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# Predictors of steroid hormone concentrations in early pregnancy: results from a multi-center cohort.

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## Abstract

**Objectives:** To identify factors predicting maternal sex steroid hormone concentrations in early pregnancy.

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**Ethical statement:** TIDES was approved by institutional review boards at all participating institutions, and all subjects signed informed consent prior to starting any study activities.

**Methods:** The Infant Development and the Environment Study (TIDES) recruited healthy pregnant women from academic medical centers in four U.S. cities. Gold standard liquid chromatography-tandem mass spectrometry was used to measure maternal sex steroids concentrations (total testosterone [TT], free testosterone [FT], estrone [E1], estradiol [E2], and estriol [E3] concentrations) in serum samples from 548 women carrying singletons (median=11.7 weeks gestation). Women completed questionnaires on demographic and lifestyle characteristics.

**Results:** In multivariable linear regression analyses, hormone concentrations varied in relation to maternal age, body mass index (BMI), race, and parity. Older mothers had significantly lower levels of most hormones; for every year increase in maternal age, there was a 1-2% decrease in E1, E2, TT, and FT. By contrast, each unit increase in maternal BMI was associated 1-2% lower estrogen (E1, E2, E3) levels, but 1-2% higher androgen (TT, FT) concentrations. Hormone concentrations were 4-18% lower among parous women, and for each year elapsed since last birth, TT and FT were 1-2% higher (no difference in estrogens). Androgen concentrations were 18-30% higher among Black women compared to women of other races. Fetal sex, maternal stress, and lifestyle factors (including alcohol and tobacco use) were not related to maternal steroid concentrations.

**Conclusions:** Maternal demographic factors predict sex steroid hormone concentrations during pregnancy, which is important given increasing evidence that the prenatal endocrine environment shapes future risk of chronic disease for both mother and offspring.

### Keywords

pregnancy; androgens; estrogens; fetal origins; steroid hormones

## Introduction

Health begins *in utero,* and there is tremendous interest in better understanding how early development contributes to our later health and disease risk. Early research relied upon size at birth as a crude proxy for the *in utero* environment, but the field has since expanded to examine a wide range of materno-feto-placental biomarkers that may confer - or protect against - future disease risk in the child. The prenatal hormonal milieu has been an area of particular interest given long-standing hypotheses that excess fetal exposure to estrogens and/or androgens may play a role in future risk of reproductive cancers (1). Additionally, recent studies link putative biomarkers of the prenatal hormonal milieu, including anogenital distance and digit ratios, to adult outcomes including polycystic ovary syndrome (PCOS) (2), endometriosis (3), prostate cancer (4), and semen quality (5). Characterizing sex steroid concentrations during pregnancy may also yield important insights into the mother's own future risk of disease including breast (6, 7) and ovarian cancer (8).

Given the important downstream health outcomes believed to be associated with the prenatal endocrine milieu, characterizing natural variation in hormone levels during pregnancy and identifying factors contributing to that variation is important. Indeed, since the 1980s, over a dozen studies have examined prenatal hormone levels in the context of maternal and infant characteristics. However the study population, sample size, timing of sample collection, laboratory techniques, and steroid hormones measured have varied considerably. For

example, although many believe that the endocrine environment during early pregnancy is arguably of greatest concern with respect to subsequent disease risk in the fetus (given the rapid cell differentiation, proliferation, and organogenesis that occurs during this period), timing of biospecimen collection for hormone measurement has ranged from early pregnancy through umbilical cord blood collection at delivery. The vast majority of these studies have relied upon immunoassays (including chemiluminescent-, electrochemiluminescent-, and radioimmunoassays) which are relatively cheap, easy, and quick to perform (6, 9–16). However in immunoassay, there is potential for the antibodies to cross-react with multiple hormones due to non-specific binding of steroids to the antibody (for example both dehydroepiandrosterone sulphate [DHEAS] and testosterone)(17), as well as with synthetic steroids (18, 19). This non-specificity may account for the higher serum sex steroid levels in pregnant women found in some studies using immunoassay (e.g. 20) compared to studies using LC/MS-MS (21). Perhaps the greatest concern is that immunoassay does not offer adequate sensitivity to measure hormones that are present in very low concentrations, such as testosterone in women (22). Newer methods like liquid chromatography-tandem mass spectrometry (LC-MS/MS), are more expensive and labor intensive, but offer greater sensitivity and specificity for steroid measurement and are therefore the current gold standard (23).

To date, only one large study has examined maternal determinants of sex steroid concentrations in early pregnancy using the preferred LC-MS/MS method. Toriola et al. (2011) measured a panel of steroid hormones (testosterone, androstenedione, estradiol [E2], progesterone, and 17-hydroxyprogesterone) in 1343 women who provided samples for a large Finnish biorepository, finding that parity, smoking, maternal age, and fetal sex predicted maternal steroid levels measured at median 11 weeks gestation (21). Here, we seek to extend this work in a more diverse, U.S. pregnancy cohort. Our objective was to identify sociodemographic predictors of early pregnancy maternal serum sex steroid concentrations, including total testosterone (TT), free testosterone (FT), estrone (E1), estradiol (E2), and estriol (E3).

## Methods

### Study participants and study overview

The Infant Development and the Environment Study (TIDES), is a multi-center longitudinal cohort study that recruited women in their first trimester of pregnancy from 2010-2012. Women were recruited from four major U.S. academic medical centers [University of California, San Francisco (UCSF), University of Minnesota (UMN), University of Rochester Medical Center (URMC), and Seattle Children’s Flospital/University of Washington (UW)]. The primary means of recruitment was through study personnel who attended obstetric clinics, approaching potentially eligible women who were awaiting their clinical appointments. Eligibility criteria included: less than 13 weeks pregnant, English-speaking, and no serious medical conditions or threats to the pregnancy. In each trimester, participants completed a questionnaire (in person or online) that included items on maternal demographics, general health, alcohol and tobacco use during pregnancy, and reproductive history. They were also asked whether any stressful life events had occurred during the

pregnancy. Items were adapted from two validated questionnaires and queried whether women had experienced job loss, serious family illness or injury, death of a close family member, relationship difficulties with their partner, serious legal/financial issues, or any other major life event during the index pregnancy (24). Participants provided a single blood sample during early pregnancy, which was generally timed to coincide with their first or second trimester clinical screening and stored at −80C until analysis. Additional information on recruitment and prenatal visits is provided elsewhere (25). Gestational dating, including gestational week at blood draw, was determined based on the first ultrasound in the medical record. When that was not available, the obstetrician's estimate of the last menstrual period in the clinical record was used to calculate gestational week at blood draw.

## Hormone Assays

Serum samples were sent overnight on dry ice to the Endocrine and Metabolic Research Laboratory at Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, where all hormone assays were performed using standard, validated protocols, as described elsewhere (26). LC-MS/MS was used to measure TT in serum using standard protocols with minor modifications that shortened the runtime and system parameters. Briefly, LC-MS/MS runs were conducted using a Shimadzu HPLC system (Columbia, MD) and an Applied Biosystems API5500 LC-MS/MS (Foster City, CA) equipped with a Turbo-Ion-Spray source that used positive mode. There was a linear response for calibration standards ranging from 2.0 ng/dL (0.069 nmol/liter) to 2000 ng/dL (69.3 nmol/liter) for testosterone. Quality control was performed on each assay run using spiked samples. Intra- and inter-run precision was less than 5% and the steroid spiked samples had an accuracy between 100 to 113% for testosterone. The limit of quantification for TT was 2 ng/dL (0.069 nmol/L). Equilibrium dialysis using labeled testosterone was used to measure FT, the unbound and biologically active form of testosterone (27).

LC-MS/MS was also used to measure serum E1, E2, and E3 concentrations in all subjects. The Shimadzu HPLC system (Columbia, MD) was again used, this time with a triple quadrupole mass spectrometer (API5000 LC-MS/MS, Foster City, CA). The system was operated in the negative mode using multiple-reaction-monitoring in order to separate the estrogens on a column, with a gradient profile from 63% to 100% methanol. For both E1 and E2, the calibration curves were linear for the range of 2 to 2000 pg/mL, and for E3, 50 to 100 pg/mL. The lower limit of quantification was 2.0 pg/mL for E1 and E2, and 50 pg/mL for E3. The within-run precision (%CV) ranged from 2.6 to 5.6 for E1, 4.3 to 5.0 for E2, and 4.1 to for E3. The between-run precision (%CV) ranged from 3.9 to 4.6 for E1, 4.6 to 5.2 for E2, and 5.2 to for E3. The accuracy was 91.9 to 101.2 for E1, 93.9 to 100.3 for E2, and 87.2 to 104.3 for E3 respectively, spanning different estrogen concentrations.

## Statistical analysis

Our main analyses considered a set of variables chosen *a priori* based on the existing literature on sex steroid hormones in pregnant and non-pregnant, cycling women (6, 7, 10, 16, 21). These variables included maternal age, maternal BMI, smoking during pregnancy (any/none), alcohol use during pregnancy (any/none), fetal sex, study center, race (Black/White/other), income, education, marital status, parity, age at menarche, stressful life events

during pregnancy (any/none), and gestational age at blood draw. Univariate statistics were calculated for all variable of interest, including counts and percentages for categorical variables as well as summary statistics for continuous variables. As expected, hormone measurements were non-normal and were therefore loglO-transformed. E3 values below the lower limit of quantification were assigned that value (20 pg/ml). Scatterplots were used to examine the relationships between the log-transformed hormone outcomes and all continuous predictors of interest. Two different model selection criteria, prediction sum of squares (PRESS) and Mallows' Cp were then used to narrow the list of potential variables of interest to a smaller set of predictors. Mallows' Cp compares how well subset models make predictions compared to the full model, while PRESS uses leave-one-out cross-validation to measure how well fitted values predict the observed responses. For each hormone outcome, both model selection methods selected the same set of predictors (though the specific predictors varied slightly by hormone). Multivariable models were then fit to include the same standard set of selected predictors for all hormone outcomes (TT, FT, El, E2, E3). The selected variables were maternal age, maternal BMI, fetal sex, study center, race, parity, and gestational age at blood draw. In secondary analyses limited to parous women, we additionally considered time since last live birth, having identified it as a predictor of sex steroid levels in non-pregnant, cycling women in previous work (28). Finally, we back-transformed the estimates from all multi-variable models so that results could be reported as percent change in hormone concentrations, facilitating interpretation. Model diagnostics were run on all models and no issues were detected with regard to normality, linearity, constant variance, or multi-collinearity. However in models for E1, E2, and E3, the residual variance was larger at URMC than the other centers. This greater variance may reflect a greater racial/ethnic diversity at that site that is not fully captured by our race variable, but is unlikely to have much impact on inferences made about the model covariates. Analyses were performed in R version 3.3.2 and p-values $< 0.05$ were considered significant.

In total, 591 women gave a blood sample in early pregnancy that was analyzed for sex steroid hormone levels (mean 11.2 weeks gestation). Two subjects, one who gave birth extremely preterm (25 weeks gestation) and another with an implausible gestational age at delivery (1 week) were excluded from subsequent analysis. The 37 women who had polycystic ovary syndrome (PCOS), a common endocrine disorder characterized by hyperandrogenemia, were also excluded. In addition, four women who were carrying multiples were excluded from analysis. After these exclusions, the final sample size was 548 women. Of these women, 41 were missing data on the outcome or on at least one predictor of interest, therefore the sample size for our main models varied from 507 to 513, depending on the specific hormone outcome.

## Results

TIDES participants were, on average, 31.0±5.4 years old with a pre-pregnancy BMI of 26.1±6.1 kg/m2 (Table 1). Most mothers were White (67.8%), well-educated (86.0% had at least some college), and 49.1% had an annual household income greater than $75,000. Over 80% of women were married or living as married and 44.9% were parous. Self-reported alcohol use and smoking during pregnancy was uncommon (4.3% and 7.5%, respectively). As expected, roughly half of mothers were carrying male fetuses (48.9%).

All hormone measurements were above the limits of quantification of the assays, with the exception of E3, for which 30.9% of values were at or below the limit. In general, samples with E3 below the LOQ were collected earlier in gestation (median 9.2 weeks; min-max: 6.4-13.0) than samples with E3 above the LOQ (median 12.2 weeks; min-max: 5-25.4). The median levels of TT, FT, E1, E2, and E3 were 63.0 ng/dL, 0.28 ng/dL, 708 pg/ml, 1440 pg/ml, and 92 pg/ml, respectively. Results of bivariate and multivariable analyses refer to log-transformed hormone values. FT and TT were highly correlated with one another (r=0.81), and TT (but not FT) was weakly correlated with E1 (r=0.24) and E2 (r=0.30). E1 and E2 were also highly correlated (r=0.89), while correlations with E3 were more modest (r=0.47 for E1; r=0.55 for E2). All of these associations were significant at $p<0.001$. In bivariate analyses (not shown), maternal age was inversely associated with FT (r=−0.34) and TT (−0.38), but not E1 (r=0.03), E2 (r=−0.04), or E3 (r=0.09). Maternal BMI was positively associated with maternal androgens (FT: r=0.32; TT: r=0.24), but showed only weak inverse associations with estrogens (E1: r=−0.18; E2: r=−0.13; E3: r=−0.06). Gestational age at blood draw was strongly associated with estrogens (E1: r=0.54; E2: r=0.58; E3: r=0.79), but not androgens (FT: r=−0.17; TT: r=0.10).

In multivariable models (including maternal age, maternal BMI, fetal sex, study center, race, parity, and gestational age at blood draw), we observed significant inverse associations between maternal age and most hormone levels (Table 2). Adjusting for other model covariates, for every year increase in maternal age, there was a 1.5% decrease in E1 (95%CI: −3.0%, −0.07%), a 1.6% decrease in E2 (95%CI: −2.8%, −0.4%), a 2.1% decrease in TT (95%CI: −3.0%, −1.2%), and a 1.3% decrease in FT (95%CI: −2.2%, −0.4%) (Table 2). This corresponds to a 6-10% decrease (depending on the particular hormone) with every five year increase in maternal age. Parous women had 12.6% lower E1 (95%CI: −23.5%, −0.1%), 18.0% lower E2 (95%CI: −26.6%, −8.4%), 13.8% lower TT (95%CI: −20.5%, −6.5%) and 16.4% lower FT (95%CI: −23.1%, −9.2%) concentrations than nulliparous women. With every unit increase in BMI, we observed increases in androgen levels [TT: 1.3% (95%CI: 0.6%, 2.0%); FT: 2.2% (95%CI: 1.5%, 2.9%)] but reductions in estrogen levels [El: −2.3% (95%CI: −3.4%, −1.3%); E2: −1.6% (95%CI: −2.5%, −0.7%); E3: −1.0% (95%CI: −1.8%, −0.3%)]. This corresponds to 5-11% lower estrogen and 6-11% higher androgen concentrations per five unit increase in BMI.

Androgen concentrations also differed by race. Compared to Black women, White women had 29.5% lower TT (95%CI: −39.0%, −18.5%) and 29.4% lower FT (95%CI: −39.1%, −18.1%), respectively, while women of “Other” races had 18.9% lower TT (95%CI: −30.5%, −5.3%) and 18.3% lower FT (95%CI: −30.2%, −4.3%). E2 concentrations were 18.0% lower (95%CI: −32.7%, −0.2%) among White women than Black women, but otherwise no significant differences in estrogens by race were noted. Estrogen concentrations were significantly higher (17-30%) with each increasing week of gestational age at the time of blood draw, but only modest differences in TT (2.6%, 95%CI: 0.8%, 4.4%) and FT (−2.8%, 95%CI: −4.5%, −1.1%) were evident. There were significant differences in hormone concentrations by study center. For example, women at the UCSF center had significantly lower FT than women at the other centers, but higher El and E2 levels than women at the other centers (with the exception of the UW center). None of the

hormones measured differed by fetal sex and only BMI and gestational age at blood draw were associated with E3 concentrations.

In secondary analyses limited to parous women only, results were largely similar (Supplemental Table 1). Flormone levels were higher with increasing time since last birth. In multivariate models, for each year elapsed since the last birth, there was 2.4% (95%CI: 0.5%, 4.3%) higher TT, 2.2% (95%CI: 0.3%, 4.3%) higher FT, 2.4% (95%CI: −0.9%, 5.8%) higher El, and 2.3% (95%CI: −0.6%, 5.3%) higher E2.

## Discussion

In a large, diverse U.S. cohort, maternal sex steroid concentrations measured by LC/MS-MS during early pregnancy were significantly predicted by maternal age, BMI, parity, and race. Specifically, concentrations of all hormones (except for E3) were lower among older mothers while BMI was associated with significantly higher androgen (FT and TT) concentrations, but lower estrogen (E1, E2, E3) concentrations. Both androgens and estrogens (with the exception of E3), were lower among parous women compared to women with no prior live birth. In secondary analyses limited to parous women, both androgen and estrogen concentrations were positively associated with the time elapsed since last live birth. In general, Black women tended to have higher androgen concentrations than women of other races. No variation in maternal hormone concentrations was observed in relation to fetal sex, stressful life events during pregnancy, or lifestyle factors including smoking and alcohol use.

Our findings of lower sex steroid levels in association with increasing maternal age are consistent with previous work (using both immunoassay and LC/MS-MS methods) examining serum hormone levels measured during early pregnancy (6, 21) and in cord blood collected at birth (16). During pregnancy, a large proportion of steroid production (particularly of estrogens and progesterone) occurs in the placenta and surprisingly, very little research has examined variation in placental function (including hormone production) in relation to maternal age. Human chorionic gonadotropin (hCG) may be reduced in older mothers, possibly indicating reduced placental capacity for hormone production and synthesis (29). Better understanding how maternal age may impact placental function and steroidogenesis is an important future direction for research given temporal trends towards delayed conception and increasing maternal age.

Adjusting for covariates including age, parous women had significantly lower androgen concentrations (as well as non-significantly lower estrogen concentrations) than women with no prior birth, a result consistent with previous work in pregnant women (6, 7, 21) and in non-pregnancy, naturally cycling women (28). These findings are robust to the specific hormone assay technique used (6, 7, 21). Other work suggests that the endocrine impact of parity extends even further, such that compared to nulliparas, women who have had a full-term pregnancy may also have lower hCG, prolactin, alpha-fetoprotein, dehydroepiandrosterone (DHEA), and DHEAS (7, 30–34). No definitive mechanistic explanation for parity-related changes in endocrine profiles during gestation has been proffered thus far, although potential explanations include changes in placental size and

activity, binding protein concentrations, receptor densities, and maternal metabolism (7). Understanding these changes is important given that parity has been linked to reproductive cancer risk (35–37) with changes in lifetime hormone exposure hypothesized to be a key mediator (7).

In the current analysis, pre-pregnancy BMI was positively associated with androgens, but inversely associated with estrogens. Somewhat surprisingly, data on maternal body size is absent from a number of studies on the prenatal hormonal milieu (21, 38), but is important to consider given that nearly 70% of U.S. women are overweight or obese, with Black and Latina/Hispanic women particularly affected (82% and 77%, respectively)(39). Troisi and colleagues noted positive associations between measures of maternal body size (height, pre-pregnancy weight, or BMI) and androgen concentrations at delivery as well as in early pregnancy (6, 16) and a recent study found positive associations between weight gain in pregnancy and T concentrations in third trimester serum as well as amniotic fluid prior to labor (40). The inverse association between BMI and estrogens is consistent with the observation that hormones of fetoplacental origin tend to be lower in the circulation of heavier mothers due to dilution (41). Androgens, by contrast, are produced primarily by the maternal ovary and adrenal gland (as well as by the male fetus though they are likely aromatized before reaching maternal circulation)(42, 43). Studies in non-pregnant cycling women and peripubertal girls also indicate that being overweight or obese is associated with higher TT and FT concentrations (44, 45). One possible mechanism for this association is that insulin, which is elevated in overweight women, stimulates the ovarian theca cells to produce androgens while also lowering sex hormone binding globulin (SHBG) production, thereby resulting in higher FT (46, 47).

In our study, Black women had significantly higher androgen concentrations and non-significantly higher estrogen concentrations than women who were White or of other races. Although several of the larger studies on this topic have focused on predominantly White, Scandinavian populations (21, 48), evidence from more diverse U.S.-based cohorts supports these findings. In an early study examining racial differences in the prenatal endocrine environment, first trimester TT was significantly higher (and E2 non-significantly higher) among 20 Black women compared to 20 White women (49). In a second cohort (n=86), TT and androstenedione concentrations (but not E2 or E3) were higher in blood collected at delivery in Black women compared to White women (6). In a larger study of 300 (150 Black/150 White) nulliparous mothers carrying male fetuses, Black women had higher first and third trimester TT and FT concentrations as well as higher first trimester E2, adjusting for covariates (20), which is consistent with the current findings. Examining racial differences in perinatal hormone exposure has been of great interest because of the dramatic differences between Whites and Blacks in the patterns and prevalence of reproductive cancers including testicular germ cell tumors (50), breast cancer (51), and prostate cancer (52). Additional work has examined the prenatal hormonal milieu among racial and ethnic groups believed to be at lower risk of endocrine-mediated cancers, such as Hispanic and Asian women living in the U.S. and elsewhere (7, 13), however our study did not have adequate representation of those and other racial and ethnic groups to provide further insight.

In contrast to some previous work in which smoking was associated with higher androgen and estradiol levels during pregnancy in Finnish women (21, 38), smoking did not predict maternal hormone concentrations in our study and was therefore not included in final models. Relatively few women in TIDES reported any smoking during pregnancy (7% compared to 14% in Toriola et al. 2011), which may account for the inconsistent findings. It is also plausible that patterns of smoking during pregnancy differ quite dramatically cross-culturally such that simply measuring “any” smoking does not adequately capture variation in smoking behaviors, particularly given that the Finnish samples were collected starting in the 1980s whereas the TIDES samples were collected starting in 2010. Cross-cultural and/or temporal variation in the social stigma against smoking during pregnancy may contribute to these disparate findings as well and measurement of cotinine levels (a urinary metabolite of nicotine and biomarker for tobacco smoke exposure) would likely yield more accurate information on smoking habits than self-report. Similar issues may explain the contrast between Troisi et al. (2008)’s findings of association between alcohol use and decreased E2 and our null findings (6).

The literature on differences in maternal sex steroids in relation to fetal sex is highly inconsistent. Amniotic fluid measurements indicate that starting in the late first trimester, the male fetus experiences much higher levels of testosterone (through his own testicular production); the low testosterone levels detected in amniotic fluid from female fetuses is presumed to be of adrenal origin (53). In theory, therefore, given the much higher androgen production in male fetuses compared to female, maternal androgen concentrations might be expected to be higher in women carrying male fetuses. Some previous work supports these differences. For instance, in a previous cohort we found that in the third (but not the second) trimester, FT and TT were significantly higher in women carrying males than in women carrying females (54) and a large Australian study found that TT and FT were higher in umbilical cord blood in males than in females at birth (55). Notably, these studies (as well as the current study) all used LC/MS-MS to measure androgens, while a number of other studies that have not detected differences in androgen levels with respect to fetal sex have used less sensitive methods like immunoassay that are not recommended for the very low levels of androgens present in females (23, 56, 57). Flere, fetal sex did not predict maternal androgen concentrations, which is consistent with reports that in women carrying male fetuses, expression of placental aromatase (converting androgens to estrogens) is greater, preventing the virilization of mothers carrying male fetuses (58).

Our study has several notable strengths. Importantly, hormone concentrations were measured using LC-MS/MS with FT evaluated using equilibrium dialysis. Of the similar studies discussed herein, only a small fraction have used LC-MS/MS technology (21), which is the gold standard for serum hormone measurement, while the remainder have used older and less sensitive immunoassay techniques (6, 7, 10, 12–16). This assay sensitivity is especially important for androgens, which are present in very low concentrations in women (23). FT is of particular interest because in contrast to TT, which includes both bound and unbound testosterone, FT represents only the unbound, bioavailable proportion of hormone. Although TT concentrations can be quite high in pregnant women, concomitant increases in SHBG, result in FT levels remaining relatively low until late pregnancy (59). As epidemiologists extend work on prenatal hormones to examine associations with subsequent

health outcomes, using gold standard measurement techniques to ensure the validity of results will be increasingly important. Another strength of our study was its recruitment of a large sample of healthy pregnant women. Some previous work on maternal endocrine profiles during pregnancy that has been under-powered or has examined special populations, such as women with pre-eclampsia or PCOS. Of the related literature, to our knowledge only Toriola et al. (2011) (n=1343) has had a sample size larger than the current study (21). The relative diversity of our sample (recruited from four U.S. cities) and our extensive questionnaires allowed us to examine race, income, stress, age at menarche and other potential predictors that could not be examined in previous work in more homogeneous cohorts reliant on birth registry data. Finally, sample collection in early pregnancy allowed us to assess the hormonal milieu at a particularly critical point during development when fetal organ systems are forming.

Our study has several limitations of note as well. At present, research on prenatal hormones is hindered by our inability to determine the relative contributions of multiple hormone sources (mother, fetus, and placenta), an advance which would be useful to better understand the mechanisms underlying observed associations in this study. An exception to this is E3, which is produced almost exclusively by the placenta based on fetal adrenal precursors. Interestingly, we observed that only BMI and gestational age at blood draw predicted E3 concentrations, suggesting that there may be little variation in placental steroidogenesis in relation to maternal sociodemographic status. Our ability to detect associations may have been hindered by the large proportion of samples below the E3 LOQ (30%), due in part to the early gestational age at sample collection.Ultimately, quantifying fetal hormone exposure is of greatest interest in the context of the developmental origins of health and disease, however given the logistical impossibilities of sampling fetal blood or amniotic fluid for research studies in healthy pregnancies, we are necessarily limited in our ability to characterize that environment. Our hormone assessments were based on a single blood draw collected opportunistically during early pregnancy and ideally, tracking trajectories of hormone concentrations over the course of an entire pregnancy (as some smaller studies have done) would be ideal and might provide more insight into vulnerable periods for future maternal disease risk (14, 38). Androgens can be converted to E1 and E2 through the enzyme aromatase; aromatase levels, which were unavailable in this study, could provide more insight into inter-individual variation in steroidogenesis. Finally, our cohort, while more diverse than many of the other populations studied in similar work, was still predominantly White, of healthy weight, and higher socioeconomic status. Thus our ability to generalize to U.S. women as a whole is limited.

In conclusion, this research in a large, diverse, multi-center U.S. pregnancy cohort adds to the body of work on predictors of maternal sex steroid concentrations measured by gold standard methods during pregnancy. Understanding how factors such as maternal age, BMI, race, and parity impact materno-feto-placental physiology is important given the extensive evidence that endocrine environment during pregnancy may have important implications for the future health of mother and child alike.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Significance statement:

**What is already known on this topic?**

Although numerous studies have examined variation in maternal hormones during pregnancy, most have had notable limitations. These limitations include small sample sizes, and demographically homogeneous populations, use of outdated or suboptimal analytic techniques for hormone measurement.

**What does this study add?**

Using gold-standard analytic techniques in a large, diverse sample, we demonstrate that maternal sex steroid profiles during pregnancy vary in relation to maternal age, body mass index (BMI), race, and parity. This work confirms and extends the previous literature on this topic.

**Table 1.**

Characteristics of the TIDES study population.

**CONTINUOUS VARIABLES.**

| Variable | Non-Missing N | Mean ± SD | Min. | Median | Max |
|---|---|---|---|---|---|
| Age (years) | 547 | 31.02±5.43 | 18.25 | 31.61 | 45.16 |
| BMI (kg/m2) | 543 | 26.06±6.11 | 17.18 | 24.33 | 56.49 |
| Age at menarche (years) | 491 | 12.63±1.58 | 7.00 | 13.00 | 18.00 |
| Time since last birth (years) (parous women only) | 228 | 4.23±3.46 | 0.10 | 3.10 | 20.50 |
| Gestational age at blood draw (wks) | 548 | 11.24 ±2.71 | 5.00 | 11.71 | 25.42 |
| Total testosterone (ng/dl) | 547 | 72.27±42.34 | 12.90 | 63.00 | 271.50 |
| Free testosterone (ng/dl) | 541 | 0.34±0.20 | 0.04 | 0.28 | 1.30 |
| Estrone (E1) (pg/ml) | 545 | 1005.95 ± 966.64 | 33.60 | 708.00 | 6875.00 |
| Estradiol (E2) (pg/ml) | 545 | 1767.74± 1382.61 | 100.00 | 1440.00 | 9325.00 |
| Estriol (E3) (pg/ml) | 543 | 195.14± 359.58 | 50.00 | 92.10 | 3870.00 |

**CATEGORICAL VARIABLES**

| Variable | Non-Missing N | N (%) |
|---|---|---|
| Race | | |
| White | 543 | 368 (67.8) |
| African-American/Black | | 62 (11.4) |
| Other | | 113 (20.8) |
| Parous | 548 | 246 (44.9) |
| Current alcohol use | 541 | 23 (4.3) |
| Current smoker | 548 | 41 (7.5) |
| Married or living as married | 547 | 459 (83.9) |
| Education | | |
| High school or less | 543 | 76 (14.0) |
| Some college or more | | 467 (86.0) |
| Annual income | | |
| <$25,000 | 530 | 126 (23.8) |
| $25,001-75,000 | | 144 (27.2) |
| >$75,001 | | 260 (49.1) |
| Study Center | | |
| San Francisco, CA | 548 | 118 (21.5) |
| Minneapolis, MN | | 159 (29.0) |
| Rochester, NY | | 147 (26.8) |
| Seattle, WA | | 124 (22.6) |
| Stressful life events during pregnancy (any) | 518 | 215 (41.5) |

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| CATEGORICAL VARIABLES | | |
|---|---|---|
| **Variable** | **Non-Missing N** | **N (%)** |
| Fetal sex- male | 548 | 268 (48.9) |

**Table 2.**

Percent change (with 95% confidence intervals) in maternal hormone concentrations in relation to maternal characteristics in the TIDES study. Each column shows results from multivariable models adjusted for all variables shown in the rows.

| Variables | E1 (pg/ml) | E2 (pg/ml) | E3 (pg/ml) | Total T (ng/dl) | Free T (ng/dl) |
|---|---|---|---|---|---|
| | N=511 | n=511 | N=509 | n=513 | n=507 |
| Age (yrs) | −1.52(−2.95, −0.07) * | −1.60(−2.78, −0.40) ** | 0.62(−0.43, 1.68) | −2.12 (−2.98, −1.24) *** | −1.27(−2.16, −0.37) ** |
| Parous | −12.61(−23.54, −0.13) * | −17.99(−26.57, −8.40) *** | −4.36(−13.09, 5.25) | −13.76 (−20.49, −6.46) *** | −16.41(−23.09, −9.16) *** |
| BMI (kg/m2) | −2.34(−3.40, −1.27) *** | −1.57(−2.45, −0.68) *** | −1.03(−1.80, −0.26) ** | 1.27 (0.61, 1.95) *** | 2.22(1.53, 2.91) *** |
| Race[1] | | | | | |
| White | −16.28(−33.98, 6.16) | −18.03(−32.66, −0.22) * | 0.41(−15.30, 19.03) | −29.48(−38.98, −18.51) *** | −29.37(−39.07, −18.13) *** |
| Other | −4.84 (−26.09, 22.52) | −3.97 (−22.10, 18.38) | 7.27(−10.49, 28.56) | −18.86 (−30.46, −5.31 ** | −18.25(−30.16, −4.32) * |
| Fetal sex[2] | 7.04(−5.73, 21.53) | 4.92(−5.56, 16.55) | −3.54(−11.94, 5.67) | 2.42 (−5.19, 10.64) | 0.57(−7.09, 8.86) |
| Gestational age at blood draw (wks) | 17.79(14.60, 21.18) *** | 17.57(14.84, 20.37) *** | 30.25(27.62, 32.93) *** | 2.56(0.80, 4.35) ** | −2.82(−4.53, −1.09) ** |
| Study center[3] | −30.90(−44.39, −14.13) *** | −16.70(−30.42, −0.28) * | 3.60(−11.35, 21.06) | 10.84(−2.88, 26.50) | 34.77(17.71, 54.31) *** |
| Minnesota, MN | −35.83(−49.05, −19.19) *** | −20.39(−34.23, −3.65) * | 12.92(−4.28, 33.21) | 22.91 (6.79, 41.45) ** | 55.44(34.68, 79.39) *** |
| Rochester, NY | −0.37 (−18.14, 21.27) | 6.58(−9.42, 25.41) | −10.94(−22.64, 2.54) | 27.80 (13.39, 44.04) *** | 96.47 (73.89, 121.97) *** |
| Seattle, WA | | | | | |

* = p<0.05;

** =p<0.01;

*** =p<0.001

[1] Reference group=Black.

[2] Reference group=female.

[3] Reference group=San Francisco, CA study center.