

# Parity-related variation in cortisol concentrations in hair during pregnancy

I Marteinsdottir,<sup>a</sup> G Sydsjö,<sup>b</sup> Å Faresjö,<sup>c</sup> E Theodorsson,<sup>d</sup> A Josefsson<sup>b</sup> 

<sup>a</sup> Department of Medicine and Optometry, Faculty of Health and Life Sciences, Linnaeus University, Kalmar, Sweden <sup>b</sup> Faculty of Medicine and Health Sciences, Department of Obstetrics and Gynaecology in Linköping and Division of Children's and Women's Health, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden <sup>c</sup> Division of Society and Health, Department of Health, Medicine and Caring Sciences, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden <sup>d</sup> Division of Clinical Chemistry, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden

Correspondence: A Josefsson, Department of Obstetrics and Gynaecology, University Hospital, 581 85 Linköping, Sweden. Email: ann.josefsson@liu.se

Accepted 23 September 2020. Published Online 25 October 2020.

**Objective** To investigate hair cortisol concentrations (HCC) monthly in pregnant women and to explore the effect of parity.

**Design** Prospective cohort study from gestational week (GW) 26, at childbirth and postpartum.

**Setting** An antenatal care clinic in southeast Sweden.

**Sample** 390 pregnant women.

**Methods** Cortisol was measured using radioimmunoassay in methanol extracts of ground hair samples.

**Main outcome measures** Hair cortisol concentrations.

**Results** Both primi- and multiparae exhibited an increase in HCC throughout pregnancy. Primiparae had significantly higher HCC in the latter part of the last trimester compared with multiparae (1 month  $P = 0.003$ , 2 months  $P = 0.038$ ). The use of psychotropic medication in the first trimester correlated to HCC postpartum ( $P < 0.001$ ). HCC in GW 14–17 was associated with HCC in GW 18–21 (primiparae and multiparae,  $P < 0.001$ ), GW 22–25 (primiparae  $P = 0.036$ , multiparae  $P = 0.033$ ), and 2 months

postpartum (primiparae  $P = 0.049$ ). HCC in GW 18–21 was associated with GW 22–25 in both primiparae ( $P < 0.001$ ) and multiparae ( $P < 0.001$ ) as well as 2 months prior to childbirth among primiparae ( $<0.037$ ). In general, all estimates of HCC in pregnancy and postpartum showed a significant association between HCC for a specific month and the HCC in the previous month (all  $P < 0.001$ ), except for the association of HCC among primiparae in GW 22–25 and 3 months prior to childbirth.

**Conclusions** Increased cortisol concentrations in hair were observed during pregnancy, which decreased 3 months prior to childbirth in multiparae. The results indicate a quicker suppression of the hypothalamic CRH (corticotropin-releasing hormone) production by placenta CRH in multiparous women.

**Keywords** Cortisol, hair, hypothalamic-pituitary-adrenal pregnancy axis, women.

**Tweetable abstract** Multiparae have a quicker suppression of hypothalamic CRH production by placenta CRH during pregnancy compared to primiparae.

Please cite this paper as: Marteinsdottir I, Sydsjö G, Faresjö Å, Theodorsson E, Josefsson A. Parity-related variation in cortisol concentrations in hair during pregnancy. BJOG 2021;128:637–644.

## Introduction

The interest in the function of the hypothalamic-pituitary-adrenal (HPA) axis of pregnant women is growing, as evidenced by the evolving literature revealing the influence of maternal stress on the physical and mental health outcomes of offspring.<sup>1–5</sup>

However, the evidence regarding whether prenatal maternal cortisol levels are the sole or main mediating link is

conflicting.<sup>6</sup> It also seems that fetal vulnerability to high maternal cortisol levels varies across the gestational phases.<sup>4,6–8</sup>

Research regarding the impact of cortisol in the mediation of maternal stress upon the fetus is complicated by the fact that the HPA-axis activity shows substantial diurnal variability<sup>9</sup> in addition to individual differences. Single cortisol measures in plasma or saliva may therefore not sufficiently reflect the overall long-term biological activity of

cortisol. Analysis of hair cortisol concentrations (HCC) is used to overcome such limitations,<sup>10–12</sup> where 1 cm of hair corresponds to about 1 month's cortisol accumulation.<sup>13</sup> This method has been validated against cortisol in saliva across the pregnancy and postpartum periods and has proved to be a reliable metric of HPA activity, enabling estimation of integrated cortisol release.<sup>14</sup>

Reports on HCC throughout pregnancy have revealed varying results, probably due to the different analysis methods used.<sup>11,14–18</sup> The research focus has also been wide-ranging and has shown several factors of importance for HCC, including season, obesity<sup>11,19</sup> and delivery mode.<sup>11</sup> In addition, some<sup>18–19</sup> researchers have noted an association between psychosocial and lifetime stress exposure on HCC during pregnancy, whereas others<sup>20</sup> have concluded that neither psychological distress nor chronic stress or psychiatric symptoms had any impact.

Higher cortisol levels have been reported in primiparae versus multiparae,<sup>21–22</sup> probably related to more pregnancy distress.<sup>23</sup> However, Federenko et al. in 2006 could not detect any effect of parity on cortisol levels.<sup>22</sup>

Hair cortisol concentrations covering single-month periods may give more precise and more coherent results when investigating correlations to childbirth and neonatal outcomes, but to our knowledge no reports of measurements conducted on a monthly basis are available regarding HCC during pregnancy and postpartum. We hypothesised that there may be variations in cortisol hair levels during pregnancy and postpartum, possibly influenced by parity. Hence, the purpose of this study is to add to the knowledge of normal HPA axis functioning during pregnancy and postpartum by determining HCC levels on a monthly basis. A second aim was to explore further differences in HCC levels associated with parity during pregnancy.

## Methods

### Sample

The Swedish antenatal healthcare system is used by almost all pregnant women in the country. The antenatal and delivery care is free of charge. At the antenatal care clinics (ANC) healthy pregnant women are recommended to attend the regular antenatal programme with seven to nine visits to a midwife during pregnancy and, if needed, extra appointments with an obstetrician and/or with the midwife. Women attending the antenatal clinic at Värnamo Hospital, a small hospital in the south of Sweden, were asked to participate. They were given written and oral information about the study by their midwife at the first visit to the antenatal clinic around gestational week 12. At the next visit (around gestational week 26) those interested in participating signed an informed consent. A total of 953 women were enrolled at the antenatal clinic during the

study period. Of these, 740 women were approached and asked to participate in the study; 154 of these were excluded due to difficulties in understanding Swedish and 186 declined to participate (Figure S1). During the study period, nine women did not provide the study nurse with any hair samples and one woman changed her mind and asked to be removed from the study. Thus, 390 women were included in the study. Samples of hair were taken at around gestational week 26, at childbirth and finally at the postpartum check-up. The numbers of hair samples of sufficient quantity and quality to perform HCC analyses were: taken around gestational week 26 ( $n = 330$ ), at childbirth ( $n = 303$ ) and at the postpartum control ( $n = 311$ ) (Figure S1). The total sample was divided into primiparous women and multiparous women in most analyses. Demographic information as well as data on mental health and use of medications are presented in Table 1.

Sample size calculations were performed prior to the execution of the study with respect to the presence of depression and anxiety symptoms. Given a power of 80% and alpha of 5%, it was found that a total of 350 women were needed in the study. A post-hoc power calculation based on the number of participating women and mean cortisol levels among primiparous and multiparous women 1 month prior to childbirth revealed that the achieved power was 20% and thus underpowered.

### Patient involvement statement

At the time of the study no patients or patient organisations were involved.

### Hair cortisol measures

Hair cortisol concentrations (HCC) were expressed as pg/mg with a method developed in-house using a competitive radioimmunoassay on methanol extracts of pulverised hair. A hair sample approximately 3 mm thick and 3 cm long was cut close to the scalp from the posterior vertex area of the head. The hair samples were further cut into 1.25-cm lengths to reflect the cortisol accumulations for each month, based on an assumption of an average hair growth rate of 1 cm per month (13). The hair samples analysed in this study weighed between 5 and 6 mg. In the laboratory, each sample was put into a 2-ml QiaGenRB sample tube along with a 0.5 mm QiaGen (Germantown, MD, USA) stainless steel bead and weighed on a Sartorius MC (Sartorius Lab Instruments GmbH & Co, Goettingen, Germany) 210p microscale. The samples were put in specially made aluminium cylinders accommodating five 2-ml Eppendorf tubes and frozen in liquid nitrogen for 2 minutes. This was followed by mincing in a Retch Tissue Lyser II at 23 Hz for 2 minutes to produce a fine hair powder. The cortisol was extracted by adding 1 ml of methanol (Chromasolv, Sigma-Aldrich, St. Louis, MO, USA) to each tube and

**Table 1.** Socio-demographic data and characteristics for primi- and multiparas

|                        | Primipara |      | Multipara |      | P-value |
|------------------------|-----------|------|-----------|------|---------|
|                        | n = 125   | %    | n = 232   | %    |         |
| Age-groups             |           |      |           |      |         |
| 15–24 years            | 25        | 20.0 | 21        | 9.1  | <0.001  |
| 25–34 years            | 94        | 75.2 | 167       | 72.0 |         |
| 35–39 years            | 6         | 4.8  | 36        | 15.5 |         |
| 40 or older            | 0         | 0.0  | 8         | 3.4  |         |
| Civil status           |           |      |           |      |         |
| Married/co-habited     | 125       | 100  | 226       | 98.7 | 0.438   |
| Partner                | 0         | 0.0  | 2         | 0.9  |         |
| Single                 | 0         | 0.0  | 1         | 0.4  |         |
| Ethnicity              |           |      |           |      |         |
| Swedish                | 115       | 93.5 | 207       | 91.2 | 0.191   |
| European               | 8         | 6.5  | 14        | 6.2  |         |
| Non-European           | 0         | 0.0  | 6         | 2.6  |         |
| Education              |           |      |           |      |         |
| Primary school         | 2         | 1.3  | 3         | 1.6  | 0.055   |
| Secondary school       | 44        | 35.5 | 110       | 47.6 |         |
| University college     | 43        | 34.4 | 51        | 22.1 |         |
| University             | 36        | 28.8 | 67        | 29.0 |         |
| Employment             |           |      |           |      |         |
| Employee               | 85        | 68.0 | 146       | 62.7 | 0.314   |
| Self-employed          | 6         | 4.8  | 12        | 5.2  | 0.885   |
| Student                | 5         | 4.0  | 9         | 3.9  | 0.949   |
| Unemployed             | 5         | 4.0  | 9         | 3.9  | 0.949   |
| Long sick-leave        | 1         | 0.8  | 1         | 0.4  | 0.654   |
| Tobacco use            |           |      |           |      |         |
| Before pregnancy       | 48        | 20.9 | 2         | 20.8 | 0.988   |
| Until GW 25            | 12        | 5.2  | 1.6       | 1.6  | 0.094   |
| Medication until GW 25 |           |      |           |      |         |
| Steroids               | 3         | 9.4  | 12        | 16.9 | 0.316   |
| Psychotropic           | 4         | 12.5 | 12        | 16.9 | 0.568   |
| Analgesic              | 3         | 9.4  | 14        | 19.7 | 0.191   |
| Other                  | 27        | 84.4 | 45        | 64.3 | 0.039   |

GW, gestational week.

Other – folic acid, iron, proton-pump inhibitors, antihistamines, aspirin, thyroxine.

placing the tubes in a metal holder on a plate with a 5° inclination on a horizontal shaker at room temperature, keeping the steel beads in constant gentle motion within the tubes for a minimum of 10 hours. Finally, the tubes were centrifuged for 1 minute at 3000g at +4°C in a micro-centrifuge, Thermo Scientific Heraeus, Picotm & Frescotm 17/21, and 800 microlitres (Thermo Fisher Scientific Inc, Bothell, WA, USA) of the supernatant was moved to another plastic sample tube for lyophilisation in a Speed-Vac Plus SC210A (Savant, Thermo Fisher Scientific Edwards Ltd, Burgess Hill, UK) using an Edwards XDS 5 vacuum pump for at least 3 hours. The samples were dissolved in radioimmunoassay buffer and analysed as

described by Morelius et al.<sup>24</sup> A hair sample of 3–10 mg is needed to maintain a total inter-assay coefficient of variation below 8% for the combination of hair extraction and measurement of cortisol by the radioimmunoassay. The intra-assay coefficient of variation for the radioimmunoassay itself was 7% at 10 nmol/l. The antiserum cross-reacts 137% with 5 $\alpha$ -dihydroxycortisol, 35.9% with 21-deoxycortisol and 35.9% with prednisolone, but less than 1% with endogenous steroids (15).

### Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 26, IBM Inc., Armonk, NY, USA). The original HCC values were both divided into quintiles and also transformed into logarithm values. The associations between socio-demographic variables and parity as well as use of medications were tested by Pearson's Chi-square test. The medians of the original values were used as measures of central tendency and variation, respectively. Due to the non-normal distribution of the original HCC values, Spearman's correlation coefficient was used when determining whether cortisol levels were correlated with possible confounding variables. Multivariate linear regression analyses were based on logarithm-transformed values and were made to assess possible associations among the nine HCC measurements for each participant. A *P*-value of <0.05 (two-sided) was considered statistically significant.

## Results

### Demographic data

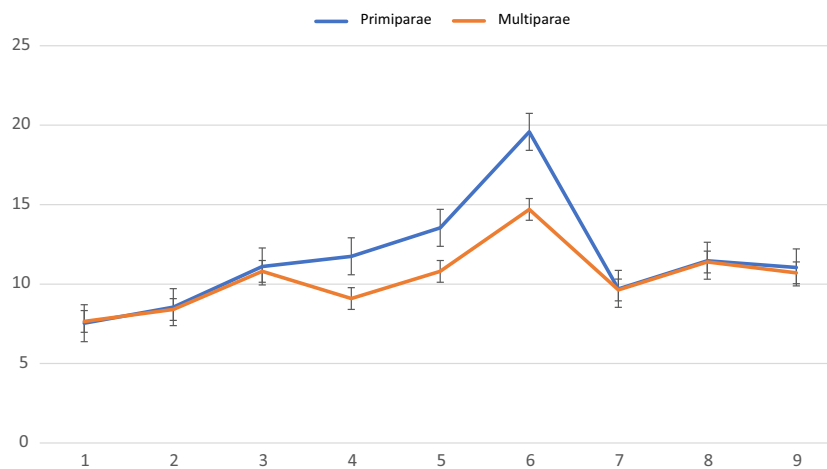
The majority of the women were married or cohabiting, had been born in Sweden, had secondary or higher education and were gainfully employed. Significant differences were seen concerning age and educational background when comparing primiparae with multiparae. First-time mothers were younger and had less education. Tobacco use decreased during pregnancy among primiparae women but remained at a low level among multiparae women (Table 1).

### Cortisol concentrations during pregnancy; impact of parity

The median HCC for the total group of pregnant women during pregnancy, at childbirth and postpartum are shown in Figure 1. Primiparae had higher levels of HCC than multiparae at 2 months (*P* = 0.038) and 1 month (*P* = 0.003) prior to childbirth (Figure 1).

### Associations between monthly cortisol levels during pregnancy

A multivariate linear analysis performed separately in all groups (Table S1) and primiparae (see Table 2a) and multiparae (see Table 2b) groups revealed significant



**Figure 1.** Median cortisol levels and corresponding 95% confidence intervals during pregnancy, at childbirth and postpartum for primiparae and multiparae. (1) Gestational week 14–17; (2) gestational week 18–21; (3) gestational week 22–25; (4) 3 months prior to childbirth; (5) 2 months prior to childbirth; (6) 1 month prior to childbirth; (7) Childbirth and 1 month postpartum; (8) 2 months postpartum; (9) 3 months postpartum.

associations between HCC at gestational weeks (GW) 14–17 with HCC at GW 18–21 (all groups:  $P < 0.001$ ) and GW 22–25 (total:  $P < 0.001$ , primiparae:  $P \leq 0.001$ ) and a tendency of association with the HCC at 2 months postpartum in the primiparae group ( $P = 0.049$ ). HCC at GW 18–21 was also significantly associated with HCC at GW 22–25 (all groups;  $P < 0.001$ ). HCC at 3 months prior to childbirth was associated with the HCC 2 months prior to childbirth (all groups:  $P < 0.001$ ). HCC at 2 months prior to childbirth was associated with HCC at 1 month prior to childbirth (all groups:  $P < 0.001$ ). HCC at 1 month prior to childbirth was associated with HCC at 1 (multiparae:  $P = 0.003$ ) and 2 months postpartum (multiparae:  $P = 0.043$ ). HCC at 1 month postpartum including childbirth was associated with HCC at 2 and 3 months postpartum ( $P < 0.001$  in all groups). Spearman's correlation revealed no associations between HCC levels with the use of steroids and analgesics or any other medications, whereas the use of psychotropic medication correlated to HCC levels in all postpartum measurements ( $r = 0.28$ ,  $r = 0.23$  and  $r = 0.30$ ).

## Discussion

### Main findings

Measures of cortisol in hair clearly demonstrate an increase in cortisol levels throughout pregnancy with a peak around childbirth. This is in line with prior reports based on salivary cortisol and supports the validity of measurement of cortisol in hair samples, as these levels mirror the activity of the HPA axis throughout pregnancy. In the third trimester, the increase in hair cortisol was significantly more pronounced in women expecting their first child than in

multiparae, who showed a decrease in hair cortisol 3 months prior to childbirth.

### Strengths and limitations

The possible uncertainty when extracting and measuring cortisol in hair samples has been minimised in this study because the applied method has previously been tested in several studies and proven valid.<sup>15,19,25</sup> Measurements of cortisol in hair instead of in saliva, blood or urine have advantages, e.g. the measurements are non-invasive and hair samples can be stored at room temperature. There is also evidence of a high level of intra-individual stability in hair cortisol concentrations.<sup>11</sup>

Several confounding factors should be discussed when interpreting cortisol levels, especially as previous research has shown conflicting results. Age and sex and, to a lesser degree, use of oral contraceptives, hair-washing frequency and hair treatment may influence hair cortisol levels.<sup>26</sup> However, in our previous studies (Å Faresjö, unpublished data) frequent washing with shampoo, and the use of hair-spray, gel and wax did not affect HCC levels, in line with a prior report.<sup>27</sup> On the other hand, the use of chemicals in hair treatments such as bleaching, dyeing, straightening or permanent waves may interfere with the cortisol concentrations,<sup>27–28</sup> although results are conflicting.<sup>11,29</sup> Our use of radioimmuno-assay minimises the risks of confounding caused by treated, e.g. coloured, hair.

To our knowledge the highest possible physiological HCC level is unknown, and no clinical standards or reference values have so far been presented. An international inter-laboratory process is ongoing to establish benchmark reference values.<sup>30</sup> The biological samples of HCC that could be considered extreme values in this study were

**Table 2a.** Multivariate analysis of cortisol concentration (HCC) in relation to each of the nine measurements, for primiparae (log values)

|                           | GW 14–17 | GW 18–21       | GW 22–25       | 3 months prior to childbirth | 2 months prior to childbirth | 1 month prior to childbirth | Childbirth and 1 month postpartum | 2 months postpartum | 3 months postpartum |
|---------------------------|----------|----------------|----------------|------------------------------|------------------------------|-----------------------------|-----------------------------------|---------------------|---------------------|
| Standardised coefficients | –        | 0.810 (<0.001) | 0.181 (0.036)  | 0.258 (0.166)                | –0.060 (0.472)               | –0.093 (0.362)              | 0.237 (0.116)                     | 0.276 (0.049)       | 0.130 (0.377)       |
| Beta (P-value)            | –        | –              | 0.705 (<0.001) | –0.072 (0.756)               | 0.085 (0.406)                | 0.103 (0.414)               | 0.263 (0.137)                     | –0.087 (0.595)      | –0.118 (0.492)      |
| Standardised coefficients | –        | –              | –              | –0.052 (0.798)               | –0.018 (0.841)               | –0.025 (0.819)              | –0.086 (0.578)                    | 0.023 (0.870)       | 0.150 (0.314)       |
| Beta (P-value)            | –        | –              | –              | 0.905 (<0.001)               | 0.119 (0.378)                | 0.241 (0.160)               | –0.256 (0.107)                    | 0.201 (0.262)       | –0.229 (0.233)      |
| Standardised coefficients | –        | –              | –              | –                            | –                            | –                           | –0.075 (0.702)                    | 0.201 (0.262)       | 0.225 (0.233)       |
| Beta (P-value)            | –        | –              | –              | –                            | –                            | –                           | 0.417 (0.003)                     | 0.273 (0.043)       | 0.187 (0.183)       |
| Standardised coefficients | –        | –              | –              | –                            | –                            | –                           | –                                 | 0.508 (<0.001)      | 0.553 (<0.001)      |
| Beta (P-value)            | –        | –              | –              | –                            | –                            | –                           | –                                 | –                   | –                   |
| Standardised coefficients | –        | –              | –              | –                            | –                            | –                           | –                                 | –                   | –                   |
| Beta (P-value)            | –        | –              | –              | –                            | –                            | –                           | –                                 | –                   | –                   |

GW, gestational week.

**Table 2b.** Multivariate analysis of cortisol concentration (HCC) in relation to each of the nine measurements for multiparae (log values)

|                           | GW 14–17 | GW 18–21       | GW 22–25       | 3 months prior to childbirth | 2 months prior to childbirth | 1 month prior to childbirth | birth and 1 month postpartum | 2 months postpartum | 3 months postpartum |
|---------------------------|----------|----------------|----------------|------------------------------|------------------------------|-----------------------------|------------------------------|---------------------|---------------------|
| Standardised coefficients | –        | 0.561 (<0.001) | 0.390 (<0.001) | 0.205 (0.033)                | –0.080 (0.232)               | 0.012 (0.822)               | 0.025 (0.809)                | 0.046 (0.388)       | 0.011 (0.879)       |
| Beta (P-value)            | –        | –              | 0.211 (<0.001) | –0.105 (0.269)               | 0.136 (0.037)                | –0.014 (0.793)              | 0.086 (0.448)                | –0.101 (0.089)      | –0.060 (0.442)      |
| Standardised coefficients | –        | –              | –              | 0.152 (0.099)                | 0.036 (0.577)                | –0.034 (0.520)              | 0.135 (0.169)                | –0.009 (0.857)      | –0.031 (0.643)      |
| Beta (P-value)            | –        | –              | –              | 0.740 (<0.001)               | 0.740 (<0.001)               | –0.037 (0.567)              | 0.138 (0.296)                | 0.037 (0.591)       | –0.115 (0.204)      |
| Standardised coefficients | –        | –              | –              | –                            | –                            | –                           | 0.230 (0.198)                | 0.013 (0.886)       | 0.002 (0.984)       |
| Beta (P-value)            | –        | –              | –              | –                            | –                            | –                           | 0.024 (0.856)                | 0.014 (0.845)       | 0.012 (0.895)       |
| Standardised coefficients | –        | –              | –              | –                            | –                            | –                           | –                            | 0.875 (<0.001)      | 0.846 (<0.001)      |
| Beta (P-value)            | –        | –              | –              | –                            | –                            | –                           | –                            | –                   | –                   |
| Standardised coefficients | –        | –              | –              | –                            | –                            | –                           | –                            | –                   | –                   |
| Beta (P-value)            | –        | –              | –              | –                            | –                            | –                           | –                            | –                   | –                   |

GW, gestational week.

replicated and analysed on two independent occasions, giving practically identical results. The cortisol values were logarithmically transformed in the statistical analyses to reduce the variation possibly caused by extreme values.

A strength of this study was that the hair was cut into pieces as part of the analytical process in the laboratory to ensure that all analysed hair samples were from the latest month. Another strength is that pulverised hair was used in our analyses. Recent research has concluded that pulverising hair prior to hormone extraction is crucial.<sup>31</sup>

In addition, the study is of a well characterised population. The attrition was mainly explained by language difficulties and therefore we assume that it had a negligible impact on data.

### Interpretation

The observed progressive increase in cortisol levels during pregnancy is consistent with previous evidence of increased maternal mean cortisol level from about gestational week 25–28 as assessed by monthly measurement of cortisol in saliva.<sup>32</sup> Our present data indicate a slightly earlier increase of cortisol concentrations during pregnancy compared with previous studies, as we found increased cortisol levels as early as gestational weeks 18–21.<sup>16</sup> This echoes the benefit of using HCC, which reflects the average cortisol concentrations over extended periods of time. The previously established transient increase in cortisol levels associated with labour, and the subsequent decrease during the subsequent 4 days,<sup>33–34</sup> was not evident in our results. This was expected, as each measurement covers 1 month, which makes short-lived fluctuations difficult to measure using hair extracts, and the hair sample taken during labour also included 1 week before until 3 weeks after childbirth. Hence, the elevation of cortisol associated with labour was probably outweighed by the downswing in cortisol in the first three postpartum weeks, in agreement with prior findings.<sup>33,35</sup> In our study, the cortisol levels in the first 3 months postpartum remained slightly higher than in the second trimester, consistent with earlier reports of prolonged cortisol elevation for two to three postpartum months.<sup>36–38</sup> This is in accordance with the reported suppressed dexamethasone test up to 6 weeks postpartum and a blunted ACTH response to CRH up to 12 weeks postpartum,<sup>39</sup> suggesting a slow restoration of the HPA system functions. It seems possible that ACTH has a role in the observed slow normalisation of cortisol level postpartum.

The present finding of higher cortisol levels among primiparous women, in line with prior reports, may reflect these women's worries, anxiety and stress about the upcoming labour.<sup>21–22,40–42</sup> It is notable that in multiparae the tendency for slowly escalating cortisol levels observed between weeks 14 and 25 is broken thereafter, and the

cortisol level becomes lower in the 3rd month before childbirth and then increases again, following the same pattern as for primiparae but remaining lower. The explanation for this could be a more rapid inhibition of the mothers' hypothalamic secretion of CRH by the placenta CRH, secondary to adaptive processes during the longstanding inhibition during previous pregnancies. The influence of parity on the cortisol level vanished directly after the childbirth and remained that way, consistent with previous observations on salivary cortisol levels.<sup>22</sup>

The postpartum cortisol levels were not related to those in the former part of the second trimester but rather to those from about GW 18 until childbirth, a period when the placenta CRH is becoming more dominant in the regulation of the rising cortisol level.<sup>32</sup> This suggests that the HPA axis has not fully normalised in the first three postpartum months but is still under the influence of pregnancy-related HPA alterations.

Taken together, the results suggest that the HPA axis may respond differently to the placenta production of cortisol in multiparae. One explanation could be that the negative feedback system is more prepared and suppresses the cortisol production of the adrenal cortex more quickly and more effectively secondary to the initiation of cortisol placenta production. Such a theory would not only explain the lower concentration of cortisol in multiparae but also the lack of association between cortisol levels in GW 14–17 and the levels found in the periods when placenta production has started. Similarly, in primiparae, the concentrations are dominated for longer by the woman's own secretion, thereby explaining the closely related monthly cortisol levels of the second trimester and the lack of association to the levels found in the third trimester.

### Conclusion

Monthly measurements of HCC appear to mirror closely the activity of the HPA axis during pregnancy. Increasing cortisol concentrations were found during pregnancy, with a decrease 3 months prior to childbirth in multiparae. The results suggest a quicker suppression of the hypothalamic CRH production by placenta CRH in multiparous women.

### Disclosure of interests

None declared. Completed disclosure of interest forms are available to view online as supporting information.

### Contribution to authorship

IM had the original idea for the study. GS, ÅF, ET and AJ planned and performed the study and interpreted the results. All authors contributed to the interpretation of the

data and revision of the manuscript, and gave input at all stages of the study.

### Details of ethical approval

The study was approved by the Regional Ethical Review Board in Linköping nr 2011/499-31 (13-03-12).

### Funding

The Medical Research Council of Southeast Sweden.

### Acknowledgements

We thank coordinating midwife Sonja Ekerstam, Department of Obstetrics and Gynaecology, Värnamo, for the excellent practical work with the study, and Marie Bladh PhD for statistical analysis and advice.

### Data availability

Data available in the supplementary material.

### Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** Study design and participation.

**Table S1.** Multivariate analysis of cortisol concentration (HCC) in relation to each of the nine measurements, total study population (log values). ■

### References

- Beydoun H, Saftlas AF. Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. *Paediatr Perinat Epidemiol* 2008;22:438–66.
- Graignic-Philippe R, Dayan J, Chokron S, Jacquet AY, Tordjman S. Effects of prenatal stress on fetal and child development: a critical literature review. *Neurosci Biobehav Rev* 2014;43:137–62.
- Rosa MJ, Lee AG, Wright RJ. Evidence establishing a link between prenatal and early-life stress and asthma development. *Curr Opin Allergy Clin Immunol* 2018;18:148–58.
- Enlow MB, Devick KL, Brunst KJ, Lipton LR, Coull BA, Wright RJ. Maternal lifetime trauma exposure, prenatal cortisol, and infant negative affectivity. *Infancy* 2017;22:492–513.
- Scharlau F, Pietzner D, Vogel M, Gaudl A, Ceglarek U, Thiery J, et al. Evaluation of hair cortisol and cortisone change during pregnancy and the association with self-reported depression, somatization, and stress symptoms. *Stress* 2018;21:43–50.
- Zijlmans MA, Riksen-Walraven JM, de Weerth C. Associations between maternal prenatal cortisol concentrations and child outcomes: A systematic review. *Neurosci Biobehav Rev* 2015;53:1–24.
- Davis EP, Head K, Buss C, Sandman CA. Prenatal maternal cortisol concentrations predict neurodevelopment in middle childhood. *Psychoneuroendocrinology* 2017;75:56–63.
- Zijlmans MAC, Beijers R, Riksen-Walraven MJ, de Weerth C. Maternal late pregnancy anxiety and stress is associated with children's health: a longitudinal study. *Stress* 2017;20:495–504.
- Spiga F, Walker JJ, Terry JR, Lightman SL. HPA axis-rhythms. *Compr Physiol* 2014;4:1273–98.
- Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, van Rossum EF. Hair cortisol, stress exposure, and mental health in humans: a systematic review. *Psychoneuroendocrinology* 2013;38:1220–35.
- Braig S, Grabher F, Ntomchukwu C, Reister F, Stalder T, Kirschbaum C, et al. Determinants of maternal hair cortisol concentrations at delivery reflecting the last trimester of pregnancy. *Psychoneuroendocrinology* 2015;52:289–96.
- Thomson S, Koren G, Fraser LA, Rieder M, Friedman TC, Van Uum SH. Hair analysis provides a historical record of cortisol levels in Cushing's syndrome. *Exp Clin Endocrinol Diabetes* 2010;118:133–8.
- Wennig R. Potential problems with the interpretation of hair analysis results. *Forensic Sci Int* 2000;107:5–12.
- D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. *Physiol Behav* 2011;104:348–53.
- Karlen J, Ludvigsson J, Frostell A, Theodorsson E, Faresjo T. Cortisol in hair measured in young adults – a biomarker of major life stressors? *BMC Clin Pathol* 2011;11:12.
- Kirschbaum C, Tietze A, Skoluda N, Dettenborn L. Hair as a retrospective calendar of cortisol production – Increased cortisol incorporation into hair in the third trimester of pregnancy. *Psychoneuroendocrinology* 2009;34:32–7.
- Kramer MS, Lydon J, Seguin L, Goulet L, Kahn SR, McNamara H, et al. Stress pathways to spontaneous preterm birth: the role of stressors, psychological distress, and stress hormones. *Am J Epidemiol* 2009;169:1319–26.
- Schreier HM, Enlow MB, Ritz T, Coull BA, Gennings C, Wright RO, et al. Lifetime exposure to traumatic and other stressful life events and hair cortisol in a multi-racial/ethnic sample of pregnant women. *Stress* 2016;19:45–52.
- Karlen J, Frostell A, Theodorsson E, Faresjo T, Ludvigsson J. Maternal influence on child HPA axis: a prospective study of cortisol levels in hair. *Pediatrics* 2013;132:e1333–e1340.
- Braig S, Grabher F, Ntomchukwu C, Reister F, Stalder T, Kirschbaum C, et al. The association of hair cortisol with self-reported chronic psychosocial stress and symptoms of anxiety and depression in women shortly after delivery. *Paediatr Perinat Epidemiol* 2016;30:97–104.
- Conde A, Figueiredo B. 24-h urinary free cortisol from mid-pregnancy to 3-months postpartum: gender and parity differences and effects. *Psychoneuroendocrinology* 2014;50:264–73.
- Federenko IS, Wolf JM, Wust S, Schlotz W, Hellhammer J, Kudielka BM, et al. Parity does not alter baseline or stimulated activity of the hypothalamus-pituitary-adrenal axis in women. *Dev Psychobiol* 2006;48:703–11.
- Gillespie SL, Mitchell AM, Kowalsky JM, Christian LM. Maternal parity and perinatal cortisol adaptation: The role of pregnancy-specific distress and implications for postpartum mood. *Psychoneuroendocrinology* 2018;97:86–93.
- Morelius E, Nelson N, Theodorsson E. Salivary cortisol and administration of concentrated oral glucose in newborn infants: improved detection limit and smaller sample volumes without glucose interference. *Scand J Clin Lab Invest* 2004;64:113–8.
- Faresjo A, Theodorsson E, Chatziarzenis M, Sapouna V, Claesson HP, Koppner J, et al. Higher perceived stress but lower cortisol levels found among young Greek adults living in a stressful social environment in comparison with Swedish young adults. *PLoS One* 2013;8:e73828.
- Stalder T, Steudte-Schmiedgen S, Alexander N, Klucken T, Vater A, Wichmann S, et al. Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology* 2017;77:261–74.

- 27 Manenshijn L, Koper JW, Lamberts SW, van Rossum EF. Evaluation of a method to measure long term cortisol levels. *Steroids* 2011;76:1032–6.
- 28 Sauve B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clin Invest Med* 2007;30:E183–E191.
- 29 Dowlati Y, Herrmann N, Swardfager W, Thomson S, Oh PI, Van Uum S, et al. Relationship between hair cortisol concentrations and depressive symptoms in patients with coronary artery disease. *Neuropsychiatr Dis Treat* 2010;6:393–400.
- 30 Russell E, Kirschbaum C, Laudenslager ML, Stalder T, de Rijke Y, van Rossum EF, et al. Toward standardization of hair cortisol measurement: results of the first international interlaboratory round robin. *Ther Drug Monit* 2015;37:71–5.
- 31 Slezak JK, Strom JO, Theodorsson E. Testosterone-like immunoreactivity in hair measured in minute sample amounts - a competitive radioimmunoassay with an adequate limit of detection. *Sci Rep* 2017;7:17636.
- 32 Allolio B, Hoffmann J, Linton EA, Winkelmann W, Kusche M, Schulte HM. Diurnal salivary cortisol patterns during pregnancy and after delivery: relationship to plasma corticotrophin-releasing-hormone. *Clin Endocrinol (Oxf)* 1990;33:279–89.
- 33 Mastorakos G, Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Ann N Y Acad Sci* 2003;997:136–49.
- 34 Thompson LA, Trevathan WR. Cortisol reactivity, maternal sensitivity, and learning in 3-month-old infants. *Infant Behav Dev* 2008;31:92–106.
- 35 Cheng CY, Pickler RH. Maternal psychological well-being and salivary cortisol in late pregnancy and early post-partum. *Stress Health* 2010;26:215–24.
- 36 Groer MW. Differences between exclusive breastfeeders, formula-feeders, and controls: a study of stress, mood, and endocrine variables. *Biol Res Nurs* 2005;7:106–17.
- 37 Jung C, Ho JT, Torpy DJ, Rogers A, Doogue M, Lewis JG, et al. A longitudinal study of plasma and urinary cortisol in pregnancy and postpartum. *J Clin Endocrinol Metab* 2011;96:1533–40.
- 38 Kammerer M, Adams D, Castelberg Bv BV, Glover V. Pregnant women become insensitive to cold stress. *BMC Pregnancy Childbirth* 2002;2:8.
- 39 Magiakou MA, Mastorakos G, Rabin D, Dubbert B, Gold PW, Chrousos GP. Hypothalamic corticotropin-releasing hormone suppression during the postpartum period: implications for the increase in psychiatric manifestations at this time. *J Clin Endocrinol Metab* 1996;81:1912–7.
- 40 Bouyou-Akotet MK, Issifou S, Meye JF, Kombila M, Ngou-Milama E, Luty AJ, et al. Depressed natural killer cell cytotoxicity against *Plasmodium falciparum*-infected erythrocytes during first pregnancies. *Clin Infect Dis* 2004;38:342–7.
- 41 Grajeda R, Perez-Escamilla R. Stress during labor and delivery is associated with delayed onset of lactation among urban Guatemalan women. *J Nutr* 2002;132:3055–60.
- 42 Valsamakis G, Papatheodorou DC, Chalarakis N, Vrachnis N, Sidiropoulou EJ, Manolikaki M, et al. In pregnancy increased maternal STAI trait stress score shows decreased insulin sensitivity and increased stress hormones. *Psychoneuroendocrinology* 2017;84:11–6.