. Additionally, we explored whether associations were different for women with low versus high socioeconomic status (SES; maternal education and annual household income) and at different stages of pregnancy.
*Method:* Pregnant women (N = 100) filled out online questionnaires during the first COVID-19 lockdown. Six months later, when most mothers were still pregnant or had just given birth, maternal hair samples were collected during home visits. When infants were six months old, mothers reported on their infant's temperament.
*Results:* Although hierarchical regression analyses revealed no associations between prenatal COVID-19 psychological stress and hair cortisol during the COVID-19 outbreak, SES proved to be a moderator in this association. Only pregnant women with higher levels of SES, not lower levels, showed a positive association between work-related and social support-related COVID-19 worries and hair cortisol. Finally, prenatal hair cortisol was not associated with later infant temperamental negative affectivity and orienting/regulation.
*Conclusion:* Although the COVID-19 outbreak proved to be a major psychological stressor worldwide, the physiological impact of the crisis might be different for pregnant women with higher SES as compared to lower SES.

## 1. Introduction

According to the *fetal programming hypothesis*, a stressful prenatal environment biologically 'programs' offspring's behavior (Seckl and Holmes, 2007). For example, research showed that maternal psychological stress during pregnancy forecasts infant behavioral development, including having a difficult temperament (for a review see: Van den Bergh et al., 2017). Specifically, elevated prenatal psychological stress has been linked to higher infant temperamental negative affectivity (i.e., fear, sadness, distress, and anger) and lower infant self-regulation (Huizink et al., 2002; Bush et al., 2017; van den Heuvel et al., 2015). The biological mechanisms underlying the associations between maternal prenatal psychological stress and infant outcomes remain unclear. Studies often focus on the maternal hypothalamus-pituitary-adrenal (HPA) axis, which becomes activated when confronted with stressors and results in the production of cortisol. However, evidence has been inconsistent about this link between psychological stress, prenatal cortisol, and infant behavior (Glover et al.,

* Corresponding author.
*E-mail address:* nina.bruinhof@radboudumc.nl (N. Bruinhof).

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2010; Beijers et al., 2014). The nature of cortisol measurements (i.e., mostly saliva) and participant samples (i.e., low-risk pregnant women) used in previous studies might have obscured the possible role of cortisol in the association between prenatal psychological stress and infant outcomes (Zijlmans et al., 2015). To address this issue, the current study aims to investigate hair cortisol in pregnant women exposed to the COVID-19 crisis and its associations with psychological stress and infant temperament. The COVID-19 crisis was chosen because it led to heightened stress in the majority of the population, including low-risk pregnant women (e.g., Lebel et al., 2020; Vacaru et al., 2021;). Because the COVID-19 pandemic is considered a chronic stressor, hair cortisol was chosen as a biomarker of maternal prenatal psychological stress capturing cortisol secretion over longer periods (Kirschbaum et al., 2009). The focus on infant temperament is due to 1) its associations with prenatal psychological stress (van den Bergh et al., 2017) and 2) having shown to be an early indicator for later psychological functioning (Rothbart et al., 2000; Gartstein et al., 2012).

Heightened levels of maternal cortisol are hypothesized to impact the intrauterine environment and affect the infant's developing neural system, with implications for later behavioral developmental outcomes (Bale et al., 2010; Bock et al., 2015; van den Bergh et al., 2018). However, psychological stress has often been found only weakly or not correlated with cortisol concentrations during pregnancy (Davis and Sandman, 2012; Werner et al., 2013; van den Heuvel et al., 2018). Cortisol is frequently measured with momentary saliva or blood samples (Zijlmans et al., 2015), reflecting acute stress and this may partly explain these weak links. In contrast, especially high and continuous levels of stress (i.e., chronic stress) are suggested to affect the developing infant (Graignic-Philippe et al., 2014). As such, hair cortisol, as a biomarker for chronic stress, would be more suitable to measure long-term and retrospective HPA axis activity (Kirschbaum et al., 2009; D'Anna--Hernandez et al., 2011). Consequently, the methodological advantages of using hair cortisol (i.e. its chronic and retrospective nature) may address some of the limitations of the previously used cortisol measures. Yet, results for hair cortisol are mixed as well (Mustonen et al., 2018). Some studies have shown that hair cortisol is positively linked to prenatal psychological stress (Kalra et al., 2007; Hoffman et al., 2016) and to maternal emotion dysregulation (albeit not to episodic and chronic stress; Conradt et al., 2020), yet others did not replicate these findings (Kramer et al., 2009; Galbally et al., 2019; Orta et al., 2019). To date, only one study investigated the associations between maternal prenatal cortisol, when measured in hair, and infant temperament (Enlow et al., 2017), revealing that maternal prenatal hair cortisol was negatively correlated with infant rate of recovery from distress. Another explanation often proposed for the weak links between psychological stress and cortisol measurements during pregnancy is that low-risk samples were used in previous studies, with stress levels that were too low to find associations (Zijlmans et al., 2015). In this study, we therefore use hair cortisol as a measure of cortisol concentration in a sample of highly stressed pregnant women during a worldwide pandemic.

Studies that assess high prenatal stress due to exposure to disasters (e.g., ice storm; King and Laplante, 2005) or severe trauma (e.g., 9/11; Yehuda et al., 2005), can be seen as natural experiments in which all participants experience the same independent stressor (King et al., 2012). Although there is some evidence for an association between elevated maternal psychological stress levels, as caused by exposure to a disaster, and difficult infant temperament, these studies missed biological measures (Laplante et al., 2016; Zhang et al., 2018). The worldwide COVID-19 outbreak is a unique disaster situation different from previously studied disasters, with high uncertainty and social distancing policies. The crisis induced heightened stress levels in pregnant women (Lebel et al., 2020; Gustafsson et al., 2021; Perzow et al., 2021; Vacaru et al., 2021). COVID-19-related psychological stress during pregnancy included worries about altered social support (e.g., not seeing friends and family), work or finances (e.g., losing one's job), and general COVID-19-related aspects (e.g., getting sick; Vacaru et al., 2021). The current study examined two preregistered associations between these three types of COVID-19 worries and hair cortisol concentrations during pregnancy.

The current study tested two preregistered associations: 1) between COVID-19-related prenatal psychological stress levels (social support-, work- and general COVID-19-related worries) and prenatal hair cortisol, and 2) between prenatal hair cortisol during the COVID-19 outbreak and infant temperament (i.e. negative affectivity and orienting/regulation). We hypothesized that higher concentrations of prenatal hair cortisol during the COVID-19 outbreak were associated with 1) higher maternal psychological stress and 2) a more difficult infant temperament, specifically higher levels of negative affectivity and lower levels of orienting/regulation. In addition, two potential moderators were studied for hypothesis 1. Because of a lack of previous studies on these moderators, these analyses were carried out in an exploratory fashion. First, pregnant women might be differently impacted by the outbreak depending on their SES. On the one hand, low SES has been observed to be a risk factor for elevated stress during the COVID-19 crisis, due to lower financial and social resources (Berthelot et al., 2020). On the other hand, women with low SES have been found to be physiologically more resilient to an uncontrollable stressor due to already having experienced or experiencing more stress and adversity throughout their daily life (Glazier et al., 2004; Goyal et al., 2010). This previously experienced adversity can result in blunted cortisol (re)activity (Desantis et al., 2015; Khoury et al., 2019; Misiak et al., 2022). Second, maternal psychological stress and hair cortisol concentrations may be different at different stages of pregnancy as cortisol is known to increase over the course of gestation (de Weerth and Buitelaar, 2005), and pregnant women progressively exhibit dampened cortisol stress reactivity (Kammerer et al., 2002; Buss et al., 2009). Therefore, we will study the potential moderating roles of maternal SES and gestational age on the association between COVID-19-related psychological stress and hair cortisol during pregnancy. The results of our study may aid our understanding of the possible impact of the COVID-19 crisis on the next (unborn) generation.

## 2. Methods

### 2.1. Participants

This study is part of the international COPE study (COVID-19 and Perinatal Experiences) and the CovGen Alliance (http://covgen.org). The COPE study longitudinally followed COVID-19 related experiences of women and men attempting to conceive, expecting a child or with an infant younger than 6 months. Materials and procedures for the international COPE study were preregistered and shared on the Open Science Framework (https://osf.io/uqhcv/). Additionally, the two main hypotheses and statistical analyses were pre-registered on the Open Science Framework (https://osf.io/bs45c/). The first wave of data was collected during the first COVID-19- lockdown in the Netherlands (April-May 2020). This lockdown entailed the closing of schools, restaurants, sports centers, and daycare. Stores were still open, but people were strongly advised to stay at home, work remotely and practice social distancing.

Pregnant participants of the COPE study (N = 1421) were recruited through social media (60%), word of mouth (15%), midwifery centers (14%), and others (11%). A subsample was contacted again in the summer of 2020 for the collection of hair samples. Due to time constraints related to lockdown restrictions before July and washout effects of hair cortisol concentrations after September (Kirschbaum et al., 2009), there was a short time window to collect these data. Therefore, we scheduled home visits that could be geographically combined to reduce driving time. These took place in different regions in the Netherlands in August and September 2020 until reaching a convenience sample of 100 participants. These participants were all pregnant during the COVID-19 outbreak and when filling in the lockdown questionnaire. We compared our subsample with the total sample of 1421 pregnant women with Welch's t-tests and found no significant difference in SES between the groups ($t$ (129.99) = −1.68, $p$ = .09). However, our subsample had lower work ($t$ (100.93) = 2.08, $p$ < .05) and COVID-19 related worries ($t$ (82.08) = 2.41, $p$ < .05) but not social support-related worries ($t$ (103.97) = 1.14, $p$ = .26).

Of the 100 participants, 3 participants had to be excluded. Exclusion criteria were excessively high hair cortisol concentrations (surpassing known prenatal hair cortisol reference ranges, see Marceau et al., 2020) due to glucocorticoid medication use ($n$ = 1), and participants being less than 6 weeks pregnant during the first online COVID-19 outbreak questionnaire ($n$ = 2). These last participants were excluded because they were a small group and their COVID-19-related worries were expected to be different from those of women further along in pregnancy. This resulted in a final sample of 97 participants. At first contact the mothers had an average age of 31.89 years (SD = 3.43; range = 22 – 41) and were on average 26.44 weeks pregnant (SD = 8.40; range = 7.43 – 40.86).

Participation in the study was voluntary and participants were provided with information about data collection and -storage, before giving informed consent. The online study was approved by The Ethics Review Board of Tilburg University (RP2019–143). The collection of biological materials, such as the hair samples, was separately approved by The Ethics Committee Faculty of Social Sciences of Radboud University (ECSW-2020–056).

### 2.2. Procedure

Psychological stress was assessed through an online questionnaire during the first COVID-19 lockdown in the Netherlands (April 4 - May 10, 2020). After giving informed consent, participants answered questions about their current COVID-19 situation, including questions about experienced worries due to COVID-19. The complete questionnaire took 30–45 min for participants to fill in. Participants received 10 euros for completing the questionnaire.

Around 6 months after the start of the COVID-19 crisis (i.e., in August and September 2020), maternal hair was collected during a home visit by a trained researcher. A hair sample of around 100–150 hairs was cut close to the scalp. Each sample was wrapped in aluminum foil and stored at room temperature. In October 2020, all hair samples were sent to the Dresden LAB Service, Germany to determine the cortisol concentrations using a column-switching LC-APCI-MS/MS method. Participants were compensated with 5 euros and a small present for their participation in the home visits. When infants were 6 months old, mothers filled in another online questionnaire about maternal wellbeing and infant development, including temperament. Filling in the questionnaire took around 30 min and mothers received 10 euros reimbursement.

### 2.3. Measures

Internal consistency of the questionnaires was assessed using Revelle's omega total ($\omega_t$; McDonald, 2013). Omega as a measure of internal consistency is especially useful for questionnaires with items varying in how strongly they relate to the construct being measured and thus in which tau equivalence is not assumed (McNeish, 2018).

#### 2.3.1. Psychological stress

Maternal prenatal COVID-19-related stress was measured with the COVID-19 and Perinatal Experiences Questionnaire (COPE questionnaire, Thomason et al., 2020). This questionnaire was created to assess the impact of the COVID-19 pandemic, such as COVID-19-related symptoms, restrictions on daily life, social support, and experienced stress. The questionnaire consists of 8 slider-questions ranging from 0.0 to 10.0. A higher score on an item indicated higher psychological stress. From these 8 items, 3 latent variables were identified by a factor analysis and principal component analysis (PCA; Vacaru et al., 2021). These variables were deemed to represent the major types of COVID-19 worries in this population and were hence chosen as the psychological stress measures in our study. The three identified factors were: 1) work and financial-related worries (worries related to current and future work and financial situation), 2) social support-related worries (worries related to changes in partner and social support), and 3) general COVID-19 related worries (worries of COVID-19 symptoms for oneself and others, caring for family, and general worries related to the COVID-19 outbreak). The components were calculated by averaging the respective items, but only when there was one or no missing values on the items. Omega for the current study population was.92 for work-related worries,.64 for social support-related worries, and.87 for general COVID-19-related worries (see Vacaru et al., 2021 for the questionnaire items and detailed PCA description).

#### 2.3.2. Hair cortisol

The hair samples were split into 3 segments of 3 cm each, as measured from the scalp (see Fig. 1). The first segment (furthest from the scalp) reflects the cortisol secretion from December 2019 to February 2020 (pre-COVID-19), the second segment reflects cortisol secretion from March to May 2020 (first COVID-19 lockdown), and the third segment reflects the cortisol from June to August 2020 (post-COVID-19 lockdown). For the analyses, we only used the first (pre-COVID-19) and second (COVID-19 lockdown) hair cortisol segment. To control for potential washout effects on the cortisol of the oldest segments (Kirschbaum et al., 2009), and following the assumption that the washout effect occurs for everyone, we exploratorily assessed the individual change by controlling for pre-COVID-19 hair in our hierarchical model. During the collection of the hair samples, 42.3% of the women were pregnant. Because women were at different stages of pregnancy when filling out the COVID-19 questionnaire, we controlled for gestational age in the analyses.

#### 2.3.3. Infant temperament

Infant temperament was measured with the Dutch version of the Infant Behavior Questionnaire-Revised Short (IBQ-R Short; Gartstein and Rothbart, 2003), which is a reliable and valid method of measuring infant temperament (Gartstein and Marmion, 2008). The IBQ-R has 91 items on a 7-point Likert scale. For this study, we assessed the temperamental factors of infant Negative Affectivity and infant Orienting/-Regulation. Negative Affectivity consisted of the scales Sadness, Distress to Limitations, Fear, and Falling Reactivity/Rate of Recovery from Distress (reversed). Higher scores on this factor mean more instances of fear, sadness, distress, anger, and discomfort. Orienting/Regulation, consisted of the scales Low Intensity Pleasure, Cuddliness, Duration of Orienting, and Soothability. Higher scores on this factor mean infants are harder to soothe and display less attentional orienting behavior. Omega of both orienting/regulation (0.88) and negative affectivity (0.93) was excellent.

#### 2.3.4. Moderators

Maternal socioeconomic status (SES) was assessed by calculating a composite score of the standardized values of maternal education (highest achieved education) and annual household income (total estimated yearly income; one missing allowed). Gestational age in weeks was reported when filling out the COPE Questionnaire during the first COVID-19 outbreak and was linked to the corresponding COVID-19 lockdown hair cortisol segment (March-May 2020 period).

#### 2.3.5. Covariates

During the home visit, participants filled in a questionnaire that included four potential hair cortisol confounders: hair product use, frequency of hair washing, heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening), and glucocorticoid use of the past 3 months (e.g., tablets, inhalation, cream, injections). For the temperament analyses infant sex was used as a covariate.

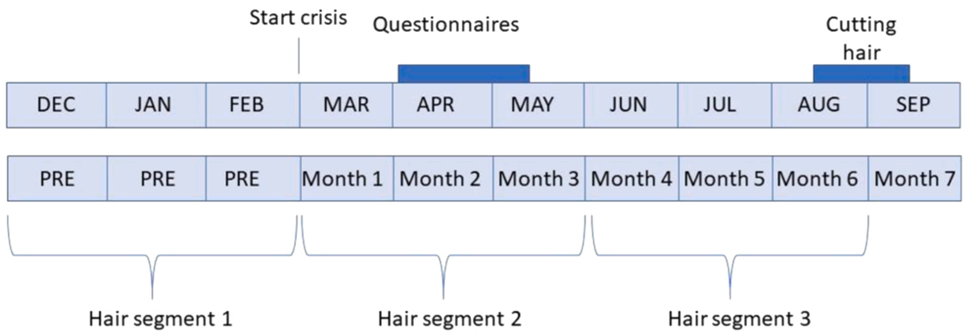


**Fig. 1.** Timeline of the 9 cm hair strand and COVID-19 outbreak.

### 2.4. Missing data

For the first hypothesis, of the 97 participants three had missingness on work-related worries, and one on social support-related worries, resulting in a remaining sample of 93 participants. For the second hypothesis, 20 participants did not provide complete data for the temperament measurement at 6 months postnatal follow-up. Of the remaining 77 participants, four participants did not fill out infant sex. As a result, the sample consisted of 73 participants for hypothesis 2. Lastly, there were 3 missing values of hair cortisol pre-COVID-19 outbreak, due to the undetectability of cortisol levels in the hair sample.

A statistical sensitivity power analysis performed in G*Power showed that with 73 participants (minimum number of participants in the analyses with hair cortisol concentrations and infant temperament), a significance level of.05, we have enough power (0.80) to detect an effect of.29 (small to moderate effect size).

### 2.5. Statistical analyses

First, all study variables were checked for outliers (greater or smaller than 3 SD from the mean). Two outliers were detected: hair cortisol COVID-19 outbreak (N = 1), hair cortisol pre-COVID-19 outbreak (N = 1). These outliers were winsorized (i.e., replaced by a value of 3 SD from the mean). The potential hair cortisol covariates were tested with multiple one-way ANOVAs. When significant, they were added as a covariate, as based on a recent hair cortisol meta-analysis (Marceau et al., 2020).

For the main analyses, hierarchical regression was performed. For hypothesis 1, we conducted a regression with maternal psychological stress during pregnancy as independent variable and prenatal hair cortisol concentrations during the COVID-19 outbreak as dependent variable. The covariates were added in step 1 and the three subscales of COVID-19 psychological stress were added in step 2. For hypothesis 2, two regression models were computed (i.e., one with infant temperamental negative affectivity as dependent variable and one with temperamental orienting/regulation as dependent variable). The covariates were added in step 1, and the hair cortisol concentrations were added in step 2. The exploratory analyses consisted of interactions of SES and the three subscales of psychological stress, and gestational age and the three subscales of psychological stress, on maternal hair cortisol. Finally, to check the cortisol stress response to the unexpected COVID-19 outbreak we reran the three hierarchical regression models of our main analyses with a relative difference score of pre-COVID-19 and COVID-19 outbreak cortisol concentrations, by additionally controlling for pre-COVID-19 hair cortisol concentrations in step 1. Potentially, not the absolute hair cortisol levels during the lockdown but increases in cortisol from pre-lockdown to lockdown, are a better representation of maternal stress. Because there are no studies yet on prenatal hair cortisol difference scores, these analyses were exploratory. All analyses were performed in R (R Core Team, 2019).

## 3. Results

### 3.1. Descriptive analyses

Descriptive statistics are shown in Table 1. Correlations between the study variables are presented in Table 2. Hair cortisol during the COVID-19 outbreak was not correlated to work-, social support- and general COVID-19-related worries. Moreover, hair cortisol during the COVID-19 outbreak was not correlated to infant negative affectivity and orienting/regulation.

The hair cortisol covariates analysis showed that hair cortisol was significantly correlated to heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening; $F(2,94) = 4.09$, $p < .05$). Therefore, the variable hair treatment was used as a covariate in all main analyses. The other three hair cortisol covariates were not significantly associated with hair cortisol concentrations: frequency of hair washing, ($F(5, 91) = 1.40$ $p$=.23), glucocorticoid use of the past 3

**Table 1**
Demographic characteristics ($N = 97$).

| | *M (SD)* / % (N) | Range |
|---|---|---|
| Infant sex (% boys) | 53.93% | |
| Parity (nulliparous) | 38.14% | |
| Hair treatment use | 39.18% | |
| Hair product use | 23.96% | |
| Glucocorticoid use | 18.56% | |
| Hair washing per week (4)<1/1-2/3-4/>) | 4/51/34/11% | |
| Dutch background | 97% | |
| **Gestational age** (in weeks, April-May 2020) | 26.44 (8.40) | 7.43 – 40.86 |
| 1st trimester | 8.25% (8) | |
| 2nd trimester | 42.27% (41) | |
| 3rd trimester | 49.48% (48) | |
| **Education** | | |
| Low | 23.71% | |
| High | 76.29% | |
| **Annual household income*** | | |
| <€40,000 | 18.39% | |
| €40,000 to €100,000 | 65.52% | |
| > €100,000 | 16.09% | |
| Pre-COVID-19 hair cortisol | 2.16 (2.63) | 0.12 – 17.59 |
| COVID-19 hair cortisol | 3.70 (3.96) | 0.06 – 17.84 |
| Work Worries | 4.08 (2.70) | 0 – 9.5 |
| Social Support Worries | 2.63 (2.41) | 0 – 8.65 |
| General COVID-19 Worries | 5.34 (2.08) | 0 – 9.1 |
| Infant Negative Affectivity | 2.71 (0.74) | 1.35 – 4.90 |
| Infant Orienting/ Regulation | 5.45 (0.51) | 4.33 – 6.46 |

*Note.* Raw hair cortisol values (pg/mg) before log transformation, hair treatment use = heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening), hair product use = temporary products present in hair at the moment (e.g., hair spray, mousse, wax), hair washing per week divided in categories: less than once a week, one to two times a week, 3–4 times a week and more than 4 times a week. Education = low: secondary education or vocational education, high: bachelor or master's degree or higher (i.e. PhD). $N$ = total sample, $M$ = mean, $SD$ = standard deviation. * 10% preferred not to answer the item on annual household income.

**Table 2**
Pearson Correlation Matrix among all variables in the study ($N = 97$).

| | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. | 9. | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. COVID-19 hair cortisol | – | .08 | .07 | -0.02 | .06 | -0.17 | -0.01 | .00 | .10 | -0.10 |
| 2. Difference hair cortisol | | – | -0.05 | -0.11 | -0.08 | -0.14 | -0.02 | .21 * | -0.02 | .01 |
| 3. Work Worries | | | – | .43 * ** | .62 * ** | .09 | .05 | -0.06 | -0.16 | -0.21 * |
| 4. Social Support Worries | | | | – | .48 * ** | .06 | -0.03 | .21 * | -0.11 | .04 |
| 5. COVID-19 Worries | | | | | – | .06 | .01 | -0.05 | -0.01 | -0.33 * ** |
| 6. Negative Affectivity | | | | | | – | -0.41 * ** | -0.12 | -0.09 | .07 |
| 7. Orienting/Regulation | | | | | | | – | .10 | .09 | -0.11 |
| 8. Gestational age | | | | | | | | – | -0.13 | -0.04 |
| 9. SES | | | | | | | | | – | .04 |
| 10. Infant sex | | | | | | | | | | – |

*Note.* * ** <0.001 * * < 0.01 * <0.05. Log transformed COVID-19 cortisol, difference hair cortisol = difference score of log transformed pre- and COVID-19 hair cortisol, SES = Socioeconomic status, $N$ = total sample. Infant sex: boys = 1, girls = 2.

months, (e.g., tablets, inhalation, cream, injections; $F(2,94) = 0.34$, $p$=.72), and hair product use, ($F(2,94) = 3.05$, $p = .052$). We followed our preregistration by only including covariates with a $p$-value smaller than.05. However, due to the almost significant $p$-value of hair product use, we re-ran the main analyses with this variable included and found similar results. Log-transformations were performed on hair cortisol concentrations since both pre-COVID-19 and COVID-19 hair cortisol variables were positively skewed. After transformation, the distributions of the hair cortisol variables met the normality assumption.

### 3.2. Main analyses

Table 3 presents the results of the hierarchical regression models. Hair cortisol concentrations during the COVID-19 outbreak were significantly predicted by the covariates gestational age, SES, and hair treatment (Step 1). Adding the three maternal COVID-19 worries variables did not significantly improve the model. Adding COVID-19 outbreak hair cortisol in step 2 did not significantly improve the model. Temperamental orienting/regulation also showed no significant relations in both steps 1 and 2. Moreover, neither associations were moderated by infant sex (see supplementary materials).

### 3.3. Exploratory regression analyses

#### 3.3.1. Moderation by SES and gestational age

Hair cortisol during the COVID-19 outbreak was significantly predicted by the interaction between SES and work-related stress ($b$ =.05, $SE$=0.02, $p$=.019) and by the interaction between SES and social support-related stress ($b$ =.14, $SE$=0.07, $p$=.034). Fig. 2 present the simple slopes associated with these interactions as a function of SES (high SES= +1 SD, low SES= −1 SD). Post hoc analyses indicated that, while hair cortisol concentrations were positively associated with work-related worries for women with high SES ($b$=.06, $SE$=.03, $p$=.03), they were not associated with work-related worries for women with low SES ($b$= −0.02, $SE$=.02, $p$=.32). The interaction between SES and social support-related worries on hair cortisol concentrations was significant, but post hoc analyses showed non-significant slopes for both low SES ($b$= −0.04, $SE$=.03, $p$=.16) and high SES ($b$=.04, $SE$=.03, $p$=.23). The association between general COVID-related worries and outbreak hair cortisol was not moderated by SES ($b$ =.03, $SE$=0.03, $p$=.20). Furthermore, the interactions between gestational age and hair cortisol were not significant: work-related worries ($b$ = −0.00, $SE$=0.00, $p$=.86), social support-related worries ($b$ = −0.01, $SE$=0.01, $p$=.32), general COVID-19-related worries ($b$ = −0.00, $SE$=0.00, $p$=.30).

#### 3.3.2. Difference score for pre-and outbreak COVID-19 hair cortisol

Table 4 summarizes that the hair cortisol difference scores from pre-COVID-19 outbreak to post COVID-19 outbreak were not significantly related to psychological COVID-19-related stress (work-, social support-, COVID-19-related worries), nor to infant negative affectivity or orienting/regulation.

**Table 3**
Results from three main Hierarchical Regression Models of COVID-19 hair cortisol on prenatal worries and two subscales of infant temperament on COVID-19 hair cortisol.

| Variables | | β | $R^2$-model | $R^2$ change | p-value |
|---|---|---|---|---|---|
| **COVID-19 outbreak hair cortisol** | | | | | |
| Step 1 | | | .12 | .12 | .02 * |
| | SES | .09 | | | .10 |
| | Hair treatment | -1.38 | | | .00 * * |
| | Gestational age | .00 | | | .40 |
| Step 2 | | | .13 | .01 | .78 |
| | Work Worries | .01 | | | .63 |
| | Social Support Worries | -0.02 | | | .43 |
| | COVID-19 Worries | .01 | | | .64 |
| **Infant negative affectivity** | | | | | |
| Step 1 | | | .05 | .05 | .57 |
| | SES | -0.08 | | | .43 |
| | Hair treatment | -0.21 | | | .29 |
| | Gestational age | -0.01 | | | .46 |
| | Infant sex | .01 | | | .94 |
| Step 2 | | | .08 | .03 | .14 |
| | COVID-19 Outbreak hair cortisol | -0.30 | | | |
| **Infant orienting/regulation** | | | | | |
| Step 1 | | | .03 | .03 | .89 |
| | SES | .04 | | | .62 |
| | Hair treatment | .05 | | | .75 |
| | Gestational age | .00 | | | .66 |
| | Infant sex | -0.11 | | | .39 |
| Step 2 | | | .03 | .00 | .82 |
| | COVID-19 Outbreak hair cortisol | -0.03 | | | |

*Note.* β = standardized regression coefficient, $R^2$-model= total explained variance by the model, $R^2$ change = partial explained variance by added predictors (step 2), $p$-value =significance level set at < 0.05. * ** <0.001 * * < 0.01 * <0.05. Log transformed COVID-19 cortisol, SES = socioeconomical status, hair treatment use = heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening).

## 4. Discussion

This study examined maternal prenatal hair cortisol concentrations during the COVID-19 outbreak and its association with maternal psychological stress during pregnancy and infant temperament at 6 months of age. The analyses did not reveal the expected association between COVID-19-related prenatal psychological stress and hair cortisol levels.

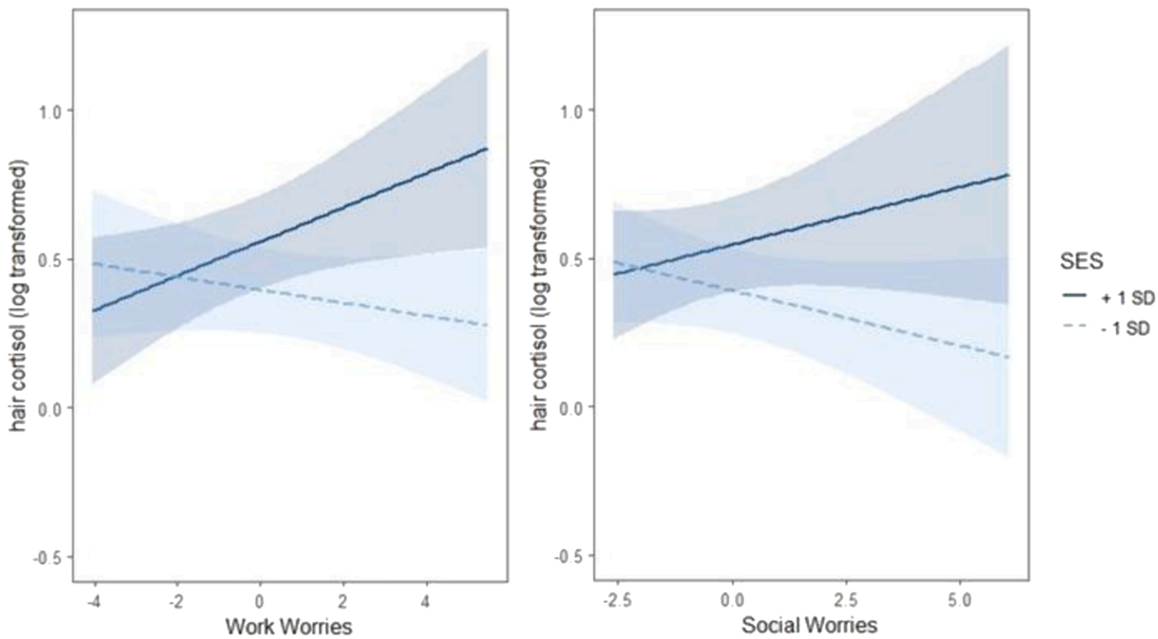


**Fig. 2.** Moderation of socioeconomic status (SES) on hair cortisol concentrations and work- and social support-related worries.

However, SES moderated this association with women with higher SES showing a positive association between work- and social support-related worries and hair cortisol during the COVID-19 outbreak, and women with lower SES levels not showing these associations. Our results showed no association between maternal prenatal hair cortisol during the COVID-19 outbreak and infant temperamental negative affectivity and orienting/regulation. Lastly, the explored difference between pre-COVID-19 and COVID-19 outbreak hair cortisol was not associated with maternal psychological stress or infant temperament.

The findings of the current study do not support those of studies reporting positive associations between hair cortisol concentrations and psychological stress during pregnancy (Kalra et al., 2007; Hoffman et al., 2016), or between hair cortisol concentrations and emotion dysregulation during pregnancy (Conradt et al., 2020). However, our results are in line with those of multiple other studies that do not find associations between hair cortisol and psychological stress during pregnancy (Kramer et al., 2009; Galbally et al., 2019; Orta et al., 2019; Conradt et al., 2020). In general, these studies, as well as our current study, used psychological stress measures that reflected daily, short-term disturbances in stress, while hair cortisol is thought to reflect more chronic cortisol secretion (Mustonen et al., 2018). Therefore, hair cortisol may be only associated with more chronic psychological stress. To test this hypothesis, studies are needed that require filling out questionnaires multiple times during the corresponding period of the hair cortisol segment.

Another explanation for the absence of a direct association is that it may not be present for the whole sample, but only for a subsample of participants. Indeed, we found that SES was a moderator, with women with a high SES showing a positive association between work- and social support-related worries and hair cortisol during the COVID-19 outbreak. Pregnant women with lower SES levels may have been differently impacted by the COVID-19 crisis than women with higher SES levels. Pregnant women with lower SES, in general, may already experience more stress (Glazier et al., 2004; Goyal et al., 2010). Therefore, an environmental stressor, such as the COVID-19 crisis, might affect them less physiologically. As a history of exposure to adversity and stress is hypothesized to result in altered HPA axis functioning with overall lower cortisol levels and lower reactivity to stressors (Desantis et al., 2015; Khoury et al., 2019; Misiak et al., 2022), this could explain the lack of an association between psychological stress and hair cortisol concentrations in pregnant low SES women. In contrast, high SES women might be expected to display a more typical physiological reaction of the HPA axis, with higher cortisol levels when experiencing heightened levels of daily stress. With the current study indicating a differential impact of the COVID-19 crisis on high and low SES groups, future research on prenatal exposure to stressors or life events should consider examining the role of SES in relation to hair cortisol.

We did not find support for our second hypothesis, that prenatal hair cortisol concentrations during the COVID-19 outbreak would be related to later infant temperament. There is little evidence for the mediating role of maternal cortisol as measured in saliva or blood in the link between maternal prenatal psychological stress and infant temperament (Zijlmans et al., 2015). Therefore, we used hair cortisol as an alternative measure of maternal cortisol that is better related to chronic stress, such as that produced by a natural disaster. However, in the current study hair cortisol concentrations during the first COVID-19 lockdown were still unrelated to maternal reports of psychological stress levels and infant temperament. Potentially, other infant outcomes such as infant health might be predicted by maternal hair cortisol concentrations during the COVID-19 outbreak and this should be further investigated. In the past, associations between maternal prenatal reported stress, cortisol and infant health, have been observed (Beijers et al., 2010; Zijlmans et al., 2017).

We additionally explored the cortisol response to the COVID-19 outbreak by examining the difference in hair cortisol levels between the pre-COVID-19 sample and the COVID-19 sample. This hair cortisol difference was not related to COVID-19-related worries and infant temperament. This exploratory analysis was based on previous studies assessing prenatal saliva cortisol changes over time. For example, a steep or blunted cortisol increase during pregnancy has been found to be associated with maternal stress, anxiety, or depression, while independent cortisol assessments were not (Kane et al., 2014). Possibly, we found no associations in the current study due to the combination of a small sample size and participants differing in gestational age. We controlled for gestational age at the time of filling in the questionnaires in the analyses, but variation in gestational age may have added noise to the natural cortisol increase during gestation (de Weerth and Buitelaar, 2005; van den Heuvel et al., 2018). Future studies with larger samples may give more power to distinguish subtle changes in chronic hair cortisol measures due to maternal psychological stress from the natural

**Table 4**
Results from 3 exploratory Hierarchical Regression Models of difference in pre-COVID and COVID-19 hair cortisol on prenatal worries and 2 subscales of infant temperament on COVID-19 hair cortisol.

| Variables | | β | $R^2$-model | $R^2$ change | *p*-value |
|---|---|---|---|---|---|
| **Work, social support, COVID-19 related stress** | | | | | |
| Step 1 | | | .03 | .02 | .55 |
| | Pre-COVID-19 cortisol | 1.11 | | | .43 |
| | SES | -1.12 | | | .15 |
| | Hair treatment | 1.38 | | | .66 |
| | Gestational age | .01 | | | .62 |
| Step 2 | | | .04 | .00 | .41 |
| | COVID-19 Outbreak hair cortisol | -2.39 | | | |
| **Infant Negative Affectivity** | | | | | |
| Step 1 | | | .07 | .07 | .44 |
| | Pre-COVID-19 cortisol | -0.18 | | | .38 |
| | SES | -0.08 | | | .43 |
| | Hair treatment | -0.27 | | | .20 |
| | Gestational age | -0.01 | | | .48 |
| | Infant sex | | | | .69 |
| Step 2 | | | .09 | .02 | .28 |
| | COVID-19 Outbreak hair cortisol | -0.41 | | | |
| **Infant Orienting/Regulation** | | | | | |
| Step 1 | | | .03 | .03 | .86 |
| | Pre-COVID-19 cortisol | .03 | | | .83 |
| | SES | .02 | | | .73 |
| | Hair treatment | .07 | | | .64 |
| | Gestational age | .00 | | | .86 |
| | Infant sex | -0.13 | | | .29 |
| Step 2 | | | .03 | .00 | .76 |
| | COVID-19 Outbreak hair cortisol | -0.08 | | | |

*Note.* M=mean, SD=standard deviation, β = standardized regression coefficient, $R^2$-model= total explained variance by the model, $R^2$ change = partial explained variance by added predictors (step 2), p-value =significance level set at < 0.05. * <0.05 * * < 0.001. Log transformed COVID-19 hair cortisol, SES = socioeconomical status, hair treatment use = heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening).

rise in cortisol over pregnancy.

This study has several strengths, such as the combination of physiological and behavioral measures, the longitudinal design, and the use of a pandemic as a global chronic stressor that induced heightened reported stress levels in pregnant women. In a previous study on the same cohort of pregnant women (N = 1421), we showed that, compared to a pre-COVID-19 pregnant sample, scores above the clinical cutoff for both depression and anxiety doubled during the COVID-19 outbreak (see Vacaru et al., 2021). A post-hoc analysis showed that our subsample of women providing hair cortisol also reported higher levels of clinically relevant depression (9% versus 6% pre-pandemic) and anxiety (40% versus 24% pre-pandemic).

However, limitations also need to be mentioned. With respect to hair cortisol, the maternal hair sample was cut in 3 × 3 cm segments, which implies that there may have been a washout effect for the pre-COVID-19 hair segment. The cortisol concentrations of the oldest hair segment are generally lower than those of newer hair segments (Kirschbaum et al., 2009). Although longer lengths of hair samples have been used and validated (Manenschijn et al., 2011), authors caution against using segments beyond the first 6 cm from the scalp because of cortisol washout (Kirschbaum et al., 2009; Orta et al., 2018). We took the washout effect into account by focusing on within-person cortisol changes, and by assuming that the washout effect occurs similarly for everyone. However, it has to be noted that, although visiting participants multiple times for hair samples collection would not have been feasible due to the then-applicable COVID-19 lockdown restrictions (e.g., social distancing), future studies should consider collecting hair segments at multiple time points, hence allowing the use of 'younger' segments (Orta et al., 2018). Moreover each hair cortisol segment reflected three months of cortisol exposure. This might have contributed to finding no associations with the difference between the pre-COVID-19 and COVID-19 samples. To assess hair cortisol trajectories, such as the impact of the COVID-19 outbreak, it could be useful to divide hair samples into smaller segments (i.e., 1 cm), to allow for more accurate measures of individual cortisol change over time.

With respect to reported worries, our subsample had lower work- and COVID-19-related worries, than the total sample of pregnant women (N = 1421), suggesting that our participants may have possibly been less representative. Accordingly, it can be argued that this sample may not have been ideal in addressing the question of whether the severity of one's stress affects how hair cortisol is related to a psychological stressor. A pre-COVID-19 control group could have been more optimal for answering this question. However, distress levels in the total sample of pregnant women were significantly higher than those of pre-COVID-19 pregnant women (Vacaru et al., 2021). In a post-hoc analysis, we assessed the distress levels in our COVID-19 subsample of women that provided hair samples (N = 97), and also found elevated percentages of women experiencing clinically relevant depression and anxiety (i.e. 9% and 40%, respectively). Moreover, the variance and ranges for the worries variables indicated that some women in our sample were extremely worried. In sum, the COVID-19 outbreak was a global stressor that apparently affected our sample psychologically in such a way that it need not be considered a low-risk participant sample that is inadequate for answering our research question (Zijlmans et al., 2015).

Next, the factors social support and work-related worries only consisted of two items, which goes against the general recommendation of a minimum of three items per factor (MacCallum et al., 1999). However, both these factors were found to predict anxiety and depressive symptoms in pregnancy (Vacaru et al., 2021) as well as insensitive parenting practices (van den Heuvel et al., 2022), which increases our confidence in their value. Nonetheless, we advise future researchers to use more items to assess maternal worries when possible. Additionally, our sample showed some variation in SES and educational level (i.e. 24% of the participants had completed lower education and 18% reported a low household income), but this was not representative of the Dutch population, thus limiting the generalizability of our results. Lastly, we used a mother-reported measure of temperament, which despite providing a knowledgeable and broad view of the child's temperament, is also subject to potential reporter bias. The use of objective behavioral measures could be an alternative for future research.

In sum, though the COVID-19 crisis affected the majority of society, the current study found no evidence for general associations between maternal prenatal psychological stress, hair cortisol concentrations, and infant temperament. However, evidence was found that COVID-19 psychological stress might have physiologically impacted pregnant women with higher SES differently than pregnant women with lower SES. Women with higher SES showed higher hair cortisol when they had more psychological stress. These results warrant further research into the mechanisms through which certain subgroups of women may be more vulnerable to chronic pregnancy stressors than others.

## Declaration of Competing Interest

The authors hereby declare no conflicts of interest.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105863.

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