ORIGINAL ARTICLE

Pregnant Women in Relation to Hair Cortisol, Stress, Pregnancy-Related Anxiety and Fear of COVID-19

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Submitted: 2023-03-28 Accepted: 2023-05-15 Published online: 2023-05-15

Key words: **pregnant women; hair cortisol; stress; pregnancy-related anxiety; fear of COVID-19**

Act Nerv Super Rediviva 2023; **65**(2): 47–54 ANSR65223A02

Abstract

BACKGROUND: The aim of the study was to examine the biological and psychological aspects of stress in pregnant women during the COVID-19 pandemic. Specifically, we focused on hair cortisol levels reflecting the second and 3rd trimester of pregnancy, perceived stress, pregnancy-related anxiety associated with the fear of COVID-19.
METHODS: The research sample comprised 40 pregnant women. The research tools consisted of a questionnaire battery: Perceived Stress Scale, Pregnancy-Related Anxiety Questionnaire, Fear of COVID-19 Scale. Another employed research tool was the hair cortisol analysis.
RESULTS: The results showed that pregnant women had higher hair cortisol levels in the 3rd trimester compared to the 2nd trimester (z=–2.850, p=0.004, r=–0.45). In regards to pregnancy-related anxiety, multiparous women had a higher score compared to primiparous women [t(28.81)=–2.90, p=0.007, d=0.48]. Perceived stress was negatively correlated with hair cortisol levels for the 3rd trimester of pregnancy (r=–0.366, p=0.01). There was no relationship between the fear of COVID-19 and pregnancy-related anxiety (r=0.301, p=0.059).
CONCLUSION: We consider that it is important to focus on the prevention of stress and pregnancy-related anxiety, especially during various demanding societal changes, and thus mitigate the potential long-term negative effects of stress on pregnant women's health and well-being.

Introduction

Pregnancy can represent a period of increased pressure and perceived stress, which affects the physiological activity of the hypothalamic-pituitary-adrenal axis (hereafter referred to as the "HPA axis") (Kalra *et al.* 2007; Musana *et al.* 2020; Romero-Gonzalez *et al.* 2020). Research shows that elevated cortisol levels occur at the final stage of pregnancy (i.e., in the third trimester) (Kirschbaum *et al.* 2009; D'Anna-Hernandez *et al.* 2011; Marteinsdottir *et al.* 2020). During pregnancy, women experience specific concerns regarding their own health and the health of their unborn child, the course of labour, and the accompanying pain (Huizink *et al.* 2016; Sheen & Slade, 2018).

Nowadays, the COVID-19 pandemic represents a new source of concern for pregnant women. Coronavirus (SARS-CoV-2) is an infectious disease accompanied by a rapid surge in cases and deaths since it had first been detected in Wuhan, China. The virus

is primarily transmitted between people through bodily fluids, such as saliva or mucous droplets. The World Health Organization declared the virus outbreak a global pandemic on 11 March 2019 (World Health Organization, 2020). The spread of the COVID-19 has impacted the lives of individuals around the world. In addition to the health-related and economic consequences, the pandemic has undoubtedly affected people's mental well-being, especially in the vulnerable population who is at increased risk, such as pregnant women (Taubman – Ben-Ari *et al.* 2020). There is relatively limited information available on the effect of the COVID-19 infection on pregnancy, which causes uncertainty and concern for many women. Studies using samples of pregnant women infected with COVID-19 have not demonstrated any risk of intrauterine transmission of the disease to the new born baby (Chen *et al.* 2020; Zhu *et al.* 2020; Fan *et al.* 2020). Nevertheless, research indicates an increased prevalence of anxiety and depression in pregnant women during the COVID-19 pandemic (Kahyaoglu & Kucukkay, 2020; Liu *et al.* 2020). A 2020 study by Kahyaoglu & Kucukkay shows that the prevalence of anxiety and depression in 403 pregnant women in Turkey during the COVID-19 pandemic was 64.5% and 56.3%, respectively. The main concerns revolved around the fear of visiting the doctor. A total of 68% of women reported that they felt discomfort when visiting the hospital or undergoing regular check-ups with a doctor. A 2020 study by Liua *et al.* examined a relatively large sample of 1947 pregnant women in two Chinese cities: Wuhan (the epicentre of the outbreak) and Chongqing. Four out of five mothers felt nervous about the consequences of the COVID-19 pandemic, mainly due to the uncontrollable nature of the situation. More than 90% of women felt vulnerable during the pandemic. The overall pregnancy-related anxiety level reached 17.2%, with higher levels (24.5%) recorded in Wuhan, where they had to follow stricter anti-pandemic measures (Liu *et al.* 2020). The study findings highlight the negative effects of the pandemic on the psychological well-being of pregnant women, which is why the present research focused on this particular high-risk group.

The present research focused on the biological and psychological aspects of stress in primiparous and multiparous women during the COVID-19 pandemic. In addition to perceived stress, the research analysed hair cortisol levels, which reflected the second and third trimesters of pregnancy. Hair cortisol is able to provide information on long-term stress exposure because the rate of hair growth averages 1 cm per month. The hair sample analysis allows retrospective assessment of cortisol at the time when the effect of the stressor was most pronounced, without the need for sampling at that particular time (Lee *et al.* 2015). D'Anna Hernandez *et al.* (2011) found a correlation between hair cortisol and salivary cortisol levels in pregnant women who experienced elevated levels during pregnancy, reaching peak levels in the third trimester. The correlation of cortisol levels in hair and saliva suggests that hair cortisol could be a useful marker of overall HPA-axis activity in pregnant women.

With the help of questionnaire methods, the pregnancy-related anxiety was examined, which is different from general anxiety and better captures specific pregnancy-related concerns (Huizink *et al.* 2004). Specifically, the pregnancy-related anxiety in primiparous and multiparous women was compared, with research indicating increased pregnancy-related anxiety and stress during the first pregnancy as women experience a completely unfamiliar situation (Conde & Figueiredo, 2014; Fenwick *et al.* 2015; Gillespie *et al.* 2018).

The present study addressed perceived stress, pregnancy-related anxiety, and hair cortisol levels in a sample of pregnant women during a pandemic.

Methods

Research sample and procedure

The research sample comprised 40 pregnant women, of whom 21 were primiparous (53%) and 19 were multiparous (48%). Thirteen women were expecting their second child and six women were expecting their third child. All women were in the third trimester of pregnancy. There were ten women in the ninth month of pregnancy, fourteen women in the eighth month and sixteen women in the seventh month of pregnancy. The age span ranged from 18 to 42 years (M=29.75). All the participants were from the Trenčín region. The participants worked in various fields. The COVID-19 pandemic affected the participants mostly in terms of family (N=12), work (N=10), financial situation (N=8), health (N=2), and eight participants used the option "other". For the purposes of hair cortisol analysis, the participants were asked about the frequency of their hair washing and hair colouring. The participants most frequently washed their hair 3–4 times a week (N=18), 2 times a week (N=16), and 1 time a week (N=6). A total of 23 women dyed their hair with chemical dye and 17 women did not dye their hair at all. The participants were also asked whether the presence of their partner during childbirth was important to them. A total of 34 participants stated that the presence of their partner during childbirth was important and 6 participants indicated that the presence of a partner was not important to them.

Data collection took place in the period from October to December 2020. Prior to the commencement of the research, a private gynaecological clinic was approached in advance with a request for permission to conduct the research at their workplace. Subsequently, pregnant women attending this outpatient clinic were approached and informed about the aim of the present research. Prior to data collection, the participants were instructed on the research process and anonymity. A Google Forms web-based question-

Tab. 1. Descriptive statistics of hair cortisol and questionnaires (The Perceived Stress Scale- PSS-10, The Pregnancy-Related Anxiety Questionnaire- PRAQ-R2, The Fear of COVID-19 Scale- FCV-19S.

	Research sample (n=40)	Primiparous (n=21)	Multiparous (n=19)
Hair cortisol (pg/mg)			
Second trimester (M, SD)	6.93 ± 4.00	6.45 ± 3.70	7.46 ± 4.35
Third trimester (M, SD)	8.16 ± 4.25	7.52 ± 4.58	8.85 ± 3.84
Questionnaires			
PSS-10 (M, SD)	18.40 ± 5.43	17.48 ± 5.35	19.48 ± 5.46
PRAQ-R2 (M, SD)	29.15 ± 7.24	26.19 ± 4.85	32.42 ± 8.12
FCV-19S (M, SD)	15.48 ± 5.21	12.81 ± 3.93	18.42 ± 4.93

naire and an instructional video of hair sampling were sent to the women's email addresses. The participants were first presented with an informed consent form followed by a series of questionnaires focusing on experiencing stress, pregnancy-related anxiety, fear of COVID-19, social support, and resilience. By filling out these questionnaires, women gave consent to the processing of the collected data. They were compensated with EUR 20 worth of meal vouchers for their participation in research.

Research sample and procedure: hair cortisol analysis

To assess HPA-axis activity, cortisol was measured via 6-cm-long hair samples (assuming an average hair rate of hair growth of 1 cm per month, a 6-cm-long segment would contain cortisol for approximately the last 6 months), with samples collected in the third trimester of pregnancy. Under established procedures, hair samples were obtained by cutting off two strands of hair from the back of the head (posterior vertex area) (Sauvé *et al.* 2007) and subsequently wrapped in aluminium foil to protect it from light and moisture (Wennig, 2000). Due to anti-pandemic measures, it was not possible to collect hair samples in person, which is why a detailed instructional video was sent to the women's email addresses, based on which the women collected the samples themselves and handed them to the nurse at the following medical examination. The samples were labelled with an identification number so that they could be reliably compared with the results of the questionnaires. Once labelled, the samples were sent to for expert examination.

Questionnaires

The questionnaires were used to collect demographic data such as age and occupation, month of pregnancy, number of children, and whether the presence of a partner at the birth was important to the participant. Other collected information included hair characteristics that could affect cortisol levels, such as hair dyeing and frequency of hair washing. Participants were given space to talk about their experience with pregnancy during the COVID-19 pandemic. This was followed by a questionnaire battery consisting of the following questionnaires:

The Perceived Stress Scale (PSS-10), in which participants indicated on a 5-point scale how often they have experienced symptoms of perceived stress in the past month. The internal consistency of the questionnaire in the present research was α=0.853.

The Pregnancy-Related Anxiety Questionnaire (PRAQ-R2) consists of ten items divided into three subscales: fear of giving birth, worries about bearing a handicapped child, and concern about one's own appearance. The scores range from 10 to 50, and the higher the score a pregnant woman achieves, the more pregnancy-related anxiety she experiences (Huizink *et al.* 2016). In the present research, the questionnaire showed good internal consistency α=0.830.

The Fear of COVID-19 Scale (FCV-19S) aims to explore information about the fear of COVID-19 in the general public. The FCV-19S is a powerful psychometric tool for assessing fear and concern about COVID-19 in individuals of both sexes and different ages. It contains seven items with statements such as "It makes me uncomfortable to think about COVID-19" or "My hands become clammy when I think about COVID-19". Participants express their level of agreement with the statements using a five-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The scores range from 7 to 37 points, the higher the score, the higher the fear of COVID-19 (Ahorsu *et al.* 2020). The internal consistency of the questionnaire in the present research was α = 0.896.

Statistical analysis

The collected data were processed using the Statistical Package for Social Sciences (SPSS, version 25) statistical software. The programme was used to determine the reliability (Cronbach's alpha) and descriptive statistics of the questionnaires and cortisol levels. Prior to selecting the appropriate statistical procedure, the Shapiro-Wilk test was performed, which is used in research samples with fewer than 50 participants. The Wilcoxon test was used to detect differences between cortisol levels in the second and third trimesters, and

the independent t-test was used to compare groups of primiparous and multiparous women. Correlation analysis, specifically Pearson and Spearman correlation coefficients, was used to determine relationships between variables.

Results

To detect changes in cortisol levels in the second and third trimesters of pregnancy, the Wilcoxon test was used. It was chosen on the basis of the non-normality of the distribution of the data. The Wilcoxon test showed that pregnant women had higher hair cortisol levels in the third trimester (M=8.16, SD=4.25) compared to the second trimester (M=6.93, SD=4.00), and this difference was significant (z=–2.850, p=0.004), with a medium effect size (r=–0.45).

To detect the difference in pregnancy-related anxiety between primiparous and multiparous women, an independent t-test was used. It was chosen based on the normality test, which indicated a normal distribution of the data. Multiparous women had higher mean scores of pregnancy-related anxiety (M=32.42, SD=8.12) compared to primiparous women (M=26.19, SD=4.85). This difference was significant (t(28,81) =–2.90, p=0.007) with a medium effect size (d=0.48).

Multiparous women scored higher on average on all pregnancy-related anxiety subscales, but a significant difference was only demonstrated in the subscale of concern about their appearance. In this subscale, multiparous women scored higher on average (M=10.58, SD=2.71) compared to primiparous women (M=6.62, SD=1.77), and this difference was significant (t(38)=–5.51, p<0.001).

To test the relationship between perceived stress and cortisol levels, the Spearman correlation coefficient was chosen as part of the correlation analysis based on the non-normality of the data distribution. The result of the analysis demonstrated a moderate negative relationship between perceived stress and cortisol levels, and this result was statistically significant (r=–0.366, p=0.01).

There was no significant relationship demonstrated between the fear of COVID-19 and pregnancy-related anxiety (r=0.301, p=0.059).

Discussion

The present research set out to examine biological and psychological aspects of stress in primiparous and multiparous women during the COVID-19 pandemic. Specifically, the study focused on hair cortisol levels reflecting the second and third trimesters of pregnancy, perceived stress, pregnancy-related anxiety associated with the fear of COVID-19.

The main findings include elevated levels of concentration in the third trimester of pregnancy, similar to other studies (Kirschbaum *et al.* 2009). There may be several reasons for elevated cortisol concentrations in the most advanced stage of pregnancy. These reasons include, for example, physiological changes during pregnancy. One of the main differences between pregnant and non-pregnant women is the significant increase in oestrogen and placental CRH that occurs mid-pregnancy. Chrousos (1999) hypothesized that the increase in the concentration of these hormones is related to increased cortisol levels at the end of pregnancy. Further insight is provided by Scott *et al.* (1990) who suggested that elevated cortisol levels during pregnancy are related to a restoration of HPA-axis sensitivity, presumably under the influence of an increase in oestrogen levels. Other factors that may have influenced the results of the present study include the stress and anxieties of pregnant women in the individual trimesters of pregnancy. The study by Da Costa *et al.* (1999) assessed the model of stress in each trimester of pregnancy. Pregnant women reported the highest anxiety and pregnancy-related fears in the third trimester of pregnancy, probably due to the approaching date of delivery. The results of the study by Chou *et al.* (2008) suggest that one of the important stressors in the third trimester of pregnancy may also be unpleasant physical symptoms such as swelling, fatigue, and nausea, which may lead to increased stress.

A factor that may contribute to lower hair cortisol levels in the hair segment farther from the scalp, in this case in the second trimester, is called the "washout effect". Older hair segments are more exposed to the external environment compared to more proximal segments. Manenschijn *et al.* (2011) assumed that distal hair segments are more damaged by UV rays, cosmetic treatments, and hair washing. The cortisol level in the most distal and damaged hair segments could be reduced as a result of these factors. Nevertheless, the effect of hair dyeing and washing on lower cortisol levels was not demonstrated in the present sample. Another potential explanation for the results of the present study is the COVID-19 situation in Slovakia. Hair samples of women were collected between October and December 2020. From September 2020 onwards, the situation in Slovakia deteriorated rapidly, recording a daily increase in infected people from an average of 1000 to more than 3000 in the course of the months of hair sampling (Worldometer, 2021). Pregnant women may have gradually realised the seriousness of the situation and the uncertainty during the pandemic. The stricter anti-pandemic measures may have had an impact not only on fears of getting infected but also on their personal and professional life, which may have led to increased stress for pregnant women and, as a consequence, an increase in cortisol levels over this period. Among the areas that negatively affected the lives of the participants as a result of the pandemic, family was the most frequently mentioned option in our questionnaire, followed by work and financial situation, restrictions on movement and leisure activities, and the consequences on people's mental well-being. Another area that was

negatively affected by the pandemic was the option of having a support person present during childbirth. In the present sample, as many as 85% of pregnant women reported that the presence of a partner or close person was important to them during childbirth. However, the presence of a support person at delivery or postpartum visits was prohibited in many hospitals due to the risk of spreading the infection, which may have led to greater concern for pregnant women at advanced stages of pregnancy.

The study also identified a difference in pregnancy-related anxiety in primiparous and multiparous women. Multiparous women scored higher in all areas of pregnancy-related anxiety – fear of giving birth, worries about bearing a handicapped child, and concern about one's own appearance, which contradicted the studies carried out by Westerneng *et al.* (2017) and Lynn *et al.* (2011), in which primiparous women showed higher pregnancy-related anxiety and pregnancy-related distress compared to multiparous women. One explanation behind this may be the experience of childbirth and pregnancy. The study carried out by Rouhe *et al.* (2009) suggests that negative experiences with childbirth and pain may have an impact on the higher level of fear of childbirth in multiparous women compared to primiparous women. Certain multiparous women may recall pregnancy and childbirth as a negative experience because of perinatal complications or adverse experiences with medical assistance (Fenwick *et al.* 2015). When analysing the results, a significantly increased mean in the subscale of concern about one's own appearance was identified among multiparous women. This implies that multiparous women are much more concerned about body changes after subsequent pregnancy and childbirth, which may have influenced the present results. As suggested by Saisto and Halmesmäki (2013), fear of childbirth and pregnancy-related concerns are not an isolated problem but are associated with women's personal characteristics, general anxiety, low self-esteem and depression, dissatisfaction with their partnerships, and lack of social support. Multiparous mothers may also face difficult challenges related to their upcoming pregnancy, such as the reorganisation of their previous parenting system, increased financial responsibilities or profession-related demands (Figueiredo & Conde, 2011).

The relationship between perceived stress and third-trimester hair cortisol levels was also examined in the present study. The results of various studies addressing this relationship are highly varied (Braig *et al.* 2016; Hoffman et. al., 2016; Kramer *et al.* 2009; Musana *et al.* 2020). A significant negative correlation between perceived stress and third-trimester hair cortisol levels was identified. The negative relationship can be explained through physiological changes resulting from pregnancy, which means that the period of pregnancy in which the hair samples are collected plays an important role. In a pilot study by Kalra *et al.* (2007), which highlights a positive relationship between the two variables, the samples were collected at the end of the first or at the beginning of the second trimester. In the present research, samples were collected in the third trimester. As outlined above, cortisol levels may increase during gestation, reaching the highest levels in the third trimester. Thus, cortisol secretion at the end of pregnancy may be strongly determined by physiological factors that make it difficult to detect even weak correlations with psychosocial constructs (Braig *et al.* 2016). As reported by Musana *et al.* (2020), the ambiguity of the relationship between hair cortisol levels and perceived stress may reflect the different time periods assessed by these methods. The Perceived Stress Scale assessed perceived stress over the past month, and hair cortisol levels reflected perceived stress ranging from three to six months.

Another explanation could be the so-called "stress-intensity hypothesis". This hypothesis predicts that only stressors of a certain intensity are capable of inducing HPA-axis activity and cortisol secretion and incorporation into scalp hair. In consequence, low-intensity stress is insufficient to increase hair cortisol levels, which reduces the probability of detecting an association between hair cortisol and subjective stress measures. In the present research, the participants' scores were in the moderate stress range (14–26 points) on the Perceived Stress Scale. Therefore, it is also possible that in many studies examining the relationship between these variables, stress was at a low level which had little effect on long-term cortisol secretion. This may change with more intense stress (e.g., after surviving trauma), which induces an intense and cumulative burden that eventually exhausts the regulatory capacities of the HPA axis and manifests itself in long-term endocrine changes (Stalder *et al.* 2017). Weckesser *et al.* (2019) in particular highlight the methodological complications that may arise when retrospective self-report questionnaires are used to assess long time periods. Mauss (2005) based on Stalder *et al.* (2017) lists individual differences in the awareness of one's own mood states, social desirability, and the aforementioned retrospective response bias among the challenges of questionnaire methods. The association between perceived and objective stress may also be influenced by environmental variables, which, when combined, influence cortisol secretion and the subjective experience of stress. An example would be an increased exposure to UV radiation, which reduces cortisol levels (Grass *et al.* 2016). However, increased exposure to sunlight may reduce perceived stress (Milaneschi *et al.* 2014). As a result of physical activity and sweating, more cortisol may be incorporated into hair (Grass *et al.* 2015). Nevertheless, there is evidence of an effect of physical activity on the reduction of perceived stress (Hackney, 2006). Based on these examples, it is clear that the ambiguous relationship between perceived stress and physiological cortisol levels may be the result of a variety of factors.

One of the limitations of the present study may be the size of the research sample. It comprised 40 participants who were unevenly distributed in the individual groups, with 21 primiparous and 19 multiparous women. In future research, it would be suitable to work with a larger number of participants to obtain more relevant results. For this reason, it is necessary to approach a higher number of health facilities. Another limitation may lie in the homogeneity of the research sample, seeing as all participants attended the same health centre and came from the same region.

Due to anti-pandemic measures and online administration, it was not possible to ensure that hair samples were collected concurrently with the completion of the questionnaire battery. As suggested by Wells *et al.* (2014), time lags between each data collection may lead to inconsistencies between physiological cortisol levels and self-assessment questionnaires.

As several studies have shown, there are various variables that can potentially influence endocrine concentrations of cortisol in the body such as frequency of exercises (Hansen *et al.* 2008), mental illnesses (Staufenbiel *et al.* 2013), chronic diseases, for instance, hypertension or diabetes mellitus (Staufenbiel *et al.* 2015), and hair characteristics, for example, hair dying or frequency of washing (Stalder *et al.* 2017). The present research did not demonstrate an effect of hair dyeing and washing on cortisol levels, but it also did not take other variables into consideration, which may also potentially influence HPA-axis activity, such as exercise, mental well-being, or chronic diseases. This could be considered another limitation of the present study.

Conclusion

In the course of the present research, various interesting results were identified. Initially, the present research focused on the differences in hair cortisol levels in the second and third trimesters of pregnancy. In the third trimester, pregnant women demonstrated significantly higher hair cortisol levels compared to the second trimester. Attention was also given to the construct of pregnancy-related anxiety, which reflects specific concerns of pregnant women regarding childbirth, child health, or changes in their physical appearance. Specifically, the present research aimed to identify differences in pregnancy-related anxiety in primiparous and multiparous women. Multiparous women demonstrated significantly higher pregnancy-related anxiety, especially in regards to the subscale of concern about their appearance. The relationship between perceived stress and objective cortisol levels in the third trimester of pregnancy was also examined. The results of studies addressing this relationship are highly variable. A significant negative relationship between perceived stress and hair cortisol levels in the third trimester of pregnancy was demonstrated in the present research sample of pregnant women. No significant relationship was identified between the fear of COVID-19 and pregnancy-related anxiety, yet pregnant women reported various concerns about family, work, or their financial situation. Therefore, it is important to focus on the prevention of stress and pregnancy-related anxiety, especially during the period of increased burden brought about by the current COVID-19 pandemic, and thus mitigate the potential long-term negative effects of stress on pregnant women's health and well-being.

Acknowledgement

This paper was supported by following grants: VEGA 1/0786/21, APVV-17-0451. Compliance with ethical standards

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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