Supplementary Table 1. Sex Steroid Hormone Concentrations in Pregnancy by Trimester in the UPSIDE Cohort.

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| --- | --- | --- | --- | --- |
| Sex Steroid Hormone | Trimester 1 (n=314) | Trimester 2 (n=287) | Trimester 3 (n=283) | *Pb* |
|  | Median (IQR)a | | |  |
| Total testosterone (TT) (ng/dL) | 63.9 (56.8) | 62.9 (59.3) | 64.3 (63.6) | <0.001 |
| Free testosterone (fT) (ng/dL) | 0.35 (0.36) | 0.31 (0.31) | 0.29 (0.34) | 0.07 |
| Estrone (E1) (pg/mL) | 936.5 (860) | 3550 (2910) | 5910 (4560) | <0.001 |
| Estradiol (E2) (pg/mL) | 1625 (1180) | 5970 (3320) | 11100 (5420) | <0.001 |
| Estriol (E3) (pg/mL) | 211.5 (405) | 3130 (1480) | 6670 (3470) | <0.001 |
| TT/E2 ratio | 0.37 (0.35) | 0.1 (0.09) | 0.05 (0.06) | <0.001 |

Note. aPresent the median and interquartile range (IQR) because sex steroid hormone levels were skewed. bMultivariate tests of means to assess the hypothesis that means of log transformed sex steroid hormones at different trimesters are equal.

Supplementary Table 2. Correlations of first trimester log-transformed sex steroid hormones (n=314).

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| --- | --- | --- | --- | --- |
|  | TT | fT | E1 | E2 |
| fT | 0.91 (p<0.001) | - | - | - |
| E1 | 0.30 (p<0.001) | 0.17 (p=0.003) | - | - |
| E2 | 0.35 (p<0.001) | 0.22 (p<0.001) | 0.81 (p<0.001) | - |
| E3 | -0.06 (p=0.27) | -0.18 (p=0.002) | 0.19 (p<0.001) | 0.30 (p<0.001) |

Note. Correlation coefficient and p value are presented for each correlation analysis.

Supplementary Table 3. Sensitivity analysis of the associations of first trimester sex steroid hormones with glucose levels and GDM measured in mid-late pregnancy.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sex Hormones | Glucose levels (n=272) | | | GDM Diagnosis (n=295) | | |
| Coefficient | 95% CI | *P* | OR | 95% CI | *P* |
| TT (ng/dL) | 5.88 | 0.45, 11.3 | 0.03 | 4.09 | 1.58, 10.56 | 0.004 |
| fT (ng/dL) | 6.65 | 1.47, 11.82 | 0.01 | 4.02 | 1.57, 10.29 | 0.004 |
| E1 (pg/mL) | 4.38 | -0.26, 9.02 | 0.06 | 3.77 | 1.55, 9.18 | 0.003 |
| E2 (pg/mL) | 5.24 | -1.56, 12.04 | 0.13 | 2.61 | 0.87, 7.82 | 0.09 |
| E3 (pg/mL) | 3.05 | -0.2, 6.29 | 0.07 | 1.07 | 0.65, 1.75 | 0.8 |
| TT/E2 | 2.44 | -2.94, 7.81 | 0.37 | 1.85 | 0.83, 4.12 | 0.13 |

Note. Sensitivity analysis excluded potential undiagnosed PCOS cases. Maternal age, race/ethnicity, parity, gestational age of blood draw, early-pregnancy BMI, and infant sex were adjusted in all models. Fertility treatment was adjusted in the models with glucose levels as the outcome. GDM: gestational diabetes; TT: total testosterone; fT: free testosterone; E1: estrone; E2: estradiol; E3: estriol.

Supplementary Table 4. The associations of first trimester sex steroid hormones with glucose levels and GDM measured in mid-late pregnancy excluding participants with history of GDM.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sex Hormones | Glucose levels (n=280) | | | GDM Diagnosis (n=304) | | |
| Coefficient | 95% CI | *P* | OR | 95% CI | *P* |
| TT (ng/dL) | 4.44 | -0.7, 9.58 | 0.09 | 3.47 | 1.34, 8.97 | 0.01 |
| fT (ng/dL) | 5.3 | 0.38, 10.22 | 0.04 | 3.77 | 1.47, 9.64 | 0.006 |
| E1 (pg/mL) | 4.6 | 0.1, 9.1 | 0.05 | 4.23 | 1.62, 11.04 | 0.003 |
| E2 (pg/mL) | 5.13 | -1.43, 11.68 | 0.13 | 2.7 | 0.85, 8.59 | 0.09 |
| E3 (pg/mL) | 2.32 | -0.81, 5.45 | 0.15 | 0.85 | 0.51, 1.41 | 0.54 |
| TT/E2 | 1.22 | -3.82, 6.26 | 0.63 | 1.61 | 0.72, 3.6 | 0.25 |

Note. Sensitivity analysis excluded participants with history of GDM. Maternal age, race/ethnicity, parity, gestational age of blood draw, early-pregnancy BMI, and infant sex were adjusted in all models. Fertility treatment was adjusted in the models with glucose levels as the outcome. GDM: gestational diabetes; TT: total testosterone; fT: free testosterone; E1: estrone; E2: estradiol; E3: estriol.

Supplementary Table 5. Odds of clinical GDM diagnosis in relation to first trimester log-transformed sex steroid hormone concentrations.

|  |  |  |  |
| --- | --- | --- | --- |
| Sex Steroid Hormones | GDM Diagnosis (n=308) | | |
| OR | 95% CI | *P* |
| TT (ng/dL) | 2.90 | 1.08, 7.83 | 0.04 |
| fT (ng/dL) | 3.11 | 1.17, 8.27 | 0.02 |
| E1 (pg/mL) | 2.77 | 1.10, 6.94 | 0.03 |
| E2 (pg/mL) | 2.24 | 0.68, 7.35 | 0.18 |
| E3 (pg/mL) | 0.93 | 0.53, 1.62 | 0.79 |
| TT/E2 | 1.53 | 0.64, 3.67 | 0.34 |

Note. Maternal age, race/ethnicity, parity, gestational age of blood draw, early-pregnancy BMI, and infant sex were adjusted in the primary models. GDM: gestational diabetes; TT: total testosterone; fT: free testosterone; E1: estrone; E2: estradiol; E3: estriol.

Supplementary Table 6. Associations of early-pregnancy BMI with first trimester sex steroid hormones.

|  |  |  |  |
| --- | --- | --- | --- |
| Sex Hormones | Model (n=299) | | |
| Coefficient | 95% CI | *P* |
| TT (ng/dL) | 0.005 | -0.005, 0.015 | 0.34 |
| fT (ng/dL) | 0.014 | 0.004, 0.025 | 0.006 |
| E1 (pg/mL) | -0.02 | -0.032, -0.009 | 0.001 |
| E2 (pg/mL) | -0.014 | -0.022, -0.006 | <0.001 |
| E3 (pg/mL) | -0.015 | -0.031, 0.001 | 0.07 |
| TT/E2 | 0.019 | 0.009, 0.029 | <0.001 |

Note. Log transformed sex steroid hormones as the outcomes, respectively. Maternal age, race/ethnicity, parity, gestational age of blood draw, infant sex, and fertility treatment were adjusted in the models. BMI: body mass index; TT: total testosterone; fT: free testosterone; E1: estrone; E2: estradiol; E3: estriol.

Supplementary Table 7. Associations of log-transformed first trimester sex steroid hormones and continuous glucose levels and GDM diagnosis in mid-late pregnancy without adjustment for early-pregnancy BMI.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sex Steroid Hormone | Glucose Levels (n=284) | | | GDM Diagnosis (n=308) | | |
| Coefficient | 95% CI | *P* | OR | 95% CI | *P* |
| TT (ng/dL) | 5.54 | 0.26, 10.81 | 0.04 | 3.97 | 1.63, 9.69 | 0.002 |
| fT (ng/dL) | 6.84 | 1.86, 11.83 | 0.007 | 4.11 | 1.76, 9.62 | 0.001 |
| E1 (pg/mL) | 2.90 | -1.61, 7.40 | 0.21 | 2.95 | 1.31, 6.64 | 0.01 |
| E2 (pg/mL) | 3.42 | -3.17, 10.01 | 0.31 | 2.25 | 0.80, 6.37 | 0.13 |
| E3 (pg/mL) | 2.39 | -0.78, 5.56 | 0.14 | 0.99 | 0.62, 1.58 | 0.98 |
| TT/E2 | 3.04 | -2.03, 8.11 | 0.24 | 1.87 | 0.91, 3.87 | 0.09 |

Note. Maternal age, race/ethnicity, parity, gestational age of blood draw, and infant sex were adjusted in all models. Fertility treatment was adjusted in the models with glucose levels as the outcome. GDM: gestational diabetes; BMI: body mass index; TT: total testosterone; fT: free testosterone; E1: estrone; E2: estradiol; E3: estriol.

Supplementary Table 8. Mixed Effects Models of the Associations of GDM Diagnosis with Sex Steroid Hormones in the 2nd and 3rd Trimesters

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sex Steroid Hormones | Primary Model (n=284) | | | Model additionally adjusting for 1st trimester SSH (n=281) | | |
| Coefficient | 95% CI | *P* | Coefficient | 95% CI | *P* |
| TT (ng/dL) | 0.12 | -0.14, 0.39 | 0.35 | -0.19 | -0.36, -0.02 | 0.03 |
| fT (ng/dL) | 0.16 | -0.12, 0.44 | 0.27 | -0.10 | -0.31, 0.12 | 0.36 |
| E1 (pg/mL) | 0.29 | 0.02, 0.56 | 0.03 | 0.01 | -0.18, 0.19 | 0.95 |
| E2 (pg/mL) | 0.14 | -0.02, 0.29 | 0.08 | 0.06 | -0.06, 0.17 | 0.35 |
| E3 (pg/mL) | 0.08 | -0.07, 0.23 | 0.27 | 0.08 | -0.07, 0.23 | 0.29 |
| TT/E2 | -0.01 | -0.28, 0.26 | 0.94 | -0.14 | -0.34, 0.06 | 0.17 |

Note. Maternal age, race/ethnicity, parity, gestational age of blood draw, fertility treatment, early-pregnancy BMI, GWG by the end of 2nd and 3rd trimesters, and infant sex were adjusted in the primary models. GDM: gestational diabetes; TT: total testosterone; fT: free testosterone; E1: estrone; E2: estradiol; E3: estriol.

SSH

GWG

GDM

Supplementary Figure 1. Mediation model of sex steroid hormones, gestational weight gain, and gestational diabetes. SSH, sex steroid hormone; GWG, gestational weight gain; GDM, gestational diabetes.

A graph of red and grey dots

Description automatically generated

Supplementary Figure 2. The relationship among early-pregnancy free testosterone, early-pregnancy BMI and GDM diagnosis. Red dots indicate participants with GDM. The horizontal blue line indicates the median level of free testosterone. The vertical blue line indicates a BMI of 25 kg/m2.