Fetal Sex-Dependent Associations Between Gestational Hormone Concentrations and Adverse Birth Outcomes

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Research Article

Keywords: birth cohort, corticotropin releasing hormone, gestational age, preeclampsia, pregnancy, preterm birth, progesterone

Posted Date: January 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-151002/v1>

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Abstract

Adverse birth outcomes remain significant public health problems that can have long-lasting impacts on mother and child. Understanding biological mechanisms underlying these outcomes, including altered endocrine function, can inform prevention efforts. The aim of this study was to evaluate associations between hormones at two time points during mid-gestation and adverse birth outcomes, and to assess effect modification by fetal sex. Repeated gestational hormone measurements (at 18 and 26 weeks) and birth outcomes were assessed among 976 women in PROTECT, a longitudinal prospective birth cohort in northern Puerto Rico, from 2011 to 2018. Birth outcomes assessed included preterm and spontaneous preterm birth (PTB), preeclampsia, gestational diabetes mellitus (GDM), small/large for gestational age (SGA, LGA), birthweight z-score, and gestational age at birth. Multivariate logistic and linear regressions were fit using visit-specific concentrations of hormones. We also conducted sensitivity analyses assessing impacts of fetal sex on observed associations. All models were adjusted for maternal age and education, and other confounders were assessed separately between birth outcomes based on *a priori* knowledge and observed associations with exposure and outcome measures. We observed increased odds of spontaneous PTB with IQR increases in progesterone (OR: 2.12, 95% CI: 1.29, 3.47), fT4 (OR: 1.73, 95% CI: 1.04, 2.86), and the ratio of progesterone to estriol (OR: 1.63, 95% CI: 1.05, 2.54) at 26 weeks. Elevated estriol was protective against preeclampsia at 26 weeks (OR: 0.42, 95% CI: 0.17, 0.99). Increases in TSH and T3 conferred greater risk of GDM at 18 weeks. Many associations were modified by fetal sex, with hormone alterations during male pregnancies conferring greater risk of PTB, spontaneous PTB, and GDM. In conclusion, associations between hormones and birth outcomes vary based on timing of hormone measurement and fetal sex. Future studies are needed to understand mechanisms involved in adverse birth outcomes and fetal sex differences.

Introduction

Preterm birth (PTB) affects approximately 11% of live births¹ and is the leading cause of neonatal mortality worldwide². Infants born preterm are at increased risk for adverse health outcomes later in life including reduced renal function³, neurodevelopmental impairments⁴, cerebral palsy⁵, and reduced myocardial function⁶. Despite being a common public health problem, the causes of PTB are largely unknown. Other rare birth outcomes are also of significant concern and present safety issues for the mother and the fetus. The spontaneous subtype of preterm birth is characterized by an inflammatory uterine environment and may arise via different mechanisms than indicated PTB⁷. Preeclampsia, a hypertensive disorder of pregnancy⁸, affects 6% of pregnancies globally⁹ and is the leading cause of maternal mortality in the United States^{10,11}. Gestational diabetes mellitus (GDM) is a disease of reduced insulin sensitivity and elevated glucose levels during gestation. High maternal glucose levels easily cross the placenta and illicit a response from the fetal pancreas. Infants born to mothers with GDM are at elevated risk for macrosomia and metabolic dysfunctions, and mothers become more likely to develop diabetes later in life¹². Very little epidemiologic work has been done to investigate these rare adverse pregnancy outcomes and so our knowledge of the mechanisms by which they occur is limited.

The maternal and fetal endocrine milieus change and interact in unique ways at different points throughout gestation. The roles of progesterone are complex, reflected by the mixed efficacy of progesterone therapy as a preventative measure for preterm birth¹³. Estrogens are responsible for uterine maintenance and increased expression of oxytocin receptors and gap junctions that are necessary for uterine contractions to occur¹⁴.

Through pregnancy, progesterone maintains uterine quiescence and keeps contractile effects of estrogens in check¹³. Thyroid hormones are critical early in pregnancy for proper brain and skeletal development of the fetus¹⁵. The maternal supply of thyroxine (T4) is particularly important in the first half of pregnancy, before the fetal thyroid gland has matured enough to produce adequate hormones¹⁶. Previous studies have demonstrated associations between clinical hyper- and hypothyroidism and adverse birth outcomes^{17–20}, but much less is known about subclinical thyroid disruption and pregnancy outcomes.

Few large epidemiological studies exist that assess a wide array of hormone concentrations and pregnancy outcomes. The majority of existing research focuses on one hormone/class of hormones, which makes it challenging to gain a broad understanding of the endocrine pathways implicated in the onset of adverse birth outcomes^{21–23}. Specifically, the spontaneous subtype of PTB has not been well studied, and current research on the rare outcomes of preeclampsia and GDM is sparse^{24–26}. Importantly, few previous studies have investigated hormone concentrations at more than one time point during gestation, nor have they assessed the impact of fetal sex on these associations. Because of these gaps in the literature, the aim of this study was to investigate associations between various hormone concentrations, measured at two time points during gestation, and adverse birth outcomes, as well as effect modification by fetal sex. Based on previous literature, we have hypothesized that increases in CRH and estriol will be associated with increased risk of early delivery, while increases in progesterone will be associated with later delivery. Further, we expect lower thyroid hormone concentrations to be associated with smaller infant size at birth. Finally, we expect to observe more significant adverse associations among male pregnancies, given previous evidence suggesting that male pregnancies are more risky than female pregnancies^{27–29}.

Methods

Study Population

Pregnant women were recruited into the PROTECT birth cohort between 2011 and 2018 at 14±2 weeks' gestation from seven hospitals and prenatal clinics in northern Puerto Rico. Study design and recruitment protocols have been described elsewhere³⁰. Demographic and self-reported health information was provided at the first clinic visit. This study was approved by the research and ethics committees of the University of Michigan School of Public Health, University of Puerto Rico, Northeastern University, and participating hospitals and clinics. All methods reported in this study were performed in accordance with relevant guidelines and regulations imposed by those institutions. All study participants provided full informed consent prior to participation.

Hormone Measurements

All women provided serum samples at their first and third clinic visits, aligning with median 18 (range 16–20) and 26 (range 24–28) weeks' gestation. Serum samples were analyzed at the Central Ligand Assay Satellite Services (CLASS) laboratory in the Department of Epidemiology at the University of Michigan School of Public Health. Progesterone, sex hormone-binding globulin (SHBG), testosterone, total triiodothyronine (T3), total thyroxine (T4), free thyroxine (fT4), and thyroid-stimulating hormone (TSH) were measured using a chemiluminescence immunoassay. Estriol and corticotropin releasing hormone (CRH) were measured using an enzyme immunoassay. Some hormone concentrations were not available for all participants due to sample volume limitations. The ratios of progesterone to estriol (Prog/E3) and T3 to T4 (T3/T4) were assessed in

addition to measured hormones because of previous research indicating that the ratios may be better indices of adverse pregnancy outcomes than single hormone measurements^{21,31,32}. All hormone concentrations below the limit of detection (LOD) were replaced by the LOD divided by the square root of two.

Birth Outcome Assessment

Based on recommendations from the American College of Obstetricians and Gynecologists, self-reported date of the last menstrual period was collected at the first study visit and used in combination with early ultrasound measurements to determine gestational age at birth³³. PTB was defined as delivery before 37 weeks' gestation. We also assessed spontaneous PTB, defined as PTB presenting with premature rupture of membranes, spontaneous preterm labor, or both⁷. Preeclampsia and GDM cases were determined based on diagnosis in the medical record by an attending physician. We calculated birthweight z-scores based on fetal sex and gestational age using widely accepted international standards³⁴. Those born with a birthweight < 10th percentile and > 90th percentile were considered small and large for gestational age (SGA and LGA), respectively.

Statistical Methods

Distributions of demographic, health, and pregnancy characteristics were calculated. Summary measures of gestational hormone concentrations were assessed using arithmetic means of all available concentrations for each study participant, or geometric means for log-normally distributed hormones. Distributions of hormone concentrations were also assessed individually at each study visit. Univariate linear models were used to test for significant differences between hormone concentrations at each study visit. Intraclass correlation coefficients (ICCs) were also used to assess between- and within-individual variability of hormone concentrations across study visits.

We utilized indicator variables for study visit and included interaction terms between each indicator and hormone concentration in final models to achieve effect estimates specific to each study visit. Sandwich estimators were used in these models to correct for biased standard errors due to the non-repeating nature of outcome variables. Gestational average hormone concentrations were not used in final statistical models because of the marked changes in some hormones that occur throughout gestation. We also conducted sensitivity analyses to assess effect modification by fetal sex. An additional interaction term was included between hormone concentration and a fetal sex indicator variable to achieve effect estimates specific to fetal sex within study visits.

Confounders were explored by evaluating their associations with exposure and outcome variables. All models adjusted for categorical forms of maternal age and maternal education. Further covariate adjustment differed between birth outcomes based on *a priori* knowledge, significant association with the outcome measure, and inclusion of the covariate impacting the hormone effect estimate by at least 10%. A list of covariates that were assessed and the outcome models in which they were included, if any, is shown in Table 1. All models assessing testosterone also included SHBG to adjust for bound testosterone.

Table 1
Inclusion of covariates between different outcome models.

	PTB	Spontaneous PTB	Gestational Age	Birthweight Z-Score	SGA	LGA	Preeclampsia	GDM
Maternal Age	X	X	X	X	X	X	X	X
Maternal Education	X	X	X	X	X	X	X	X
Employment Status								
Annual Household Income								
Marital Status	X	X	X					
Smoking Status					X	X		
Environmental Tobacco Smoke Exposure	X	X	X					X
Alcohol Usage			X					X
Parity								
Pre-Pregnancy BMI			X	X			X	
Infant Sex								

Results

Demographics of the study population are shown in Table 2. The majority of mothers were under the age of 30 (67.1%), had at least some college education (79%), were employed (63%), had an annual household income under \$30,000 (63.1%), were married (53.1%), had never smoked (86%) or been exposed to environmental tobacco smoke (88.7%), did not drink alcohol during pregnancy (93.6%), had given birth to less than 2 previous children (86.9%), and had a pre-pregnancy BMI of less than 25 (56.1%).

Table 2
Maternal demographic characteristics of the study population (N = 976)

	N (%)
Maternal Age (years)	
18–24	354 (36.3%)
25–29	301 (30.8%)
30–34	206 (21.1%)
35–41	115 (11.8%)
Maternal Education	
GED or less	203 (21%)
Some College	331 (34.2%)
Bachelors or Higher	433 (44.8%)
Employment Status	
No	357 (37%)
Yes	608 (63%)
Annual Household Income	
< 10k	269 (31.6%)
10k-<30k	268 (31.5%)
30k-<50k	203 (23.8%)
>=50k	112 (13.1%)
Marital Status	
Single	197 (20.4%)
Married	521 (53.9%)
Cohabiting	249 (25.7%)
Smoking Status	
Never	833 (86%)
Ever	121 (12.5%)
Current	15 (1.55%)
Daily Environmental Tobacco Smoke Exposure	
Never	808 (88.7%)
1 Hour or less	40 (4.39%)

	N (%)
> 1 Hour	63 (6.92%)
Alcohol Use	
Never	504 (52.2%)
Yes, before Pregnancy	400 (41.4%)
Yes, currently	62 (6.42%)
Number of Previous Children	
0	355 (42.7%)
1	367 (44.2%)
2 to 5	109 (13.1%)
Pre-Pregnancy BMI	
[0,25]	520 (56.1%)
(25, 30]	240 (25.9%)
Above 30	167 (18%)
Fetal Sex	
Female	464 (48%)
Male	502 (52%)

Distributions of hormone concentrations are shown in Supplementary Table S1. Most hormone concentrations were significantly different at 18 and 26 weeks' gestation, with notable increases occurring with estriol (median 15.1 and 38.2 ng/mL at 18 and 26 weeks, respectively) and progesterone (median 39.3 and 73.5 ng/mL at 18 and 26 weeks, respectively). ICCs for all other hormones ranged from 0.647 (T4) to 0.856 (testosterone).

Distributions of birth outcomes are shown in Table 3. PTB and spontaneous PTB occurred in 9.9% and 5.8% of the study population, respectively. Preeclampsia and GDM were less prevalent (2.9% and 1.9%, respectively). Occurrences of SGA and LGA births were similar (8.9% and 9.6%, respectively). Median gestational age of the study population was 39.1 weeks (IQR: 38.1–40).

Table 3
Distributions of continuous and binary birth outcomes

	Min	10th	25th	50th	75th	90th	Max
Gestational Age (wks)	20.3	36.7	38.1	39.1	40	40.7	42.7
Birthweight Z-Score (ounces)	-5.34	-1.19	-0.571	-0.00005	0.707	1.25	9.70
	(19.0)	(91.0)	(102)	(113)	(123)	(133)	(224)
N (%)							
Preterm Birth							
No	867 (90.1%)						
Yes	95 (9.88%)						
Spontaneous Preterm Birth							
No	883 (94.2%)						
Yes	54 (5.76%)						
Preeclampsia							
No	947 (97.1%)						
Yes	28 (2.87%)						
Gestational Diabetes							
No	900 (98.1%)						
Yes	17 (1.85%)						
Small for Gestational Age							
No	842 (91.1%)						
Yes	82 (8.87%)						
Large for Gestational Age							
No	835 (90.4%)						
Yes	89 (9.63%)						

Figure 1 shows the associations between hormone concentrations and birth outcomes at each study visit (all effect estimates and p-values are shown in Supplementary Table S2). There were greater odds of spontaneous PTB with increasing progesterone concentrations at 26 weeks (OR: 2.12, 95% CI: 1.29, 3.47) and fT4 concentrations at both study visits (18wk OR: 1.60, 95% CI: 1.07, 2.39; 26wk OR: 1.73, 95% CI: 1.04, 2.86). The risk of spontaneous PTB was significantly different between study visits with an IQR increase in Prog/E3 (interaction $p = 0.026$), a null association observed at 18 weeks and increased odds observed at 26 weeks (OR: 1.63, 95% CI: 1.05, 2.54). Reductions in gestational age at birth were observed with increased concentrations of progesterone (β : -3.56 days, 95% CI: -6.02, -1.10), fT4 (β : -2.22 days, 95% CI: -3.84, -0.61), and T4 (β : -1.87 days, 95% CI: -3.62, -0.11) around 18 weeks, and with prog/e3 at both study visits (18wk β : -1.77 days, 95% CI: -3.36, -0.19; 26wk β :

-1.98 days, 95% CI: -3.58, -0.37). Notably, the effect of progesterone was significantly different between study visits (interaction $p = 0.044$).

Results at 18 weeks suggested that elevated progesterone and reduced estriol are associated with increased risk of having an SGA infant (E3 OR: 0.66, 95% CI: 0.45, 0.97; progesterone OR: 1.53, 95% CI: 1.09, 2.17; prog/E3 OR: 1.77, 95% CI: 1.29, 2.44). This trend remained at 26 weeks for only prog/E3 (OR: 1.53, 95% CI: 1.07, 2.17). Similarly, prog/E3 at 18 weeks was inversely associated with birthweight z-score (β : -0.12, 95% CI: -0.23, -0.02) and estriol at 26 weeks was positively associated with birthweight z-score (β : 0.21, 95% CI: 0.01, 0.41).

A protective effect against preeclampsia was observed with increases in SHBG at 18 weeks (OR: 0.55, 95% CI: 0.30, 0.99) and estriol (OR: 0.42, 95% CI: 0.17, 0.99) and SHBG (OR: 0.46, 95% CI: 0.25, 0.83) at 26 weeks. Conversely, elevated risk of preeclampsia was observed with an increase in TSH at 26 weeks (OR: 2.18, 95% CI: 1.19, 3.99). The odds of GDM increased with an IQR increase in TSH (OR: 1.67, 95% CI: 1.02, 2.72), T3 (OR: 2.83, 95% CI: 1.04, 7.68), and T3/T4 (OR: 2.97, 95% CI: 1.20, 7.35) at 18 weeks, and increased with higher estriol at 18 weeks (OR: 5.95, 95% CI: 1.27, 27.8). None of the associations with preeclampsia or GDM were significantly different between study visits.

Sensitivity analyses revealed that many associations were significantly different between male and female pregnancies (Fig. 2; all effect estimates and p-values are shown in Supplementary Table S3). The most compelling effect modification by fetal sex was observed for preterm birth; the interaction term between hormone concentration and fetal sex indicator was significant among 7 out of 11 hormones and hormone ratios assessed. SHBG was protective against PTB at 26 weeks among female (OR: 0.60, 95% CI: 0.37, 0.96), but not male, pregnancies (interaction $p = 0.032$). Higher testosterone at both study visits was associated with increased odds of PTB among female pregnancies and reduced odds of PTB among male pregnancies (interaction $p < 0.001$). Notably, increased odds of PTB were observed among only male pregnancies with elevated concentrations of CRH (OR: 1.82, 95% CI: 1.09, 3.05; interaction $p = 0.002$), estriol (OR: 1.81, 95% CI: 1.07, 3.06; interaction $p = 0.022$), progesterone (OR: 1.88, 95% CI: 1.16, 3.04; interaction $p = 0.011$), and fT4 (OR: 1.63, 95% CI: 1.06, 2.51; interaction $p = 0.115$) at 18 weeks. Assessment of gestational age as a continuous variable did not provide such compelling results, but it did provide additional evidence of fetal sex modifying the association with progesterone at 18 weeks (male pregnancy β : -4.9 days, 95% CI: -2.73, -7.07 days; interaction $p = 0.015$).

The spontaneous subtype of PTB also showed several cases of effect modification by fetal sex. An IQR increase in CRH at 18 weeks was associated with greater odds of spontaneous PTB among only male pregnancies (OR: 2.73, 95% CI: 1.38, 5.43; interaction $p = 0.003$). Increases in testosterone at both visits were protective against spontaneous PTB among only male pregnancies (interaction $p = 0.001$). Increases in T3 and fT4 at both study visits were associated with increased odds of spontaneous PTB among only male pregnancies, but effect modification was significant only for T3 (interaction $p = 0.013$). Finally, higher progesterone at 26 weeks was associated with increased odds of spontaneous PTB among only male pregnancies (OR: 2.34, 95% CI: 1.36, 4.03).

Fetal sex modified the association between SGA and only the ratio prog/E3 (interaction $p = 0.022$), which was positive among only male pregnancies at both 18 weeks (OR: 2.39, 95% CI: 1.59, 3.60) and 26 weeks (OR: 1.98, 95% CI: 1.29, 3.05). Accordingly, increased estriol resulted in increases in birthweight z-score at both 18 weeks (β : 0.19, 95% CI: 0.02, 0.36) and 26 weeks (β : 0.31, 95% CI: 0.08, 0.53) among only male pregnancies (interaction $p = 0.030$). Fetal sex did not modify any associations between hormones and odds of LGA.

Though there was no evidence of effect modification by fetal sex on associations between hormones and preeclampsia, significant effects were observed only among female pregnancies with increases in SHBG (OR: 0.34, 95% CI: 0.14, 0.81), TSH (OR: 2.41, 95% CI: 1.11, 5.23), and fT4 (OR: 0.40, 95% CI: 0.17, 0.92) at 26 weeks. Conversely, there was significant evidence of effect modification by fetal sex on the association between various hormones and odds of GDM. Elevated thyroid hormones were observed to be protective against GDM among female pregnancies [(fT4 at 18wks OR: 0.29, 95% CI: 0.10, 0.85; interaction $p = 0.001$), (T4 at 18wks OR: 0.32, 95% CI: 0.11, 0.90; interaction $p = 0.002$)], but positively associated with GDM among male pregnancies [(T3 at 18wks OR: 6.04, 95% CI: 1.72, 21.3; interaction $p = 0.028$), (fT4 at 26wks OR: 4.87, 95% CI: 1.53, 15.5), (T4 at 26wks OR: 3.05, 95% CI: 1.02, 9.13)]. A similar trend was observed for the ratio of prog/E3; there was a protective effect at 18 weeks among female pregnancies (OR: 0.25, 95% CI: 0.09, 0.71) and a positive association at 26 weeks among male pregnancies (OR: 2.93, 95% CI: 0.99, 8.69; interaction $p = 0.004$).

PTB and Gestational Age

We observed greater odds of PTB and spontaneous PTB with increasing progesterone concentrations (when fetal sex was male), but other studies demonstrating similar significant associations are lacking. One study observed progesterone concentrations measured between 28 and 32 weeks' gestation to be higher among women who delivered preterm compared to full term²². We observed higher progesterone concentrations among PTB cases when fetal sex was male, but only around 18 weeks' gestation. We also observed higher progesterone concentrations around 26 weeks among women who spontaneously delivered preterm compared to women who carried to term.

Previous work has shown that a ratio favoring estriol in mid-pregnancy²¹ and at delivery³⁵ is associated with earlier time of labor. Progesterone concentrations rise steadily during pregnancy, contributing to uterine quiescence, downregulation of prostaglandin production, and immune tolerance of the fetus^{36,37}. At the onset of human labor, progesterone concentrations do not notably decrease; rather, the body's response to progesterone is dampened. It is not clear exactly how this occurs, but possibilities include reduction in progesterone receptor expression, changes in receptor isoforms, and local progesterone metabolism³⁸. As term approaches, the ratio of progesterone to estriol shifts to favor estrogens, with the functional decrease in progesterone driving initiation of labor³⁹. The new dominance of estrogens promotes an increase in prostaglandin and oxytocin receptors and enzymes responsible for muscle contractions, which work together to help promote labor⁴⁰. We observed a positive association between odds of PTB and estriol concentrations (when fetal sex was male), but we also unexpectedly observed later gestational age at birth with higher concentrations of estriol at 26 weeks' gestation when the fetus was female. In contrast with previous studies, we observed that higher prog/E3 was associated with reduced gestational age and increased odds of SGA. Interestingly, among women who delivered preterm, a previous study observed lower prog/E3 among only those without premature rupture of membranes⁴¹, possibly implicating different endocrine pathways in the occurrence of PTB with and without premature rupture of membranes.

Decreased odds of PTB have been shown with increased concentrations of fT4 in the second²³ and third⁴² trimesters, which contradicts our finding that fT4 was inversely associated with gestational age at birth (among the whole study population and when the fetus was male), and increased odds of PTB (when the fetus was male) and spontaneous PTB. One prior study also found increased odds of PTB with greater T3 concentrations at 10

and 26 weeks gestation⁴². Similarly, we found that T3 was associated with spontaneous PTB when the fetus was male. Mechanisms of the association between thyroid hormones and PTB are poorly understood, but previous research has suggested that altered thyroid hormone concentrations may be involved in other disease states or exposures for which we have evidence of associations with PTB such as oxidative stress and inflammation^{43–45}, or environmental exposures such as phthalates^{46–48}.

Several previous studies have observed that male fetal sex is associated with a greater risk of delivering preterm. Proposed biological explanations for this observation include a pro-inflammatory environment generated by a male fetus²⁷ and larger size at birth for males relative to females²⁸. Increased risk of PTB when the fetus was male among only Caucasian women has also been observed, suggesting a potential interaction between race and fetal sex²⁹. We observed significant associations with PTB unique to women carrying a male fetus for CRH, estriol, progesterone, and fT4, providing further evidence that the effect of fetal sex on the occurrence of PTB is complex, possibly involving diverse endocrine pathways.

Preeclampsia

Among all pregnancies, we observed reduced odds of preeclampsia with an increase in estriol at 26 weeks. In accordance with our findings, another study showed that estriol concentrations in the second trimester⁴⁹ were lower among women with preeclampsia than women with normal pregnancies. Previous studies have also found increased odds of preeclampsia with higher second trimester fT4 concentrations^{23,50}, and lower third trimester fT4 concentrations⁵¹. All associations we observed between fT4 and preeclampsia were inverse, and the inverse association at 26 weeks among female pregnancies was significant. The association between fT4 and preeclampsia has been shown to be modified by human chorionic gonadotropin (hCG) concentrations, with high fT4 positively associated with preeclampsia only when hCG is low⁵². This effect modification may be due to the known angiogenic role of hCG during early pregnancy⁵³.

Hormonal involvement in the etiology of preeclampsia is complex due to the angiogenic dysfunction of the affected uterus. In preeclampsia cases, proper remodeling and infiltration of blood vessels by placental extravillous trophoblasts does not occur, and this can be observed before the onset of clinical symptoms^{54,55}. It is unclear whether endocrine disruption plays a causal role in initiation of uterine dysfunction, or if uterine dysfunction triggers a maternal endocrine response in an attempt to adapt to the hypoxic state⁵⁶.

Gestational Diabetes Mellitus

A previous epidemiology study has demonstrated associations between high second trimester estriol concentrations and greater odds of GDM²⁵. We also observed increased odds of GDM with estriol at 26 weeks. Testosterone concentrations were inversely associated with odds of GDM among male pregnancies in our study, which differs from previous research that showed higher testosterone concentrations among women with GDM²⁶ and with greater insulin resistance⁵⁷ compared to women with normal pregnancies.

Previous work has suggested that fT4 concentrations early in pregnancy are inversely associated with odds of GDM^{51,58}. In accordance with those findings, the ratio of fT3 to fT4 has been observed to be positively associated with odds of GDM⁵⁹, suggesting that increased conversion of T4 to biologically active T3 may play a role in the onset of GDM. In alignment with those findings, we observed greater odds of GDM among all pregnancies with increased T3 concentrations at 18 weeks, and greater odds of GDM among male pregnancies

with increased T3 at both study visits. We also observed an inverse association between fT4 and odds of GDM at 18 weeks among female pregnancies, while that association was positive among male pregnancies at 26 weeks. Previous work has shown that women with GDM have higher circulating concentrations of inflammatory cytokines such as IL-6 and TNF-alpha⁶⁰, which have been observed to be inversely associated with T3 concentrations⁶¹. These inflammatory markers may increase insulin resistance during pregnancy and, mediated by alterations in thyroid hormone concentrations, contribute to higher circulating glucose levels and increased odds of GDM. Several previous studies have observed greater risks for GDM among women carrying a male fetus⁶²⁻⁶⁴, possibly due to poorer beta-cell function among male fetuses⁶⁵.

Birth Size

We observed that decreased birthweight among females was marginally associated with elevated T4 at 26 weeks. Previous work found similar inverse associations, but with fT4 instead of total T4^{66,67}. Thyroid hormones are critical for fetal growth, possibly via their influences on fetal insulin-like growth factor, leptin, or the placenta's abilities to transfer nutrients⁶⁸. Even in the case of nearly identical patterns of thyroid hormone concentrations throughout gestation between mothers, differences in expression of hormone transporters in the placenta and intracellular receptors in fetal tissues can result in different thyroid hormone exposure profiles for the fetus and, consequently, varying effects on fetal growth and development⁶⁹. Assessment of thyroid hormone effects on birth outcomes in the second half of gestation is even more complex as the fetal thyroid gland begins to produce hormones and the fetus relies less on maternal supply of T4⁶⁹. Conflicting results on the relationship between thyroid hormones and birthweight between studies may be due in part to unmeasured differences in fetal thyroid function.

Strengths and Limitations

The present study was subject to several limitations. We were not able to measure hCG or assess thyroid autoantibody status. Thus some of our results could be biased due to unmeasured confounding variables. Some critical changes in the maternal endocrine environment occur later in gestation than we were able to measure, such as the exponential increase in CRH right before the onset of labor. Although the goal of this study was to determine whether mid-pregnancy hormone levels were indicative of increased risk of adverse pregnancy outcomes, measurements at later time points could shed additional light on the various endocrine pathways implicated in adverse birth outcomes. We observed low rates of preeclampsia and GDM, which reduces the reliability of effect estimates. However, these lower rates were observed because we excluded women with preexisting conditions from our cohort to allow more precise examination of associations between hormone concentrations and birth outcomes, since preexisting conditions can influence hormone concentrations and susceptibility to adverse birth outcomes. Furthermore, excluding women with preexisting conditions may limit the generalizability of our findings. Finally, some results assessing preeclampsia and GDM may be subject to reverse causation bias if the disease state, before clinical observation, resulted in the hormonal changes that we observed.

Despite the aforementioned limitations, this study was also strong in various ways. This is one of few studies to assess a broad panel of hormone concentrations at more than one time point during gestation to investigate relationships with various birth outcomes and different windows of susceptibility. Many epidemiological studies limit their analytical panel to either thyroid or steroid hormones, or do not assess the spontaneous subtype of PTB. We are also one of few groups to assess interactions between gestational hormone concentrations and

fetal sex. Finally, our study was strengthened by a higher sample size of mothers than was seen in most previously published cohorts, which is particularly important when studying rare outcomes occurring in less than 5% of the population.

Conclusions

In conclusion, we observed a range of associations between hormones and adverse birth outcomes. We found differences based on the timing of hormone assessment, and many significant findings were unique to mothers carrying a male fetus. Future work will attempt to place these findings in the context of relevant environmental contaminants on the island of Puerto Rico by exploring possibilities of endocrine disruption as a mediator between chemical exposures and pregnancy outcomes. Additional studies are needed to more fully elucidate the role of altered hormone concentrations in the etiology of adverse birth outcomes.

Declarations

Author Contributions

ALC analyzed and interpreted data and drafted the original article. JDM, JFC, ANA, RLC, and CMV made substantial contributions to the conception and design of the study. DJW and ZYR contributed to acquisition of data. BM, RLC, and MSO assisted with interpretation of data. All authors provided substantial revisions and gave approval for the final version to be published.

Acknowledgements

We would like to extend our gratitude to all PROTECT study participants and their families. The authors also thank the nurses and research staff who participated in cohort recruitment and follow up, as well as the Federally Qualified Health Centers (FQHC) in Puerto Rico that facilitated participant recruitment, including Morovis Community Health Center, Prymed in Ciales, Camuy Health Services, Inc. and the Delta OBGyn Group in Manati, as well as the Manati Medical Center and the Metro Pavia Hospital in Arecibo.

Additional Information

This study was supported by the Superfund Research Program of the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH; grant number P42ES017198). Additional support was provided from NIEHS grant number P30ES017885 and the Environmental influences on Child Health Outcomes (ECHO) program grant number UH3OD023251. ECHO is a nationwide research program supported by the NIH, Office of the Director to enhance child health.

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Figures

Figure 1

Differential associations between birth outcomes and hormones measured at 18 and 26 weeks' gestation. Dark green boxes represent the effect estimates for hormones measured at 18 weeks, light green circles represent the effect estimates for hormones measured at 26 weeks, and black bars represent the 95% confidence interval. The vertical red line represents the null value. IQR: Interquartile range.

Figure 2

Differential associations between birth outcomes and hormones measured at 18 and 26 weeks based on fetal sex. Pink boxes represent the effect estimates for hormones measured at 18 weeks among female pregnancies, red circles represent the effect estimates for hormones measured at 26 weeks among female pregnancies, light blue boxes represent the effect estimates for hormones measured at 18 weeks among male pregnancies, dark

blue circles represent the effect estimates for hormones measured at 26 weeks among male pregnancies, and black bars represent the 95% confidence interval. The vertical red line represents the null value. IQR: Interquartile range.

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