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## Prenatal Sex Hormones and Behavioral Outcomes in Children

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### Abstract

Abnormal sex hormone levels in utero have been associated with child behavioral problems, but it is unclear if normal variation in prenatal sex hormones is associated with subsequent behavior in childhood. We assessed maternal sex hormones, including serum estrone (E1), estradiol (E2), estriol (E3), free testosterone (FT), and total testosterone (TT), during early pregnancy (gestational week 6–21 (mean = 11.1)) and evaluated child behavior at ages 4–5 using the Behavioral Assessment System for Children (BASC-2) and Social Responsiveness Scale (SRS-2) in 404 mother/child pairs (211 girls, 193 boys) within The Infant Development and Environment Study, a multi-site pregnancy cohort study. Associations between hormones and composite scores were evaluated using multiple linear regressions in both sexes combined, and separate models assessed effect modification by sex with the addition of interaction terms. A 10-fold increase in maternal FT or TT was associated in both sexes with a 4.3-point (95% CI: 0.5, 8.2) or 4.4-point (0.8, 8.0) higher BASC-2 internalizing composite T score, respectively. In addition, a 10-fold increase in FT or TT was associated with a 3.8-point (0.04, 7.5) or 4.0-point (0.5, 7.5) higher behavioral symptoms index composite score. In models evaluating effect modification by sex, a 10-fold increase in E1 was associated with a 4.3-point (1.2, 7.4) decrease in adaptive skills composite score in girls only (interaction  $p=0.04$ ). We observed associations between testosterone and internalizing behaviors and behavioral symptoms index in both sexes, as well as a female-specific association between E1 and adaptive skills. Sex hormones during pregnancy may play a key role

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#### Author Contributions

The TIDES team was responsible for the development of the original study aims, study design, implementation, data collection, and management. DBD conducted the statistical analysis and drafted the manuscript. All authors provided critical revisions and approved the manuscript for submission.

#### Conflicts of Interest

The authors have no conflicts of interest to declare with respect to this manuscript.

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in influencing later-life behavior, and additional studies should further examine different periods of susceptibility to hormonal signals.

## Keywords

Estrogen; Testosterone; Child Behavior; Neurodevelopment

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## 1. Introduction

Sex hormones play an important role in regulating the development of sex-differentiated brain structures and sex-specific reproductive behaviors (Cohen-Bendahan 2005, Auyeung 2013, Martel 2013). Their role in child behavioral problems and psychiatric disorders is suggested by sex differences in the prevalence of these disorders, with some disorders found to be more common in males (e.g., autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD)) (Werling and Geschwind 2013, Arnett 2015) and others more common in females (e.g., anxiety disorders) (Eaton 2012, Altemus 2014). Given that sex hormone levels remain low until puberty (Sisk and Zehr 2005), pre-pubescent behavioral effects of sex hormones are likely programmed during fetal development (Miranda and Sousa 2018). A key period for this programming is likely in early pregnancy, which is thought to be a critical period for neurological sex differentiation as this is when male fetal testosterone surges, driving subsequent masculinization of brain structures and gender-stereotyped behavior (Hines 2002, McCarthy 2016).

Prenatal sex hormone associations with child pathologic behavior have primarily been evaluated in the context of androgens (Gore 2014), with several studies observing associations between second trimester amniotic fluid testosterone and a variety of problematic behaviors, including attention problems in boys (Korner 2019) and autistic traits in both sexes (Auyeung 2012). Studies of disorders of sex development such as congenital adrenal hyperplasia (CAH), a disorder that results in abnormally high androgen concentrations in utero and throughout life, have observed higher scores for assessments of later-life autism-like behaviors (Knickmeyer 2006) or increased incidences of anxiety and depression-related disorders and substance abuse in later life in both males and females with CAH (Falhammar 2014, Engberg 2015). Maternal hyperandrogenic endocrinopathies such as polycystic ovarian syndrome (PCOS) have also been associated with neurobehavioral outcomes in children lacking CAH or any other salient endocrine disorders, including higher odds of ASD, ADHD, and their comorbidity in children (Kosidou 2017, Cherskov 2018, Cesta 2019). Another study evaluating behavioral assessments in the context of maternal PCOS and directly measured amniotic testosterone levels found that increased autism-like behaviors and lower empathetic behaviors were associated with higher testosterone in both boys and girls and with maternal PCOS in girls (Palomba 2012). In contrast to these findings related to hyperandrogenism, boys with reproductive disorders associated with lower prenatal testosterone levels, such as hypospadias and cryptorchidism, have also been shown to have higher odds for ASD (Rotem 2018).

Compared with androgens, fewer studies have examined the relationship between prenatal estrogens and child behavior problems. Although estrogen levels have been associated with inhibition and related internalizing features, most of these studies focus on activational effects in adolescence, when sex differences in internalizing disorders tend to emerge (Martel 2013). In the adult brain, estrogens are considered neuroprotective, in part due to neuronal trophic and anti-apoptotic effects (Kajta and Beyer 2003). Increased ASD risk was observed in association with decreases in maternal serum E3 sampled between gestational weeks 15–20, which coincides with the protective effect of E3 observed for birth outcomes like pre-term birth (Windham 2016). By contrast, a recent pilot study observed the opposite, with greater maternal serum E2 in gestational weeks 15–18 associated with an increased ASD risk in offspring (Bilder 2019). Though an earlier study of second trimester amniotic E2 found no association with autistic traits in toddlers (Auyeung 2012), a more recent male-only study of second trimester amniotic estrogens found increased risk of ASD associated with E1, E2, E3, and progesterone but not testosterone; however, those models did not adjust for covariates (Baron-Cohen 2019). Beyond direct estrogen measures, recent studies of fetal single nucleotide polymorphisms (SNPs) in sex hormone receptors have yielded mixed results and some evidence of effect modification by sex (Miodovnik 2012).

Most of the existing research on the relationship between psychopathologic behaviors and sex hormones has either been conducted in animal models or in the context of maternal conditions known to affect prenatal sex hormones. Despite the evidence for the role of prenatal sex hormones in the development of child behavior (Auyeung 2013), little is known about the organizational role maternal sex hormones during pregnancy play in behaviors related to psychopathologic disorders, and few studies have evaluated the relationship between these aspects of child behavior and levels of maternal prenatal sex hormones in healthy individuals. The current study examines the relationship between maternal sex hormone levels measured during early pregnancy in healthy women and behavioral outcomes in children aged 4–5 years old. These outcomes encompass several childhood behavioral dimensions, broadly categorized as autism-related, externalizing, internalizing, adaptive behaviors, and other behavior problems as measured by the behavioral symptoms index. Based on existing literature, we hypothesized that prenatal maternal sex hormones would be associated with child behaviors, with prenatal testosterone expected to be associated with externalizing behaviors, autism-related behaviors, and behavioral symptoms index and prenatal estrogen predicted to be associated with internalizing behaviors. Given the potential sex-specificity of sex hormone associations with behavior, we also examined whether these associations are modified by sex.

## 2. Methods

### 2.1 Study participants

From 2010 to 2012, pregnant women were recruited in their first trimester at the University of California San Francisco (UCSF), University of Minnesota (UMN), University of Rochester Medical Center (URMC), and Seattle Children’s Hospital/University of Washington (UW) to take part in The Infant Development and Environment Study (TIDES). Eligibility criteria included that participants were 18 years of age, English speaking, and

<13 weeks pregnant, as well as that they had no severe threat to pregnancy and intended to deliver at one of the study hospitals. TIDES was approved prior to recruitment by the institutional review boards at each center, and informed consent was obtained from all participants. Questionnaires containing demographic, medical history, physiological, and behavioral questions (e.g., pertaining to alcohol and tobacco use) were administered in early pregnancy, usually on the same day as serum collection or completed online at the participants' convenience. Gestational dating was based on the first ultrasound in the medical record. 626 women completed screening and had serum samples drawn during early pregnancy. This study is limited to 403 mothers and 404 children for whom complete early pregnancy serum sex hormone, child 4–5-year behavioral assessment, and covariate data were available (including data from both members of a single twin pair).

## 2.2 Serum sex hormone measurements

A single serum sample was obtained in early pregnancy between gestational week 6 and 21, with most samples drawn in the first trimester (median = 11.6 weeks gestation), and stored at  $-80^{\circ}\text{C}$  until analysis. The Endocrine and Metabolic Research Laboratory at the Los Angeles Biomedical Research Institute (now renamed Lundquist Institute) at the Harbor-UCLA Medical Center performed all serum hormone assays using validated methods. Serum E1, E2, and E3 were separated on a column with a gradient profile from 63% to 100% methanol using a Shimadzu (Columbia, MD) high performance liquid chromatography (HPLC) system and a triple quadrupole mass spectrometer (Applied Biosystems (Foster City, CA) API5000 liquid chromatography tandem mass spectrometer (LC-MS/MS)) operated in the negative mode using multiple-reaction monitoring. For both E1 and E2, the calibration curve was linear over a range of 2 to 2000 pg/mL with the lower limit of quantification (LLOQ) was 2.0 pg/mL, and the calibration curve for E3 was linear from 50 to 5000 pg/mL with a LLOQ of 50 pg/mL. The within-run and between-run precisions (% coefficient of variation) were 2.6%–5.6% and 3.9%–4.6% for E1, 4.3%–5.0% and 4.6%–5.2% for E2, and 4.1%–5.7% and 5.2%–8.7% for E3, respectively. The accuracy ranged from 91.9–101.2 for E1, 93.9–100.3 for E2, and 87.2–104.3 for E3.

Serum TT LC-MS/MS runs were performed as previously described (Shiraishi 2008) with a Shimadzu HPLC system attached to an Applied Biosystems API5500 LC-MS/MS equipped with a turbo-ion-spray source and operated in the positive mode. The calibration curve was linear from 2 ng/dL to 2000 ng/dL, the LLOQ for TT was 2.0 ng/dL, the within- and between-run precisions were less than 5%, and the accuracy of spiked samples was between 100% and 113%. Percent FT, representing unbound testosterone, was measured by equilibrium dialysis using radiolabeled testosterone as described previously, and the LLOQ is 0% (Qoubaitary 2006). Then FT was calculated by multiplying the percent FT with the TT concentration. Both FT and TT measurements were included in our analyses because evidence suggests that FT may be a more clinically relevant measure of testosterone activity (Tsuji-mura 2012, Antonio 2016), but it remains unclear if FT is the only bioactive form of testosterone (Goldman 2017). Therefore, analyzing both provides a more comprehensive examination of testosterone effects.

### 2.3 Neurobehavioral Assessments

TIDES children completed study visits between 4 and <6 years of age (median = 4.5 years). Mothers were asked to complete a study questionnaire in person or, when this was not possible, by mail. Mothers completed the Behavioral Assessment System for Children, 2<sup>nd</sup> Edition (BASC-2), and Social Responsiveness Scale, 2<sup>nd</sup> Edition (SRS-2). For the BASC-2, Likert scale (“never”, “sometimes”, “often”, or “always”) responses on 138 items were combined into raw subscale scores, which were again summed to get four raw composite scores: externalizing, internalizing, behavioral symptoms index, and adaptive skills composite scores. The BASC-2 externalizing composite score is comprised of hyperactivity and aggression subscale scores; the internalizing composite is comprised of anxiety, depression, and somatization subscale scores; the behavioral symptoms index (BSI) composite is comprised of attention problems, atypicality, and withdrawal subscale scores; and the adaptive skills composite is comprised of activities of daily living, adaptability, functional communication, and social skills subscale scores.

The SRS-2 is comprised of 65 questions on a 0–3 Likert scale, which are summed to become one of the subscale scores of social awareness, social cognition, social communication, social motivation, and restricted interests and repetitive behaviors (RRB). The SRS-2 has been validated to distinguish children with ASD from healthy children (Frazier et al., 2012). The SRS-2 total score is calculated as the sum of all subscale scores. For both BASC-2 and SRS-2, raw scores were converted into sex-standardized, norm-referenced T scores (mean = 50, SD = 10) based on each assessment’s manualized procedures. As per the conventions of both tests, higher values indicate more problematic behaviors for all scores except for the BASC-2 adaptive skills composite score.

### 2.4 Statistical methods

The BASC-2 and SRS-2 composite scores were chosen as the primary outcomes for this analysis, and they were analyzed as sex-standardized T scores rather than raw scores because T scores, not raw scores, are utilized in clinical applications. Summary statistics were computed for all relevant measures. The twins were treated as independent participants since including a random intercept or other means of nesting this single pair of twins within the same mother did not change results. Maternal sex hormone concentrations were right-skewed and  $\log_{10}$ -transformed in all models. Hormone concentrations below the LLOQ were imputed using the LLOQ divided by  $\sqrt{2}$ . Multivariable linear regression was used to examine associations between maternal hormones and BASC-2 and SRS-2 outcomes using the “HC0” Huber-White heteroskedasticity-consistent standard error sandwich estimator to avoid potential heteroskedasticity-induced bias (White 1980). To examine potential effect modification by sex, multiple linear regressions including a maternal sex hormone by child sex interaction term were also evaluated. Each model only evaluated one sex hormone predictor and one behavioral outcome at a time. Due to issues with the theory behind p-value adjustment (Rothman 1990), the novelty of the relationships we are examining, and the exploratory nature of our analysis, we decided not to adjust for multiple comparisons. All models adjusted for the a priori-selected covariates maternal and child age, study center, race, income and education categories, child sex, and alcohol and cigarette consumption in the week prior to the serum draw. Covariates were chosen a priori because of their potential

confounding effect on one or more outcome measures (Fox 1995, Herrmann 2008, O'Connor and Paley 2009). We chose to analyze composite behavior scores rather than subscale scores due to their increased reliability and clinical relevance, but we calculated subscale T score summary statistics and regression results for reference.

### 3. Results

#### 3.1 Study Participants

Table 1 summarizes the demographic and pregnancy characteristics for study participants. Participants were recruited in similar numbers across study centers. Mothers were primarily white (75%) and most had some level of college or graduate education (89%). Few mothers reported using tobacco cigarettes (3%) or drinking alcohol (4%). Self-reported pre-pregnancy and early pregnancy (median 11.6 weeks gestation) BMI were similar, with 19% of mothers reporting an obese pre-pregnancy BMI > 30 and 22% reporting a pre-pregnancy BMI of 25–30. Most serum samples (92%) were drawn in the first trimester. Few children (5%) were born with a low birth weight (< 2.5 kg), and only 1 (0.25%) was born with an extremely low birth weight (< 1 kg). Forty (9.9%) of the births were preterm (<37 weeks), and 1 (0.25%) was extremely preterm (<28 weeks). Twenty-eight (7%) mothers reported having polycystic ovary syndrome (PCOS). Of the original 626 women recruited, those included in the final analysis were more likely to be slightly older, white, higher income, more educated, and from the UCSF or UMN study sites (see Table A.3).

#### 3.2 Sex Hormone Levels

Table 2 shows maternal sex hormone summary statistics and the results of t-tests comparing hormones by fetal sex. Sex hormone levels fell within typical ranges (Oleary 1991, Schock 2016) and did not differ significantly by child sex. Log<sub>10</sub>-transformed E1 and E2 were strongly correlated ( $r = 0.89$ ), as were FT and TT ( $r = 0.82$ ) (see Figure A.1). Hormone measurements were below the LLOQ only for E3 (34%).

#### 3.3 Neurobehavioral Assessment Outcomes

Sex-standardized BASC-2 and SRS-2 composite T scores are summarized in Table 3. The distributions for all T scores were similar to the normative sample (i.e., mean of 50 and SD of 10). Though BASC-2 and SRS-2 T scores are sex-standardized, there were still significant differences in T scores between sexes among the study participants. Males had significantly lower BASC-2 internalizing and higher SRS-2 total composite T scores.

Pearson correlation coefficients between BASC-2 and SRS-2 composite T scores are shown in Figure A.2. The strongest correlations were between BASC-2 externalizing and BSI scores ( $r = 0.84$ ) and BSI and SRS-2 total score ( $r = 0.72$ ), and correlations between the other scores were moderate, ranging in magnitude from  $r = -0.32$  between BASC-2 internalizing and adaptive skills scores to  $r = 0.69$  between BSI and internalizing score.

#### 3.4 Linear Regressions

The results of linear regressions associating maternal sex hormone levels and behavioral test composite scores are shown in Figure 1A and Table A.1 for models without the hormone by



sex interaction term and in Figure 1B and Table A.2 for models with the interaction term. In both sexes combined, each ten-fold increase in TT is significantly associated with a 4.40 (95% CI: 0.83, 7.97) point and 4.01 (95% CI: 0.52, 7.50) point increase in BASC-2 internalizing and BSI composite scores, respectively. A ten-fold increase in FT is significantly associated with a 4.35 (0.54, 8.16) point increase in internalizing composite score and a 3.75 (0.05, 7.46) point increase in BSI composite score. In the hormone by sex interaction models (Figure 1B), the only association with a significant sex-specific hormone main effect coefficient and a significant hormone by sex interaction term is the female-specific adverse association between E1 and BASC-2 adaptive skills composite score ( $-4.26$  ( $-7.37, -1.16$ ); interaction  $p=0.035$ ). Subscale score summary statistics and regression results are shown in Section 5 of Appendix A.

#### 4. Discussion

The findings of this study suggest that maternal testosterone levels during pregnancy are positively associated with internalizing behaviors and behavioral symptoms index in 4–5-year-old children of both sexes. Our results are somewhat inconsistent with findings from the literature observing associations between PCOS and ASD (Kosidou 2016, Cherskov 2018, Cesta 2019) or ADHD (Kosidou 2017, Cesta 2019), although there was a significant association between FT and TT and BSI score, which includes the attention problems subscale score. Although the estimates were imprecise and not statistically significant, male-specific FT associations of a similar nature were observed with SRS-2 total score and BSI. Associations between behavior and the highly correlated FT and TT measures were similar in effect size, suggesting that these measures both similarly indicate prenatal testosterone activity. For the estrogens, there was a sex-specific association between prenatal E1 and worse adaptive skills in girls, but not boys. All significant associations were in the adverse direction, and the magnitudes of these associations were all around 4 points per 10-fold increase in hormone. Given that each T score unit can be interpreted as 0.1 SD of the population distribution for that outcome and each sex hormone vary over a range of 1–2  $\log_{10}$  units, these results suggest that natural variation in early pregnancy maternal sex hormones could have meaningful impacts on behavioral outcomes in children when considered at the population-level.

Few previous studies support the observed associations in both sexes between maternal testosterone and internalizing behaviors and BSI score, which encompasses some behavior problems related to both internalizing problems (e.g., social withdrawal) and externalizing problems (e.g., attention problems). However, rodent models of maternal hyperandrogenism suggest female-specific internalizing behavioral effects of testosterone. Female mice born to dams treated with dihydrotestosterone (DHT) during pregnancy exhibited increased adrenoceptor  $\alpha 1B$  expression in the amygdala and corticotropin-releasing hormone in the hypothalamus, possible markers of anxiety (Manti 2018), and female rats born to dams injected with testosterone during pregnancy exhibited anxiety-like behaviors and changes in amygdala receptor gene expression consistent with anxiety (Hu 2015). Social vocalizations decreased for both sexes of rat pups born to dams treated with the aromatase inhibitor letrozole, but only female offspring also exhibited decreased social and sexual interaction (Xu 2015). In contrast, in humans it was male infants with higher amniotic testosterone

levels during pregnancy who exhibited greater fear responses, not females (Bergman 2010). Both males and females with CAH exhibited greater lifetime rates of psychiatric disorders related to anxiety and depression (Falhammar 2014, Engberg 2015), though this may not reflect the influence of prenatal hyperandrogenic exposures specifically since CAH may also affect these outcomes through impaired adrenaline production and other mechanisms. In relation to other psychopathological behavioral outcomes, studies suggest that testosterone associations with behavior are not sex-specific, as this was the case for amniotic testosterone associations with autism-related behaviors (Kosidou 2016, Kosidou 2017, Cherskov 2018), and maternal PCOS associations with child ASD and ADHD (Kosidou 2016, Kosidou 2017, Cherskov 2018). There is also a lack of sex-specificity observed for correlations between maternal plasma testosterone and fetal plasma testosterone and cortisol later in pregnancy (Gitau 2005), as well as between amniotic testosterone and cortisol from gestational week 15 to 37 (Sarkar 2008). These correlations suggest that maternal testosterone directly or indirectly plays a role in regulation of the fetal hypothalamic-pituitary-adrenal (HPA) axis, which has already been suggested in adult studies showing crosstalk between the HPA and hypothalamic-pituitary-gonadal (HPG) axes, such as testosterone and E2 increasing arginine vasopressin signaling that leads to increased adrenal cortisol release (Grassi 2013). Dysregulation of the fetal HPA has been associated with later-life anxiety and depression (Ansoerge 2007), and so perhaps our observed maternal testosterone associations with internalizing and BSI-related behaviors may result, in part, through testosterone effects on the fetal HPA axis. We also used more accurate methods to measure TT and FT, LC-MS and equilibrium dialysis, respectively, than other groups who largely used immunoassays and equations to measure TT and FT, respectively. This may have contributed to some of the differences between our observed testosterone associations and those of previous studies.

Few studies have examined maternal estrogens in relation to child behavioral outcomes, and none thus far have observed the sex-specific association between E1 and adaptive skills that we observed in our results. Perhaps reflecting a similar mechanism, we also observed a suggestive but nonsignificant association between E1 and BSI, with some suggestion of a greater association in females. Miodovnik et al. observed adverse associations with adaptive skills in males born to mothers with a functional CYP1B1 SNP, and studies have shown that a functional increase in CYP1B1 leads to decreased testosterone signaling and increased E2 (Miodovnik 2012). These results suggest that males, but not females, may have increased behavioral problems with increasing maternal estrogen exposure. The adaptive skills composite score covers some behaviors that may overlap with autistic traits such as social skills, and the recent finding of an increased risk of autism in association with second trimester maternal E2 may reflect similar processes to our observations regarding E1 (Bilder 2019). The observed estrogen association with these adverse behavioral outcomes may be a compensatory response given the neuroprotective and uteroprotective role of estrogens, including maintaining normal development of the fetal adrenal cortex, which is vital in assuring placental steroid production and fetal survival (Kaludjerovic and Ward 2012). A similar compensatory mechanism may also underlie the amniotic E2 associations with ASD risk observed by Baron-Cohen et al. (Baron-Cohen 2019). However, these authors suggest that prenatal estrogen increases GABAergic signaling, which subsequently increases cerebrocortical excitatory synapses, a theorized mechanism of autism (Sellers 2015). It is



unclear why we only observe the adverse association with E1 in females. E2 is the predominant form of estrogen in women until menopause and is the most biologically active form of estrogen (Rothman 2011), and so it is surprising that we would see an association with E1 rather than E2. The primary role of E1 is to act as a pro-estrogen, able to be converted into the more potent E2 or to a lesser extent E3 (Kuhl 2005). In addition, 17 $\beta$ -hydroxysteroid dehydrogenase 2 prevents excess active estrogen delivery to the fetus by catalyzing the conversion of E2 back into E1 in the placental fetal endothelial cells (Drolet 2007). If the observed association with E1 reflects a compensatory response, perhaps E1 is being upregulated as a pro-estrogen pool, ready to be rapidly converted to E2, though it is also possible that these associations instead reflect adverse neurological effects of estrogens.

Our results differ from several studies evaluating hormones in amniotic fluid, and this may be due to a difference in hormone action or transport between the maternal and fetal compartments. Amniotic fluid levels are thought to more directly reflect fetal exposure than maternal serum, and the two compartments have been shown to be uncorrelated for FT and TT and moderately correlated for E2 in the second trimester (van de Beek 2004). However, direct sampling of paired fetal and maternal blood samples from the mid-second to late third trimester demonstrated correlations and similar concentrations between maternal and fetal blood TT in both sexes (Gitau 2005), and our own group previously found increased maternal testosterone in mothers of male fetuses later in pregnancy, suggesting testosterone transfer between the fetus and mother (Sathyanarayana 2014). Fetal testosterone is primarily synthesized by the fetus itself, either primarily by the testes in males or the fetal adrenal cortex in females, and protective mechanisms such as aromatase exist to prevent excessive maternal testosterone delivery to the fetus by transforming it to estrogen (Makieva 2014). It is possible that observed maternal testosterone associations with neurobehavioral outcomes are at least partially mediated by aromatization of maternal testosterone into fetal estrogen after crossing the placenta, which could explain differences in associations between maternal serum and amniotic testosterone, as well as the recent Baron-Cohen et al. 2019 findings regarding amniotic estrogen and ASD. Another possibility is that maternal testosterone may influence development without directly translocating to the fetus, such as through reductions in uterine blood flow and placental amino acid transport observed in rodent experiments (Sathishkumar 2011, Gopalakrishnan 2016). As E2 has been shown to be moderately correlated between amniotic fluid and maternal serum, maternal estrogen levels may more directly reflect fetal exposure. During pregnancy, maternal and fetal estrogens are both primarily synthesized in the placenta with approximately equal contribution of the estrogen precursor dehydroepiandrosterone from both the mother and the fetus (Siiteri and MacDonald 1966), and placental E1 and E2 are secreted preferentially into the fetus and mother, respectively (Gurpide 1982). Despite this common source for maternal and fetal estrogen, maternal estrogen may still act on fetus via indirect pathways, such as by enhancing uteroplacental blood flow as suggested by animal studies (Albrecht and Pepe 2010). Future studies further examining maternal versus amniotic hormone level associations with child behavioral problems are necessary to determine if they differ in magnitude, direction, or sex-specificity.

Strengths of this study include the prospective, multi-site design; a relatively large sample, allowing for examination of sex-specific effects of sex hormones; examination of both



## 5. Conclusions

In one of the only studies to examine maternal sex hormone levels in early pregnancy in relation to child behavioral outcomes in early childhood, we find associations between testosterone and internalizing and BSI-related behaviors in both sexes and a negative association between E1 and adaptive skills in girls. Maternal sex hormones may influence child neurobehavioral outcomes, and so it is important to understand factors that affect hormones during pregnancy as these could have lasting impacts on neurobehavioral programming.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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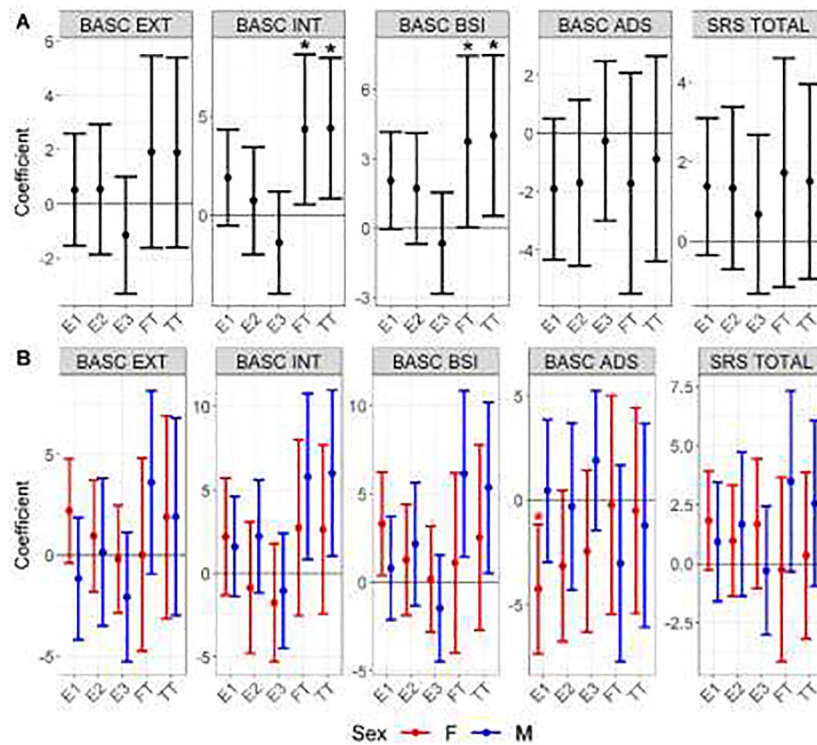
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### Highlights

- Early pregnancy maternal serum sex hormones are associated with 4–5y child behavior
- Maternal testosterone was associated with internalizing and behavioral symptoms
- Maternal estrone was associated with poorer adaptive skills in girls



**Figure 1: Mean and 95% CI Changes in Child BASC and SRS Composite Scores Associated with Maternal Sex Hormones**

Figure 1A shows results from linear regressions without a hormone by sex interaction term, and Figure 1B shows the sex-specific marginal coefficients from linear regressions including the interaction term. All coefficients are point changes in scores associated with a ten-fold increase in hormones. Estimates with an asterisk above them indicate  $p < 0.05$  in Figure 1A and that both marginal sex-specific and relevant interaction term  $p$ -values are  $< 0.05$  in Figure 1B. EXT: Externalizing composite score, INT: internalizing composite score, BSI: behavioral symptoms index composite score, ADS: adaptive skills composite score, SRS TOTAL: SRS total composite score.

**Table 1:**

Demographic Characteristics of 404 Mother-Child Dyads in the TIDES Study with Maternal Sex Hormone and Child Neurobehavioral Outcome Measures

| Characteristic                        | Mean (SD)    | Median (Range)        |
|---------------------------------------|--------------|-----------------------|
| Maternal age (years)                  | 31.50 (5.08) | 31.87 (18.25 – 44.26) |
| Pre-pregnancy BMI <sup>a</sup>        | 25.57 (6.15) | 23.65 (17.36 – 50.65) |
| Early-pregnancy BMI                   | 26.00 (6.10) | 23.65 (17.36 – 50.65) |
| Gestational age at serum draw (weeks) | 11.11 (2.64) | 11.57 (5.71 – 20.29)  |
| Gestational age at birth (weeks)      | 39.27 (1.88) | 39.57 (25.00 – 42.43) |
| Birth weight (kg)                     | 3.37 (0.57)  | 3.35 (0.55 – 5.15)    |
| Child age (years)                     | 4.53 (0.34)  | 4.53 (3.93 – 5.95)    |
| Characteristic                        | N (%)        |                       |
| Child sex                             |              |                       |
| M                                     | 193 (47.77%) |                       |
| F                                     | 211 (52.23%) |                       |
| Center                                |              |                       |
| UCSF                                  | 99 (24.50%)  |                       |
| UMN                                   | 131 (32.43%) |                       |
| URMC                                  | 92 (22.77%)  |                       |
| UW                                    | 82 (20.30%)  |                       |
| Race                                  |              |                       |
| Asian                                 | 20 (4.95%)   |                       |
| Black                                 | 34 (8.42%)   |                       |
| Other                                 | 47 (11.63%)  |                       |
| White                                 | 303 (75.00%) |                       |
| Income                                |              |                       |
| \$25000                               | 78 (19.31%)  |                       |
| \$25001 – \$75000                     | 108 (26.73%) |                       |
| > %75000                              | 218 (53.96%) |                       |
| Education                             |              |                       |
| High school                           | 43 (10.64%)  |                       |
| College                               | 158 (39.11%) |                       |
| Graduate school                       | 203 (50.25%) |                       |
| Early pregnancy alcohol consumption   |              |                       |
| No                                    | 390 (96.53%) |                       |

|                               |              |
|-------------------------------|--------------|
| Yes                           | 14 (3.47%)   |
| Early pregnancy cigarette use |              |
| No                            | 386 (95.54%) |
| Yes                           | 18 (4.46%)   |

<sup>a</sup>One participant was missing pre-pregnancy BMI data.

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**Table 2:**

Maternal Serum Sex Hormone Concentrations between Mothers of Female versus Male Children

| Hormone    | Fetal Sex | Mean (SD)   | Median (Range)     | N missing (%) | N < LLOQ (%) | t-test p-value <sup>a</sup> |
|------------|-----------|-------------|--------------------|---------------|--------------|-----------------------------|
| E1 (pg/mL) | Total     | 1031 (952)  | 736 (62.7 – 6590)  | 2 (0.50%)     | 0 (0%)       | 0.21                        |
|            | M         | 1000 (964)  | 690 (87.3 – 5110)  | 2 (1.04%)     | 0 (0%)       |                             |
|            | F         | 1060 (942)  | 774 (62.7 – 6590)  | 0 (0.00%)     | 0 (0%)       |                             |
| E2 (pg/mL) | Total     | 1763 (1354) | 1440 (100 – 9300)  | 2 (0.50%)     | 0 (0%)       | 0.42                        |
|            | M         | 1784 (1498) | 1310 (170 – 9300)  | 2 (1.04%)     | 0 (0%)       |                             |
|            | F         | 1744 (1214) | 1460 (100 – 6060)  | 0 (0.00%)     | 0 (0%)       |                             |
| E3 (pg/mL) | Total     | 183 (313)   | 89.3 (50 – 2360)   | 4 (0.99%)     | 136 (34.00%) | 0.26                        |
|            | M         | 202 (340)   | 94.1 (50 – 2230)   | 2 (1.04%)     | 62 (32.46%)  |                             |
|            | F         | 167 (285)   | 86.1 (50 – 2360)   | 2 (0.95%)     | 74 (35.41%)  |                             |
| FT (ng/dL) | Total     | 0.33 (0.21) | 0.27 (0.04 – 1.69) | 5 (1.24%)     | 0 (0%)       | 0.43                        |
|            | M         | 0.33 (0.23) | 0.27 (0.04 – 1.69) | 3 (1.55%)     | 0 (0%)       |                             |
|            | F         | 0.33 (0.20) | 0.28 (0.06 – 1.11) | 2 (0.95%)     | 0 (0%)       |                             |
| TT (ng/dL) | Total     | 71.7 (43.6) | 61.8 (12.9 – 288)  | 0 (0.00%)     | 0 (0%)       | 0.22                        |
|            | M         | 70.9 (46.7) | 61.2 (12.9 – 263)  | 0 (0.00%)     | 0 (0%)       |                             |
|            | F         | 72.5 (40.6) | 64.2 (15.9 – 288)  | 0 (0.00%)     | 0 (0%)       |                             |

<sup>a</sup> t-tests evaluated differences in hormone concentrations between mothers of female and male children. Hormones were log<sub>10</sub>-transformed prior to analysis with the t-test due to right skew. Percent values for missing data counts reflect the percent of the total for that fetal sex group. Percent values below the lower limit of quantification (LLOQ) are out of the non-missing total for that fetal sex group. E1 = estrone, E2 = estradiol, E3 = estriol, FT = free testosterone, TT = total testosterone.



**Table 3:****BASC-2 and SRS-2 Composite T Scores in Male and Female Children**

| Score                | Child Sex | Mean (SD)  | Median (Range) | N missing (%) | t-test p-value <sup>a</sup> |
|----------------------|-----------|------------|----------------|---------------|-----------------------------|
| BASC Externalizing   | Total     | 49.4 (8.0) | 48 (34 – 78)   | 1 (0.25%)     | 0.89                        |
|                      | M         | 49.4 (7.9) | 48 (35 – 73)   | 0 (0.00%)     |                             |
|                      | F         | 49.3 (8.1) | 48 (34 – 78)   | 1 (0.47%)     |                             |
| BASC Internalizing   | Total     | 48.9 (9.1) | 48 (30 – 78)   | 1 (0.25%)     | 0.02*                       |
|                      | M         | 47.8 (8.9) | 47 (30 – 77)   | 0 (0.00%)     |                             |
|                      | F         | 49.9 (9.2) | 49.5 (31 – 78) | 1 (0.47%)     |                             |
| BASC BSI             | Total     | 48.9 (8.3) | 48 (30 – 85)   | 1 (0.25%)     | 0.76                        |
|                      | M         | 48.7 (8.2) | 48 (30 – 77)   | 0 (0.00%)     |                             |
|                      | F         | 49.0 (8.4) | 49 (34 – 85)   | 1 (0.47%)     |                             |
| BASC Adaptive Skills | Total     | 51.1 (8.7) | 52 (20 – 72)   | 1 (0.25%)     | 0.14                        |
|                      | M         | 51.8(9.1)  | 52 (20 – 70)   | 0 (0.00%)     |                             |
|                      | F         | 50.5 (8.6) | 51 (25 – 72)   | 1 (0.47%)     |                             |
| SRS Total            | Total     | 44.9 (6.9) | 44 (35 – 81)   | 12 (2.97%)    | 0.03*                       |
|                      | M         | 45.6 (7.1) | 44 (35 – 74)   | 6 (3.11%)     |                             |
|                      | F         | 44.2 (6.6) | 43 (35 – 81)   | 6 (2.84%)     |                             |

<sup>a</sup> t-tests evaluated differences in BASC-2 and SRS-2 composite T scores between female and male children. Percent values for missing data counts reflect the percent of the total for that child sex group. BSI = behavioral symptoms index.