Fetal Dehydroepiandrosterone from Hair Samples at Birth Predicts Language Development

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ABSTRACT

Objective. Sex hormones testosterone and estradiol have been related to children's language development. The shared precursor hormone, dehydroepiandrosterone (DHEA), however, has not yet been considered as a biological marker of language ability, despite emerging evidence that fetal DHEA plays a critical role in the organization of the neonatal brain. The present study investigated whether fetal DHEA, compared to fetal testosterone, is associated with infant language development.

Design and methods. DHEA and testosterone concentrations were measured in newborn hair strands (n = 56) collected two weeks after birth, capturing fetal long-term hormone secretions during the third trimester of pregnancy. At six months of age, children's language abilities were assessed using the German version of the Bayley Scales of Infant Development.

Results. Linear regression analysis revealed fetal DHEA levels to be significantly associated with language abilities at six months of age, with lower DHEA levels corresponding to higher language scores. Control analyses assessing general cognitive abilities showed no association of fetal DHEA levels with infant cognitive function. Testosterone levels were not associated with language nor general cognitive skills.

Conclusions. The current study identifies fetal DHEA levels extracted from newborn hair samples as a potential biological factor influencing infant language development.

KEYWORDS: Fetal Testosterone; Fetal Dehydroepiandrosterone (DHEA); Language Development, Biomarker; Hair Hormone Concentration

1 INTRODUCTION

Sex hormones have an essential impact on the hemispheric organization and functional lateralization of the developing brain, thus affecting language processing (Auyeung et al., 2013; Friederici et al., 2008; McCarthy, 2008). Previous research on the role of sex hormones in language development has focused on both prenatal and postnatal levels of testosterone and estradiol. At the prenatal stage, fetal testosterone levels extracted from amniotic fluid were linked to language comprehension in girls at four years of age, with an inverted U-shaped relationship observed (Finegan et al., 1992). In boys, higher perinatal testosterone concentrations from umbilical cord blood indicated smaller vocabulary at two years of age (Hollier et al., 2013) as well as an increased risk of language delay at one and three years of age (Whitehouse et al., 2012), possibly explained by higher prenatal testosterone exposure leading to atypical cerebral lateralization (Hollier et al., 2014b). The impact of postnatal sex hormone concentration on language development has received even more attention. Across sexes, early postnatal testosterone levels from saliva have been reported to negatively relate to later expressive vocabulary at 18 to 30 months of age (Kung et al., 2016) and, when measured from blood samples, to language comprehension at four years of age (Schaadt et al., 2015). For female sex hormones, evidence suggests estradiol to be positively linked to language development. In boys and girls, postnatal estradiol levels from blood samples have been positively associated with infants' cry melody patterns and babbling skills during the first year of life (Quast et al., 2016; Wermke et al., 2014) and with language comprehension at four years of age (Schaadt et al., 2015). Notwithstanding these findings, the predictive value of prenatal and postnatal sex hormones for language abilities has not been consistently substantiated across all studies (Auyeung et al., 2009; Farrant et al., 2013). For example, Knickmeyer et al. (2005) reported that fetal testosterone drawn from amniotic fluid was not related to language abilities at four years of age. In sum, initial evidence partly suggests that testosterone and estradiol contribute to early language development. Yet, studies differ with respect to method and time point of hormone extraction and deliver inconsistent results.

From an endocrinological perspective, testosterone and estradiol are products of the shared hormonal precursor dehydroepiandrosterone (DHEA) along the hormonal pathway. DHEA is the most abundant steroid hormone in the fetal organism, along with its sulfate ester dehydroepiandrosterone sulfate (DHEA-S) (jointly referred to as “DHEA(S)”) (Parker, 1999; Stárka et al., 2015). DHEA(S) is mainly produced by the fetal adrenal glands beginning in the eighth to tenth week of gestation (Ishimoto and Jaffe, 2011; Mesiano and Jaffe, 1997). DHEA(S) production increases throughout the second and third trimesters, highly exceeding maternal synthesis during late pregnancy (Coulter and Jaffe, 1998; Ishimoto and Jaffe, 2011; Mesiano and Jaffe, 1997; Sucheston and Cannon, 1968). Following parturition, the structure of the adrenal cortex is dramatically remodeled: Initiated by an apoptotic process, a programmed cell death, the fetal zone atrophies within the first weeks postpartum. As the fetal zone disappears, DHEA(S) levels decline rapidly, reaching a nadir six months postnatally (Mesiano and Jaffe, 1997; Sucheston and Cannon, 1968).

As a neuroactive steroid, fetal DHEA(S) has the potential to shape later development. This is evidenced by animal studies which have demonstrated DHEA(S)’s critical role in the organization of the neonatal brain as early as in the embryonic phase (Baulieu, 1998; Compagnone and Mellon, 2000, 1998). Similarly, in the human fetal organism, DHEA(S) has been found to promote neural growth and to regulate inflammatory processes that affect early neurodevelopment, already evident in the prenatal period (Lee et al., 2024; Suzuki et al., 2004). Nevertheless, DHEA(S) has been barely addressed in research on child neurodevelopment (Greaves et al., 2019; Kamin and Kertes, 2017), and not yet studied with respect to language development. The elevated levels of DHEA(S) concentration during the fetal period directs research attention to this stage of development, which can be captured using newborn hair samples as a retrospective method of fetal hormone analysis that is not influenced by circadian or situational variations (Gao et al., 2013; Gareri and Koren, 2010).

The current study investigated whether fetal DHEA extracted from newborn hair is associated with infants’ language development at six months of age. We hypothesized fetal DHEA to be

related to early language abilities, based on its function as a neurosteroid that holds the potential to shape early development (Lee et al., 2024; Suzuki et al., 2004). As the hormone derivatives of DHEA, estradiol and testosterone, appear to have opposite effects on language development, we did not have hypotheses about the direction of the relationship. For comparison, we also investigated infants' language development in relation to fetal testosterone. Based on previous evidence suggesting high testosterone levels to negatively contribute to early language development (Hollier et al., 2013; Kung et al., 2016), we expected a significant negative association between fetal testosterone and later language skills.

2 METHODS

2.1 Participants

Participants were mother-infant dyads from an ongoing longitudinal study funded by the Austrian Science Fund (FWF), which examined the effects of biological and environmental factors on infant cognitive, and socio-emotional development. To this aim, pregnant women and their offspring were followed from the 34th week of gestation until 12 months postpartum. Prior to participation, mothers provided informed consent and the experimental procedure was approved by the ethics committee of the University of Salzburg (EK-GZ: 12/2013). For this study, the criteria for maternal inclusion were: (a) German as native language, (b) no high-risk pregnancy, (c) age of 18 years or older. None of the women reported smoking or drinking alcohol during pregnancy. The infant inclusion criteria were: (a) typical development, (b) gestational age of more than 37 weeks at birth, (c) German as environmental language, (d) no hearing impairment at the age of six months, (e) absence of family history of language impairments (first- and second-degree). These criteria were ensured via a study-specific anamnesis questionnaire, along with questions on demographic information, such as the mother's relationship status with the child's father and maternal education. The demographic information and characteristics of the final sample are provided in Table 1 with gestational age at birth, infant age at hair collection, and infant sex being statistically considered as covariates.

A total of 67 mother-infant dyads initially participated in the project. Infants were included in the final study sample if data from at least one of the two tested sex hormones (testosterone and DHEA) were available from the newborn hair sample. In n = 9 infants, testosterone or DHEA was not detectable. Two further participants were excluded, one due to parental refusal to provide hair samples and one due to the mother receiving progesterone supplementation during pregnancy. Moreover, as reference values for hormone concentrations extracted from newborn hair samples are yet to be established, children's data were excluded if either their testosterone (n = 3) or DHEA (n = 2) or both levels (n = 1) exceeded two standard deviations of the average values of the total sample. Note that the total sample's average values were considered for reference, as there were no statistically significant sex differences (Wilcoxon rank-sum test; testosterone: Males: M = 1.63 (SD = 1.43) pg/mg, Females: M = 2.19 (SD = 2.26) pg/mg; W = 323, p = .45; DHEA: Males: M = 19.56 (SD = 12.07) pg/mg, Females: M = 21.56 (SD = 11.76) pg/mg; W = 395, p = .43). Following these criteria, the final participant sample was n = 56 (29 females), including DHEA levels from 50 children and testosterone levels from 44 children, with an overlapping sample of 39 children (flow chart presented in Figure 1). As the effect of fetal DHEA, the hormonal precursor to both estradiol and testosterone, on infant language abilities has not yet been tested, we estimated relevant effect sizes on these downstream hormones in order to approximate the effect sizes required for our power analysis. Specifically, we referred to Finegan et al. (1992), the only study providing detailed statistical analysis on prenatal testosterone, and Wermke et al. (2014) and Quast et al. (2016) for estradiol's role in early language development, providing the basis for defining the necessary sample size.

2.2 Determination of fetal hair testosterone and DHEA

To determine fetal endogenous testosterone and DHEA levels, we measured hormone concentrations in newborn hair strands collected two weeks after birth (M_{age} = 16.3 d, SD = 2.9 d). We chose to measure DHEA rather than DHEA-S because unlike DHEA-S, it can cross the blood-brain barrier (Stárka et al., 2015). Scalp hair samples were cut at the posterior vertex,

wrapped in aluminum foil, and stored in the dark. The hair sample was analyzed by the Dresden LabService GmbH at the Technische Universität Dresden, using column-switching liquid chromatography tandem mass spectrometry assay with atmospheric-pressure chemical ionization (LC-APCI-MS/MS) (Gao et al., 2013): Contrary to the procedure in adults, entire hair strands were examined in our sample, as newborn hair is in the early growth stage. Hair samples were washed with isopropanol and methanol (Carl Roth GmbH & Co. KG; Karlsruhe, Germany) incubation was used to extract testosterone and DHEA from the whole, unpowdered hair. A column switching strategy for on-line solid phase extraction (SPE) was applied for subsequent analyte detection on an AB Sciex API 5000 QTrap mass spectrometer. Stock solutions were prepared with testosterone and DHEA from Sigma-Aldrich (Hamburg, Germany), and standard solutions with testosterone-d5 and DHEA-d4 from Biocrates Life Sciences AG (Innsbruck, Austria) as deuterated internal standards.

In contrast to hormone analyses from saliva or umbilical cord blood, the analysis of newborn hair provides a retrospective index of integrated long-term hormone secretion (Gao et al., 2013). As research on prenatal drug exposure indicates that neonate scalp hair reflects metabolic function after the 28th week of gestation (Gareri and Koren, 2010), the current values can be considered as indicators of fetal hormone concentration in the third trimester of pregnancy.

2.3 Infant language and cognitive development

At six months of age (*M*age = 6.2 M, *SD* = 0.2 M), children's neurocognitive development was assessed using the German version of the Bayley Scales of Infant Development (BSID-III) (Bayley, 2006a, 2006b; Reuner and Rosenkranz, 2014). The assessment was carried out by a trained female examiner in the presence of the parent. To test infants' language skills, the language scale with the two subtests receptive and expressive communication was used. The receptive communication subtest measures auditory acuity, joint attention, and responsiveness to language. The expressive communication assesses preverbal communication skills, including babbling, gesturing, joint referencing, and turn-taking. The raw

scores from the subscales were first transformed into standardized scores (M = 10, SD = 3). The total score for the language scale, calculated as the sum of both standardized scores for receptive and expressive communication, was then converted to age-normed scores (M = 100, SD = 15).

To control for the influence of general cognitive abilities on language development, we additionally used the cognitive scale of the BSID-III. The cognitive scale for six-month-olds targets sensorimotor development and infants' exploration and manipulation of objects. The raw score was transformed into a standardized, age-normed score (M = 100, SD = 15).

2.4 Data Analysis

Multiple linear regression analyses were used to evaluate the association between fetal DHEA and language development at six months of age with sex as a main effect alongside the hormone variable. To control for general cognitive abilities, we additionally conducted the analysis with infant cognitive development at six months of age as dependent variable. For comparison, we applied the same approach to fetal testosterone levels. Thus, in total four multiple regression analyses were performed. As supplementary analysis, Spearman's rank correlation coefficients were calculated to provide a more robust assessment, examining the strength of monotonic relationships between hormone levels and developmental outcomes. Prior to statistical analysis, a log-transformation was applied due to the significant skewness of the obtained testosterone values (W = .89, p < .001). The same approach was applied to the DHEA values to ensure comparability (W = .97, p = .32). As hair length and infant age at hair sample extraction (see Table 1) were considered as potential confounders of hormone levels, we tested their associations with both hormone measures using bivariate Pearson correlations. Since neither was significantly associated with DHEA or testosterone levels (all p > 0.05), hair length and infant age at hair sample extraction were not included as covariates in the regression analyses. To reassure the reliability of our findings, all analyses were repeated with the subset of children (n = 39) for whom both hormone values were available. All statistical

analyses were conducted using RStudio 2024.04.2+764, with a significance level of p = .05 (see Tables S1-S8 for comprehensive statistics including 95% confidence intervals).

3 RESULTS

Concentrations of DHEA and testosterone, along with the performance in the language and cognitive scales of the BSID-III, are presented in Table 2, with no statistically significant sex differences observed in any of the variables.

Multiple linear regression analysis showed that the model including fetal DHEA and infant sex significantly predicted language abilities at six months of age (*adjusted* R^2 = .13, $F(2,47)$ = 4.63, p = .01, see Table S1). Note, that fetal DHEA levels (β = -0.38, $p < .01$) but not infant sex served as a significant predictor (β = -0.18, p = .17) in this model. Specifically, fetal DHEA was found to be negatively associated with infants' language abilities at six months of age suggesting that the higher the fetal DHEA levels, the lower the later language abilities (see Figure 2; confirmed by spearman correlation, ρ = -.32, p = .02). The control analysis, using general cognitive abilities at six months as dependent variable in the model (*adjusted* R^2 = .02, $F(2,47)$ = 1.49, p = .24, see Table S2), did not reveal any predictive value of fetal DHEA levels (β = 0.20, p = .16, spearman ρ = .14, p = .33) or infant sex (β = -0.12, p = .39).

In contrast to fetal DHEA, fetal testosterone (β = 0.08, p = .62, see Figure 3, spearman ρ = .01, p = .94) was not statistically significantly associated with infants' language abilities, nor was infant sex (β = -0.06, p = .69), as shown in multiple linear regression analysis (*adjusted* R^2 = -0.04, $F(2,41)$ = 0.20, p = .82, see Table S3). Also for general cognitive abilities, fetal testosterone levels (β = 0.08, p = .60, spearman ρ = .11, p = .47) and infant sex (β = -0.06, p = .69) showed no statistically significant predictive value (*adjusted* R^2 = -0.04, $F(2,41)$ = 0.22, p = .80, see Table S4).

When the analyses were repeated for the sample of children for whom values for both hormones were available (n = 39), the results confirmed the statistically predictive value of fetal

DHEA for later language abilities, but not for general cognitive abilities (see Tables S5, S6). Moreover, fetal testosterone did not statistically contribute to the prediction of either language or cognitive abilities at six months of age in this participant sample (see Tables S7, S8).

4 DISCUSSION

The present study examined the impact of fetal DHEA, in comparison to testosterone, on behavioral language outcome during infants' first year of life and found that fetal DHEA was related to lower language outcome at six months of age. This finding suggests fetal DHEA concentration in the third trimester to serve as an early biological predictor of infant language abilities. To test for the observed effect's language-specificity, we also evaluated general cognitive abilities, finding no significant association with DHEA. In contrast to DHEA, fetal testosterone was neither associated with either early language abilities nor cognitive functioning. In addition, our study highlights the use of newborn hair samples for hormone extraction as a method to retrospectively infer fetal hormone concentrations during the third trimester even months (here about the third trimester) before sampling is possible in newborns.

In light of previous studies on the role of sex hormones, which reported either negative or positive associations with language outcomes (Quast et al., 2016; Schaadt et al., 2015; Whitehouse et al., 2012), and given the lack of studies on fetal DHEA as contributing factor to infant language outcome, we did not have hypotheses on the direction of the postulated relationship with DHEA. Our results revealed a significant negative association, with higher fetal DHEA levels leading longitudinally to lower language abilities. The finding that fetal DHEA levels in infant fingernails have been shown to reflect antenatal stress (Tegethoff et al., 2011) may provide an explanation for the observed direction, as maternal stress during pregnancy has been found to be negatively associated with two-year-old children's language skills (Laplante et al., 2004). Evidence from animal studies demonstrates that maternal stress during pregnancy is related to changes in the hypothalamic-pituitary-adrenal (HPA) activity (Kapoor et al., 2008), providing further explanation of the pathway from prenatal stress to DHEA levels, as fetal DHEA(S) production is regulated by the HPA axis (Mesiano and Jaffe, 1997; Smith,

2022; Smith et al., 1998; Wadhwa, 2005). During the fetal period, the HPA axis is particular sensitive to environmental factors (Howland et al., 2017; Sandman et al., 2012), which is reflected in the concept of fetal programming: The rapidly developing fetal organism is particularly susceptible to both organizing and disorganizing influences, such that environmental factors exert long-lasting effects on fetal neurodevelopmental pathways (Demers et al., 2021; Nathanielsz et al., 2003; Seckl and Meaney, 2004). In conclusion, it could be postulated that higher fetal DHEA levels, resulting from increased HPA activity triggered by elevated maternal stress, may negatively influence infant language development. Considering perceived maternal stress during pregnancy potentially mediated by DHEA could provide further insights into this relationship in future studies.

An intriguing observation in our study is that fetal DHEA is specifically associated with language abilities, but not with general cognitive abilities. This finding corresponds to previous reports on the contribution of sex hormones to language development, where estradiol and testosterone levels have been found to relate to infants' babbling (Quast et al., 2016), expressive vocabulary (Kung et al., 2016), and sentence comprehension (Schaadt et al., 2015). Furthermore, these sex hormones have been reported to be involved in language function and organization of the infant brain (McCarthy, 2008), affecting the brain-responses of infants as young as four weeks old (Friederici et al., 2008). Our study offers new insights by suggesting that also DHEA, the precursor hormone of testosterone and estradiol, may contribute to early language development. Yet, the underlying mechanisms of how DHEA affects the brain and the long-term trajectory of language development require further investigation. Additional studies will be needed to validate the predictive value of fetal DHEA as a potential biological marker for language abilities in the course of development.

In contrast to DHEA, testosterone levels have previously been investigated with respect to later language development employing different extraction methods which have, however, yielded contradictory results. Studies using amniotic fluid from the second trimester reported a negative association with vocabulary at age two years (Lutchmaya et al., 2001), and, in girls

only, an inverted U-shaped relationship with sentence comprehension at age four years (Finegan et al., 1992). In follow-up studies with the same cohort as Lutchmaya, Baron-Cohen, and Ragatt's (2001), the association between fetal testosterone and language abilities was no longer detectable at age four years (Auyeung et al., 2009; Knickmeyer et al., 2005). Moreover, studies examining perinatal testosterone levels from umbilical cord blood consistently reported a negative association with language abilities in boys, but not in girls (Hollier et al., 2013; Whitehouse et al., 2012). For example, Whitehouse and colleagues (2012) discussed higher perinatal testosterone levels as a potential risk factor for language delay in boys, and as a potential protective factor in girls. In sum, most of the studies assessing the statistical predictive value of prenatal or perinatal testosterone levels on later language outcomes found sex-specific differences, not only in terms of an association per se but also the direction of this association (Finegan et al., 1992; Whitehouse et al., 2012). In our study, however, sex did not significantly add to explaining later language outcomes. Our findings may be influenced by the time window covered by hormone extraction from hair: The sex-specific predictive value of testosterone may depend on the stage of development at which it is measured, as testosterone levels in boys rise prenatally from gestational weeks eight to 24, peaking at 16 weeks, and rise again postnatally during the so-called mini-puberty from weeks four to 12 postpartum (Hines, 2020). Therefore, these time windows may be particularly valuable for assessing the potential sex-specific effects of testosterone on later development in boys and girls. Since we assessed testosterone levels during the last trimester, but not in early pregnancy, this may explain the absence of sex differences within our sample.

In assessing fetal hormone exposure, there is currently no methodological gold standard and different measures may lead to inconsistent results, making comparisons across studies challenging (Hollier et al., 2014a; van de Beek et al., 2004). Our study used newborn hair samples for sex hormone extraction as a novel method to reflect fetal hormone concentrations, capturing metabolic activity from the 28th week of fetal development (Gareri and Koren, 2010). The fact that we were able to extract sex hormone levels for nearly 90% of our participants renders this method feasible and promising (see Figure 1). Apart from hair-sample analysis,

hormone extraction from amniotic fluid is the only other method previously used to investigate the association between fetal hormone metabolism from the second trimester onwards and child behavior. However, as amniocentesis is mainly performed in high-risk pregnancies to diagnose fetal defects or genetic diseases, this method has clear limitations in the study of general development (Knickmeyer et al., 2005). Hormone extraction from umbilical cord blood is neither an option to capture fetal hormone levels as it reflects perinatal concentrations of sex hormones influenced by birth (Taylor et al., 2000; van de Beek et al., 2004). Apart from the invasiveness required to access fetal body fluids, these methods' limitation lies in their representation of only single time points of fetal hormone levels, which vary throughout the day (Bates and Herzog, 2020; Granger et al., 1999; Taylor et al., 2000). In contrast, as a non-invasive method, hair-sample analysis provides a retrospective index of long-term hormone secretion (Gao et al., 2013), offering insight into fetal endocrinological development during the third trimester.

5 CONCLUSIONS

The current study identifies fetal DHEA levels extracted from neonate hair samples as a relevant candidate biological marker of infant language development, with potential lasting effects for child development. Given the evidence for the central role of the fetal HPA axis in shaping fetal development, DHEA, which is produced by activation of the HPA axis, should be targeted in studies on prenatal programming and its impact on infant development.

Declarations

The authors have declare no conflict of interest. This study adhered to the Declaration of Helsinki. Prior to participation, mothers provided informed consent and the experiment was approved by the ethics committee of the Paris Lodron University of Salzburg (EK-GZ: 12/2013, approved on 23rd of January, 2014, Addendum: 17th November, 2021).

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Data availability

Non-sensitive data is available from the corresponding author upon reasonable request.

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TABLES

Table 1: Participant and demographic information of final sample (*n* = 56)

Variable	
Maternal and family characteristics	
Maternal age at delivery, mean (SD) [range]	33.16 y (4.31) [23-42]
Maternal relationship status at the 34th GW (%)	
Married to the child's father	53.57
In a relationship with the child's father	41.07
Single	5.36
Professional qualification of mothers (%)	
Without vocational qualification	1.79
Completed vocational training	17.86
Higher vocational education	19.64
University degree (or higher)	60.71
Infant characteristics	
Sex (% female)	51.8
Gestational age at birth in weeks, mean (SD) [range]	39+6 (7d) [38-42]
Delivery mode (%)	
Vaginal delivery	62.50
Operative vaginal delivery (forceps or vacuum)	12.50
C-section	25.00
Birth weight, mean (SD) [range]	3382 g (434) [2540-4740]
Apgar Score, 10 min post-birth, mean (SD) [range]*	9.93 (0.33) [8-10]
Age at hair collection, mean (SD) [range]	16.34 d (2.90) [12-26]
Child age at six months, mean (SD) [range]	6.22 M (0.24) [5.90 – 6.83]
Hair mass, mean (SD) [range]	2.51 mg (2.06) [0.2-7.5]
Hair length, mean (SD) [range]*	2.00 cm (0.73) [1.0-3.5]

Note. Maternal and family characteristics include maternal age, relationship status, and professional qualification. Infant characteristics comprise sex, age at birth, age at hair collection, age at the six-month BSID-III assessment, birth details, hair mass, and hair length (* one missing value).

Table 2: Fetal hormone concentrations and performance on the language and cognitive scales (*n* = 56).

	Final sample (*n* = 56)	males (*n* = 26)	females (*n* = 29)	*p*
Fetal testosterone level (*n* = 44, 22f) (pg/mg)	1.48 (1.21) [0.05-4.97]	1.63 (1.43) [0.05-4.97]	1.34 (0.94) [0.24-3.26]	.88
Fetal DHEA level (*n* = 50, 26f) (pg/mg)	18.78 (9.30) [3.70-41.67]	18.06 (9.66) [3.70-38.80]	19.44 (9.09) [3.74-41.67]	.51
BSID-III Cognitive scale	113.60 (15.16) [80-135]	112.20 (15.34) [80-130]	114.80 (15.15) [95-135]	.55
BSID-III Language scale	114.20 (11.87) [85-141]	112.40 (13.32) [85-141]	116.00 (10.29) [94-141]	.19
Receptive subscale raw scores, mean	9.55 (1.52) [7-14]	9.33 (1.64) [7-14]	9.76 (1.41) [8-13]	.17
Expressive subscale raw scores	9.77 (1.60) [6-13]	9.63 (1.62) [6-13]	9.90 (1.59) [7-13]	.54

Note. For the final sample (*n* = 56), the mean, standard deviation, and range for fetal testosterone and DHEA levels, as well as for the scores on the BSID-III cognitive and language scales at six months of age, are provided for each sex, with no statistically significant sex differences observed according to the Wilcoxon rank-sum test.

FIGURES

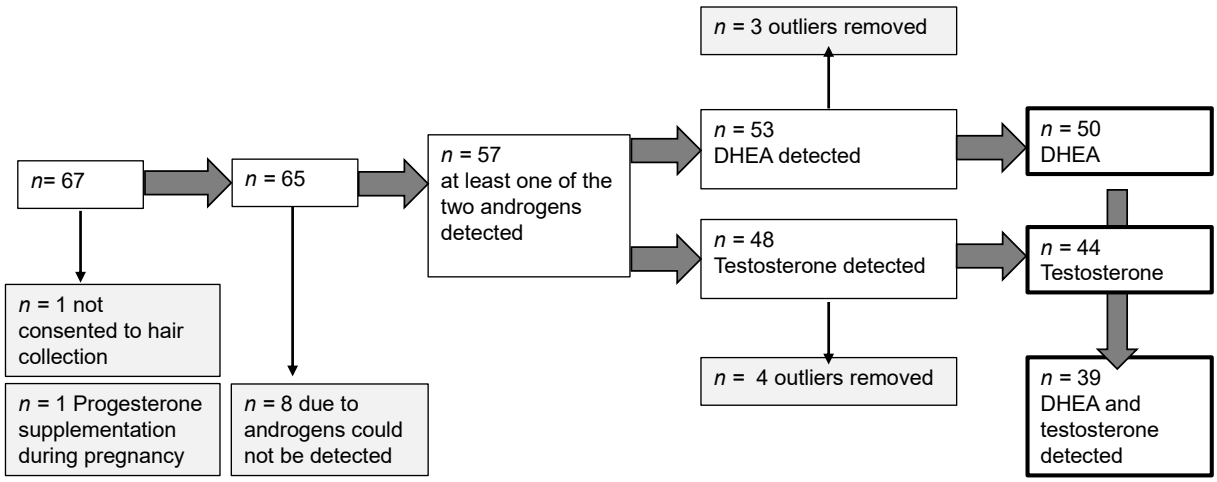

Figure 1. Flow chart of sample inclusion and exclusion

Note. Of the 65 hair samples analyzed, testosterone or DHEA was detected in 57 hair samples, with DHEA observed in 53 samples and testosterone in 48 samples. After excluding outliers (defined as 2 SD above the mean, one participant identified as an outlier for both hormones), the final sample size was n = 56 (DHEA sample: n = 50, testosterone sample: n = 44, overlapping sample of n = 39).

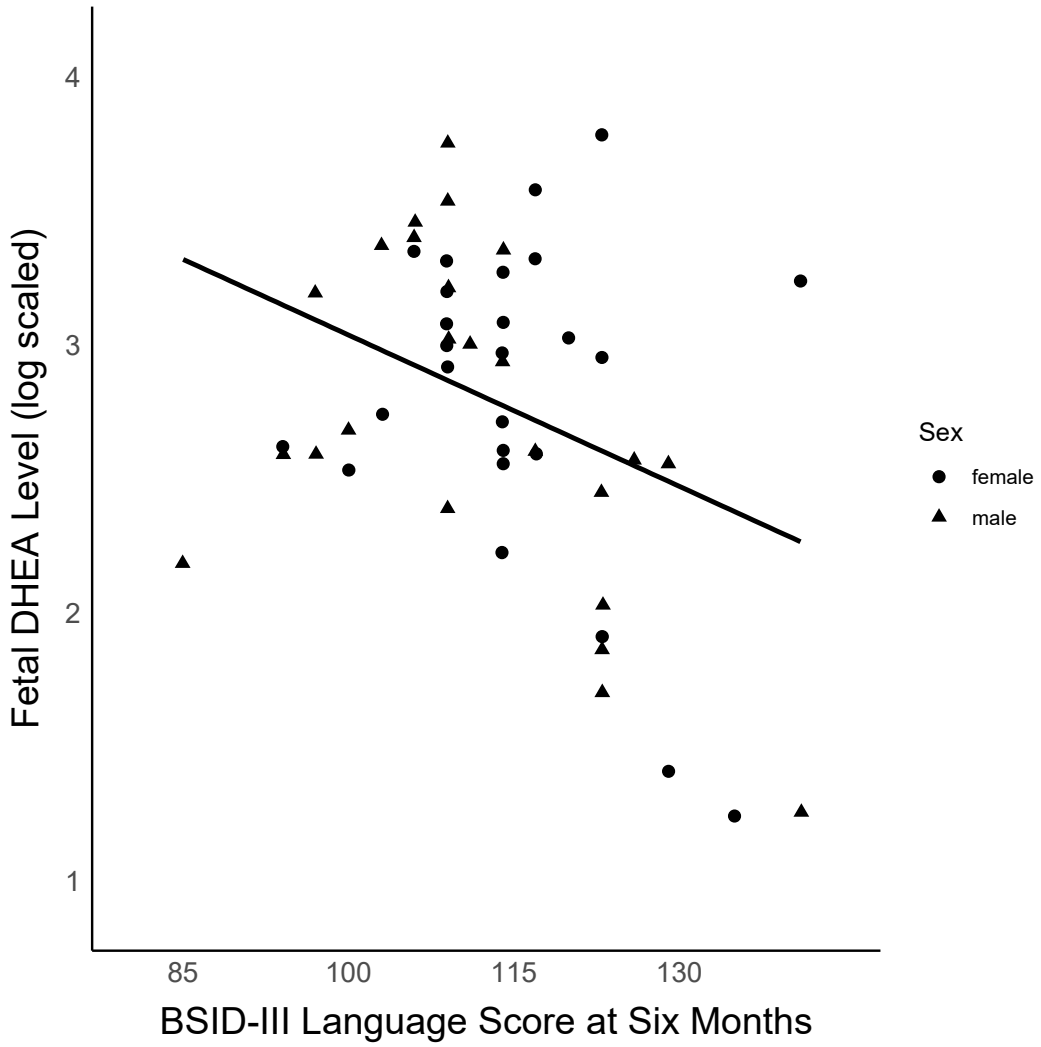

Figure 2. Association between fetal DHEA levels and language abilities at six months assessed using the Bayley Scales of Infant Development (BSID) III (n = 50)

Note that lower language skills at six months were associated with higher levels of fetal DHEA extracted from hair at birth (ρ = -.32, p = .02), reflecting the hormone concentration in the third trimester of pregnancy (with outliers excluded, data jittered to prevent overlapping points).

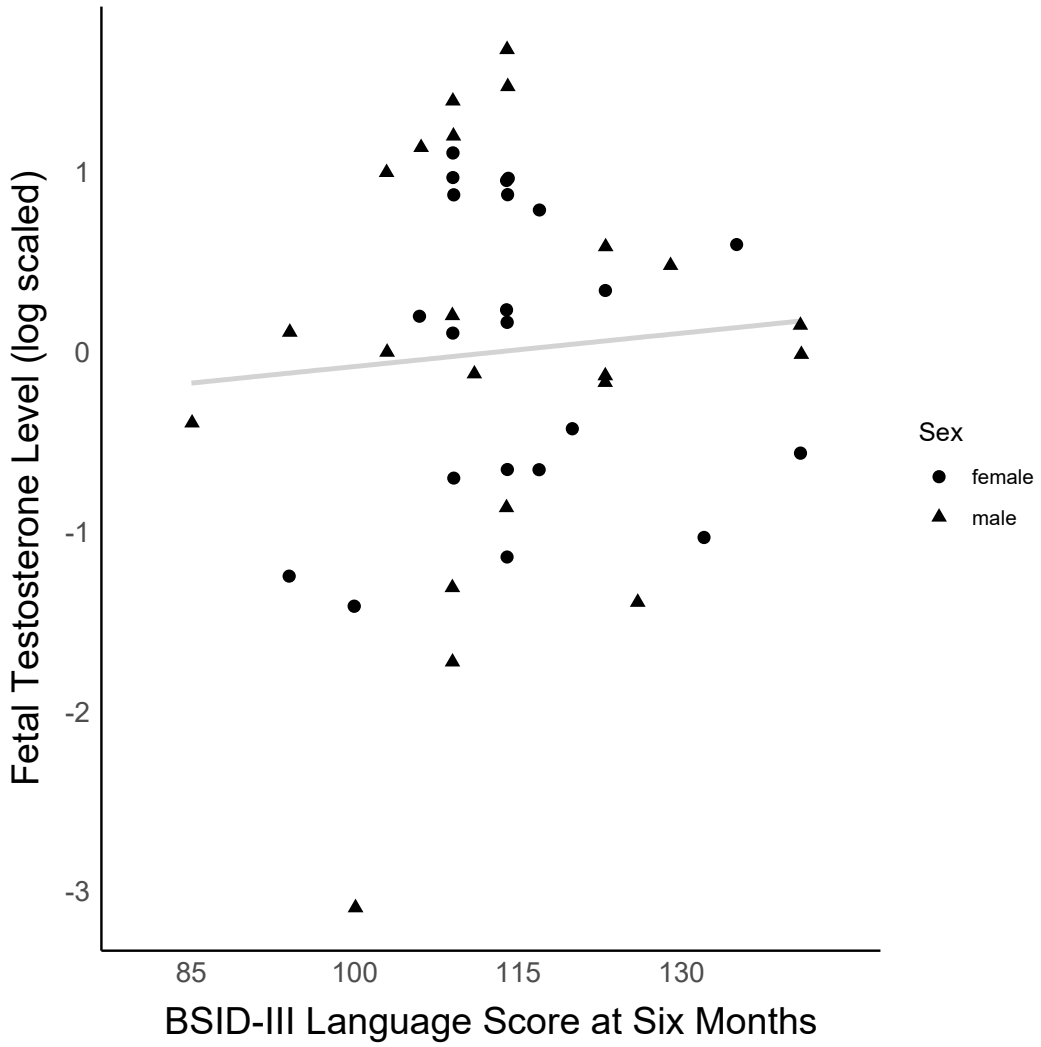

Figure 3. Association between fetal testosterone levels and language abilities at six months assessed using the Bayley Scales of Infant Development (BSID) III (n = 44)

Note that language skills at six months were not significantly associated with fetal testosterone levels extracted from hair at birth (ρ = .01, p = .94), reflecting the hormone concentration in the third trimester of pregnancy (with outliers excluded, data jittered to prevent overlapping points).