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How prenatal cortisol levels may differentially affect the neurodevelopment of boys and girls

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ABSTRACT

Background: Prenatal stress could have serious consequences on maternal and fetal health. In this sense, some studies have stated that maternal HCC during pregnancy could contribute to sex-specific effects on infant neurodevelopment, following the Developmental Origins of Health and Disease Hypothesis. Aim: This study aimed to determine whether maternal hair cortisol concentration (HCC) during each trimester of pregnancy and postpartum could predict the neurodevelopmental outcomes of their 12-month-old offspring, with sex-specific differences considered. Study design: longitudinal. Subjects: The study involved 93 pregnant women and their babies. Outcome measure: Hair samples collected during each trimester and postpartum and The Bayley Scales for Infant Development III was used to assess the infants' abilities. Results: The results showed that maternal HCC during the first and second trimesters could predict language and motor abilities. However, when discriminated by sex, only females' cognitive, expressive language, and fine and gross motor skills were predicted by cortisol, not males. Conclusions: These findings support the idea that non-toxic levels of cortisol can positively influence infants' neurodevelopment.

1. Introduction

Child development is a complex process that can be affected by many factors, including prenatal exposure to stressful events and a stressful environment [1,2]. The impact of maternal prenatal distress on child development can be supported by the Developmental Origins of Health and Disease Hypothesis (DOHaD) [3,4] which states that the fetus' environment, either positive or negative, can consequently influence the baby's development and is also dependent on sensitive periods during pregnancy. Thus, a stressful prenatal environment could result in altered birth and developmental outcome [5,6].

This influence can be assessed either through psychological questionnaires or endocrine biomarkers such as cortisol [7,8]. Taking into account the DOHaD a high level of stress during pregnancy reflected in increased cortisol can affect the baby through various mechanisms [6,9]. Firstly, elevated anxiety and stress can modify the protective placental enzyme barrier (11β-HSD2) that transforms active cortisol into its inactive form (cortisone and 11β-dehydrocorticosterone), allowing maternal cortisol into the fetus' bloodstream [10]. Additionally, the placental secretion of corticotropin-releasing hormone (CRH) generates positive feedback of cortisol production on the mother and the baby, potentially altering the development of the central nervous system as well as the infant's hypothalamic-pituitary-adrenal axis (HPA). Finally, maternal increased levels of cortisol, in conjunction with increased catecholamines, can reduce the placental blood flow, which would decrease the fetus' blood flow as well and could alter its growth and development [6,11].

The measurement of this hormone can be done through salivary, urine, plasma or hair samples [12]. Since the time and quality of exposure to the stressor and cortisol levels are determinants of the influence it can have on both mother and child, hair cortisol concentrations (HCC) as a biomarker for chronic stress is an optimal retrospective measure during pregnancy [7,13].

A negative relationship has been found between maternal HCC during the first trimester of pregnancy and newborn's HCC, which could be evidence that supports the Developmental Origins of Health and

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Disease Hypothesis [8]. This fact could support the theory, as it allows us to observe the influence of the prenatal environment on potential fetal physiological alterations. However, it would be just a potential indicator since it must be linked to the later health conditions of the offspring. It is worth mentioning that cortisol is a hormone required for the regulation of adaptative processes, its variations related to the mother's HCC could have a negative impact on the infant's neurodevelopment [8]. In this line, studies linking different types of maternal distress during pregnancy and child's development have inconclusive and conflicting results [14,15], but they seem to be related to the time of exposure during gestation [16,17]. It appears, during early and late gestation the fetus is less protected from the effects of stress and cortisol [18], in particular, elevated stress during late gestation, measured by HCC has been related to worse motor development at 6 months of age [1]. Moreover, similar negative relations exist between high cortisol in plasma during the second trimester and lower cognitive development at 17 months, nevertheless, they appear to be moderated by a positive maternal postnatal rearing [19].

Conversely, positive direct associations have also been identified. Even though previous theories have stated that cortisol during pregnancy can be detrimental to the offspring's health and development, its important role in metabolic function and organ growth needs to be taken into account [20,21]. Therefore, healthy and moderate levels of glucocorticoids have been related to an adequate development of cognitive and emotional brain networks during certain sensitive periods [16,22], since these levels are not considered harmful for the infant. Accordingly, studies differentiating cortisol's influence on development at 12 months old by trimester have found that high levels of maternal salivary cortisol during early gestation can negatively affect offspring's cognitive development, but in late gestation, it reflects better cognitive development [16]. Moreover, some recent studies have shown that maternal cortisol during conception and early pregnancy (HCC and serum cortisol) could have an impact on fetal sex, being high maternal HCC related to the probability of having a female [23,24]. In this sense, maternal HCC during pregnancy could contribute to sex-specific effects on infant neurodevelopment making it worthwhile to explore this area in more depth.

Undoubtedly, stress and cortisol levels during gestation can influence offspring's outcome, yet few studies have analyzed the relation between HCC as a stable biomarker for chronic stress considering sensitive periods during pregnancy and postpartum, and the baby's neurodevelopment, analyzing whether there is a sex-specific differential effect. Hence, the main objective of this study was to analyze longitudinally if maternal HCC during each trimester of pregnancy and postpartum could predict cognitive, language and motor neurodevelopmental outcomes of their offspring at 12 months of age. In addition to this, the second objective was to check if maternal HCC during pregnancy and postpartum could have a differential effect on male or female infants' neurodevelopment.

In accordance with the influence of prenatal stress on child development, as elucidated by the DOHaD, it is hypothesized that maternal stress will negatively impact the baby's neurodevelopment at 12 months of age.

2. Material and methods

2.1. Participants

The initial sample consisted of 101 pregnant women and their 12-month-old babies. Two of the dyads were excluded for incomplete neurodevelopmental information from the babies and the other six were excluded as outliers with either extremely high or extremely low concentrations on the cortisol measurements. The outliers were detected by the difference between the first quartile (Q1) and the third quartile (Q3), or interquartile range, as a reference. In a box plot, a value is considered an outlier if it is located 1.5 times that distance from one of those quartiles (mild outlier) or 3 times that distance (extreme outlier) [25]. Thus, the final sample was made up of 93 pregnant women ($M = 33.57$ years old, $SD = 3.94$) with their respective 93 12-month-old babies ($M = 12.06$ months old, $SD = 0.615$). Inclusion criteria for the pregnant women required a) being 18 (in Spain this age is considered of legal age) y/o and older, b) being in the first trimester of pregnancy at the moment of enrollment, in order to carry out a complete pregnancy follow-up, and c) being able to read and write in Spanish. On the other hand, the exclusion criteria were a) being in treatment with corticosteroids and b) suffering any type of psychological illness. Participants were recruited at different health centers in the city of Granada.

This study was approved by the Human Research Ethics Committee of (masked for peer review). The study protocol was implemented between February 2018 and June 2018 following the guidelines of the Declaration of Helsinki (AMM, 2008) and the Good Clinical Practice Directive (Directive 2005/28/EC) of the European Union. Participation was voluntary, and each participant signed a written informed consent document.

2.2. Instruments

2.2.1. Sociodemographic variables, obstetric information and birth questionnaire

Sociodemographic and obstetric information such as age, educational level, marital status, and pregnancy method among others, were collected through a physical questionnaire during the first visit to the midwife. Birth information such as the baby's birthweight, gestational age and sex, was collected during postpartum through an online questionnaire.

2.2.2. Hair Cortisol levels (HCC)

Hair cortisol levels were obtained from approximately 150 strands of hair taken from the posterior part of the head, taking into account the assumption that hair grows approximately 1 cm per month [26], the first 3 cm closest to the scalp were referred as the cortisol released during the previous three months [12]. The samples of hair were obtained once in every trimester and at postpartum and reserved in an aluminum foil envelope for later analysis.

Following sample collection, the samples underwent initial washing with isopropanol twice to eliminate any externally deposited cortisol, originating from sweat or sebum, on the hair shaft. Subsequently, the samples were air-dried, weighed, and finely powdered using a ball mill (Bullet Blender Storm, Swedesboro NJ, USA) to break up the protein matrix of the hair and enhance the surface area for extraction. Cortisol present within the hair shaft was then extracted into HPLC-grade methanol through a 72-hour incubation in darkness at room temperature with constant inversion using a rotator. After incubation, centrifugation was performed on the samples, and the resulting supernatant was evaporated to complete dryness using a vacuum evaporator (Centrivac, Heraeus, Hanau, Germany). The resulting extract was reconstituted in 150 uL of phosphate buffered saline (PBS) at pH 8.0. The reconstituted sample was promptly frozen at −20 °C for subsequent analysis [27–29]. Finally, the Hair Cortisol Concentration (HCC) of each sample was assessed using the Cortisol Salivary ELISA kit (Alpco Diagnostics) with phosphate buffered saline (PBS) at pH 8.0. Manufacturer-provided instructions for proper usage were followed. The reported cross reactivity by the manufacturer is as follows: Prednisolone 13.6 %, Corticosterone 7.6 %, Deoxycosticosterone 7.2 %, Progesterone 7.2 %, Cortisone 6.2 %, Deoxycortisol 5.6 %, Prednisone 5.6 %, and Dexamethasone 1.6 %. No cross-reaction was observed with DHEAS and Tetrahydrocortisone.

The intra- and inter-assay variations were analyzed on internal quality controls used for routine salivary cortisol measurement, measured in duplicate in eight consecutive assays. The intra-assay coefficients of variance (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 [7]."

2.2.3. Neurodevelopmental assessment

The Bayley Scales for Infant Development III (BSID-III) [30] was implemented for the assessment of infant's neurodevelopment at 12 months old. This scale can be used with children from 16 days to 42 months of age and measures three general aspects of neurodevelopment: cognitive skills, language skills (receptive and expressive communication), and motor skills (fine and gross motor abilities). The Bayley-III Scales provide four different types of normative reference scores: raw scores, scaled scores, composite scores, and percentiles. Raw scores are converted into scaled scores ranging from 1 to 19, with a mean of 10 and a standard deviation of 3. Composite scores are derived from the sums of scaled scores from the subtests and are distributed on a metric scale with a mean of 100 and a standard deviation of 15, ranging from 40 to 160. Finally, scaled scores can be transformed into percentiles using the standardized norms provided by the test. It has adequate internal validity, with a reliability coefficient ranging from 0.86 (for the fine motor subscale) to 0.91 (for the gross motor, cognitive and expressive communication subscale; the remaining receptive language subscale is 0.87). Additionally, it has been previously used to evaluate potential links between prenatal stress and infant neurodevelopment [1,31,32].

2.3. Procedure

Pregnant women were informed about the study during their first control visit ($M = 11.94$ weeks of pregnancy, $SD = 3.89$) to the midwife, where if agreed, they would sign the consent form, fill out the sociodemographic questionnaire and a hair sample was collected and stored in an aluminum foil envelope for further analysis. The conception date was taken from the Pregnancy Health Document, and it was previously determined by the midwife by asking the woman about her last menstrual period, along with an ultrasound measurement, in order to make the calculation as accurate as possible, especially in cases where women had irregular periods. The collection of maternal hair samples was made afterward on the second ($M = 23.55$ weeks of pregnancy, $SD = 4.21$) and third trimester ($M = 33.54$ weeks of pregnancy, $SD = 2.83$), as well as at postpartum (approximately 2 weeks after childbirth), during their follow-up visits, where they would also provide birth and obstetric information through questionnaires. Once the childbirth date was given on the postpartum questionnaire, the baby's neurodevelopment assessment was established and a couple of days before their first birthday, their mothers were contacted to schedule it. The infant's neurodevelopment was assessed always by the same specialized evaluator in the same room, at the (masked for peer review) at 12 months of age using the BSID-III.

2.4. Data analysis

Descriptive and frequency analyses were performed on the sociodemographic and obstetric variables to describe the general sample. A natural logarithmic transformation was performed on the maternal hair cortisol concentration scores to adjust to the normal distribution [33], and afterward, descriptive analyses of the average of maternal hair cortisol and neurodevelopmental scores from the BSID-III were made. Pearson's partial correlations were made to analyze the relation between confounders (age, maternal education in years and birthweight), predictor (HCC on each trimester and postpartum), and predictive variables (Neurodevelopment scores). Followed by three linear regression analyses to identify whether confounding variables previously registered in literature (age, educational level and baby's weight at birth) [19] would affect the infant's neurodevelopment scores. Finally, to identify if maternal hair cortisol concentrations during pregnancy and postpartum could predict infant's neurodevelopment, various independent linear regressions were made entering neurodevelopment scores obtained through the BSID-III as the dependent variable, and maternal hair cortisol concentrations by trimester individually as independent variables.

The absence of multicollinearity was confirmed with a VIF <10 between the predictor and predicted variables.

As for the second objective, newborn infants were divided into two groups according to sex. Dependent *t*-tests were performed to check if there were differences in neurodevelopment among male and female infants. This comparison had two levels of independent variables (boy versus girl) and as dependent variables were the three neurodevelopmental subscale scores. After that, same as in the general objective, various independent linear regressions were made entering neurodevelopment scores obtained through the BSID-III as the dependent variable for each group (male and female infants), and maternal hair cortisol concentrations by trimester individually as independent variables.

The Statistical Package for the Social Sciences 26.0 for Macintosh (SPSS, Armonk, NY) was used to perform the analyses.

3. Results

3.1. Sample description

A total of 93 pregnant women and their 12-month-old babies participated in this study. Women presented a mean age of 33.7 years ($SD = 3.94$), 66.7 % of them were married and 69.9 % had a university level of education. As for the 93 babies, their mean age was 12.06 months ($SD = 0.615$), 49.5 % were male and 50.5 % were female. The main sociodemographic and obstetric information can be found in Table 1.

The HCC can be observed in Table 2, where the averages and standard deviations of both the raw and logarithmic transformation scores are presented. Mother's HCC increased progressively between trimesters, reaching its highest rise at postpartum. The scores of raw HCC

Table 1
Descriptive and frequency data for sociodemographic and obstetric variables.

Sociodemographic variables		
Variables		M (DT)/N (%)
Age		33.57 (3.94)
Level of education (years)		17.8 (3.73)
Nationality	Spanish	88 (94.6 %)
	Immigrant	5 (5.4 %)
Marital status	Married/cohabitant	79 (85 %)
	Single/Divorced/widowed	9 (9.7 %)
Employment status	Unemployed	19 (20.4 %)
	Working	69 (74.2 %)
	Studying	5 (5.4 %)
Hair	Natural	35 (37.6 %)
	Dyed	57 (61.3 %)
Smoker	Yes	5 (5.4 %)
	No	88 (94.6 %)
Alcohol	Yes	1 (1.1 %)
	No	92 (98.9 %)
Obstetric variables		
Primiparous	Yes	51 (54.8 %)
	No	42 (45.2 %)
Wanted pregnancy	Yes	84 (90.4 %)
	No	9 (9.7 %)
Pregnancy method	Spontaneous	79 (84.9 %)
	Fertility treatment	14 (15.1 %)
Previous miscarriages	0	37 (39.8 %)
	1	30 (32.3 %)
	≥ 2	26 (28 %)
Delivery method	Eutocic	70(75.3 %)
	Instrumental	10(10.8 %)
	C-section	13(14 %)
Birthweight (grams)		3296.4 (433.05)
Gestational age (weeks)		39.58 (1.21)
Length (cm)		50.80 (2.01)
Sex of the fetus	Female	47 (50.5 %)
	Male	46 (49.5 %)

Table 2
Cortisol measures and neurodevelopment results.

Cortisol measures				
		Raw data		Log values
		M(SD)	Median(range)	M(SD)
Trimester of measurement	T1	161.85 (127.04)	123.15 (57.70–361.80)	4.91 (0.58)
	T2	174.78 (100.46)	158.5 (55.70–410.50)	5.03 (0.51)
	T3	261.89 (260.43)	198.6 (58.80–747.40)	5.24 (0.81)
	T4	506.67 (265.55)	527.2 (109.40–925.90)	6.05 (0.64)

Bayley Scales for Infant Development - III scores			
Scale	Measures		M(SD)
Cognitive		Total	44 (3.80)
		Scaled	12.1 (2.3)
		Composite	110.2 (11.5)
		Percentile	70.5 (21.5)
Language	Receptive communication	Total	14.9 (2.3)
		Scaled	10.25 (2.7)
	Expressive communication	Total	15.9 (2.6)
		Scaled	10.9 (2.0)
	Scaled		21.2 (4.3)
	Composite		103.6 (12.5)
	Percentile		57.5 (25.3)
Motor	Fine motor	Total	30.2 (3.1)
		Scaled	11.3 (2.9)
	Gross motor	Total	41.8 (4.7)
		Scaled	10.0 (3.2)
	Scaled		21.4 (4.9)
	Composite		104.2 (14.8)
	Percentile		57.4 (28.6)

Note: T1 = First trimester; T2 = Second trimester; T3 = Third trimester; T4 = Postpartum.

ranged from 57.70 to 361.80 in the first trimester, between 55.70 and 410.50 in the second trimester, and between 58.80 and 747.40 in the third trimester; finally, it ranged between 109.40 and 925.90 in the postpartum period. The logarithmic transformation of cortisol ranged from 2.98 to 6.91 in the first trimester, between 3.75 and 6.36 in the second trimester, and between 3.59 and 7.42 in the third trimester; finally, it ranged between 4.75 and 7.13 on the postpartum period.

Finally, the total mean scaled scores on the BSID-III subscales had normal scores. Specifically, cognitive scaled scores were from 7 to 18; the receptive communication scores ranged from 4 to 17, expressive language scores ranged from 9 to 24; motor scaled scores were the lowest, ranging from 5 to 19 on the fine motor scale and from 4 to 19 on the gross motor scale.

3.2. Preliminary analyses

Pearson correlation analyses were made between the confounding, predictor and predictive variables. Finding a significant positive correlation between HCC collected in the second trimester with various language and motor scores and the HCC collected during the third trimester and gross motor total score. A correlation matrix can be found on Fig. S1 whereas the scatterplots for the correlations mentioned can be consulted on Fig. S2.

Afterward, simple linear regressions were performed to identify if confounding variables accounted for changes in the neurodevelopment of the child. Regressions were made with age, educational level in years and birthweight, separately as independent variables and the BSID-III results as dependent variables, finding that none of them predicted any of the neurodevelopment scales.

3.3. Linear regressions using maternal HCC as a predictor of baby's neurodevelopment at 12 months

In order to assess the main objective of this study, simple linear regressions were made to identify if HCC during each trimester of pregnancy and postpartum could predict infant's neurodevelopment at 12 months. The results indicate that HCC in the third trimester could only predict the total gross motor scores ($\beta = 1.245$; SE = 0.553; $R^2 = 0.048$; $p < 0.05$). On the other hand, HCC levels measured during the second trimester significantly predicted receptive (β=1.172; SE = 0.548; $R^2 = 0.048$; $p < 0.05$) and expressive language ($\beta = 1.038$; SE = 0.517; $R^2 = 0.042$; $p < 0.05$) development scores as well as fine ($\beta = 1.682$; SE = 0.576; $R^2 = 0.086$; $p < 0.005$) and gross motor scores (β=1.613; SE = 0.648; $R^2 = 0.064$; $p < 0.05$). Finally, HCC levels during the second trimester could predict the highest variance on the general motor scores including scaled motor score predicting 15 % of the variance, the composed motor score predicting 15 % of the variance, and the motor percentile score, predicting 12 % of the variance. Maternal HCC levels during the first trimester and postpartum did not show significant predictions of infant's neurodevelopment. Other results regarding linear regressions of HCC during the second trimester as a predictor of infant's neurodevelopment can be found in Table 3.

Table 3
Linear regressions with hair cortisol concentrations during the second trimester of pregnancy as predictor of infant neurodevelopment.

IV. Cortisol T2		R^2	F	β(SE)	t	*p*
Cognitive	Total score	0.018	2.655	1.265 (0.776)	1.629	0.107
	Scaled score	0.031	2.946	0.810 (0.472)	1.716	0.089
	Composite	0.020	2.848	3.941 (2.335)	1.688	0.095
	Percentile	0.036	3.397	8.028 (4.356)	1.843	0.069
Receptive Language	Total score	0.053	5.074	1.056 (0.469)	2.253	**0.027***
	Scaled score	0.048	4.575	1.172 (0.548)	2.139	**0.035***
Expressive Language	Total score	0.042	4.025	1.038 (0.517)	2.006	**0.048***
	Scaled score	0.032	3.025	0.706 (0.406)	1.739	0.085
Language	Scaled score	0.049	4.718	1.868 (0.860)	2.172	**0.032***
	Composite	0.049	4.691	5.475 (2.528)	2.166	**0.033***
	Percentile	0.046	4.404	10.709 (5.103)	2.099	**0.039***
Fine Motor	Total score	0.013	1.238	0.702 (0.631)	1.113	0.269
	Scaled score	0.086	8.516	1.682 (0.576)	2.918	**0.004****
Gross Motor	Total score	0.096	9.718	2.848 (0.914)	3.117	**0.002****
	Scaled score	0.064	6.198	1.613 (0.648)	2.490	**0.015***
Motor	Scaled score	0.116	11.890	3.293 (0.955)	3.448	**0.001*****
	Composite	0.116	11.943	9.924 (2.872)	3.456	**0.001*****
	Percentile	0.120	12.416	19.567 (5.553)	3.524	**0.001*****

Note: T2 = Second trimester of pregnancy. Data in bold indicate statistically significant associations.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

3.4. Differential effect of maternal cortisol during gestation on the child's neurodevelopment as a function of sex

In order to fulfill the second objective, dependent *t*-tests were performed in the first place, to check if there were differences in main maternal sociodemographic and obstetric variables, as well as maternal HCC in each trimester of pregnancy and postpartum. Differences were found in newborn length ($t = -2.363$; $p = 0.02$), being longer male ($M = 51.28$ cm, $SD = 2.05$) than female newborns (Table 4).

Secondly, dependent *t*-tests revealed no differences in infant neurodevelopment among male and female infants. Finally, linear regression revealed that maternal HCC during the second trimester could predict cognitive neurodevelopment in female infants, specifically in total score ($\beta = 3.195$; SE = 1.012; $R^2 = 0.181$; $p < 0.05$), scaled ($\beta = 1.905$; SE = 0.610; $R^2 = 0.178$; $p < 0.05$), and composite score ($\beta = 9.526$; SE = 3.048; $R^2 = 0.178$; $p < 0.05$), and percentile score ($\beta = 12.281$; SE = 6.380; $R^2 = 0.154$; $p < 0.05$). There also were differences in receptive language (total score; $\beta = 1.483$; SE = 0.715; $R^2 = 0.087$; $p < 0.05$); and fine motor scaled score ($\beta = 2.187$; SE = 0.952; $R^2 = 0.105$; $p < 0.05$), gross motor in total score ($\beta = 4.579$; SE = 1.357; $R^2 = 0.202$; $p < 0.05$) and scaled score ($\beta = 2.184$; SE = 0.999; $R^2 = 0.096$; $p < 0.05$); and general motor neurodevelopment in scaled score ($\beta = 4.427$; SE = 01.512; $R^2 = 0.160$; $p < 0.05$), composite score ($\beta = 13.383$; SE = 4.535; $R^2 = 0.162$; $p < 0.05$) and percentile score ($\beta = 24.684$; SE = 8.524; $R^2 = 0.157$; $p < 0.05$). Those data could be found in Table 5.

4. Discussion

The first objective of this study was to identify if maternal HCC during each trimester of pregnancy and postpartum could predict infants' development at 12 months, in the areas of cognitive, receptive and expressive language, and fine and gross motor skills. Our results show that expressive and receptive language, as well as fine and gross motor skills, were positively predicted by cortisol released between the first and second trimesters, meaning pregnant women who displayed higher levels of cortisol in the first and second trimesters, had children with higher scores in said developmental scales at 12 months old. These findings were obtained considering that there were no associations between potential influencing factors (such as age, maternal education in years, and birthweight) and the outcome variables.

These results may be controversial and although some studies are inconsistent with our results, others go in the same line. Some have stated that following the DOHaD [3,9], high levels of cortisol can have a detrimental effect on offspring's neurodevelopment, relating higher HCC during late gestation to decreased motor development at 6 months of age [1]. The difference presented in our results could be related to the difference in cortisol concentrations, being higher in the study of Caparros-Gonzalez et al. [1] in comparison to the ones presented in this study, as well as the difference in the infant's age at the neurodevelopmental assessment, being at 12 months in this study and at 6 months in the previous one. Nevertheless, some studies have found similar results to ours stating that higher perceived stress during early gestation could be beneficial to motor development at 12 months old [34], as well as increased levels of cortisol in saliva during late stages are related to enhanced cognitive development at 12 months [20].

In this matter, it must be considered that the Fetal Origins of Health and Disease hypothesis states that changes during the maternal prenatal period can affect both, negatively and positively [35] the offspring's health and development, thus moderate levels of cortisol could be potentiating an ideal development of language and motor abilities. The improvement in development related to high levels of stress can be explained by evolutionary adaptation, where if during gestation, fetuses perceived the environment as threatening, they must adapt to thrive [36]. Therefore, our results can be supported by the theory that non-neurotoxic levels of cortisol, are not harmful to offspring's development [16,20], in fact, mild to moderate levels of distress can even

Table 4
Mean differences of sociodemographic, obstetric variables and maternal hair cortisol concentrations between female and male infants.

Sociodemographic variables					
Variables		Female M (DT)/N (%)	Male M (DT)/N (%)	t/χ^2	*p*
Age		33.36 (4.47)	33.78 (3.36)	−0.513	0.610
Level of education (years)		16.64 (3.50)	17.74 (3.90)	−1.432	0.155
Nationality	Spanish	44 (93.6 %)	44 (95.7 %)	0.989	0.610
	Immigrant	3 (6.4 %)	2 (4.3 %)		
Marital status	Married/ cohabitant	42 (89.3 %)	37 (80.5 %)	4.337	0.227
	Single/ Divorced/ widowed	5 (10.6 %)	9 (19.5 %)		
Employment status	Unemployed	8(17 %)	11 (23.9 %)	5.294	0.258
	Working	37 (78.7 %)	32 (69.5 %)		
	Studying	2 (4.3 %)	3 (6.5 %)		
Hair	Natural	17 (36.2 %)	18 (39.1 %)	1.035	0.596
	Dyed	30 (63.8 %)	28 (60.9 %)		
Smoker	Yes	4 (8.5 %)	1 (2.2 %)	1.835	0.361
	No	43 (91.5 %)	45 (97.8 %)		
Alcohol	Yes	0 (0 %)	1 (2.2 %)	1.033	0.495
	No	47 (100.0 %)	45 (97.8 %)		
Obstetric variables					
Primiparous	Yes	28 (59.6 %)	23 (50 %)	0.860	0.236
	No	19 (40.4 %)	23 (50 %)		
Wanted pregnancy	Yes	41 (87.3 %)	43 (93.5 %)	4.101	0.251
	No	6 (12.8 %)	3 (6.5 %)		
Pregnancy method	Spontaneous	40 (85.1 %)	39 (84.8 %)	2.336	0.311
	Fertility treatment	7 (14.9 %)	7 (15.2 %)		
Previous miscarriages	0	18 (38.3 %)	19 (41.3 %)	0.170	0.918
	1	15 (31.9 %)	15 (32.6 %)		
	≥2	14 (29.8 %)	12 (26.1 %)		
Birthweight (grams)		3230.21 (473.29)	3364.02 (381.00)	−1.500	0.137
Gestational age (weeks)		39.55 (1.12)	39.61 (1.31)	−0.220	0.826
Length (cm)		50.32 (1.88)	51.28 (2.05)	−2.363	**0.02***
		Log maternal HCC			
T1		4.81 (0.59)	5.01 (0.58)	1.604	0.112
T2		4.99 (0.48)	5.08 (0.54)	0.788	0.433
T3		5.12 (0.68)	5.36 (0.92)	1.376	0.172
T4		5.98 (0.69)	6.13 (0.59)	1.161	0.249

Note: T1 = First trimester; T2 = Second trimester; T3 = Third trimester; T4 = Postpartum.

* p < 0,02.

Table 5
Linear regressions with hair cortisol concentrations during the second trimester as predictor of infant neurodevelopment, selecting by sex.

		R^2		F		β(SE)		t		*p*	
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
Cognitive	Total score	0.181	0.003	9.970	0.111	3.195(1.012)	−0.378(1.135)	3.157	−0.333	**0.003**	0.741
	Scaled score	0.178	0.001	9.764	0.036	1.905(0.610)	−0.132(0.697)	3.125	−0.189	**0.003**	0.851
	Composite	0.178	0.001	9.764	0.061	9.526(3.048)	−0.845(3.419)	3.125	−0.247	**0.003**	0.806
	Percentile	0.154	0.000	8.209	0.006	18.281(6.380)	−0.451(5.846)	2.865	−0.077	**0.006**	0.939
Receptive Language	Total score	0.087	0.027	4.298	1.213	1.483(0.715)	0.695(0.631)	2.073	1.101	**0.044**	0.277
	Scaled score	0.071	0.029	3.451	1.295	1.529(0.823)	0.854(0.750)	1.858	1.138	0.070	0.261
Expressive Language	Total score	0.071	0.016	3.452	0.716	1.688(0.909)	0.467(0.552)	1.858	0.846	0.070	0.402
	Scaled score	0.057	0.010	2.733	0.431	1.161(0.702)	0.294(0.448)	1.653	0.657	0.105	0.515
Language	Scaled score	0.074	0.026	3.609	1.175	2.666(1.403)	1.148(1.059)	1.900	1.084	0.064	0.284
	Composite	0.074	0.026	3.586	1.170	7.798(4.118)	3.373(3.118)	1.894	1.082	0.065	0.285
	Percentile	0.067	0.025	3.248	1.134	14.484(8.036)	7.005(6.578)	1.802	1.065	0.078	0.293
Fine Motor	Total score	0.003	0.058	0.115	2.713(0.952)	0.400(1.180)	0.958(0.582)	0.339	1.647	0.736	0.107
	Scaled score	0.105	0.064	5.279	3.032	2.187(0.952)	1.210(0.695)	2.298	1.741	**0.026**	0.089
Gross Motor	Total score	0.202	0.024	11.389	1.092	4.579(1.357)	1.260(1.205)	3.375	1.045	**0.002**	0.302
	Scaled score	0.096	0.033	4.782	1.520	2.184(0.999)	1.049(0.851)	2.187	1.233	**0.034**	0.224
Motor	Scaled score	0.160	0.072	8.578	3.393	4.427(1.512)	2.220(1.205)	2.929	1.842	**0.005**	0.072
	Composite	0.162	0.071	8.708	3.339	13.383(4.535)	6.623(3.624)	2.951	1.827	**0.005**	0.074
	Percentile	0.157	0.082	8.385	3.909	24.684(8.524)	14.414(7.290)	2.896	1.977	**0.006**	0.054

promote the maturation of the fetus and their development [22,37].

Moreover, as mentioned before, our sample appears to have lower average levels of HCC throughout the whole pregnancy than what has been previously registered on another Spaniard sample [7,8], which could also support the fact that adequate levels of maternal cortisol during pregnancy could lead to an ideal development of the offspring.

Considering that during early gestation the fetus is less protected from the effects of stress and cortisol [18] and that our sample seems to have moderate levels of HCC, both fine and gross motor skills seem to be benefited. Also, better expressive and receptive language abilities at 12 months appear to be a novel result since previous studies have not found any differences on this scale [34]. The influence of cortisol changes during early to mid-gestation could relate to the process of neuronal development, where stages of neuronal proliferation and migration occur and establish the basis of neuronal connections in the future [38,39].

Regarding the second objective, maternal HCC between the first and second trimester positively predicted neurodevelopmental scores in female but not in male infants, more specifically in cognitive, motor, and receptive language development. Although this is an emerging research field, some researchers have pointed in the same direction.

For example, Hicks et al. [40] performed a meta-analysis in which we conclude that maternal activation of the HPA axis during pregnancy could have a sex-specific impact on the offspring's risk of psychopathology. Concretely, a higher risk was found in developing internalizing problems in female offspring, associated with higher levels of prenatal stress. Similar results were found in a recent study, evidencing that female offspring have also shown higher reactivity to stress [41]. Hypothetic pathways to explain the influence are in maternal HPA activation, which could alter fetuses' HPA triggering the activation or deactivation of different mechanisms implicated [42].

Returning to female internalizing problems, [43] found that a relationship between higher maternal cortisol and stronger connectivity of the amygdala in newborns could be a possible explanation for these differences. Nevertheless, more research is needed in this field to link possible biological pathways to these discoveries.

Despite the interesting results found, this study has some limitations, as it should be noted that the generalization of these results must be handled cautiously since the pregnant women sample only accounts for women with a high educational level, which could be playing a role in the positive relationship between cortisol and neurodevelopment. We should take into account that pregnant women living in situations of hardship are likely to have different outcomes. Moreover, paternal variables that could influence the results have not been taken into account, so future studies could include variables such as paternal stress, tobacco and alcohol consumption, or perceived social support. Finally, general knowledge about the influence of maternal cortisol's underlying mechanisms of cortisol on baby's development is yet to be fully understood. For future research, it could be beneficial to incorporate more measurements throughout the entire pregnancy, potentially taking at least one measurement per month. This would expand the longitudinally obtained information.

In conclusion, better development of language and motor skills linked to a higher, yet moderate, level of maternal HCC highlight the need to continue to explore the normative levels of cortisol during gestation as well as its neurotoxic influence on pregnant women and their offspring's development. Moreover, this study enhances the importance of recognizing sensitive periods during pregnancy that can influence the offspring's development to provide adequate mental and health care to pregnant women during these stages. Specifically, our results support the idea that early to mid-stages of gestation are critical, taking into account that HCC measures taken during the second-trimester account for cortisol released from late first trimester and early second trimester. In addition, the findings regarding the different relationships between cortisol levels according to trimester and neurodevelopment based on the child's sex open up an interesting field that is necessary for a better understanding and approach to the consequences of maternal stress on offspring.

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Declaration of competing interest

The authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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