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ARTICLE

Pregnancy outcome in women with polycystic ovary syndrome in relation to second-trimester testosterone levels

BIOGRAPHY

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KEY MESSAGE

Women with polycystic ovary syndrome (PCOS) have higher levels of plasma testosterone and free androgen index during pregnancy compared with women without PCOS. High testosterone levels in the early second trimester is potentially associated with increased risk of preeclampsia in women with PCOS. Findings are significant but further studies are warranted.

ABSTRACT

Research question: Do women with polycystic ovary syndrome (PCOS) have higher testosterone levels during pregnancy and what role does high testosterone play in the development of obstetric complications?

Design: Retrospective cohort study from Uppsala University Hospital, Sweden. The study population consisted of women with PCOS (n = 159) and a comparison group of women without PCOS matched for body mass index (n = 320). Plasma testosterone levels were measured in the early second trimester by liquid chromatography with tandem mass spectrometry, and women with PCOS were grouped into tertiles according to their testosterone levels. Possible associations with obstetric complications, maternal metabolic factors and offspring birth weight were explored by multivariable logistic and linear regression models.

Results: Compared with women who do not have PCOS, women with PCOS had higher total testosterone (median 1.94, interquartile range [IQR] 1.21–2.64 versus 1.41, IQR 0.89–1.97; $P < 0.001$), and free androgen index (median 0.25, IQR 0.15–0.36 versus 0.18, IQR 0.11–0.28; $P < 0.001$). Women with PCOS who had the highest levels of testosterone had increased risk for preeclampsia, even when adjusted for age, parity, country of birth and smoking (adjusted OR 6.16, 95% CI 1.82 to 20.91). No association was found between high testosterone in women with PCOS and other obstetric complications.

Conclusions: Women with PCOS have higher levels of total testosterone and free androgen index during pregnancy than women without PCOS matched for body mass index. Preliminary evidence shows that women with PCOS and the highest maternal testosterone levels in early second trimester had the highest risk of developing preeclampsia. This finding, however, is driven by a limited number of cases and should be interpreted with caution.

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KEYWORDS
Birth weight
Maternal metabolic factors
Polycystic ovary syndrome
Pregnancy complications
Testosterone

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Declaration: The authors report no financial or commercial conflicts of interest.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of childbearing age, with a prevalence between 5–13% depending on the population studied and definition used (*Bozdag et al., 2016*). The syndrome is characterized by hyperandrogenism, anovulation and polycystic ovaries (*Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome [PCOS], 2004*), and the polycystic ovarian morphology is associated with high levels of anti-Müllerian hormone (AMH) (*Indran et al., 2018*). In childbearing age, infertility is one of the most common problems in women with PCOS, but with assisted reproductive technology, longitudinal studies suggest overall high fecundity (*Persson et al., 2019*). Once pregnant, women with PCOS face a higher risk of miscarriage, gestational diabetes, gestational hypertension, preeclampsia and preterm birth (*Palomba et al., 2015*; *Yu et al., 2016*; *Bahri Khomami et al., 2018*; *Bahri Khomami et al., 2019*). According to a recent meta-analysis, these risks are independent of obesity, even though obesity is a major risk factor on its own for these complications (*Bahri Khomami et al., 2019*). High levels of AMH, in contrast, seem protective of gestational hypertension, and unrelated to other obstetric and neonatal complications (*Shand et al., 2014*; *Valdimarsdottir et al., 2019*).

Findings on the role of hyperandrogenism for the obstetric complications that women with PCOS may encounter are less conclusive, as these also seem to depend on PCOS phenotype and maternal ethnicity, but only a few high-quality studies have been published (*Falbo et al., 2010*; *Palomba et al., 2010*; *2014*; *Bahri Khomami et al., 2018*). Women with PCOS have higher total testosterone and free androgen index during pregnancy (*Glintborg et al., 2018*; *Valdimarsdottir et al., 2019*), and testosterone levels are positively correlated with AMH levels (*Detti et al., 2019*; *Piltonen et al., 2019*; *Valdimarsdottir et al., 2019*). The specific role of testosterone in the development of obstetric and neonatal complications has been assessed in a relatively limited number of studies, but rarely in PCOS populations. Previously published findings consistently suggest that high testosterone and high placental expression of androgen receptors are features of manifest preeclampsia (*Acromite et al., 1999*; *Salamalekis et al., 2006*; *Ghorashi and Sheikhvatan, 2008*; *Hsu et al., 2009*; *Sathishkumar et al., 2012*; *Sharifzadeh et al., 2012*), potentially owing to a dysregulation of the placental aromatase levels (*Sathishkumar et al., 2012*). It is not known, however, if high testosterone causes preeclampsia or if it is a result of manifest preeclampsia, as the predictive potential of testosterone levels in early pregnancy in relation to later development of preeclampsia has not been ascertained (*Carlsen et al., 2005*; *Tuutti et al., 2011*).

Most meta-analyses of neonatal outcomes do not suggest increased risk of giving birth to small for gestational age (SGA) or large for gestational age infants among women with PCOS (*Yu et al., 2016*; *Bahri Khomami et al., 2018*). Elevated maternal testosterone levels at gestational week 17 and 33 have been associated with lower birth weight (*Carlsen et al., 2006*), whereas other studies have found no such association (*Gustin et al., 2012*; *Cho et al., 2017*). If maternal testosterone affects birth weight, this should only be detectable in female offspring as male fetuses produce much higher levels of testosterone than their mothers. To the best of our knowledge, previous studies on the relationship between maternal testosterone levels and birth weight have not taken offspring sex into account. In addition, most previous studies in this field have used immunoassays, which are no longer considered to be gold standard in steroid hormone analysis (*Taieb et al., 2002*; *Kushnir et al., 2011*). Given the heterogeneous presentation of women with PCOS, and the relative lack of understanding of pathophysiological mechanisms that mediate PCOS-related pregnancy complications, the aim of this study was to evaluate if women with PCOS have increased testosterone levels during pregnancy, and what role high testosterone levels play in women with PCOS in the development of gestational diabetes, gestational hypertension, preeclampsia, preterm birth and offspring birth weight.

MATERIALS AND METHODS

Study population

This is a retrospective cohort study, including women who have donated blood samples to the population-based Uppsala Biobank of Pregnant Women at Uppsala University Hospital, Sweden. The Biobank collects blood samples from pregnant women for multiple scientific purposes and is a dedicated effort aimed at future research. Since 31 May 2007, all Swedish-speaking pregnant women, aged 18 years and older, without blood-borne diseases who attended the second-trimester routine ultrasound scan (gestational weeks 18–19) were invited to participate in the Biobank. The Biobank is population-based, as 97% of the pregnant population participate in routine ultrasound examinations, and all routine ultrasound examinations in Uppsala County are carried out at Uppsala University Hospital. Furthermore, Uppsala University Hospital is the only delivery ward within the county, leading to excellent follow-up of participants. Participation in the Biobank, however, is dependent upon a research nurse being available, and it is estimated that the Biobank covers about one-half of the pregnant population of Uppsala County (*Granfors et al., 2014*). Upon inclusion, brief demographic data are collected, including previous and ongoing chronic disorders, ongoing medication and smoking in early pregnancy.

All women diagnosed with PCOS (E282), with the International Classification of Diseases 10 (ICD-10), between 2003 and 2015 were identified in the hospital patient register in Uppsala and linked to the Biobank database. A PCOS diagnosis was made according to Rotterdam criteria (*Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome [PCOS], 2004*), i.e. requiring the presence of two of the following three criteria: polycystic ovaries on transvaginal ultrasound; oligomenorrhoea or amenorrhoea; or signs of hyperandrogenism, either biochemical, i.e. elevated testosterone levels, elevated free androgen index (>5.0) or elevated androstenedione levels, or clinical, i.e. as presence of hirsutism, expressed as Ferriman–Gallwey score over eight or as needed for specific anti-hirsutism treatment. All women with PCOS had normal prolactin levels and thyroid function tests. The medical records of all women with PCOS were scrutinized to ensure a correct diagnosis and to obtain information on obstetric and perinatal outcomes. By September 2015, 174 pregnant women with PCOS

had participated in the Biobank. Fifteen women with PCOS were excluded; four women were pregnant with twins; one miscarried late; one had a stillbirth; seven deliveries took place outside Uppsala County; and two women were misdiagnosed with PCOS, leaving 159 women with PCOS available for hormonal analysis. Most women (n = 99) had received the PCOS diagnosis as part of an infertility work-up, whereas the remaining women had consulted a physician for PCOS symptoms, such as menstrual disturbances and hirsutism. Unfortunately, the diagnostic evaluation for infertility at the time did not include analysis of testosterone plasma concentrations. The women with PCOS were grouped into testosterone tertiles, with the following cut-offs: less than 1.44 (low); 1.44–2.36 (medium); and greater than 2.36 (high) nmol/l. Fifty-two women were assigned to each tertile as testosterone values were available for 156 women with PCOS.

For each pregnant woman with PCOS, two women without PCOS, matched for body mass index (BMI), with singleton pregnancies, were chosen as comparison. Each woman without PCOS had donated a blood sample during the same calendar week as the corresponding PCOS woman. In addition, the non-PCOS women were healthy according to self-reports collected in conjunction with the blood sampling. The medical records of the women in the comparison group, who became pregnant after assisted reproductive technology, were reviewed to ensure than none of them had been previously diagnosed with PCOS or had an anovulation-related infertility factor. The two controls selected for the patient with PCOS who was excluded owing to stillbirth were kept in the study.

Information on maternal clinical variables, pregnancy complications and perinatal outcomes was derived from the standardized antenatal, obstetric and paediatric medical records. Information on first-trimester weight, height, BMI, parity, maternal country of birth and history of miscarriages was obtained from the first antenatal visit. Information on previous miscarriage was available for 350 women. The gestational weight gain was defined as difference in weight between first and last antenatal visit, available for 197 women. Blood pressure at first antenatal visit was missing for two women and blood pressure at last antenatal visit was available for 340 women. The highest non-fasting glucose concentration, measured in gestational weeks 10–36, was available for 474 women.

Obstetric diagnoses according to ICD-10 recorded in the obstetric medical records were noted. Obstetric outcomes of interest were gestational diabetes (O244), preeclampsia (O14), gestational hypertension (O13) and preterm birth (O609). Birth weight was expressed as standardized birth weight scores, i.e. SD in relation to gestational length and offspring sex (*Marsal et al., 1996*). Large for gestational age was defined as having a birth weight of more than two SD above the mean birth weight for gestational age according to the reference curve (*Marsal et al., 1996*). No cases of SGA were detected in the PCOS group and consequently SGA was excluded as outcome.

Methods

Blood samples for the Biobank were collected at the early second-trimester anomaly scan, usually carried out at 18–19 completed gestational weeks. The samples were collected in tubes containing ethylenediaminetetraacetic acid and centrifuged at 1500 g for 10 min. Plasma and buffy coat were separated within 2 h and stored at –70°C. All hormone analyses were analysed in one batch. Testosterone and sex hormone binding globulin (SHBG) were analysed at Analytical Chemistry, Biomedical Centre at Uppsala University.

Analysis of testosterone by ultra-performance supercritical fluid chromatography–tandem mass spectrometry

The validated protocol for analysis of testosterone in human plasma was used. Samples were prepared by spiking with 50 µl of 75 ng/ml corresponding $^{13}C_3$-testosterone into 50 µl plasma followed by liquid-liquid extraction with methyl tert-butyl ether after protein precipitation with methanol before the analysis. During the extraction, the analyte was protected against oxidation by the addition of 0.05 mg/ml butylated hydroxytoluene to the methyl tert-butyl ether.

Ultra-performance supercritical fluid chromatography (Waters ACQUITY® UPC^2™) (Milford, MA, USA) was used to assay the testosterone coupled with tandem mass spectrometry (XEVO® TQ-S) (Milford, MA, USA). Chromatographic separation was achieved on ethylene bridged hybrid column (150 mm 3.0 mm, 1.7 µm Waters, Milford, MA, USA) at 40°C. The mobile phase consisted of supercritical CO_2 (A, 99.999%) and 0.1% formic acid in methanol: isopropanol (B, 1:1, volume per volume) at a flow rate 1.5 ml. The isocratic elution was carried out with 5% of B mobile phase and total analytical time was less than 2 min. The mass spectrometric detection was carried out using electrospray ionization in the positive ionization mode (ESI^+) with nitrogen and argon serving as desolvation and collision gas, respectively. Data acquisition range was 100–600 m/z. Quantification was based on a multiple reaction monitoring method with a suitable isotope internal standard. Two specific transitions were chosen, one for confirmation (the 'qualifier') and one for quantification (the 'quantifier'), 289.2 > 109.1; 289.2 > 97.1, respectively. The linearity of the testosterone was evaluated over a range of concentrations (0.1–50 nmol/l) and correlation coefficients (R^2) were 0.998. The limit of quantification (signal to noise ratio = 10) and coefficient of variation of testosterone assay was 0.2 nmol/l, less than 10%, respectively. Precision was estimated by running quality-control samples in five replicates on the same day and three independent days, intra–assay coefficient of variation ranged from 5.52–7.64%, whereas inter–assay coefficient of variation was 2.46–5.30%. The recovery of the testosterone assay was 87%. All data were acquired in centroid mode, analysed and processed using the MassLynx™ 4.1 software (Waters, Milford, MA, USA). Duplicate analyses of each sample were carried out and the average values were reported (coefficient of variation <3%). For technical reasons (insufficient amount of sample), testosterone analysis could not be carried out on samples from seven women, leaving 472 testosterone values available for analysis (n = 156 in PCOS group).

Analysis of sex hormone binding globulin

The peptide hormones SHBG (catalogue number DSHBG0B) were measured by using immuno-assay kits from R&D Systems (Minneapolis, MN, USA), which use ^{125}I-labelled SHBG and polyethylene glycol-complexed second antibody. The plasma samples required a 100-fold dilution, 10 µl of plasma plus 990 µl of Calibrator Diluent RD5-24.

The assay was of the sandwich-type using a pre-coated 96 well plate and a supply of enzyme-labelled secondary antibody, and standard according to the manufacturer's instructions. The resulting absorbance was read in a BioRad Model 680 Microplate Reader at 450 nm with 595 nm as background. The goodness of fit was verified by the r^2 values. The limit of quantification was 2.0 nM for SHBG. Repeatability of the assay was checked and the median repeatability SD relative SD for SHBG was 13.5%. Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision, coefficient of variation ranged from 3.6–5.7%. Three samples of known concentration were tested in 20 separate assays to assess inter-assay precision and coefficient of variation ranged from 4.8–7.6%. The recovery of human SHBG spiked to levels throughout the range of the assay was evaluated and was 87–96%. The accuracy is presented by the manufacturer using an in-house preparation calibrated against NIBSC/WHO International Standard 08/266.

Free androgen index

The ratio of total testosterone to SHBG is referred to as free androgen index and is used as an estimate of free testosterone (*Stanczyk, 2006*). Free androgen index was calculated by the formula (testosterone [nmol/l]/SHBG [nmol/l]) x 100. For technical reasons, SHBG analysis could not be conducted in one sample, resulting in 471 free androgen index values (n = 156 in the PCOS group).

Statistics

Anthropometric, metabolic variables and obstetric and neonatal complications were compared between women with PCOS and women without PCOS by linear or logistic regression, with adjustment for age. Mann–Whitney U test was used to compare hormone levels between the groups. Analysis of variance was used to compare metabolic variables between women with PCOS in the three testosterone tertiles. Obstetric and neonatal outcomes were compared between groups with logistic regression, adjusting for age, parity, maternal country of birth and smoking. These co-variables were chosen on the basis of differences between women with PCOS and controls, but also on previously published data (*Bahri Khomami et al., 2018*). Assisted reproductive technology was not adjusted for because it is considered to be a mediator, not a confounder.

To evaluate the effect of high testosterone on birth weight, multivariable linear regression was used. In this analysis, women with pre-pregnancy diabetes (n = 4), gestational diabetes (n = 6) and preeclampsia (n = 17) were excluded, as these have strong influences on birth weight, leaving 445 and 444 women in the testosterone and free androgen index analysis, respectively. The analysis was adjusted for age, maternal BMI, maternal height, parity, maternal country of birth and smoking. These co-variables were chosen on the basis of findings in bivariate and correlational analyses, but also on information from published research (*Cnattingius et al., 1993*; *Kallak et al., 2017*; *Rochow et al., 2018*; *Spada et al., 2018*). IBM SPSS statistics version 26.0 (Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Maternal characteristics, metabolic variables, obstetric complications and hormone levels are presented in TABLE 1. Women with PCOS were older (31.7 ± 4.6 versus 30.2 ± 5.1; P = 0.002), more often had history of previous miscarriage (n = 38 [37.3%] versus n = 48 [19.4%]; P < 0.001) and had more often conceived through assisted reproduction technology (n = 78 [49.1%] versus n = 11 [3.4%]; P < 0.001). Even though BMI, systolic and diastolic blood pressures were similar at the beginning of pregnancy, women with PCOS developed preeclampsia more often than women who did not have PCOS (n = 11 [6.9%] versus n = 6 [1.9%]; P = 0.005). No differences between the groups were observed for gestational diabetes, gestational hypertension, preterm birth or LGA.

Women with PCOS had higher early second-trimester total testosterone (median 1.94 nmol/l, IQR 1.21–2.64 versus 1.41 nmol/l, IQR 0.89–1.97; P < 0.001) and free androgen index (median 0.25, IQR 0.15–0.36 versus 0.18, IQR 0.11–0.28; P < 0.001) compared with women who did not have PCOS. No woman in the study population had free androgen index above 5.0. The SHBG levels were similar in women with PCOS and in women without PCOS (median 789 nmol/l, IQR 604 – 975 versus 782 nmol/l, IQR 584–1013; P = 0.9).

Anthropometric and metabolic variables according to second-trimester testosterone levels in controls and women with PCOS are presented in TABLE 2. No differences in age, BMI, gestational weight gain or blood pressure at first or last antenatal visit were demonstrated between the women with PCOS who had low, medium or high testosterone levels.

Obstetric and neonatal complications in women without PCOS and women with PCOS grouped according to testosterone tertiles are presented in TABLE 3. Women with highest levels of testosterone within the PCOS group had increased risk for preeclampsia (OR 6.74, 95% CI 2.09 to 21.78), even when adjusted for age, parity, country of birth and smoking (adjusted OR 6.16, 95% CI 1.82 to 20.91). No association was found between high testosterone in women with PCOS and gestational diabetes, gestational hypertension, preterm birth or LGA.

The multiple linear regression models on how total testosterone and free androgen index predict standardized birth weight in female and male offspring, respectively, are presented in TABLE 4. Total testosterone levels were not associated with standardized birth weight in either female or male offspring. In contrast, free androgen index was positively associated with standardized birth weight in male offspring (B = 0.12, 95% CI 0.01 to 0.23). The inclusion of PCOS diagnosis in the models was also tested, but PCOS was not associated with standardized birth weight, and also did not change the estimates for either total testosterone or free androgen index.

DISCUSSION

The main findings of the present study were that women with PCOS had higher testosterone levels during early second trimester of pregnancy compared with the women who did not have PCOS. Women with PCOS more commonly developed preeclampsia, and high testosterone in women with PCOS is potentially associated with increased risk of preeclampsia. High testosterone during pregnancy in women with PCOS, however, was not associated with maternal metabolic factors during pregnancy, other obstetric complications or female offspring birth weight.

The strengths of this study include the population-based design, the

TABLE 1 MATERNAL CHARACTERISTICS, CLINICAL VARIABLES, OBSTETRIC COMPLICATIONS AND HORMONE LEVELS IN RELATION TO POLYCYSTIC OVARY SYNDROME

	PCOS (*n* = 159), %	Non-PCOS (*n* = 320), %	*P*-value
Age, years	31.7 ± 4.6	30.2 ± 5.1	0.002
Primipara	90 (56.6)	141 (44.1)	0.01
Nordic country of origin	148 (93.1)	304 (95.0)	0.4
Previous miscarriage[a]	38 (37.3)	48 (19.4)	<0.001
Assisted reproductive technology	78 (49.1)	11 (3.4)	<0.001
Smoking during early pregnancy	5 (3.1)	19 (5.9)	0.2
BMI, kg/m^2	26.2 ± 5.8	26.1 ± 5.4	0.9
Underweight, <18.5 kg/m^2	0	1 (0.3)	0.9
Normal weight, 18.5–24.9 kg/m^2	85 (53.5)	174 (54.4)	
Overweight, 25–29.9 kg/m^2	38 (23.9)	75 (23.4)	
Obese, >30 kg/m^2	36 (22.6)	70 (21.9)	
Gestational weight gain, kg[b]	12.6 ± 4.9	13.0 ± 5.2	0.7
Systolic blood pressure at first antenatal visit, mmHg[c]	117 ± 12	116 ±11	0.2
Diastolic blood pressure at first antenatal visit, mmHg[c]	72 ± 9	71 ± 8	0.1
Systolic blood pressure at last antenatal visit, mmHg[d]	124 ± 13	123 ± 12	0.6
Diastolic blood pressure at last antenatal visit, mmHg[d]	78 ± 10	78 ± 8	0.9
Highest non-fasting glucose, mmol/l[e]	6.5 ± 1.2	6.4 ± 1.0	0.9
Gestational diabetes	4 (2.5)	3 (0.9)	0.2
Gestational hypertension	7 (4.4)	15 (4.7)	0.9
Preeclampsia	11 (6.9)	6 (1.9)	0.005
Preterm birth	7 (4.4)	12 (3.8)	0.7
Birth weight, g	3606 ± 548	3596 ± 543	0.9
Large for gestational age	7 (4.4)	21 (6.6)	0.3
Testosterone, nmol/l	1.94 (1.21–2.64)	1.41 (0.89–1.97)	<0.001
SHBG, nmol/l	789 (604–975)	782 (584–1013)	0.9
Free androgen index	0.25 (0.15–0.36)	0.18 (0.11–0.28)	<0.001

Statistical analyses by independent t-test, Mann–Whitney U test and Pearson Chi-Square test.

Data expressed as mean ± SD, number (%) or median and interquartile range.

Information available for [a]350; [b] 197; [c] 477; [d] 340 and [e]474 women.

BMI, body mass index; PCOS, polycystic ovary syndrome; SHBG, sex hormone binding globulin.

relatively large sample size, the detailed information on the study participants and the use of liquid chromatography with tandem mass spectrometry for testosterone analyses (*Kushnir et al., 2011*). Further, obstetric data were collected prospectively by healthcare professionals in a standardized manner, contributing to strengthened data reliability and limiting the risk for recall bias. Limitations include a small number of women with PCOS when subdivided according to testosterone tertiles and the single time-point of blood sampling. Another limitation is that PCOS cases were identified from an electronic patient record, based on ICD-10 diagnoses. A substantial number of women received their diagnosis as part of an infertility work-up and treatment, and therefore the clinical evaluation did not include full assessment of pre-pregnancy hyperandrogenism. Similarly, by including non-PCOS women merely matched for BMI and sampling date, misclassification of PCOS may have occurred in the comparison group that could have led to an underestimation of the association between PCOS and pregnancy outcomes. We also noted that the present PCOS study population seemed relatively healthy, with almost normal BMI and few obstetric complications. As a result of BMI-matching, the proportions of overweight and obese women in the PCOS versus non-PCOS groups were similar. In addition to the relatively low BMI of our study population, this could explain the low prevalence and the absent difference between groups in gestational diabetes. Potentially, a more severely affected group of women with PCOS would have yielded different results.

Women with PCOS have higher testosterone levels during pregnancy compared with women without PCOS. This finding is in line with previous studies in pregnant women with PCOS, in which high testosterone levels also in late pregnancy have been noted (*Glintborg et al., 2018*; *Detti et al., 2019*; *Piltonen et al., 2019*). In pregnancy, maternal testosterone levels depend mainly on the feto–placental unit. The ovaries, the adrenals and the adipose tissue, however, may also contribute to the circulating testosterone levels in women with PCOS (*Maliqueo et al., 2013*; *Kallak et al.,*

TABLE 2 ANTHROPOMETRIC AND METABOLIC VARIABLES ACCORDING TO SECOND TRIMESTER TESTOSTERONE LEVELS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

	Non-PCOS	PCOS	PCOS	PCOS	*P*-value[a]
Testosterone tertiles		**Low <1.44 nmol/l**	**Medium 1.44–2.36 nmol/l**	**High >2.36 nmol/l**	
Testosterone nmol/l	1.41 (0.89–1.97)	1.00 (0.67–1.21)	1.95 (1.64–2.08)	3.05 (2.61–3.95)	0.09
Age, years	30.2 ± 5.1	31.5 ± 5.0	31.6 ± 4.3	31.9 ± 4.5	0.9
BMI, kg/m^2	26.1 ± 5.4	26.8 ± 5.9	25.0 ± 4.6	26.8 ± 6.7	0.2
Gestational weight gain, kg	13.0 ± 5.2	13.0 ± 5.6	13.5 ± 4.4	11.7 ± 4.5	0.6
Systolic blood pressure at first antenatal visit, mmHg	116 ±11	118 ± 11	116 ± 13	119 ± 11	0.3
Diastolic blood pressure at first antenatal visit, mmHg	71 ± 8	72 ± 8	72 ± 9	73 ± 9	0.7
Systolic blood pressure at last antenatal visit, mmHg	123 ± 12	122 ± 14	127 ± 14	124 ± 10	0.4
Diastolic blood pressure at last antenatal visit, mmHg	78 ± 8	76 ± 9	81 ± 11	79 ± 8	0.1
Highest non-fasting glucose (mmol/l)	6.4 ± 1.0	6.6 ± 1.3	6.6 ± 1.0	6.3 ± 1.2	0.4

[a] Statistical analyses compared the three polycystic ovary syndrome (PCOS) tertiles with analysis of variance.

Fifty-two women with PCOS in each tertile.

Testosterone levels expressed as median and interquartile range and other values as mean ± SD.

BMI, body mass index.

2017). We and other investigators have previously demonstrated that women with PCOS have higher AMH levels during pregnancy (Detti et al,. 2019; Piltonen et al., 2019; Valdimarsdottir et al., 2019). As AMH is produced by the antral follicles in the ovary and by the placenta (Novembri et al., 2015), and as AMH is correlated with testosterone levels (Detti et al., 2019; Piltonen et al., 2019; Valdimarsdottir et al., 2019), it may be speculated that the ovaries continue contributing to the overall testosterone synthesis in pregnant women with PCOS.

Women with PCOS had increased risk of preeclampsia, in line with previous findings (Palomba et al., 2015; Yu et al., 2016). Even though several obstetric complications have been described in women with PCOS (Palomba et al., 2015; Yu et al., 2016; Bahri Khomami et al.,

TABLE 3 OBSTETRIC AND NEONATAL COMPLICATIONS IN WOMEN WHO DO NOT HAVE PCOS AND WOMEN WHO HAVE PCOS GROUPED ACCORDING TO TESTOSTERONE TERTILES

		Testosterone tertile	*n* (%)	OR (95% CI)	Adjusted OR (95% CI)
Gestational diabetes	Non-PCOS		3 (0.9)	Reference	Reference
	PCOS	Low	1 (1.9)	2.05 (0.21 to 20.05)	1.42 (0.13 to 15.63)
	PCOS	Medium	1 (1.9)	2.05 (0.21 to 20.05)	2.83 (0.25 to 32.45)
	PCOS	High	2 (3.8)	4.17 (0.68 to 25.60)	6.44 (0.84 to 49.62)
Gestational hypertension	Non-PCOS		15 (4.7)	Reference	Reference
	PCOS	Low	(0)	–	-
	PCOS	Medium	4 (7.7)	1.67 (0.53 to 5.25)	1.34 (0.40 to 4.42)
	PCOS	High	3 (5.8)	1.23 (0.34 to 4.40)	1.12 (0.31 to 4.13)
Preeclampsia	Non-PCOS		6 (1.9)	Reference	Reference
	PCOS	Low	3 (5.8)	3.16 (0.77 to 13.06)	2.79 (0.66 to 11.80)
	PCOS	Medium	2 (3.8)	2.07 (0.41 to 10.53)	1.53 (0.29 to 8. 12)
	PCOS	High	6 (11.5)	6.74 (2.09 to 21.78)	6.16 (1.82 to 20.91)
Preterm birth	Non-PCOS		12 (3.8)	Reference	Reference
	PCOS	Low	1 (1.9)	0.50 (0.06 to 3.90)	0.38 (0.05 to 3.06)
	PCOS	Medium	1 (1.9)	0.50 (0.06 to 3.90)	0.33 (0.04 to 2.71)
	PCOS	High	5 (9.6)	2.70 (0.91 to 8.00)	2.18 (0.71 to 6.73)
Large for gestational age	Non-PCOS		21 (6.6)	Reference	Reference
	PCOS	Low	2 (3.8)	0.56 (0.13 to 2.47)	0.53 (0.18 to 2.37)
	PCOS	Medium	2 (3.8)	0.56 (0.13 to 2.47)	0.71 (0.16 to 3.22)
	PCOS	High	3 (5.8)	0.86 (0.25 to 2.99)	0.91 (0.26 to 3.25)

Logistic regression with adjustments for maternal age, parity, country of birth and smoking.

Testosterone tertiles (52 women with polycystic ovary syndrome [PCOS] in each tertile): low <1.44 nmol/l, medium 1.44–2.36 nmol/l, high >2.36 nmol/l.

TABLE 4 MULTIPLE LINEAR REGRESSIONS OF TOTAL TESTOSTERONE AND FREE ANDROGEN INDEX, AS PREDICTORS OF STANDARDIZED BIRTH WEIGHT, IN FEMALE AND MALE OFFSPRING

Covariate	Unstandardized *B* (95% CI)	*P*-value	R^2
Female offspring			
Total testosterone	0.14 (–0.02 to 0.29)	0.082	0.21
Free androgen index	0.08 (–0.07 to 0.24)	0.289	0.20
Male offspring			
Total testosterone	0.05 (–0.06 to 0.16)	0.341	0.06
Free androgen index	0.12 (0.01 to 0.23)	0.034	0.07

Adjusted for maternal age, body mass index, height, parity, country of birth and smoking.

Women with pre-pregnancy diabetes (n = 4), gestational diabetes (n = 6) and preeclampsia (n = 17) are excluded from this analysis, leaving 445 women for testosterone analysis and 444 women for free androgen index analysis.

2019), no associations with gestational diabetes, gestational hypertension or preterm birth were noted in this study population. Reasons for this discrepancy could be overall lower prevalence rates of these obstetric complications than in the general population, and the relatively healthy population of women with PCOS, mostly seeking care for infertility and with a mean BMI of 26 kg/m^2.

We found preliminary evidence that women with PCOS who had the highest maternal testosterone levels in the early second trimester were those who had the highest risk of developing preeclampsia. This finding is driven by a few cases (with resulting wide confidence intervals), however, and should be interpreted with caution. This relationship could be explained by the significant difference between the risks of preeclampsia in the groups of women with PCOS versus women without PCOS. The association between testosterone and preeclampsia has been described (*Carlsen et al., 2005*; *Sathishkumar et al., 2012*), although most studies have evaluated the relationship once preeclampsia has developed (*Acromite et al., 1999*; *Salamalekis et al., 2006*; *Ghorashi and Sheikhvatan, 2008*; *Hsu et al., 2009*; *Sharifzadeh et al., 2012*; *Sathishkumar et al., 2012*). It is still not clear if testosterone has a causal role in the pathophysiology of preeclampsia, and prediction studies have been both positive (*Carlsen et al., 2005*) and negative (*Tuutti et al., 2011*). In non-pregnant women with PCOS, the hyperandrogenic phenotype has been associated with more severe metabolic disturbances (*Daan et al., 2014*; *Jeanes and Reeves 2017*), which could explain the increased risk of preeclampsia in women with high testosterone (*Sharifzadeh et al., 2012*; *Kumar et al., 2018*). We found no association, however, between high testosterone during pregnancy in women with PCOS and several maternal metabolic factors collected throughout pregnancy. Given the relatively weak evidence provided by this study, further prospective studies in pre-pregnancy phenotyped women with PCOS are needed to elucidate whether the hyperandrogenic phenotypes are associated with increased risk of preeclampsia as well as other PCOS-related obstetric complications.

High testosterone during pregnancy in women with PCOS was not associated with female offspring birth weight, but we noted a weak association with male offspring birth weight. Maternal testosterone levels are unaffected by fetal sex, but slight increases in testosterone levels in women carrying male fetuses may be seen under certain circumstances. We have previously shown that women carrying the CC genotype of the single nucleotide polymorphism rs700815 in the aromatase (*CYP19A1)* gene have higher testosterone levels during pregnancy if they carry a male fetus (*Kallak et al., 2017*). Further, animal studies on AMH exposure during pregnancy suggest impaired placenta aromatase function in an animal model of PCOS, opening up for transfer of testosterone levels between the mother and the fetus and *vice versa* (*Tata et al., 2018*). We speculate that the association between maternal free androgen index and male offspring birth weight is a reflection of higher testosterone levels in the male fetuses with greater growth potential, spilling over to the maternal circulation.

In conclusion, women with PCOS had higher levels of plasma testosterone during early second trimester pregnancy compared with women who do not have PCOS. Also, women with PCOS who had the highest maternal testosterone levels were those who had the highest risk of developing preeclampsia. Further prospective studies of women with PCOS who have been phenotyped before the pregnancy are warranted.

ACKNOWLEDGEMENTS

The authors express their gratitude to the research nurses who helped in the recruitment and blood sampling of participants and all the women who participated. The Biobank and the specific sub-study were approved by the Regional Ethical Review Board in Uppsala (Dnr 2007/181 date of approval 7 August 2007 and 2017/029 date of approval 22 March 2017). Written informed consent was obtained from all women upon acceptance to participate in the Biobank. The Swedish Research Council grant 2015-4870 (JB) is acknowledged.

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Received 7 May 2020; received in revised form 25 August 2020; accepted 21 September 2020.