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Maternal androgens and autism spectrum disorder in the MARBLES prospective cohort study

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Abstract

Background: Maternal hormonal risk factors for autism spectrum disorder (ASD) in offspring could intersect genetic and environmental risk factors.

Objectives: This analysis explored ASD risk in association with maternal testosterone, androstenedione, and dehydroepiandrosterone (DHEA) measured in first, second, and third trimesters of pregnancy.

Methods: MARBLES is a prospective pregnancy cohort study based at the MIND Institute in Northern California that enrolls mothers who have at least one child previously diagnosed with ASD and are expecting, or planning to have another child. At 36 months the younger sibling is clinically classified as having ASD, or as non-typically developing (Non-TD), or typically developing (TD). Maternal androgens during pregnancy were measured in serum samples from 196 mothers. Multivariable logistic regression models estimated risk of ASD and Non-TD in offspring compared to TD, in relation to the log-transformed maternal androgen concentrations, at each trimester.

Results: Non-significant associations were observed, and borderline significant associations were only observed in some stratified unadjusted models. Second trimester maternal testosterone

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: RJ Schmidt receives research funding from the Simons Foundation, was a paid consultant for a research study at Drexel University, and has received honoraria from NIH for grant reviews. A-M Iosif has received honoraria for reviewing activities from Elsevier, NIH and Department of Defense. L Granillo and NW Snyder declare no competing financial interests.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rasd.2022.102054.

was non-significantly associated with ASD in female offspring, although not after adjustment, aRR 1.54 (95% CI 0.71, 3.33), and second trimester maternal DHEA was non-significantly associated with non-TD in male offspring, again not after adjustment, aRR 0.50 (95% CI 0.21, 1.21). Secondary analysis suggested that third trimester androgen concentrations in mothers with male offspring had significant or near significant associations with their child's Social Responsiveness Scale score.

Conclusion: No significant associations were found between maternal androgen concentrations and risk of ASD or Non-TD in the child.

Keywords

Testosterone; Androstenedione; Autism; Pregnancy; Prospective study

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by difficulty in communication and social reciprocity, restricted interest, and repetitive behavior (Croen, Grether, Hoogstrate, & Selvin, 2002). It can be accompanied by co-morbidities including anxiety, depression, gastrointestinal disorders, and other chronic conditions that might include hormonal dysregulation (Manikkam et al., 2004). The etiology of ASD is unknown, but growing evidence supports the hypothesis that genetic and environmental factors both play roles in increasing risk of ASD (Lyall et al., 2017) and that gestation is a critical window for developmental processes that are abnormal in ASD and non-typical development (Non-TD) (Auyeung, Lombardo, & Baron-Cohen, 2013; Newschaffer et al., 2007). Hormone regulation can be influenced by both genetic (Coviello et al., 2011) and environmental factors (Hampl, Kubátová, & Stárka, 2016), and be a mediating factor for abnormalities during pregnancy (Varshavsky et al., 2019).

The 4:1 ratio of males to females with ASD under the age of 8 (Redfield et al., 2014) raises questions concerning different developmental trajectories for males and females and sex hormones (Werling & Geschwind, 2013). The Extreme Male Brain theory has been proposed as an explanation for the skewed sex ratio in ASD (Baron-Cohen, 2002), and also has been criticized (Krahn & Fenton, 2012; Ridley, 2019). Sex-differential biology and underdiagnosis of ASD in females are other proposed explanation for the skewed ratio (Begeer et al., 2013; Rynkiewicz et al., 2016; Werling, 2016). Additionally, maternal androgen concentrations during pregnancy have been hypothesized to influence ASD risk, the exact mechanisms through which this increased risk could happen have been broadly hypothesized, including intrauterine growth restriction (Carlsen, 2006) and DNA disruption (Xu et al., 2015).

During pregnancy, testosterone plays several roles, including driving developmental programming to masculinize and de-feminize the brain (Gore, Martien, Gagnidze, & Pfaff, 2014; McCarthy, 2016), and fetal testosterone begins to increase more in male fetuses during sexual differentiation (Hines, Constantinescu, & Spencer, 2015), estimated around 8–16 weeks of gestation (Finegan, Bartleman, & Wong, 1989; Reyes, Boroditsky, Winter, & Faiman, 1974; Reyes, Winter, & Faiman, 1973).

Two studies that examined either late first trimester or early second trimester maternal serum steroid levels found no significant association between maternal testosterone and risk of ASD traits or risk of ASD diagnosis (Bilder et al., 2019; Tsompanidis et al., 2021). However, maternal estradiol was associated with increased risk of ASD in offspring (Bilder et al., 2019), and was also predictive of infant Quantitative Checklist for Autism in Toddlers (Q-CHART) scores in male offspring (Tsompanidis et al., 2021).

Studies have examined the association between hormone conditions in mothers and ASD diagnosis and perceived ASD traits in offspring. Children born to mothers with polycystic ovary syndrome (PCOS) had increased odds of an ASD diagnosis (Abu-Zaid et al., 2022; Cherskov et al., 2018; Katsigianni, Karageorgiou, Lambrinoudaki, & Siristatidis, 2019; Kosidou et al., 2016; May, Yi, Loveland, Vollenhoven, & Williams, 2021; Rotem et al., 2021). This relationship was not observed to be as strong or consistent in all studies, however (Hisle-Gorman et al., 2018; Schieve et al., 2017). Further research specific to women with ASD has shown that women with ASD are at higher odds of steroid-related conditions and symptoms (Pohl, Cassidy, Auyeung, & Baron-Cohen, 2014; Simantov et al., 2022), including PCOS (Cherskov et al., 2018). Additionally, women with PCOS had increased odds of ASD (Cherskov et al., 2018; May et al., 2021). It is important to note that PCOS is not exclusively associated with ASD, but also is associated with increased risk of psychiatric disorders, such as mood, anxiety, and eating disorders in offspring, which are not exclusively hormone driven, nor are more commonly occurring in males (Berni, Morgan, Berni, & Rees, 2018; Chen, Kong, Piltonen, Gissler, & Lavebratt, 2020). Other disorders that are characterized by decreased androgen concentrations were also associated with increased risk of ASD (May et al., 2021). There is potential that other androgens besides those in this analysis may be involved in ASD risk.

One study on intrauterine testosterone showed that higher testosterone *in-utero* was associated with increased risk of ASD traits as measured by Q-CHAT. However, some studies examining cord blood or amniotic testosterone concentrations did not find an association with ASD (Park et al., 2017; Whitehouse et al., 2012). Furthermore, prenatal testosterone from amniotic fluid in the 2nd trimester did not associate with ASD traits in later in adolescence (Dooley et al., 2022). One study examined both girls with congenital adrenal hyperplasia (CAH) and a cohort of typically-developing children who had amniotic testosterone measurements, and neither analysis presented an association between prenatal testosterone and autistic traits as measured by Childhood Autism Spectrum Test (CAST) (Kung et al., 2016, p. 12602). EARLI (Early Autism Risk Longitudinal Investigation), an enriched-risk pregnancy cohort, examined androgens found in meconium (Terloyeva et al., 2020). EARLI reported some positive associations between androgen levels in meconium and ASD-related traits in children at 12 and 36 months of age, although they were found in stratified analyses. Testosterone was found to predict higher Social Responsiveness Scale (SRS) scores in males with male probands.

Prenatal estrogens and steroidogenic activity have emerged as a potential driver of ASD risk. Prenatal estradiol, estrone, and progesterone each were associated with increased odds of ASD (Baron-Cohen et al., 2020), and a study examining multiple prenatal steroids proposed that a generalized latent steroidogenic factor could be elevating steroids in children who later

were diagnosed with ASD compared to children typically developing (Baron-Cohen et al., 2015).

Recent research in phenotypic androgenic effects have used facial morphology as a marker of prenatal hormone influence. One study found that there may be a phenotype for autism that can be identified in facial features (Tan et al., 2020), and another found facial landmark masculinity was associated with neurodevelopmental disorders (NDD) diagnosis including ASD (McKenna et al., 2021).

The association between risk of ASD and androgens has been explored in multiple facets. This study adds to the current knowledge by examining maternal serum androgens at each pregnancy trimester in relation to ASD diagnosis in offspring.

1.1. Objective

This study explored the risk of ASD and Non-TD in association with maternal testosterone, androstenedione, and dehydroepiandrosterone (DHEA) measured at the first, second, and third trimester of pregnancy. This study also examined the association between maternal androgen concentrations and SRS scores, and maternal androgen concentrations and Autism Diagnostic Observation Schedule (ADOS) scores in offspring.

2. Methods

2.1. Participants

Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) is an on-going high-risk pregnancy cohort comprised of mother-child pairs. Families are eligible for MARBLES if they have at least one child already diagnosed with ASD and are expecting, or planning to have another and live within 2.5 driving hours from the University of California (UC) Davis MIND Institute (Hertz-Picciotto et al., 2018). Mothers are recruited before and throughout pregnancy. Questionnaires and biologic specimens are collected before, during, and after pregnancy, but not all mothers have specimens available in all timepoints. This study included MARBLES children born after the start of the study in 2006 until December 31, 2015, whose mothers had at least 4.2 ml of serum available at any trimester of pregnancy for a measurable sample. For families with more than 1 eligible child, we used only the first-born child data. A total of 196 mother-child pairs met all inclusion criteria, of which 49 children were diagnosed with ASD, 27 with Non-TD, and 120 as typically developing (TD). Of these, 64 mother-child pairs had first trimester measurements, 131 had second trimester measurements, and 189 had third trimester measurements (Fig. 1). This study has been approved by the UC Davis Institutional Review Board, and informed and written consent was obtained before collection of biospecimens and data.

3. Materials and design

3.1. Androgen measurement

Initially 409 samples, from 208 mother-child pairs, were pulled and aliquoted into 0.2 ml samples. Earliest blood draw available for the trimester was selected. All samples had unconjugated testosterone, androstenedione, and DHEA quantified, except for one where

testosterone was below the level of detection. If the child did not have their final diagnosis by 6 months after their 3rd birthday or the child was the second born in a twin birth or later pregnancy, the mother-child pair was not included in the statistical analysis (n = 25 samples from 12 mother-child pairs). All three trimesters are included to provide insight on androgens and risk at specific times in a high-familial-risk pregnancy cohort.

Unconjugated testosterone, androstenedione, and DHEA were measured using liquid chromatography–high-resolution mass spectrometry (LC-MS/HRMS) using the procedure detailed in Supplemental Material 1 as previously described (Frey et al., 2016).

3.2. Covariates

A directed acyclic graph (DAG) (Shrier & Platt, 2008) was developed and five covariates were identified as potential confounders based on previous literature regarding their relationships with androgens and ASD: parity (Cheslack-Postava et al., 2014; Toriola et al., 2011), maternal age (Carlsen, Jacobsen, & Bjerve, 2003; Sandin et al., 2012), maternal race and ethnicity (Keen, Reid, & Arnone, 2010), maternal pre-pregnancy BMI (Getz, Anderka, Werler, & Jick, 2016), and maternal stress (Kinney, Munir, Crowley, & Miller, 2008) (Fig. 2). The child's sex was considered a potential effect modifier (Krishnan, 2018). The Perceived Stress Scale, a measure of maternal stress was collected throughout pregnancy (Cohen, Kamarck, & Mermelstein, 1983), with some mothers having up to 3 questionnaires completed during one trimester. In cases of multiple questionnaires in one trimester, the perceived stress score was averaged across all questionnaires completed that trimester.

3.3. Primary outcome

Child's developmental outcome of ASD, Non-TD or TD clinically-classified at age 3 years was the primary outcome of interest. Trained personnel at the MIND Institute, who have obtained research reliability, assessed each child within 6 months of turning 3 years old. The outcome classification was based on an algorithm incorporating ADOS, Mullen Scales of Early Learning (MSEL), and clinical diagnosis (Ozonoff et al., 2014). For classification of ASD, the child needed to have a scores over the ADOS cutoff and an ASD classification based on DSM-5. For a Non-TD classification, the child needed to not meet criteria for ASD and have either two or more MSEL scores 1.5 SD below the normative mean, one MSEL score 2 SD below the normative mean, or high ADOS scores. A child with an ADOS score of 1 or 2 points, all MSEL scores within 2.0 SD of the normative mean, and at most one MSEL subscale score below 1.5 SD of the normative mean, was considered TD.

3.4. Secondary outcome

The SRS was used as a dimensional, quantitative measure of severity of non-typical reciprocal social behavior on a continuous scale (Constantino et al., 2003). The total SRS score, completed between 34 and 51 months of age, was examined in relation to testosterone, androstenedione, and DHEA, with higher scores indicating greater symptomatology and severity of social impairment. Only a subset of the MARBLES cohort completed the SRS evaluation.

ADOS communication and social total score was also examined in secondary analysis. ADOS calibrated severity scores were used as a measure of ASD symptom severity with higher scores indicating greater severity (Gotham, Pickles, & Lord, 2009).

4. Procedures and analysis

4.1. Statistical analysis

The linearity of the relationship between each androgen and the log-odds for ASD and Non-TD were examined separately by timepoint using empirical logit plots. To improve the linearity of these associations, exposure variables were transformed by taking the natural logarithm.

Multivariable logistic regression was used to evaluate the risk of ASD or Non-TD compared to TD using SAS 9.4 software's PROCGENMOD. Risk ratios (RR) and 95% confidence intervals were used to estimate effects and assess statistical significance. Testosterone, androstenedione, and DHEA were examined separately at each trimester for confounding and effect modification.

An unadjusted model and two adjusted models were developed for each androgen and each outcome (ASD, Non-TD). Model building was completed separately for ASD compared to TD and Non-TD compared to TD. In the first adjusted model, each confounder needed to be generally associated (p-value ≤ 0.3) with the exposure and outcome variables individually, and the exposure of interest's RR value needed to change by 10% when the covariate was added to the model. For a covariate to be considered an effect modifier, the product term between the exposure and covariate needed a p-value ≤ 0.2. Step-wise selection was used to build the first adjusted model. One covariate was added at a time starting with the crude model. In the case of missing observations for a covariate, the adjusted model was compared to a crude model which excluded participants missing the covariate. Maternal pre-pregnancy BMI met all criteria as a confounder in both ASD compared to TD and Non-TD compared to TD. For consistency, we provided models adjusted for maternal pre-pregnancy BMI in all trimesters for all androgens, although maternal pre-pregnancy BMI was not a confounding covariate at each trimester in each androgen. The first adjusted model is the model referred to throughout the results. The second adjusted model included all confounders identified in the DAG (pre-pregnancy BMI, parity, maternal race/ethnicity, maternal stress, and maternal age), but due to the sample size, some models did not converge. There is biological plausibility for effect modification by child's sex. If the product term between child's sex and testosterone, androstenedione, or DHEA had a p-value ≤ 0.2, stratified models were examined. One sample was missing the concentration of testosterone and was excluded from the statistical analysis.

SRS scores were transformed for analyses by taking the square-root value of the T-score. Linear regression evaluated the relationship between each log-transformed androgen concentration and the square-root transformed SRS T-score. Model diagnostic plots suggested a quadratic relationship between exposure and outcome; therefore, a quadratic term was added to the model.

Count regression models examined the relationship between ADOS score and each log-transformed androgen concentration. A value of 1 was subtracted from the total ADOS score, and a negative binomial distribution was used in the regression model.

4.2. Sensitivity analysis

Demographic characteristics of those with samples who were included in the analysis and those who were not included were compared using chi-square, two-sample t, or Wilcoxon rank sum tests, as appropriate. E-values were calculated for unadjusted RRs that were borderline significant. This was done to see if an unmeasured confounder could wash out the association observed (VanderWeele & Ding, 2017).

5. Results

The number of specimens provided varied across mother-child pairs, with some providing a sample in all three trimesters, and others providing a sample in one or two trimesters. Maternal androgen concentration in the second trimester was stratified by participant characteristics in Table 1. There were statistically significant differences in maternal testosterone and androstenedione between mothers younger than 35 years old and mothers 35 years or older, and in maternal testosterone between mothers who completed college and mothers who did not complete college. Maternal androgen concentration stratified by participant characteristics in the first and third trimesters are provided in eTables 1 and 2. A Spearman correlation coefficient heatmap of maternal androgen concentrations over the pregnancy is available in Fig. 3. Maternal testosterone and androstenedione concentrations were highly correlated at each trimester with a correlation coefficient in the first trimester of 0.86, in the second trimester of 0.82, and in the third trimester of 0.85. Participant characteristics were also stratified by child's clinical outcome. Each mother-child pair was included in eTable 3 regardless of number of samples and timepoint. Among the 196 mother-child pairs, there were 49 children with ASD, 27 with Non-TD, and 120 were TD at age 3 years. Maternal education was significantly different across child diagnostic outcomes. Missing observations were noted for paternal age, maternal metabolic condition status, parity, maternal cigarette smoking, insurance provider, and maternal perceived stress in all trimesters.

Distribution of maternal androgens collected over pregnancy are available in eTable 4.

Borderline significant associations ($p < 0.1$) were observed in unadjusted models for: testosterone in the second trimester with risk of Non-TD compared to TD, RR 0.48 (95% CI 0.21, 1.08) (Table 2), and DHEA in the second trimester with Non-TD risk compared to TD, RR 0.52 (95% CI 0.26, 1.05) (Table 3). These associations were attenuated after adjustment (adjusted p-values 0.35 and 0.21 respectively).

When p-values for the interaction between androgens and child's sex were < 0.2, child's sex was considered an effect modifier. Logistic regression models stratified by child's sex are available in eTables 5–7.

A borderline significant association was observed for testosterone in the second trimester with risk of Non-TD compared to TD before adjustment, RR 0.48 (95% CI 0.21, 1.08) (Table 2). In analysis stratified by child's sex (eTable 5), there were borderline significant associations in the unadjusted models between testosterone and the risk of ASD in female offspring in the second trimester prior to adjustment for pre-pregnancy maternal BMI, but the association did not hold after adjustment aRR 1.54 (95% CI 0.71, 3.33).

Null associations were observed for androstenedione in both the full model (Table 4) and the stratified models (eTable 6).

No significant associations were observed between DHEA and ASD. A borderline significant association was observed for DHEA in the second trimester with risk of Non-TD compared to TD before adjustment, RR 0.52 (95% CI 0.26, 1.05) (Table 3). A borderline significant association was also seen between DHEA and risk of Non-TD in male offspring in the second trimester prior to adjustment, with an adjusted risk ratio of 0.50 (95% CI 0.21, 1.21) (eTable 7).

5.1. SRS results

Results of secondary analysis completed on the subsample with SRS scores, including 74 mother-child pairs in the second trimester (18 with ASD, 9 with Non-TD, and 47 with TD) and 101 mother-child pairs in the third trimester (22 with ASD, 18 with Non-TD, and 60 with TD), are shown in eTable 8 and Table 5 for all children, stratified by child sex, and stratified by both the sex of the older sibling with ASD (proband) and child sex. No significant associations were observed with second trimester concentrations. Third trimester concentrations in mothers with male offspring had significant or near significant associations between each androgen and their child's SRS score. The following associations were observed in models for male offspring, adjusted for maternal pre-pregnancy BMI in the third trimester, and not stratified by proband gender (Table 5): higher testosterone concentrations were associated with lower SRS scores, Beta −7.09 (95% CI −12.49, −1.68); higher androstenedione concentrations were associated with lower SRS scores, Beta −2.00 (95% CI −2.84, −1.17); and higher DHEA concentrations were associated with lower SRS scores, Beta −1.71 (95% CI 3.58, 0.17).

5.2. ADOS results

Analyses examining ADOS scores and maternal androgen concentration was done on mother-child pairs in the second and third trimesters. After adjustment for maternal pre-pregnancy BMI and stratification by child's sex, there were positive associations between second trimester maternal testosterone and female child's ADOS score, Beta 1.08 (95% CI 0.13, 2.03), and second trimester maternal androstenedione and female child's ADOS score, Beta 1.00 (95% CI 0.11, 1.88) (eTable 9). No significant associations were observed in the third trimester (eTable 10).

5.3. Sensitivity analysis results

A higher percentage of mothers included in the analysis identified their race as white, compared to mothers not included in the analysis (73.5% vs. 60.2%). No other demographic

characteristics were significantly different. After evaluating the e-values, a weak confounder could remove borderline associations observed.

6. Discussion

6.1. Principle findings

This study provides weak evidence for a relationship between maternal androgen concentrations and risk of ASD. Results might not be generalizable as the cohort represents a unique population of families at increased genetic risk of ASD (Hertz-Picciotto et al., 2018). Investigating risk factors in these younger siblings can provide insights for a growing percentage of families, as ASD prevalence continues to increase (Redfield et al., 2014).

An interesting, yet non-significant, result was seen in the stratified risk models. The non-significant direction of risk associated with ASD differed between male and female offspring for testosterone and androstenedione during the second trimester. Although increased androgens were expected to increase risk of ASD in males and females, only in female children there was some evidence for an association, which was attenuated with adjustment. Though not significant, during the same trimester, increased testosterone and androstenedione suggested a protective effect for male offspring. The critical time periods for ASD etiology remains unclear; however, fetal brain development occurs at a rapid rate in the first and second trimesters of pregnancy (Budday, Steinmann, & Kuhl, 2015), and the different directions of association could also potentially be due to an interaction of sex and trimester. The potential biological mechanisms through which this difference across sex could represent is not certain, and is more likely due to small cell sizes upon stratification and resultant unstable estimates. In this analysis, lack of significant associations in the first trimester could also be due to the limited sample size and power to detect modest effects.

In the third trimester, testosterone, androstenedione, and DHEA all had a significant inverse association with SRS scores for male offspring, after adjustment for maternal pre-pregnancy BMI. This finding could suggest a protective effect of maternal androgens in the third trimester for male offspring.

6.2. Strengths of the study

The high-familial risk MARBLES cohort study aims to understand the exposures that these families of Northern California face, and the mechanisms through which they could affect the risk of a child developing ASD. This study obtained serum testosterone concentrations during pregnancy, rather than proxy indicators of testosterone, such as PCOS diagnosis, used in some previous studies. Additionally, testosterone was not the only androgen examined. Two precursors, androstenedione and DHEA, were also included to enhance the overall picture of hormonal risk. Androgen concentrations were within the range, though on the lower end of the range, of other pregnancy studies obtaining androgen concentrations (Kuijper, Ket, Caanen, & Lambalk, 2013).

6.3. Limitations of the data

The sample size available for this analysis limited the statistical power to test weak associations and precluded stratification with simultaneous adjustment for multiple confounding factors in some cases. Low sample sizes in some models precluded the ability to present adjusted results. Missingness in some covariates also hindered the ability to run logistic regression models with all observations, providing a possibility for residual confounding and selection bias. Multiple comparisons were made, and when multiplicity was taken into account, there was no association of even of borderline significance. In light of the role that genetics has in ASD etiology (Bai et al., 2019), this study would benefit from a gene-environment interaction analysis. There was insufficient power to address interactions between gene variants and concentration of androgens. We did not have concentrations of other hormones, proteins, and enzymes, and we did not have recorded medical conditions related to hormones, such as PCOS, that could play a role in overlapping or relevant pathways. Therefore, we were unable to examine ratios of different hormones and rates of hormone binding, and we were unable to control for other hormone concentrations and hormone related medical conditions. In future studies, it would be advantageous to obtain this data and build upon the current analysis. Additionally, although most mothers in this analysis had multiple hormone measurements during pregnancy, there were not enough with measurements from each trimester to complete a longitudinal study.

6.4. Interpretation

This study provides weak evidence of a relationship between elevated serum androgen concentrations in mothers during pregnancy and the risk of ASD in children. Associations with androgens could be modified by the sex of the offspring, particularly associations between maternal androgen concentrations and ASD traits, as measured in SRS and ADOS. This analysis provided limited insight to the relationship between maternal androgens and risk of ASD in females, and observed no relationship between maternal androgens and risk of ASD in males.

7. Implications

This study attempted to both bring light to the gap in knowledge regarding maternal androgens measured during pregnancy and developmental outcomes in offspring. It provided insight for pregnancies at high-risk for later child neurodevelopmental disorders. None of the crude borderline significant associations observed remained in adjusted analyses. We found that maternal testosterone and androstenedione during the second trimester had different directions of risk of ASD in male and female offspring, although the risk observed was non-significant. Because we focused on ASD-affected families, these findings should be interpreted in light of this specific high-risk population. This work would benefit from additional research in the general population with larger sample sizes and measurements at multiple time points in pregnancy to allow for more powerful examination of associations by child sex.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data will be made available on request.

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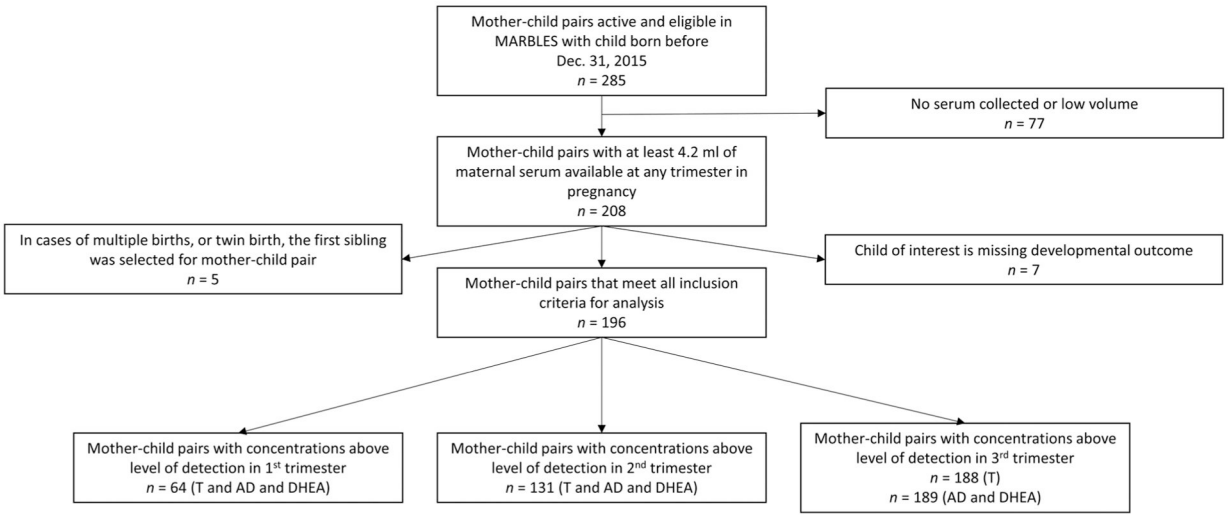

Fig. 1.

Flow diagram of MARBLES mother-child pairs included in the final analysis.

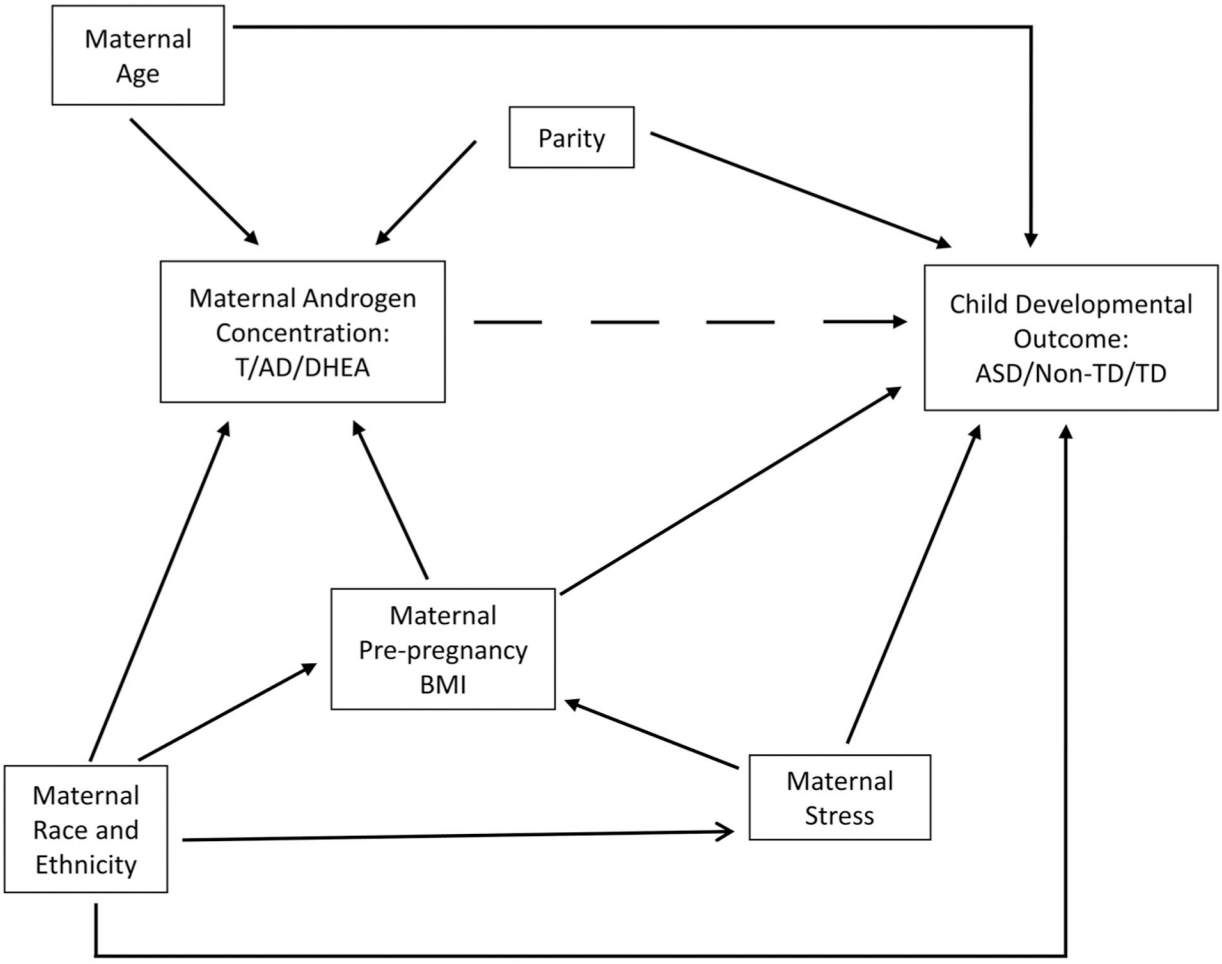

Fig. 2.

Directed acyclic graph (DAG) identifying potential confounding variables of the relationship between maternal testosterone (T), androstenedione (AD), and dehydroepiandrosterone (DHEA) concentrations and child's developmental outcome.

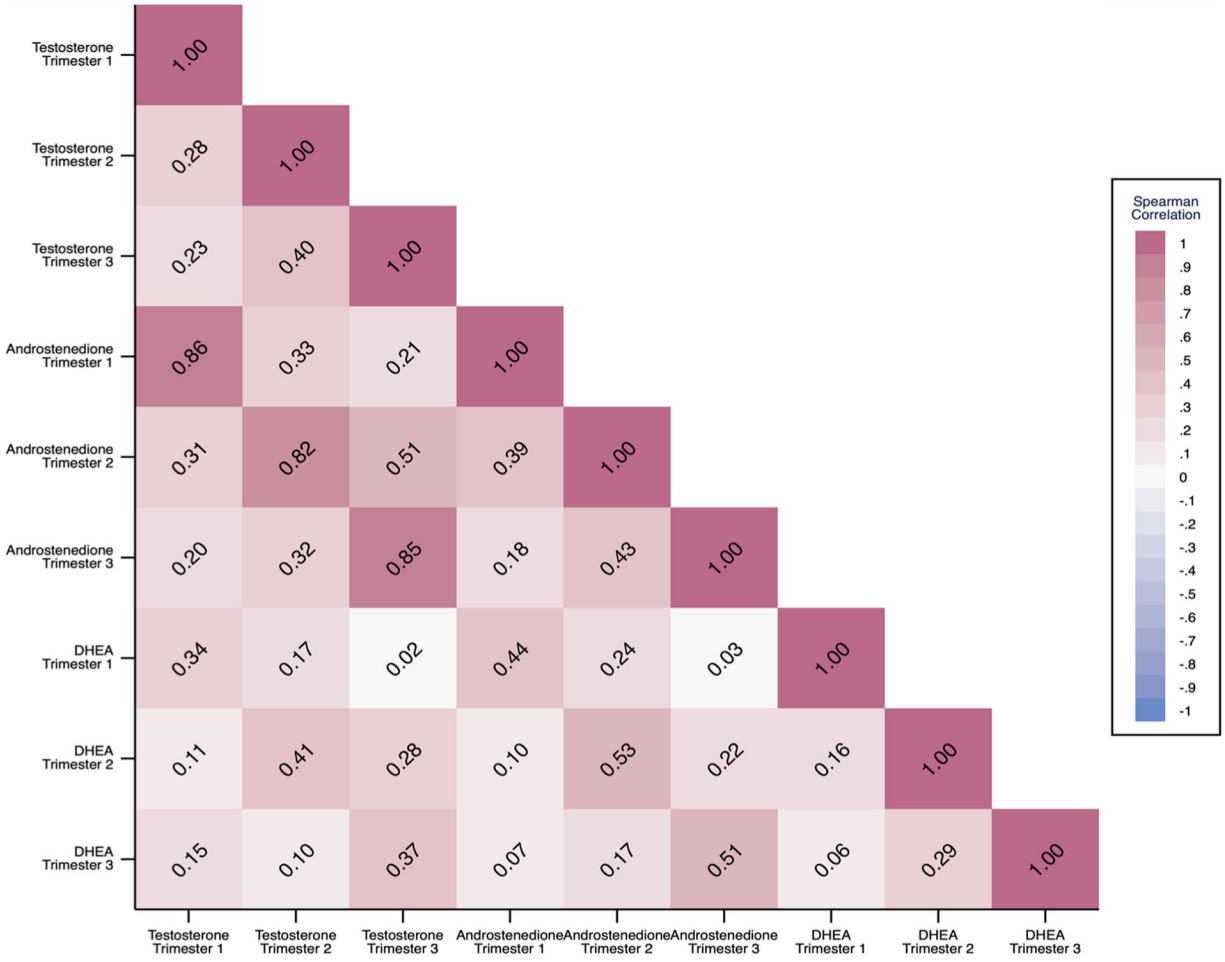

Fig. 3.

Spearman correlation coefficient heatmap of maternal androgen concentrations over time.

Table 1

Summary of maternal androgen concentrations in the second trimester of pregnancy stratified by demographic characteristics of children and their mothers in the MARBLES Study.

	Maternal Androgen Concentration in the Second Trimester					
	Testosterone (*pg/ml*) Median (IQR)	***P*-value**	**Androstenedione (*pg/ml*) Median (IQR)**	***P*-value**	**DHEA (*pg/ml*) Median (IQR)**	***P*-value**
Child's sex		0.75		0.64		0.95
Male child (n = 77)	448.73 (333.44, 712.65)		1088.40 (742.08, 1407.34)		1332.35 (854.63, 2134.51)	
Female child (n = 54)	436.47 (338.63, 608.62)		977.60 (780.88, 1383.88)		1317.22 (756.96, 2198.53)	
Child's birthweight		0.36		0.22		0.96
Birthweight < 5 lb 8 oz (n = 4)	580.69 (393.68, 1029.52)		1681.13 (983.26, 2368.85)		1368.16 (605.77, 3230.20)	
Birthweight ≥ 5 lb 8 oz (n = 4)	441.65 (333.44, 693.62)		1036.26 (734.84, 1396.95)		1332.35 (798.50, 2146.63)	
Maternal race/ethnicity		0.86		0.46		0.74
White, Non-Hispanic (n = 93)	437.03 (343.02, 693.62)		1017.87 (780.88, 1383.88)		1332.35 (818.77, 2019.67)	
Non-White (n = 38)	488.76 (287.57, 752.92)		1069.96 (650.17, 2039.67)		1295.12 (792.74, 2282.10)	
Maternal age at pregnancy		0.01 *		0.01 *		0.08
Maternal age < 35 years (n = 68)	535.45 (346.93, 744.87)		1193.76 (792.01, 1744.25)		1476.01 (836.70, 2384.30)	
Maternal age ≥ 35 years (n = 63)	386.69 (328.68, 517.06)		951.03 (690.49, 1230.53)		1197.07 (703.46, 1873.11)	
Maternal pre-pregnancy BMI		0.16		0.35		0.71
Normal[a] (n = 58)	415.07 (322.82, 616.18)		980.89 (787.55, 1383.88)		1318.74 (791.43, 2177.91)	
Overweight (n = 42)	511.73 (335.20, 810.87)		1152.52 (913.67, 1723.52)		1344.52 (965.20, 2198.53)	
Obese (n = 31)	443.77 (343.02, 608.62)		1036.26 (645.95, 1363.54)		1438.73 (750.70, 2134.51)	
Maternal education		0.02 *		0.21		0.06
Partial college or less (n = 59)	524.68 (348.64, 731.37)		1116.85 (689.19, 1723.52)		1411.14 (942.59, 2411.33)	
Completed college (n = 72)	412.31 (322.32, 559.78)		978.99 (788.68, 1355.42)		1213.92 (699.45, 1899.37)	
Maternal has metabolic condition[b]		0.98		0.58		0.70
Metabolic condition (n = 53)	433.14 (338.77, 688.40)		1036.26 (689.19, 1384.35)		1438.73 (798.50, 2274.69)	
No metabolic condition (n = 78)	449.59 (333.44, 698.08)		1073.30 (796.47, 1424.76)		1297.15 (810.70, 2062.39)	
Maternal cigarette smoking ever[c]		0.76		0.99		0.28
Has smoked (n = 27)	470.93 (358.27, 698.66)		1070.83 (734.84, 1384.35)		1862.80 (877.05, 2469.49)	
Has not smoked (n = 102)	442.71 (333.44, 698.08)		1041.66 (780.88, 1570.56)		1292.50 (791.43, 2004.59)	

	Maternal Androgen Concentration in the Second Trimester					
	Testosterone (*pg/ml*) Median (IQR)	***P*-value**	**Androstenedione (*pg/ml*) Median (IQR)**	***P*-value**	**DHEA (*pg/ml*) Median (IQR)**	***P*-value**
Parity		0.18		0.31		0.96
1–2 children (n = 105)	428.92 (330.37, 698.08)		1017.87 (689.19, 1407.34)		1392.52 (791.43, 2177.91)	
More than 2 children (n = 26)	481.80 (420.06, 693.62)		1184.76 (869.21, 1396.95)		1212.21 (1020.20, 2144.10)	
Insurance delivery type		0.63		0.39		0.15
Private (n = 99)	437.03 (333.44, 698.08)		1017.87 (727.50, 1388.12)		1227.34 (791.43, 2144.10)	
Public (n = 32)	449.59 (336.98, 674.57)		1131.66 (813.10, 1793.30)		1455.36 (956.13, 2343.55)	

Note. IQR = interquartile Range; *P*-value from Wilcoxon rank sum tests for categorical variables with 2 levels and Kruskal-Wallis tests for variables with more than 2 levels.

[a] Underweight mothers were included with normal weight mothers.

[b] Metabolic conditions include hypertension, preeclampsia, diabetes mellitus 1, diabetes mellitus 2, gestational diabetes, and obesity.

[c] Frequency missing = 2.

* p-value < 0.05.

Table 2

Unadjusted and adjusted associations between maternal testosterone concentration and child ASD and Non-TD outcomes.

	Testosterone[a], *25th- 75th Percentile Range (pg/ml)*			ASD vs. TD		Non-TD vs. TD	
Variable	**TD**	**ASD**	**Non-TD**	**RR (95% CI)**	***P*-Value**	**RR (95% CI)**	***P*-Value**
Trimester 1							
Crude model	40	18	6	1.23 (0.74, 2.06)	0.43	1.45 (0.52, 4.02)	0.47
Adjusted Model[b]	(270.1–689.9)	(350.6–664.7)	(327.5–578.2)	1.11 (0.65, 1.92)	0.70	1.28 (0.43, 3.85)	0.66
Adjusted Model[cd]				1.15 (0.62, 2.70)	0.66	No Convergence	
Trimester 2							
Crude model	82	33	16	1.11 (0.64, 1.91)	0.71	0.48 (0.21, 1.08)	0.08[e]
Adjusted Model[b]	(338.6–688.4)	(352.4–810.9)	(298.3–519.2)	1.16 (0.68, 1.97)	0.59	0.70 (0.33, 1.48)	0.35
Adjusted Model[cf]				1.23 (0.73, 2.08)	0.43	No Convergence	
Trimester 3							
Crude model	116	46	26	1.13 (0.75, 1.70)	0.56	0.76 (0.46, 1.26)	0.30
Adjusted Model[b]	(305.9–608.5)	(282.2–648.2)	(254.1–514.0)	1.23 (0.82, 1.84)	0.32	0.82 (0.49, 1.37)	0.45
Adjusted Model[cg]				1.44 (0.90, 2.31)	0.12	No Convergence	

Note. ASD = Autism Spectrum Disorder; Non-TD = Non-Typical Development; TD = Typical Development; RR = Risk Ratio.

[a] Natural-log transformed for analyses.

[b] Adjusted for maternal pre-pregnancy BMI.

[c] Adjusted for all DAG covariates (maternal pre-pregnancy BMI, parity, maternal race/ethnicity, maternal stress, maternal age).

[d] Missing 1 observation in ASD group.

[e] *p*-value < 0.10.

[f] Missing 1 observation in TD group, 1 observation in ASD group, and 1 observation in Non-TD group.

[g] Missing 3 observations in the TD group, 2 observations in the ASD group, and 1 observation in Non-TD group.

Table 3

Unadjusted and adjusted associations between maternal dehydroepiandrosterone (DHEA) concentration and child ASD and Non-TD outcomes.

	DHEA[a], *25th- 75th Percentile Range (pg/ml)*			ASD vs. TD		Non-TD vs. TD	
Variable	**TD**	**ASD**	**Non-TD**	**RR (95% CI)**	***P*-Value**	**RR (95% CI)**	***P*-Value**
Trimester 1							
Crude model	40	18	6	1.46 (0.80, 2.65)	0.22	2.29 (0.77, 6.84)	0.14
Adjusted Model[b]	(1131.1–2113.1)	(1296.0–3034.3)	(1757.3–4030.5)	1.43 (0.74, 2.76)	0.29	3.38 (0.78, 14.70)	0.10
Adjusted Model[c]				1.38 (0.62, 3.08)	0.42	No Convergence	
Trimester 2							
Crude model	82	33	16	1.27 (0.77, 2.10)	0.35	0.52 (0.26, 1.05)	0.07[d]
Adjusted Model[b]	(818.8–2004.6)	(977.9–2392.1)	(587.8–1879.4)	1.47 (0.87, 2.48)	0.15	0.60 (0.26, 1.35)	0.21
Adjusted Model[c]				1.39 (0.82, 2.36)	0.22	No Convergence	
Trimester 3							
Crude model	117	46	26	1.01 (0.72, 1.41)	0.96	0.98 (0.62, 1.57)	0.94
Adjusted Model[b]	(732.6–1784.0)	(894.9–1631.4)	(773.4–2012.7)	1.05 (0.77, 1.45)	0.75	1.03 (0.65, 1.63)	0.91
Adjusted Model[c]				1.04 (0.74, 1.47)	0.82	No Convergence	

Note. ASD = Autism Spectrum Disorder; Non-TD = Non-Typical Development; TD = Typical Development; RR = Risk Ratio.

[4]Missing 1 observation in ASD group.

[5]Missing 1 observation in TD group, 1 observation in ASD group, and 1 observation in Non-TD group.

[6]Missing 3 observations in the TD group, 2 observations in the ASD group, and 1 observation in Non-TD group.

[a]Natural-log transformed for analyses.

[b]Adjusted for maternal pre-pregnancy BMI.

[c]Adjusted for all DAG covariates (maternal pre-pregnancy BMI, parity, maternal race/ethnicity, maternal stress, maternal age).

[d]*p*-value < 0.10.

Table 4

Unadjusted and adjusted associations between maternal androstenedione concentration and child ASD and Non-TD outcomes.

	Androstenedione[a], *25th- 75th Percentile Range (pg/ml)*			ASD vs. TD		Non-TD vs. TD	
Variable	**TD**	**ASD**	**Non-TD**	**RR (95% CI)**	***P*-Value**	**RR (95% CI)**	***P*-Value**
Trimester 1							
Crude model	40	18	6	1.34 (0.69, 2.62)	0.38	No Convergence	
Adjusted Model[b]	(707.7–1649.4)	(911.6–1675.7)	(1163.2–1499.9)	1.16 (0.56, 2.40)	0.69	No Convergence	
Adjusted Model[c]				1.17 (0.51, 2.64)	0.71	No Convergence	
Trimester 2							
Crude model	82	33	16	1.09 (0.65, 1.83)	0.74	0.62 (0.31, 1.24)	0.18
Adjusted Model[b]	(787.5–1383.9)	(650.2–1786.0)	(563.4–1291.7)	1.17 (0.69, 1.98)	0.56	0.80 (0.38, 1.71)	0.57
Adjusted Model[c]				1.30 (0.73, 2.30)	0.37	No Convergence	
Trimester 3							
Crude model	117	46	26	1.09 (0.75, 1.58)	0.66	0.89 (0.60, 1.30)	0.54
Adjusted Model[b]	(820.7–1467.0)	(788.7–1797.0)	(688.0–1372.5)	1.18 (0.81, 1.74)	0.39	No Convergence	
Adjusted Model[c]				1.32 (0.84, 2.09)	0.23	No Convergence	

Note. ASD = Autism Spectrum Disorder; Non-TD = Non-Typical Development; TD = Typical Development; RR = Risk Ratio.

[4]Missing 1 observation in ASD group.

[5]Missing 1 observation in TD group, 1 observation in ASD group, and 1 observation in Non-TD group.

[6]Missing 3 observations in the TD group, 2 observations in the ASD group, and 1 observation in Non-TD group.

[a]Natural-log transformed for analyses.

[b]Adjusted for maternal pre-pregnancy BMI.

[c]Adjusted for all DAG covariates (maternal pre-pregnancy BMI, parity, maternal race/ethnicity, maternal stress, maternal age).

Table 5

Unadjusted and adjusted associations between maternal testosterone, androstenedione, DHEA and child Social Responsiveness Score (SRS) in the 3rd trimester.

Hormones	Proband's sex	Participant's sex	Outcome = SRS[a] (3rd Trimester Observations)							
			Unadjusted model				Adjusted model[b]			
			Beta	95% CI	*p*	R-square	Beta	95% CI	*p*	R-square
ln(T)	All	Female (*n* = 50)	0.40	−2.07, 2.87	0.75	0.01	0.55	−1.92, 3.03	0.65	0.03
		Male (*n* = 49)	**−7.35**	**−12.69, −2.01**	**0.01***	**0.20**	**−7.09**	**−12.49, −1.68**	**0.01***	**0.21**
		All (*n* = 99)	−1.86	−4.07, 0.35	0.10	0.06	−1.65	−3.87, 0.58	0.14	0.07
	Male	Female (*n* = 42)	−0.12	−2.40, 2.16	0.92	0.02	−0.14	−2.49, 2.22	0.91	0.02
		Male (*n* = 40)	**−8.04**	**−13.92, −2.15**	**0.01***	**0.22**	**−7.65**	**−13.58, −1.71**	**0.01***	**0.24**
		All (*n* = 82)	−2.07	−4.32, 0.17	0.07	0.08	−1.95	−4.26, 0.36	0.10	0.08
	Female	Female (*n* = 8)	5.03	−29.66, 39.72	0.72	0.17	–	–	–	–
		Male (*n* = 9)	−3.67	−30.28, 22.94	0.75	0.22	–	–	–	–
		All (*n* = 17)	−2.01	−18.91, 14.88	0.80	0.01	–	–	–	–
ln(AD)	All	Female (*n* = 50)	−0.15	−3.88, 3.59	0.94	0.00	−0.06	−3.79, 3.67	0.97	0.03
		Male (*n* = 50)	**−2.08**	**−2.90, −1.26**	**< 0.001***	**0.36**	**−2.00**	**−2.84, −1.17**	**< 0.001***	**0.37**
		All (*n* = 100)	**−1.70**	**−2.37, −1.04**	**< 0.001***	**0.21**	**−1.68**	**−2.34, −1.02**	**< 0.001***	**0.23**
	Male	Female (*n* = 42)	−0.93	−4.36, 2.50	0.59	0.03	−0.94	−4.43, 2.56	0.59	0.03
		Male (*n* = 41)	**−2.12**	**−3.02, −1.22**	**< 0.001***	**0.38**	**−2.01**	**−2.93, −1.09**	**< 0.001***	**0.40**
		All (*n* = 83)	**−1.81**	**−2.48, −1.15**	**< 0.001***	**0.27**	**−1.79**	**−2.46, −1.12**	**< 0.001***	**0.27**
	Female	Female (*n* = 8)	−8.73	−73.40, 55.93	0.74	0.29	–	–	–	–
		Male (*n* = 9)	−8.81	−33.98, 16.35	0.42	0.26	–	–	–	–
		All (*n* = 17)	−10.25	−33.52, 13.02	0.36	0.07	–	–	–	–
ln(DHEA)	All	Female (*n* = 50)	2.24	−1.51, 5.98	0.24	0.04	2.57	−1.19, 6.33	0.18	0.07
		Male (*n* = 50)	**−1.91**	**−3.76, −0.05**	**0.04***	**0.10**	−1.71	−3.58, 0.17	0.07	0.13
		All (*n* = 100)	**−1.58**	**−3.02, −0.15**	**0.03***	**0.05**	**−1.46**	**−2.90, −0.02**	**0.05***	**0.07**
	Male	Female (*n* = 42)	2.06	−1.43, 5.55	0.24	0.06	2.12	−1.51, 5.74	0.24	0.06

Hormones	Proband's sex	Participant's sex	Outcome = SRS[a] (3rd Trimester Observations)							
			Unadjusted model				Adjusted model[b]			
			Beta	**95% CI**	***p***	**R-square**	**Beta**	**95% CI**	***p***	**R-square**
		Male (*n* = 41)	**−2.42**	**−4.65, −0.19**	**0.03** *	**0.13**	−2.12	−4.37, 0.12	0.08	0.17
		All (*n* = 83)	**−1.94**	**−3.48, −0.40**	**0.01** *	**0.07**	**−1.85**	**−3.41, −0.29**	**0.02** *	**0.08**
	Female	Female (*n* = 8)	6.41	−39.50, 52.32	0.73	0.04	–	–	–	–
		Male (*n* = 9)	11.26	−12.36, 34.88	0.29	0.20	–	–	–	–
		All (*n* = 17)	2.62	−8.15, 13.38	0.61	0.03	–	–	–	–

Note. T = testosterone, AD = androstenedione; DHEA = dehydroepiandrosterone.

[a] Square-root transformed.

[b] Adjusted for maternal pre-pregnancy BMI.

* *p*-value < 0.05.