Hair cortisol levels in pregnancy as a possible determinant of fetal sex: a longitudinal study

Borja Romero-Gonzalez1,2, Jose A. Puertas-Gonzalez1,2, Raquel Gonzalez-Perez3, Marta Davila4 and Maria Isabel Peralta-Ramirez2

1Mind, Brain and Behaviour Research Centre, Granada, Spain; 2Personality, Assessment and Psychological Treatment Department, School of Psychology, University of Granada, Granada, Spain and 3Department of Pharmacology, CIBERehd, School of Pharmacy, Instituto de Investigación Biosanitariaibs.GRANADA, University of Granada, Granada, Spain

Abstract

Stress during pregnancy has been widely studied and associated to different variables, usually with negative results for the health of the mother and the newborn, such as having a higher risk of suffering postpartum depression, premature birth, obstetrics complications or low birthweight, among others. However, there are not many lines of research that study the role that the sex of the baby plays on this specific stress and vice versa. Thus, the main objective was to analyse the relationship between the sex of the offspring and the stress of the mothers in the first trimester of pregnancy. In order to achieve this, 108 women had their biological stress measured (trough hair cortisol levels) and psychological stress evaluated (the Prenatal Distress Questionnaire (PSS), the Perceived Stress Scale (PDQ) and the Stress Vulnerability Inventory (IVE)). The results revealed significant differences in maternal hair cortisol levels in the first trimester based on the sex of the baby they had given birth to (<i>t</i> = −2.04; <i>P</i> < 0.05): the concentration of the hormone was higher if the baby was a girl (164.36: 54.45–284.87 pg/mg) than if it was a boy (101.13: 37.95–193.56 pg/mg). These findings show that the sex of the future baby could be conditioned, among many other variables, by the mother’s stress levels during conception and first weeks of pregnancy. Further research is needed in this area to support our findings.

Introduction

Pregnancy is a stressful period. Not only do expectant mothers undergo physical changes, they also fear childbirth and are preoccupied about the baby’s health as well as their motherhood. They may suffer strains in personal relationships. The worries and stress experienced during pregnancy activate the hypothalamic–pituitary–adrenal axis (HPA), leading to the release of cortisol, a hormone with the ability to cross the placenta and condition fetal development.

Cortisol levels have traditionally been evaluated using blood, urine, saliva or amniotic fluid samples. These measurements, however, are punctual and highly affected by the sleep–wake cycle. An alternative is the extraction of hair cortisol: indeed, hair cortisol is the only retrospective biomarker of chronic stress that is unaffected by contextual variables such as noise, temperature or social interaction. It enables retrieving cortisol measurements over the 3 months prior to the date of extraction.

During pregnancy, high concentrations of prenatal hair cortisol have been associated with preterm birth, childbirth complications, maternal psychological diseases and wellbeing, post-partum depression and the baby’s neurological development. Nevertheless, the relationship between cortisol at the time of conception and the baby’s future sex has been scarcely studied. Studies conducted on the relationship between cortisol and the sexual predisposition of the fetus found high levels of salivary cortisol in the third trimester of pregnancy in women who gave birth to a girl. In the same vein, other authors showed that women who had higher cortisol concentrations in their saliva before conception were less likely to have a male baby.

These findings are in fact in line with numerous studies showing the decline of male births in the population following exposures to stressful stimuli such as earthquakes, murders, terrorist acts, stressful work or life-changing events. One hypothesis is that parents’ stress modifies the concentration of sex hormones through the activation of the HPA axis and has implications regarding sex allocation. This approach is supported by several experimental studies that have found that the sex of the zygotes is influenced by the stress of both parents around the moment of conception: the higher the stress level, the more likely of giving birth to a girl.

However, other authors have failed to find such a relationship between cortisol and the baby’s sex. The studies linking stress to biological measures and the sex of future babies may be promising, but they are in fact few and far between.
Therefore, further tests are necessary to show whether cortisol concentrations during pregnancy influence the sex of the offspring and whether they do so during conception. In fact, to the best of our knowledge, no study, that is not merely punctual (cortisol in saliva, urine or blood), has yet been carried out using a retrospective measure (the mother’s hair cortisol) on the relationship between cortisol and the baby’s sex.

Therefore, the study’s main objective was to check whether a relationship existed between the sex of the offspring and the cortisol secretion in the mother’s hair before and during the baby’s conception, as well as in the first weeks of pregnancy. In a complementary way, a second aim was to know whether psychological stress could be related to sex of the offspring, the levels of psychological and specific stress were evaluated in those weeks of pregnancy.

**Methods**

**Sample size estimation**

There are some studies whose aim was to find out the relationship between salivary cortisol and sex of the fetus. Sample size estimation was calculated using this variable in Giesbrecht et al.\(^16\). G * Power\(^2\) was used to calculate the sample size to achieve 80% power and contrast the null hypotheses \( H_0: \mu_1 = \mu_2 \) at the 5% alpha level. Comparing two independent means (t-test) using mean scores and deviation standards of Giesbrecht et al.\(^16\) of two groups (male and female), the sample size required were 72 participants, 37 for each group (Cohen’s \( d = 0.77 \)).

**Participants**

The total sample was made up of 108 expectant women in weeks 8 and 10 of their pregnancy and there were seven women who were pregnant using a fertility treatment. They were recruited in various health centres of the province of Granada (Spain).

The inclusion criteria were as follows: being an expectant woman in weeks 7 to 10 of pregnancy; aged over 18 years and having a minimum of an average level in Spanish. The exclusion criterion was: having a pre-pregnancy illness or taking corticosteroids.

Participation was voluntary, and an informed written consent document was read and signed by every participant. This study followed the guidelines of the Helsinki Declaration (AMM, 2008) and the Good Clinical Practice Directive (Directive 2005/28/EC) of the European Union and was approved by the Human Ethics Research Committee of the University of Granada (reference 881).

**Instruments**

Sociodemographic and obstetric data were collected from the 2010 Pregnant Woman’s Health Document\(^35\), which is the official health record for pregnant women and their newborns. The included variables were: age, marital status, educational level, employment status, smoking or not, maternal body mass index, type of pregnancy, number of children, number of previous abortions, whether or not the pregnancy was desired, as well as whether or not it was risky.

**Psychological Assessment**

Perceived Stress Scale (PSS)\(^34,35\). The PSS provides information on the perception of general stress during the preceding month.

It consists of 14 items scores on a 5-point Likert scale (0 = never, 1 = almost never, 2 = once in a while, 3 = often, 4 = very often). Spanish reliability alpha’s Cronbach coefficient is 0.81.

Pregnancy Distress Questionnaire (PDQ)\(^36,37\): this is a 12-item scale that measures pregnancy-specific stress related to maternal concerns about pregnancy, such as medical problems, labour and delivery, physical symptoms, bodily changes and the baby’s health. Responses are given using a 5-point Likert-type scale where 0 = not at all and 4 = very much. The Cronbach’s alpha reliability coefficient is 0.71.

**Chronic stress biomarker: hair cortisol levels**

The cortisol evaluation consisted in taking a lock of hair containing approximately 150 strands from the rear corner of the skull, as close as possible to the scalp\(^38\). A maximum length of 3 cm was set for each sample to reflect cortisol levels during the preceding 3 months\(^39\). The samples were wrapped in aluminium foil to be adequately protected from light and humidity and were kept at room temperature until further analysis using the salivary kit ELISA. The analysis protocol was published in Romero-Gonzalez et al.\(^1\) The lower detection limit was 12.5 pg/mg and the cross-reactivity reported by the manufacturer was as follows: prednisolone 13.6%, corticosterone 7.6%, deoxycorticosterone 7.2%, progesterone 7.2%, cortisone 6.2%, deoxycortisol 5.6%, prednisone 5.6% and dexamethasone 1.6%. No cross-reactions were detected with dehydroepiandrosterone or tetrahydrocortisone.

The intra- and inter-assay precisions were analysed on internal quality controls used for routine salivary cortisol measurement, measured in duplicate in eight consecutive assays. The intra-assay coefficients of variation (CV) were 2.7% at 10.7 ng/ml and 4.3% at 43.9 ng/ml. The inter-assay CVs were 4.4% and 6.3%, respectively\(^3\).

**Procedure**

Women were told about the study when they attended their first prenatal appointment with their midwife, 7 to 10 weeks into their pregnancy. Those who agreed to participate were given an information sheet and then signed the informed consent document. Each participant was assigned an identification code in order to ensure anonymity throughout the study. Subsequently, sociodemographic and obstetric information was collected, psychological questionnaires were completed in paper format (PDQ, EEP-1, IVE), and hair samples were then taken applying the established sample collection protocol\(^40\). Once they gave birth, they were contacted and asked the sex of their baby. This study followed the The Strengthening the Reporting of Observational studies in Epidemiology standards for cohort studies.

**Data analysis**

The averages and percentages of the most relevant sociodemographic and obstetric variables (age, marital status, educational level, employment status, type of pregnancy, number of children, number of previous abortions and whether the pregnancy was desired, as well as whether it was risky or not) were calculated first.
The participants were then divided into two groups according to the sex of their babies (male or female). Subsequently, in order to check any differences regarding major sociodemographic and obstetric variables between both groups, the t-Student (quantitative variables) and Chi-square (categorical variable) tests were performed.

The data met the assumptions of normality and uniformity of variances (tests of Kolmogorov–Smirnov and Shapiro–Wilk of normality \( P > 0.05 \); Levene test to evaluate the homogeneity of variances \( P > 0.05 \)).

To verify the presence of significant differences in maternal hair cortisol levels between women who had given birth to a girl and those who had given birth to a boy, a comparison was made of independent samples using non parametric Mann–Whitney test. The variable ‘baby’s sex’ was the independent variable, with two levels (female and male). The dependent variables were maternal hair cortisol levels. Besides, to know whether psychological variables were different regarding baby’s sex, a comparison was made of independent samples using the t-Student test. The variable ‘baby’s sex’ was the independent variable, with two levels (female and male) and dependent variables were the scores on perceived stress (PSS), vulnerability to stress (SVS) and pregnancy-specific stress (PDQ).

In addition, calculations were performed, using Cohen’s \( d \), to determine whether the differences between the groups were clinically relevant. We took into account the considerations regarding the interpretation of the effect size magnitudes: \( d \) 0.20: size of the small effect; \( d \) 0.50: medium effect size; \( d \) 0.80: large effect size\(^4\).

The analyses were conducted using the IBM SPSS Statistics for Windows version 25.0. (Armonk, New York).

Results

Sample description

Initially, a total of 178 pregnant women were willing to take part in the study, of which 164 met the inclusion criteria. It was not possible to know the sex of the baby of 21 of them, another 12 decided to leave the study due to a lack of time, and 15 had a miscarriage. In the case of 8 babies (four girls and four boys) of the remaining 16 mothers, the amount of baby hair was deemed insufficient for cortisol analysis, so they were removed from the sample. The final total sample consisted of 108 pregnant women in their first trimester of gestation, aged between 22 and 43 years (\( M = 33.73; SD = 4.37 \)).

The participants were divided into two groups based on the sex of the baby they gave birth to (male or female). Thus, 46 women (\( M = 33.41 \) years of age; \( SD = 3.83 \)) were included in the baby boy group and a total of 62 women (\( M = 33.98 \) years of age; \( SD = 4.78 \)) were included in the baby girl group.

Table 1 shows the main sociodemographic and obstetric differences depending on the newborn’s sex. No significant differences were found between the two groups in terms of age, marital status, educational level, employment status, type of pregnancy, number of children, number of previous abortions, pregnancy risk or desired/non-desired pregnancy.

Relationship between the baby’s sex and hair cortisol levels representing the preconceptional period and the first trimester of pregnancy

Statistically significant differences in maternal cortisol levels were found in the first trimester, depending on the baby’s sex (\( U = 1097.50; P < 0.05 \)). The levels were higher in the case of baby girls (expressed in median:lower quartile-upper quartile) (164.36: 54.45-284.87 pg/mg) compared to that of baby boys (101.13: 37.95-193.56 pg/mg). Cohen’s \( d \) reported an effect size of 0.40 (Fig. 1).

\[
\text{Table 1. Differences in sociodemographic variables and obstetric information between pregnant women who had a girl or boy baby}
\]

| Sociodemographic variables | Girl baby \((N=62)\) | Boy baby \((N=46)\) | Test \((\chi^2/t)\) | \(P\) |
|----------------------------|----------------------|----------------------|---------------------|-------|
| Age                        | 33.98 (4.78)         | 33.41 (3.83)         | 0.61                | 0.55  |
| Marital status             |                      |                      |                     |       |
| Married/cohabitant         | 60 (96.8%)           | 46 (100%)            | 2.46                | 0.65  |
| Single/divorced/widow      | 2 (3.2%)             | -                    |                     |       |
| Level of education         |                      |                      |                     |       |
| Primary                    | 4 (6%)               | 3 (7.1%)             | 2.42                | 0.29  |
| Secondary                  | 15 (24%)             | 11 (23.8%)           |                     |       |
| University                 | 43 (70%)             | 32 (69.1%)           |                     |       |
| Employment situation       |                      |                      |                     |       |
| Unemployed                 | 14 (22%)             | 7 (15.4%)            | 1.78                | 0.78  |
| Working                    | 47 (76%)             | 37 (79.5%)           |                     |       |
| Student                    | 1 (2%)               | 2 (5.1%)             |                     |       |
| Smoking                    |                      |                      |                     |       |
| Yes                        | 9 (14.5%)            | 6 (13%)              | 0.048               | 0.827 |
| No                         | 53 (85.5%)           | 40 (87%)             |                     |       |
| Body Mass Index            | 23.40 (4.46)         | 24.90 (5.03)         | 1.41                | 0.161 |

Obstetric information

| Type of pregnancy          |                      |                     |                     |       |
|----------------------------|----------------------|----------------------|---------------------|-------|
| Spontaneous                | 53 (86%)             | 43 (93.5%)           | 3.31                | 0.19  |
| Artificial insemination    | 4 (6%)               | 3 (6.5%)             |                     |       |
| Previous children          |                      |                      |                     |       |
| 0                          | 28 (45.2%)           | 21 (45.7%)           | 1.64                | 0.44  |
| 1                          | 28 (45.2%)           | 17 (37%)             |                     |       |
| ≥2                         | 6 (9.7%)             | 8 (17.4%)            |                     |       |
| Previous miscarriages      |                      |                      |                     |       |
| 0                          | 36 (58.1%)           | 29 (63%)             | 1.41                | 0.49  |
| 1                          | 18 (29%)             | 9 (19.6%)            |                     |       |
| ≥2                         | 8 (12.9%)            | 8 (17.4%)            |                     |       |
| Pregnancy desired          |                      |                      |                     |       |
| Yes                        | 53 (85.7%)           | 44 (95.7%)           | 3.94                | 0.27  |
| No                         | 9 (14.3%)            | 2 (4.3%)             |                     |       |

Note: Significance level at \( P \leq 0.05 \). Student \( t \)-test used for continuous variables and Chi-squared test for categorical variables.
Relationship between the Baby’s Sex and Stress and Pregnancy Concerns during the First Trimester

There were no significant differences regarding the baby’s sex based on the results obtained in the PDQ ($t = 1.46; P > 0.05$) or were there any differences in the results of EEP-1 ($t = 70; P > 0.05$) or in the IVE ($t = .08; P > 0.05$).

Discussion

The study’s objective was to understand the relationship between the baby’s sex and the level of cortisol in the mother’s hair before conception and in the first weeks of the first trimester, as well as between the baby’s sex and the psychological stress perceived by mothers in the first trimester of gestation.

The results showed differences in maternal hair cortisol levels in the first trimester of pregnancy among women who gave birth to a baby boy compared to those who delivered a baby girl. Specifically, cortisol levels were higher in the first trimester of pregnancy when the fetus was a girl. Therefore, the level of this hormone in the hair in the first trimester of pregnancy seems to be related to the baby’s sex.

When interpreting the results of this study, we must remember that the hormone was extracted from women’s hair between weeks 8 to 10 of their pregnancy. In this way, retrospective information on maternal cortisol levels was obtained during the conception and in the first weeks of pregnancy. Thus, maternal stress was shown to be higher over that period in mothers who later gave birth to a girl as opposed to those who gave birth to a boy.

Previous studies on links between maternal stress during conception and the baby’s sex have produced similar findings: women who presented higher concentrations of cortisol in their saliva prior to conception were less likely to deliver a male baby. Nevertheless, our study seems to contradict other results, such as those of Bosquet Enlow et al., who have found that maternal hair cortisol levels were higher across the three trimesters when mother have a boy. Future research should focus on this aspect in order to increase knowledge on this topic.

On the other hand, the results showed that there was no difference in perceived psychological stress in the first trimester between mothers who had a boy and those who had a girl. That is, neither the specific stress of pregnancy nor the levels of perceived stress, nor the levels of vulnerability to stress influenced the sex of the baby. These results are in line with that of a previous longitudinal study, in which maternal stress was measured using a series of psychological instruments, and no significant differences were found between the scores of women who gave birth to a boy and those who gave birth to a girl.

Thus, the results of the study seem to support the explanatory theories according to which this hormone plays an important role in determining the sex of the baby, both during conception and in pregnancy.

A possible explanation would be that the activation of the HPA axis modifies sex hormone concentrations at the time of conception. However, the mechanisms underlying this modification are unclear. On the one hand, there is evidence that testosterone functions as a mechanism when determining the baby’s sex, since the greater the prenatal stress levels, the higher the levels of female testosterone. Some studies have also focussed on the role of the father’s stress at the time of conception, although we did not take such research into account in the present study. Song et al. found that the proportion of sex chromosomes in ejaculated sperm may be altered by exposure to stress, reducing the viability of Y chromosomes, thus affecting the distribution of sexes at birth. Moreover, the X sperm are better at passing through cervical mucus, so when hormonal changes occur, caused by stress, these sperm are greater achievers than the Y sperm.

On the other hand, another possible explanation for the results is the theory according to which selective male miscarriages take place during pregnancy. Our sample, however, did not include pregnant women who subsequently aborted, so we were not able to learn more about this latter relationship.

What does seem clear – and this has been shown in a number of studies – is that fetuses are vulnerable and that stress plays a role. For example, it has been shown that Y fetuses mature more slowly than X fetuses; they tend to present pregnancy complications and preterm birth and at birth, they are more likely to have shorter telomeres. The study presented a number of limitations. For example, the role that fathers’ stress may have at the time of conception, which seems relevant in determining the sex of the baby, was not taken into account. Therefore, it would be interesting to include measurements of cortisol in fathers’ hair in subsequent studies. Besides, some authors have detected in older adults that hair cortisol levels are slightly higher in dark brown hair, so it could be worth obtaining this information to know if this phenomenon could occur also in pregnant women.

It would also be relevant, in the future, to follow up female participants who were discarded because they aborted. Examining these cases would allow checking whether women expecting boys undergo more stress abortions than women expecting girls, as suggested by recent research. Including this data in future studies would thus lead to a deeper understanding of the mechanisms underlying deviations in the sex of the offspring.

Finally, a future line of research could also be that of reproducing the study with samples of women who present high-risk pregnancies (such as pregnancies resulting from in-vitro fertilisation or artificial insemination). Such populations experience high levels of stress and would allow us to better understand the relationship between stress and the child’s sex.

The findings of the present study suggest that a relationship exists between cortisol levels in mothers at the time of conception and in the first trimester of gestation and the sex of their future babies. Thus, giving birth to a girl would imply higher levels of maternal hair cortisol at the time of conception and in the first weeks of pregnancy. However, no relationship was found between the sex of the offspring and perceived psychological stress levels in the first trimester.
To conclude, the research presented here is pioneering to the extent that it links prenatal stress to the sex of newborns. This was done by measuring pregnant women’s hair cortisol, the only longitudinal measure that is suitable for this purpose. Therefore, this work contributes to an open field of research which requires further studies to explain the role played by prenatal stress and cortisol on sex allocation.

Acknowledgments. Thank you to every pregnant woman who participated in the study. This study is part of a Doctoral Thesis of Mr. Jose A. Puertas-Gonzalez.

Financial support. This work was supported by the Frontier Project “A-CTS-229-UGR18” of the Ministry of Economy, Knowledge, Business and University of the Junta de Andalucía, co-supported by funds/European Regional Development Fund (ERDF) – a way to build Europe. Besides, Mr. Jose Antonio Puertas-Gonzalez has been awarded with an individual research grant (Spanish Ministry of Science, Innovation and Universities, FPU program, reference number 18/00617), as well as Dr. Borja Romero-Gonzalez (Spanish Ministry of Economy, Industry and Competitiveness, FPI Program, reference number BES-2016-077619).

Conflict of interest. None.

Ethical statement. Participation was voluntary, and an informed written consent document was read and signed by every participant. This study followed the guidelines of the Helsinki Declaration (AMM, 2008) and the consent document was read and signed by every participant. This study followed the guidelines of the Helsinki Declaration (AMM, 2008) and the consent document was read and signed by every participant.

References

1. Lobel M, Dunkel Schetter C. Pregnancy and prenatal stress. Environ Mental Health. 2016; 2016, 318–329. doi: 10.1016/B978-0-12-397045-9.00164-6
2. Karlén J, Frostell A, Theodorsson E, Fareto J, Ludvigsson J. Maternal influence on child HPA axis: a prospective study of cortisol levels in hair. Pediatrics. 2013; 132(5), e1333–e1340. doi: 10.1542/peds.2013-1178
3. Romero-Gonzalez B, Caparros-Gonzalez RA, Gonzalez-Perez R, Delgado-Puertas P. Peralta-Ramirez MI. Newborn infants’ hair cortisol levels reflect chronic maternal stress during pregnancy. PLoS One. 2018; 13(7), e0200279. doi: 10.1371/journal.pone.0200279
4. Bergman K, Sarkar P, Glover V, O’Connor TG. Maternal prenatal cortisol and infant cognitive development: moderation by infant-mother attachment. Biol Psychiatry. 2010; 67(11), 1026–1032. doi: 10.1016/j.biopsych.2010.01.002
5. de Weerth C, Buitelaar JK, Beijers R. Infant cortisol and behavioral habituation to weekly maternal separations: links with maternal prenatal cortisol and psychosocial stress. Psychoneuroendocrinology. 2013; 38(12), 2863–2874. doi: 10.1016/j.psyneuen.2013.07.014
6. Jung C, Ho JT, Torpy DJ, et al. A longitudinal study of plasma and urinary cortisol in pregnancy and postpartum. J Clin Endocrinol Metab. 2011; 96(5), 1533–1540. doi: 10.1210/jc.2010-2395
7. Jahangard L, Mikoteit T, Bahiraei S, et al. Prenatal and postnatal hair steroid levels predict post-partum depression 12 weeks after delivery. J Clin Med. 2019; 8(9), 1290. doi: 10.3390/jcm9091290
8. Wosu AC, Valimiradottir U, Shields AE, Williams DR, Williams MA. Correlates of cortisol in human hair: implications for epidemiologic studies on health effects of chronic stress. Ann Epidemiol. 2013; 23(12), 797–811. doi: 10.1016/j.annepidem.2013.09.006
9. Caparros-Gonzalez RA, Romero-Gonzalez B, Strivens-Vilchez H, Gonzalez-Perez R, Martinez-Augustin O, Peralta-Ramirez MI. Hair cortisol levels, psychological stress and psychopathological symptoms as predictors of postpartum depression. PLoS One. 2017; 12(8), 1–17. doi: 10.1371/journal.pone.0182817
10. Caparros-Gonzalez RA, Romero-Gonzalez B, Gonzalez-Perez R, et al. Maternal and neonatal hair cortisol levels are associated with infant neurodevelopment at six months of age. J Clin Med. 2019; 8(11), 2015. doi: 10.3390/jcm8112015
11. García-León MÁ, Caparros-Gonzalez RA, Romero-Gonzalez B, Gonzalez-Perez R, Peralta-Ramirez I. Resilience as a protective factor in pregnancy and puerperium: its relationship with the psychological state, and with Hair Cortisol Concentrations. Midwifery. 2019; 75, 138–145. doi: 10.1016/j.midw.2019.05.006
12. Hoffman MC, D’Anna-Hernandez K, Benitez P, Ross RG, Laudenslager ML. Cortisol during human fetal life: characterization of a method for processing small quantities of newborn hair from 26 to 42 weeks gestation. Dev Psychobiol. 2016; 59(1), 123–127. doi: 10.1002/dev.21433
13. Romero-Gonzalez B, Caparros-Gonzalez RA, Gonzalez-Perez R, Coca-Arco S, Peralta-Ramirez MI. Hair cortisol levels, psychological stress and psychopathological symptoms prior to instrumental deliveries. Midwifery. 2019; 77, 45–52. doi: 10.1016/j.midw.2019.06.015
14. Anderssen MS, Jensen RC, Schmedes AV, et al. Third trimester cortisol status is associated with offspring sex and polycystic ovary syndrome status: Odense Child Cohort. Fertil Steril. 2019; 112(4), 764–772. doi: 10.1016/j.fertnstert.2019.05.013
15. DiPietro JA, Costigan KA, Kivlighan HT, Chen P, Laudenslager ML. Maternal salivary cortisol differs by fetal sex during the second half of pregnancy. Psychoneuroendocrinology. 2011; 36(4), 588–591. doi: 10.1016/j.psyneuen.2010.09.005
16. Giesbrecht GF, Campbell T, Letourneau N, et al. Sexually dimorphic adaptations in basal maternal stress physiology during pregnancy and implications for fetal development. Psychoneuroendocrinology. 2015; 56, 168–178. doi: 10.1016/j.psyneuen.2015.03.013
17. Walsh K, McCormack CA, Webster R, et al. Maternal prenatal stress phenotypes associate with fetal neurodevelopment and birth outcomes. Proc Natl Acad Sci U S A. 2019; 116(48), 23996–24005. doi: 10.1073/pnas.1905890116
18. Chason RJ, McLain AC, Sundaram R, et al. Preconception stress and the secondary sex ratio: a prospective cohort study. Fertil Steril. 2012; 98(4), 937–941. doi: 10.1016/j.fertnstert.2012.06.037
19. Bruckner TA, Catalano R, Ahern J. Male fetal loss in the U.S. following the terrorist attacks of September 11, 2001. BMC Public Health. 2010; 10, 276 (1–6). doi: 10.1186/1471-2458-10-273
20. Catalano R, Bruckner T, Marks AR, Eskenazi B. Exogenous shocks to the human sex ratio: the case of September 11, 2001 in New York City. Hum Reprod. 2006; 21(12), 3127–3131. doi: 10.1093/humrep/de283
21. Catalano R, Bruckner T, Gould J, Eskenazi B, Anderson E. Sex ratios in California following the terrorist attacks of September 11, 2001. Hum Reprod. 2005; 20(5), 1221–1227. doi: 10.1093/humrep/deh763
22. Grech V, Zammitt D. The President Kennedy assassination and the male to female birth ratio. Early Hum Dev. 2016; 103, 119–121. doi: 10.1016/j.ehud.2016.08.008
23. Hansen D, Möller H, Olsen J. Severe periconceptional life events and the sex ratio in offspring: Follow up study based on five national registers. Br Med J. 1999; 319(7209), 548–549. doi: 10.1136/bmj.319.7209.548
24. Ruckstuhl KE, Colijn GP, Amiot V, Vinish E. Mother occupation and sex ratio deviations? J Theor Biol. 2012; 278, 558–567. doi: 10.1016/j.jtbi.2011.07.002
25. Navara, KJ. The sex ratio deviations? J Theor Biol. 2012; 278, 558–567. doi: 10.1016/j.jtbi.2011.07.002
26. Grant VJ. Could maternal testosterone levels govern mammalian sex ratio deviations? J Theor Biol. 2007; 246(4), 708–719. doi: 10.1016/j.jtbi.2007.02.005
27. James WH, Grech V. A review of the established and suspected causes of variations in human sex ratio at birth. Early Hum Dev. 2017; 109, 50–56. doi: 10.1016/j.earlhumdev.2017.03.002
28. Song WH, Mohamed EA, Pang WK, et al. Effect of endocrine disruptors on the ratio of X and Y chromosome-bearing live spermatozoa. Reprod Toxicol. 2018; 82, 10–17. doi: 10.1016/j.reprotox.2018.09.002
31. Bae J, Lynch CD, Kim S, Sundaram R, Sapra KJ, Buck Louis GM. Preconception stress and the secondary sex ratio in a population-based preconception cohort. *Fertil Steril*. 2017; 107(3), 714–722. doi: 10.1016/j.fertnstert.2016.12.011

32. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods*. 2009; 41(4), 1149–1160. doi:10.3758/BRM.41.4.1149

33. Andalusian Ministry of Health. Pregnancy health document. 2010. http://www.juntadeandalucia.es/salud/sites/csald/galerias/documentos/c_3_c_1_vida_sana/embarazo_y_salud/lactancia_materna/cartilla_embarazo.pdf.

34. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983; 24(4), 385–396. doi:10.2307/2136404

35. Remor E. Psychometric Properties of a European Spanish Version of the Perceived Stress Scale (PSS). *Span J Psychol*. 2006; 9(1), 86–93. doi: 10.1017/S1138741600006004

36. Caparros-Gonzalez RA, Perra O, Alderdice F, et al. Psychometric validation of the Prenatal Distress Questionnaire (PDQ) in pregnant women in Spain. *Women Heal*. 2019; 59(8), 937–952. doi:10.1080/03630242.2019.1584143

37. Yali AM, Lobel M. Coping and distress in pregnancy: an investigation of medically high risk women. *J Psychosom Obstet Gynecol*. 1999; 20(1), 39–52. doi: 10.3109/01674829909075575

38. Beech, HR, Laurence E, Burns, Sheffield, BF. Tratamiento del estrés: un enfoque comportamental. 1986. Alhambra, Madrid.

39. Robles-Ortega, H, Peralta-Ramírez, MI, Navarrete-Navarrete, N. Validación de la versión española del inventario de vulnerabilidad al estrés de Beech, Burns y Sheffield. *Av Pri Sal*. 2006, 62.

40. Sauvé B, Koren G, Walsh G, Tokmakjian S, Van Uum SHM. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clin Investig Med*. 2007; 30(5), 183–191.

41. Staldt T, Kirschbaum C. Analysis of cortisol in hair - State of the art and future directions. *Brain Behav Immun*. 2012; 26(7), 1019–1029. doi:10.1016/j.bbi.2012.02.002

42. Cohen J. 2.2. The Effect Size Index: d. In: *Statistical Power Analysis for the Behavioral Sciences*. 1988.

43. Bosquet Enlow M, Sideridis G, Bollati V, Hoxha M, Hacker MR, Wright RJ. Maternal cortisol output in pregnancy and newborn telomere length: evidence for sex-specific effects. *Psychoneuroendocrinology*. 2019; 102, 225–235. doi:10.1016/j.psyneuen.2018.12.222

44. Bale TL. The placenta and neurodevelopment: sex differences in prenatal vulnerability. *Dialogues Clin Neurosci*. 2016; 18(4), 459–464.

45. Rosa MJ, Nentin F, Bosquet Enlow M, et al. Sex-Specific associations between prenatal negative life events and birth outcomes. *Stress*. 2019; 22(6), 647–653. doi: 10.1080/10253890.2019.1608944

46. Zeitlin J. Fetal sex and preterm birth: are males at greater risk? *Hum Reprod*. 2002; 17(10), 2762–2768. doi:10.1093/humrep/17.10.2762

47. Zeitlin J, Ancel PY, Larroque B, Kaminski M. Fetal sex and indicated very preterm birth: results of the EPIPAGE study. *Am J Obstet Gynecol*. 2004; 190(5), 1322–1325. doi:10.1016/j.ajog.2003.10.703

48. Bosquet Enlow M, Bollati V, Sideridis G, et al. Sex differences in effects of maternal risk and protective factors in childhood and pregnancy on newborn telomere length. *Psychoneuroendocrinology*. 2018; 95, 74–85. doi:10.1016/j.psyneuen.2018.05.025

49. Lin J, Sun J, Wang S, et al. In vitro proinflammatory gene expression predicts in vivo telomere shortening: a preliminary study. *Psychoneuroendocrinology*. 2018; 96, 179–187. doi:10.1016/j.psyneuen.2018.06.020

50. Lanfear JH, Voegel CD, Binz TM, Paul RA. Hair cortisol measurement in older adults: influence of demographic and physiological factors and correlation with perceived stress. *Steroids*. 2020; 163, 108712. doi:10.1016/j.steroids.2020.108712