Endocrine and cardiometabolic cord blood characteristics of offspring born to mothers with and without polycystic ovary syndrome

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Objective: To compare the endocrine and cardiometabolic cord blood characteristics of offspring of mothers with polycystic ovary syndrome (PCOS) with those of healthy controls.
Design: Cross-sectional case control study.
Setting: University medical centers.
Patient(s): Offspring from mothers with PCOS (n = 61) and healthy controls (n = 82).
Intervention(s): Cord blood withdrawal from neonates.
Main Outcome Measure(s): Cord blood estradiol, androstenedione, dehydroepiandrosterone sulfate (DHEAS), testosterone, sex hormone-binding globulin, free androgen index (FAI), insulin, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, c-reactive protein, adiponectin, and leptin.
Result(s): Androstenedione and leptin concentrations were increased in the offspring of women with PCOS compared with the controls: androstenedione median 2.9 (interquartile range [IQR] 2.3–3.9) nmol/L vs. 2.2 [IQR 1.6–2.7] nmol/L; and leptin median 13.6 [IQR 8.3–22.9] μg/L vs. 9.8 [IQR 6.0–16.5] μg/L. After adjusting for maternal and pregnancy-related confounders (such as maternal age, gestational age, birth weight), androstenedione appeared associated with PCOS in both male (relative change 1.36 [1.04; 1.78]) and female offspring (relative change 1.40 [1.08; 1.82]). Similarly, in male offspring the leptin concentrations appeared associated with PCOS after correction for confounders (relative change 1.55 [1.12; 2.14]). After correction for multiple testing, these associations attenuated.
Conclusion(s): Observed results suggest that androstenedione concentrations are increased in the cord blood of male and female offspring of women with PCOS, although this requires confirmation. This finding would support the hypothesis that a maternal hyperandrogenic environment during pregnancy in women with PCOS may predispose their offspring to fetal hyperandrogenism. The potential associations between fetal hyperandrogenism and long-term health effects remain to be elucidated.
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Key Words: Androgens, cord blood, PCOS offspring

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Polycystic ovary syndrome (PCOS), a heterogeneous condition characterized by ovulatory dysfunction, polycystic ovary morphology, and/or hyperandrogenism, affects up to 15% of the general female population (1, 2). Frequently PCOS is accompanied by various metabolic abnormalities, including obesity, hyperinsulinemia and dyslipidemia, which may result in the development of type 2 diabetes mellitus, atherosclerosis, and

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cardiovascular disease in later life (2–5). Moreover, pregnancies in women with PCOS are more often complicated by gestational diabetes, pregnancy-induced hypertension, preeclampsia, and premature delivery (6).

Family studies, including twin studies, demonstrate a distinct heritability of PCOS, particularly of hyperandrogenism (7, 8). It is likely that PCOS originates from a complex interaction between an inherent genetic predisposition along with environmental factors (9, 10). Intrauterine conditions may potentially contribute to the development of PCOS and associated complications in the later life of exposed offspring (11). Studies in primates have established that excess prenatal exposure to androgens may induce the development of PCOS in offspring (12, 13).

Circulating androgen levels may be increased in pregnant women with PCOS, providing a potential source of fetal androgen excess (14, 15). Moreover, hyperinsulinemia in pregnant women with PCOS may contribute to fetal androgen excess through the inhibition of placental aromatase activity, which decreases the conversion of maternal and fetal androgens to estrogens (16, 17).

Umbilical cord blood characteristics reflect maternal, placental, and fetal conditions, and may therefore indicate potential derangements in the endocrine or metabolic intrauterine fetal environment. Few studies with limited sample size have previously compared cord blood characteristics of offspring of mothers with PCOS with non-PCOS controls, generating conflicting outcomes. Some studies have reported increased androgens levels in the cord blood of offspring of mothers with PCOS (18, 19), but others have observed decreased androgen concentrations (15, 20) or no differences compared with controls (21). The current study was undertaken to compare both endocrine and cardiometabolic cord blood characteristics between a carefully phenotyped population of offspring of mothers with PCOS and non-PCOS controls. Due to the expected differences in sex hormone levels in male and female offspring, and potential differences in correlations with metabolic biomarkers, subanalyses for gender were performed.

MATERIALS AND METHODS

Study Population

Cord blood was collected from children born to PCOS mothers who were included in a multicenter study that was conducted in the Netherlands between April 2008 and April 2012 (clinicaltrials.gov, trial number NCT00821379). The primary aim of this study was to design a multivariable prediction model for maternal and perinatal complications in women with PCOS (22). Women diagnosed with PCOS according to the Rotterdam criteria and who wanted to conceive underwent a standardized preconception screening before inclusion (4, 23). Subsequently, most of the women underwent ovulation induction or in vitro fertilization as infertility treatment (24). Once a pregnancy was established, the women were observed through repeated antenatal care visits and a postpartum visit at 6 weeks. All study procedures have previously been described in detail elsewhere (22).

For the current study we included mixed arteriovenous cord blood samples of singleton PCOS pregnancies that were obtained and stored at the University Medical Center Utrecht (n = 61). After withdrawal, cord blood samples were processed and first stored at −20°C (for a maximum of 3 years), and thereafter stored at −150°C.

For the control population, cord blood samples were provided from the Rotterdam Periconceptional Cohort Study, the design of which has been described in detail elsewhere (25). This study represents an ongoing prospective birth cohort, which was initiated in 2009 within the Erasmus Medical Center Rotterdam (www.birthcohorts.net, PREDICT). Among other goals, this study focuses on determinants of periconceptional health, reproductive performance, pregnancy course, and outcomes. All women scheduled for a first antenatal visit at the outpatient clinic of the Erasmus Medical Center with a gestational age less than 12 weeks were invited to participate. Upon inclusion, the women completed questionnaires that included preconceptional data on menstrual cycle regularity and duration of menstrual cycles. Furthermore, all women underwent a standardized examination followed by a three-dimensional ultrasound examination. For the current study, women previously diagnosed with PCOS were excluded based on their preconception medical records (25). We included all available mixed arteriovenous cord blood samples of singleton non-PCOS pregnancies (n = 82) that were collected between August 2011 and May 2014. After withdrawal the cord blood samples were processed and stored at −80°C.

Both studies were conducted with permission of local institutional ethical review boards. Written, informed consent was obtained from all participants.

Endocrine Assessments

Dehydroepiandrosterone sulfate (DHEAS) was measured using an electrochemiluminescence immunoassay on the Cobas E411 (Roche Diagnostics GmbH); the lower limit of detection was 0.05 μmol/L, and the interassay variation ranged from 6% to 4.5% at 0.5–17 μmol/L. We measured sex hormone-binding globulin (SHBG) using an electrochemiluminescence immunoassay on the Cobas E411 (Roche Diagnostics GmbH); the lower limit of detection was 2 nmol/L, and the interassay variation was <4% in the range of 10–120 nmol/L. Estradiol was measured using an electrochemiluminescence immunoassay on the Cobas E411 (Roche Diagnostics GmbH), and the lower limit of detection was <40 pmol/L. Samples were diluted 11 times, and the interassay variation ranged from 11% at 70 pmol/L to <4% at 190–560 pmol/L.

Testosterone and androstenedione were measured on the Thermo Vantage liquid chromatography with tandem mass spectrometer (LC-MS/MS) (Thermo Fisher Scientific BV). The samples were extracted using tert-butylmethyl ether, after which the components were separated on a RP C18 Hypersil Gold column and then injected into the LC-MS/MS using atmospheric pressure chemical ionization. The interassay coefficient of variations were as follows: androstenedione: 8% at 1.0 nmol/L and <5% at 3–23 nmol/L; and testosterone 7.4% at 0.95 nmol/L and <3.8% at

4–23 nmol/L. The lower limit of detection was <0.1 nmol/L for testosterone as well as androstenedione using 0.5 mL of sample volume.

Cardiometabolic Assessments

C-reactive protein (CRP), triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured using a Beckman Coulter AU5811 analyzer. The interassay coefficients of variations were as follows: CRP 6.5% at 0.9 mg/L and <2% at >2.5 mg/L; triglycerides: <2.1% at 1–2 mmol/L; cholesterol: <1.4% at 3.5–7 mmol/L; and HDL cholesterol: <1.4% at 1–2.2 mmol/L. Low-density lipoprotein (LDL) cholesterol was calculated, using the Friedewald formula (26). Insulin was measured using an electrochemiluminescence immunoassay on the Cobas E411 (Roche Diagnostics GmbH); the lower limit of detection was 2 mE/L, and the interassay variation was <4.4%.

Adiponectin was determined in a Quantikine ELISA (Acrp30; R&D Systems); the lower limit of detection was 3.9 ng/mL for undiluted samples. Plasma samples were diluted 500-fold, and the interassay variation coefficients were <6.5% at 30–175 ng/mL (n = 9 undiluted control samples).

Leptin was determined using the Human Leptin radioimmunoassay #HL-81HK (EMD Millipore); the lower limit of detection was 0.7 ng/mL. The interassay coefficients of variation were <6% at 4–60 μg/L (n = 7 undiluted control samples).

Definitions of Pregnancy Complications

Gestational diabetes mellitus was defined as two or more plasma glucose levels exceeding a given threshold after a 100-g glucose challenge: fasting glucose ≥5.3 mmol/L, 1 hour glucose ≥10.0 mmol/L, 2 hour glucose ≥8.6 mmol/L, 3 hour glucose ≥7.8 mmol/L (27). Pregnancy-induced hypertension was defined as blood pressure exceeding 140/90 mm Hg occurring in previously normotensive women beyond 20 weeks of gestation. Preeclampsia was defined as pregnancy-induced hypertension with proteinuria (≥300 mg/24 hours). Premature delivery was defined as a delivery before 37 weeks of gestational age. Birth weight percentiles were calculated using reference values for the Dutch population, which have been adjusted for gestational age, gender, parity, and ethnicity (28). Small for gestational age was defined as a birth weight below the 10th percentile, and large for gestational age was defined as a birth weight above the 90th percentile.

Statistical Analyses

Due to the non-normal distribution of baseline, endocrine and cardiometabolic characteristics, values were depicted as the median with interquartile range (IQR) or as absolute numbers with percentages. Maternal and neonatal baseline characteristics and prevalence of pregnancy complications were compared between the PCOS and the non-PCOS control populations using Mann-Whitney *U* tests for continuous variables and chi-square tests for categorical variables. Due to expected differences in sex hormone levels in male and female offspring, and potential differences in possible correlations with metabolic biomarkers, subanalyses for gender were performed.

Biomarker values under the detection limit were imputed as values at 50% of the lowest limit of detection. Biomarkers were excluded from all statistical analyses when >50% of measured values were under the lower limit of detection. Mann-Whitney *U* tests were used to assess differences in median cord blood concentrations between PCOS and control offspring, also stratified for gender. Linear regression analysis was used to assess the potential associations between log-transformed cord blood concentrations and PCOS status in both male and female offspring.

Analyses were adjusted for potential confounding factors that were known or expected to be related to either PCOS or the assessed cardiometabolic/endocrine biomarkers using three different models. Model 1 (maternal characteristics) was adjusted for maternal ethnicity, maternal age at delivery, maternal body mass index (BMI) in the first trimester of pregnancy, parity at inclusion, and mode of conception. Model 2 (pregnancy characteristics) was adjusted for birth weight, gestational age at birth, presence of gestational diabetes and/or large for gestational age, presence of maternal placental syndromes (i.e., pregnancy-induced hypertension and/or preeclampsia and/or small for gestational age), infection, and mode of delivery. Model 3 was adjusted for all the variables in Model 1 + Model 2.

The results of the linear regression analyses were back-transformed to show relative changes in cord blood parameters among the groups. Due to the exploratory design of the current investigation, false-discovery rates were calculated to allow correction for multiple testing. Finally, bivariate Pearson's correlation coefficients between log-transformed cord blood parameters were calculated within groups. All statistics were performed using SPSS, version 21.0 (IBM). $P<.05$ was considered statistically significant.

RESULTS

The maternal and neonatal baseline characteristics are shown in Table 1. Parity at inclusion was higher in the controls compared with the women with PCOS ($P=.002$). Pregnancies in women with PCOS were more often established through infertility therapy compared with the controls ($P<.001$), and the treatment consisted mostly of ovulation induction (64% of PCOS pregnancies). At delivery, the women with PCOS were slightly younger compared with the controls (31.4; IQR 28.6–33.2) years versus 32.8 (IQR: 29.6–36.4) years ($P=.048$). Furthermore, pregnancies in women with PCOS were more often complicated with gestational diabetes mellitus (26% vs. 12%, $P=.047$).

The median cord blood concentrations in the offspring of women with PCOS and the controls are shown in Table 2, stratified for gender. The CRP measurements were excluded from all analyses because over 90% of measured values were under the lower limit of detection. Less than 4% of all reported values were below the lower limit of detection, and hence were input (Materials and Methods).

TABLE 1

Maternal and neonatal baseline characteristics in 61 PCOS cases and 82 controls.

Characteristic	PCOS offspring (n = 61)	Control offspring (n = 82)	*P* value
Maternal ethnicity			
Caucasian	58 (95)	73 (89)	.20
Maternal parity at inclusion	0 (0–0)	0 (0–1)	.002
Method of conception			
Spontaneous	13 (21)	58 (71)	< .001
Ovulation induction	39 (64)	—	
IVF/ICSI	8 (13)	22 (27)	
Other	1 (2)	2 (2)	
Maternal body mass index first trimester	24.2 (21.1–28.2)	24.4 (22.5–28.2)	.62
Ever smoked during pregnancy	6 (10)	9 (11)	.48
Maternal age at delivery (y)	31.4 (28.6–33.2)	32.8 (29.6–36.4)	.048
Gender offspring			
Boys	36 (59)	39 (48)	.18
Girls	25 (41)	43 (52)	
Ethnicity offspring[a]			
Caucasian	56 (92)	68 (84)	.16
Gestational age at birth (d)	278 (272–284)	276 (266–280)	.08
Birthweight (g)	3,465 (3,103–3,755)	3,300 (3,014–3,714)	.30
Mode of delivery			
Vaginal unassisted	38 (62)	51 (63)	.005
Vaginal assisted	15 (25)	7 (9)	
Planned cesarean delivery	3 (5)	10 (12)	
Unplanned cesarean delivery	5 (8)	13 (16)	
Maternal pregnancy complications			
Gestational diabetes	16 (26)	10 (12)	.047
Pregnancy induced hypertension	8 (13)	7 (9)	.38
Premature delivery	3 (5)	6 (7)	.56
Preeclampsia	1 (2)	2 (2)	.74
Infection	1 (2)	1 (1)	.83
SGA	3 (5)	5 (6)	.68
LGA	7 (12)	9 (11)	.86

Note: Values represent median (interquartile range), or absolute number (percentage). Differences were assessed using chi-square tests for categorical variables, and Mann-Whitney *U* tests for continuous variables. Variables contained a maximum of 7% missing values. ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; LGA = large for gestational age, defined as birthweight >P90; PCOS = polycystic ovary syndrome; SGA = small for gestational age, defined as birthweight <P10.

[a] Based on both maternal and paternal ethnicity.

Daan. Cord blood characteristics with PCOS. Fertil Steril 2016.

Androstenedione and leptin concentrations were higher in the PCOS offspring compared with the controls (androstenedione: 2.9 [2.3–3.9] nmol/L vs 2.2 [1.6–2.7] nmol/L, $P<.001$; leptin: 13.6 [8.3–22.9] μg/L vs. 9.8 [6.0–16.5] μg/L, $P=.01$). The androstenedione concentrations were statistically significantly higher in both the male and female PCOS offspring compared with the controls ($P<.001$ and $P=.004$, respectively; Supplemental Fig. 1, available online, for the box plots). Other cord blood parameters did not statistically significantly differ between the cases and controls.

Adjusted associations between PCOS and cord blood parameters are depicted in Table 3 for male offspring and in Table 4 for female offspring. After adjustment for maternal and pregnancy related confounders, the associations between cord blood androstenedione concentrations and PCOS appeared statistically significant in all models in male and female offspring (fully adjusted model 3: boys, relative change 1.36 (1.04; 1.78); $P=.028$; girls, relative change 1.40 (1.08; 1.82); $P=.012$). Similarly, in male PCOS offspring, the leptin concentrations appeared statistically significantly associated with PCOS after correction for confounders in all models (fully adjusted model 3: relative change 1.55 (1.12; 2.14), $P=.009$). After correction for multiple testing, all associations attenuated and lost statistical significance.

Finally, correlations between all cord blood parameters within the groups were assessed (Supplemental Figs. 2 and 3, available online). Correlations between the cord blood parameters differed within the gender groups. In the male PCOS offspring, statistically significant correlations were observed between DHEAS and lipids (triglycerides: $r=-0.39$, $P=.02$; total cholesterol: $r=-0.45$, $P=.01$; LDL cholesterol: $r=-0.44$, $P=.01$), and between leptin and adiponectin ($r=0.40$, $P=.02$). In the female PCOS offspring, no statistically significant correlations were observed between androgens and cardiometabolic cord blood parameters, including leptin and adiponectin. In the male and female control offspring, androstenedione was correlated with triglycerides (male: $r=0.39$, $P=.02$; female: $r=0.36$, $P=.02$). In the male controls, leptin was correlated with insulin ($r=0.44$, $P=.01$) and CRP ($r=0.33$, $P=.045$), whereas in female controls leptin was correlated only with estradiol ($r=-0.37$, $P=.02$).

TABLE 2

Differences in median serum parameters between 61 PCOS offspring and 82 controls.

	Male offspring						Female offspring			
Parameter[a]	PCOS offspring (n = 61)	Control offspring (n = 82)	*P* value	PCOS (n = 36)	Control (n = 39)	*P* value	PCOS (n = 25)	Control (n = 43)	*P* value
E_2 (nmol/L)	17.3 (11.4–24.7)	17.6 (10.3–23.2)	.76	18.5 (13.2–26.0)	19.9 (10.3–27.2)	.60	15.6 (10.8–21.7)	15.9 (9.9–22.7)	.69
AD (nmol/L)	2.9 (2.3–3.9)	2.2 (1.6–2.7)	<.001[b]	3.2 (2.3–4.5)	2.2 (1.6–2.6)	<.001[b]	2.8 (2.3–3.7)	2.3 (1.7–2.7)	.004[b]
DHEAS (μmol/L)	11.0 (8.7–14.0)	10.0 (8.3–14.3)	.57	12.0 (8.2–15.0)	11.0 (9.1–16.0)	.83	11.0 (8.8–13.0)	9.7 (6.5–14.0)	.33
T (nmol/L)	0.4 (0.3–0.5)	0.3 (0.2–0.5)	.15	0.4 (0.3–0.6)	0.4 (0.3–0.7)	.37	0.3 (0.2–0.3)	0.3 (0.1–0.4)	.39
SHBG (nmol/L)	37.0 (29.0–44.5)	35.0 (27.8–46.0)	.73	38.0 (29.0–45.8)	35.0 (27.0–40.0)	.20	33.5 (29.5–41.0)	35.0 (28.0–49.0)	.70
FAI	0.9 (0.6–1.5)	0.8 (0.6–1.4)	.27	1.2 (0.8–1.8)	1.2 (0.8–2.0)	.98	0.7 (0.5–1.0)	0.7 (0.5–0.9)	.36
Insulin (μU/mL)	5.5 (2.7–8.3)	4.2 (1.9–8.1)	.39	4.9 (2.2–7.9)	3.9 (2.3–7.7)	.94	5.7 (2.8–9.0)	4.2 (1.6–8.4)	.22
Total C (mmol/L)	1.7 (1.5–2.1)	1.6 (1.4–2.0)	.51	1.7 (1.4–2.0)	1.6 (1.4–1.9)	.48	1.7 (1.5–2.1)	1.7 (1.4–2.1)	.74
LDL-C (mmol/L)	0.8 (0.7–1.0)	0.7 (0.6–1.0)	.18	0.9 (0.7–1.0)	0.7 (0.6–0.9)	.14	0.9 (0.7–1.0)	0.8 (0.6–1.1)	.58
HDL-C (mmol/L)	0.7 (0.6–0.9)	0.7 (0.6–0.9)	.92	0.7 (0.6–0.8)	0.7 (0.6–0.8)	.71	0.7 (0.7–0.9)	0.7 (0.6–0.9)	.54
Triglycerides (mmol/L)	0.4 (0.3–0.6)	0.4 (0.3–0.6)	.73	0.4 (0.3–0.5)	0.4 (0.2–0.5)	.37	0.4 (0.3–0.6)	0.4 (0.3–0.6)	.62
Adiponectin (μg/mL)	36.8 (28.8–43.2)	32.6 (25.1–42.3)	.15	38.4 (27.7–46.5)	32.3 (23.5–41.8)	.14	36.0 (29.8–41.2)	32.9 (25.2–43.5)	.69
Leptin (μg/L)	13.6 (8.3–22.9)	9.8 (6.0–16.5)	.01[b]	11.7 (8.3–21.8)	8.7 (5.1–14.7)	.05	14.4 (9.2–27.6)	10.6 (6.6–18.4)	.07

Note: Values represent median concentrations (interquartile ranges), differences are assessed using Mann-Whitney *U* tests. AD = androstenedione; C = cholesterol; DHEAS = dehydroepiandrosterone sulfate; E_2 = estradiol; FAI = free androgen index (testosterone/SHBG × 100); HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PCOS = polycystic ovary syndrome; SHBG = sex hormone binding globulin; T = testosterone.

[a] Conversion factors from SI units to conventional units when applicable: estradiol x 1000/3.671 = concentration in pg/dL; androstenedione/0.0349 = concentration in ng/dL; DHEAS/0.027 = concentration in μg/dL; testosterone/0.0347 = concentration in ng/dL; SHBG/8.896 = concentration in μg/mL; total cholesterol/0.0259 = concentration in mg/dL; LDL-C/0.0259 = concentration in mg/dL; HDL-C/0.0259 = concentration in mg/dL; triglycerides/0.0113 = concentration in mg/dL.

[b] Significant (*P*<.05).

Daan. Cord blood characteristics with PCOS. Fertil Steril 2016.

DISCUSSION

Our current study compared both endocrine and cardiometabolic cord blood characteristics between the offspring of women with PCOS and non-PCOS controls. We observed that androstenedione and leptin concentrations were increased in PCOS offspring compared with controls. After adjusting for potential maternal and pregnancy confounders (such as maternal age, gestational age, and birth weight), the androstenedione concentrations appeared to be associated with PCOS in both male and female offspring; however, these associations attenuated after correction for multiple testing. Similarly, leptin concentrations appeared associated with PCOS in male offspring after adjusting for confounders, although statistical significance was lost after correction for multiple testing.

Few studies have been published regarding the cord blood characteristics of the offspring of women with PCOS, and they have yielded conflicting results. Two previous investigations reported increased cord blood testosterone concentrations in the offspring of women with PCOS (18, 19). In both reports androstenedione concentrations were not assessed, and different testosterone immunoassays were employed. One previous report observed no differences in cord blood androgens when comparing the offspring of women with PCOS and controls, despite increased maternal serum testosterone and androstenedione concentrations at midgestation and at birth (21). Although highly sensitive mass spectrometry methods were used, the sample size was limited to only 20 PCOS offspring.

Contrary to our findings, two previous studies reported lower cord blood androstenedione concentrations (assessed by mass spectrometry or immunoassay) in female offspring of women with PCOS compared with controls (15, 20). In one of these reports, maternal androstenedione and testosterone concentrations were found to be increased in women with PCOS at late gestation (15). Furthermore, altered placental aromatase activities were reported in women with PCOS, which may increase circulating androgen concentrations (15). The investigators hypothesized that the decrease in cord blood androstenedione could reflect fetal modifications in steroid metabolism (15). Hence, the differences in the results between our investigation and previous reports could potentially be influenced by variations in placental tissue activity among women with PCOS, along with differences in fetal adaptation. Furthermore, potential differences in the applied diagnostic criteria for PCOS, the presence of maternal hyperandrogenism, maternal BMI and ethnicity, and differences in laboratory assays and statistical analyses may account for the wide variations in the reported results.

The results from our investigation might support the hypothesis that a hyperandrogenic environment during pregnancy in women with PCOS may predispose the offspring to fetal hyperandrogenism. Previous studies in primates established that fetal exposure to exogenous testosterone during early to midgestation induces endocrine, reproductive,

TABLE 3

Associations between PCOS and cord blood parameters in male offspring.

	Model 1[b]			Model 2[c]			Model 3[d]		
Parameter[a]	Relative change	*P* value	FDR	Relative change	*P* value	FDR	Relative change	*P* value	FDR
E_2 (nmol/L)	1.01 (0.77; 1.32)	.93	.51	1.06 (0.80; 1.41)	.70	.48	0.99 (0.72; 1.36)	.97	.51
AD (nmol/L)	1.41 (1.12; 1.77)	.004[e]	.05	1.41 (1.11; 1.80)	.006[e]	.08	1.36 (1.04; 1.78)	.028[e]	.09
DHEAS (μmol/L)	0.85 (0.67; 1.07)	.16	.29	0.77 (0.60; 0.99)	.044[e]	.11	0.75 (0.57; 0.98)	.035[e]	.09
T (nmol/L)	1.03 (0.73; 1.44)	.87	.51	1.02 (0.73; 1.43)	.89	.49	0.93 (0.64; 1.36)	.72	.51
SHBG (nmol/L)	1.14 (0.93; 1.38)	.20	.29	1.01 (0.83; 1.23)	.92	.49	0.98 (0.80; 1.21)	.89	.51
FAI	0.91 (0.62; 1.32)	.60	.46	1.01 (0.69; 1.48)	.95	.49	0.95 (0.62; 1.45)	.80	.51
Insulin (μU/mL)	0.83 (0.48; 1.45)	.51	.44	1.12 (0.64; 1.97)	.68	.48	0.88 (0.47; 1.67)	.69	.51
Total cholesterol (mmol/L)	1.07 (0.93; 1.24)	.32	.32	1.04 (0.90; 1.20)	.58	.48	1.05 (0.89; 1.23)	.56	.51
LDL-C (mmol/L)	1.22 (0.97; 1.52)	.08	.21	1.15 (0.93; 1.43)	.19	.35	1.19 (0.92; 1.54)	.17	.32
HDL-C (mmol/L)	1.00 (0.85; 1.17)	.98	.51	0.95 (0.81; 1.12)	.57	.48	0.97 (0.81; 1.17)	.79	.51
Triglycerides (mmol/L)	1.04 (0.81; 1.32)	.77	.51	1.06 (0.83; 1.35)	.65	.48	1.00 (0.78; 1.29)	.98	.51
Adiponectin (μg/mL)	1.13 (0.92; 1.39)	.26	.31	1.05 (0.85; 1.29)	.68	.48	1.06 (0.84; 1.33)	.61	.51
Leptin (μg/L)	1.52 (1.09; 2.14)	.016[e]	.07	1.47 (1.07; 2.02)	.018[e]	.08	1.55 (1.12; 2.14)	.009[e]	.09

Note: Values represent relative changes (confidence intervals), with female non-PCOS offspring as the reference population. AD = androstenedione; BMI = body mass index; DHEAS = dehydroepiandrosterone sulfate; E_2 = estradiol; FAI = free androgen index (testosterone/SHBG × 100); FDR = false-discovery rate, which reflects the *P* value after correction for multiple testing; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PCOS = polycystic ovary syndrome; SHBG = sex hormone binding globulin; T = testosterone.

[a] Conversion factors from SI units to conventional units when applicable: estradiol × 1000/3.671 = concentration in pg/dL; androstenedione/0.0349 = concentration in ng/dL; DHEAS/0.027 = concentration in μg/dL; testosterone/0.0347 = concentration in ng/dL; SHBG/8.896 = concentration in μg/mL; total cholesterol/0.0259 = concentration in mg/dL; LDL-C/0.0259 = concentration in mg/dL; HDL-C/0.0259 = concentration in mg/dL; triglycerides/0.0113 = concentration in mg/dL.

[b] Model 1: adjusted for maternal ethnicity, maternal age at delivery, maternal BMI, parity, and mode of conception.

[c] Model 2: adjusted for birthweight. gestational age, presence of gestational diabetes and/or large for gestational age, presence of maternal placental syndromes(i.e., pregnancy-induced hypertension and/or preeclampsia and/or small for gestational age), infection, and mode of delivery.

[d] Model 3: Model 1 + Model 2.

[e] Significant ($P < .05$).

Daan. Cord blood characteristics with PCOS. Fertil Steril 2016.

and metabolic PCOS traits in the offspring (11, 13, 29). These observations strongly suggest that aside from an inherent genetic predisposition, androgen excess in utero may affect multiple organ systems, thereby supporting a potential fetal origin for the development of PCOS and cardiometabolic complications in later life (11).

Increased androgen concentrations (androstenedione, testosterone, DHEAS) during pregnancy have also been reported in women with PCOS (14, 21, 29). Maternal androgens might cross the placental passage and contribute to higher fetal androgen levels. Moreover, the placenta itself produces androgens (androstenedione, testosterone),

TABLE 4

Associations between PCOS and cord blood parameters in female offspring.

	Model 1[b]			Model 2[c]			Model 3[d]		
Parameter[a]	Relative change	*P* value	FDR	Relative change	*P* value	FDR	Relative change	*P* value	FDR
E_2 (nmol/L)	0.81 (0.57; 1.15)	.23	.24	0.88 (0.65; 1.19)	.39	.40	0.78 (0.53; 1.15)	.21	.32
AD (nmol/L)	1.39 (1.02; 1.91)	.040[e]	.16	1.30 (1.05; 1.62)	.019[e]	.25	1.40 (1.08; 1.82)	.012[e]	.16
DHEAS (μmol/L)	1.15 (0.89; 1.51)	.28	.24	1.10 (0.85; 1.42)	.47	.40	1.16 (0.85; 1.57)	.35	.41
T (nmol/L)	0.98 (0.67; 1.43)	.93	.51	1.10 (0.80; 1.49)	.58	.40	1.01 (0.69; 1.47)	.96	.50
SHBG (nmol/L)	0.80 (0.64; 1.01)	.06	.16	0.97 (0.81; 1.17)	.76	.43	0.86 (0.68; 1.09)	.22	.32
FAI	1.22 (0.86; 1.75)	.27	.24	1.11 (0.81; 1.53)	.51	.40	1.17 (0.78; 1.74)	.44	.44
Insulin (μU/mL)	2.07 (1.10; 3.93)	.025[e]	.16	1.33 (0.78; 2.27)	.29	.40	1.80 (0.94; 3.45)	.07	.30
Total cholesterol (mmol/L)	1.00 (0.85; 1.18)	.98	.51	1.06 (0.91; 1.22)	.51	.40	1.02 (0.84; 1.23)	.87	.49
LDL-C (mmol/L)	1.04 (0.83; 1.30)	.74	.46	0.92 (0.70; 1.21)	.56	.40	1.07 (0.92; 1.14)	.59	.45
HDL-C (mmol/L)	1.03 (0.88; 1.20)	.73	.46	1.02 (0.89; 1.17)	.73	.43	0.98 (0.83; 1.17)	.86	.49
Triglycerides (mmol/L)	0.84 (0.63; 1.13)	.24	.24	1.11 (0.83; 1.47)	.48	.40	0.91 (0.66; 1.26)	.57	.45
Adiponectin (μg/mL)	0.93 (0.71; 1.22)	.59	.45	0.99 (0.78; 1.26)	.92	.48	1.05 (0.77; 1.42)	.75	.49
Leptin (μg/L)	1.43 (0.90; 2.25)	.12	.22	1.31 (0.92; 1.86)	.13	.40	1.35 (0.84; 2.15)	.21	.32

Note: Values represent relative changes (confidence intervals), with female non-PCOS offspring as the reference population. AD = androstenedione; BMI = body mass index; DHEAS = dehydroepiandrosterone sulfate; E_2 = estradiol; FAI = free androgen index (testosterone/SHBG × 100); FDR = false-discovery rate, which reflects the *P* value after correction for multiple testing; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PCOS = polycystic ovary syndrome; SHBG = sex hormone binding globulin; T = testosterone.

[a] Conversion factors from SI units to conventional units when applicable: estradiol × 1000/3.671 = concentration in pg/dL; androstenedione/0.0349 = concentration in ng/dL; DHEAS/0.027 = concentration in μg/dL; testosterone/0.0347 = concentration in ng/dL; SHBG/8.896 = concentration in μg/mL; total cholesterol/0.0259 = concentration in mg/dL; LDL-C/0.0259 = concentration in mg/dL; HDL-C/0.0259.

[b] Model 1: adjusted for maternal ethnicity, maternal age at delivery, maternal BMI, parity, and mode of conception.

[c] Model 2: adjusted for birthweight. gestational age, presence of gestational diabetes and/or large for gestational age, presence of maternal placental syndromes (i.e., pregnancy-induced hypertension and/or preeclampsia and/or small for gestational age), infection, and mode of delivery.

[d] Model 3: Model 1 + Model 2. = concentration in mg/dL; triglycerides/0.0113 = concentration in mg/dL.

[e] Significant ($P < .05$).

Daan. Cord blood characteristics with PCOS. Fertil Steril 2016.

which—under normal circumstances—are rapidly converted to estrogens due to placental aromatase activity (14). Reduced placental aromatase activity in women with PCOS—for instance, through the inhibitory effects of insulin—may contribute to increased circulating androgen levels (14, 17).

Cord blood estrogens and androgens are derived from both fetal adrenal as well as placental steroidogenesis (20, 30). Placental steroidogenesis depends on maternal and fetal steroid precursor hormones (20, 30). The fetal adrenals actively produce DHEAS in utero, which is converted to androstenedione, testosterone, and estradiol by the placenta. Hence, cord blood endocrine characteristics represent both the fetal and maternal endocrine environments (20, 30, 31). In a post hoc analysis to assess the potential effect of the maternal endocrine environment, we compared cord blood androstenedione concentrations between the offspring of mothers with PCOS with a hyperandrogenic mother (n = 25, 41%), and the offspring of mothers with PCOS with a normoandrogenic mother; we found no statistically significant differences for either male and female offspring (data not shown). Although the maternal androgen levels were not reassessed during pregnancy, this finding suggests that maternal androgens may not be the sole determinant of fetal androgen production in PCOS offspring.

To our knowledge only a single previous study assessed leptin and adiponectin concentrations in the cord blood of the offspring of mothers with PCOS; that study reported a higher leptin concentration in these offspring, although correction for multiple testing was not applied (29). In the aforementioned investigation, Maliqueo et al. (29) found that leptin cord blood concentrations appeared to be associated with the mother's BMI at midpregnancy and the birth weight, and thus may be a reflection of fetal adiposity. In our investigation, leptin was statistically significantly associated with birth weight (r=0.44, P<.001) as well as birth percentile (r=0.26, P=.04) in the offspring of mothers with PCOS, but not with maternal BMI during the first trimester (data not shown).

Out of all the androgens we assessed, only crude androstenedione concentrations showed a statistically significant difference between the cases and controls. In current routine clinical practice, androstenedione is inconsistently assessed due to the remaining uncertainties concerning its diagnostic potential in PCOS (32). It is known that patterns of hyperandrogenism may differ among women with PCOS, as they are influenced by factors such as age, BMI, and insulin levels (33, 34). A recent observation has suggested that serum androstenedione may be the most sensitive indicator of androgen excess in women with PCOS because more than two-thirds of women with PCOS who were categorized as normoandrogenic based on testosterone levels exhibited increased androstenedione levels (35). Furthermore, that study observed a strong negative relationship between androstenedione (not testosterone) and insulin sensitivity, independent of age and BMI (35). These results emphasize the diagnostic potential of androstenedione when evaluating hyperandrogenism and associated cardiometabolic abnormalities in PCOS. The results from our study further support those observations.

We observed differences in correlations between cord blood hormone levels and cardiometabolic parameters among the groups. In our study, cord blood androgens were statistically significantly associated with lipid levels in the male offspring of mothers with PCOS (DHEAS), and in the male and female offspring of our controls (androstenedione). Cholesterol serves as a precursor for androgen synthesis, which may explain this association. Differences in these associations between groups may be influenced by variations in body composition, which if persistent may influence future cardiovascular risk. Hyperandrogenism and adiposity are closely related because adiposity increases circulating insulin levels, which inhibits the hepatic production of SHBG and leads to higher levels of bioactive testosterone (36). Moreover, insulin and insulin-like growth factor I directly stimulate the ovarian synthesis of androgens (37).

To the best of our knowledge, ours is the largest study in which cord blood characteristics were compared between offspring of women with PCOS and non-PCOS controls, including stratification by gender. A highly sensitive LC-MS/MS method was used for the measurement of cord blood androgen levels, resulting in high assessment accuracy for androgen levels. Furthermore, the included study population was extensively phenotyped in a standardized fashion, allowing correction for numerous maternal and pregnancy-related confounders. Whether these confounders are causative for or are a result of PCOS remains unsettled due to the unknown etiology of this condition and the cross-sectional design of our study investigation.

A potential limitation of the current investigation is that mixed arteriovenous cord blood samples were used, as in the vast majority of other studies. Although small variations may exist between arterial and venous steroid concentrations, strong correlations have been previously described (38). Another potential limitation could be the difference in mode of conception between the two groups, although this was adjusted for in the statistical analyses. Furthermore, small differences in sample handling between the groups were present. However, based on the known stability of the assessed biomarkers, it is unlikely that this exerted a substantial effect on the reported results. Due to the cross-sectional design of this study, we could not assess the potential associations between increased androstenedione in cord blood and the future development of PCOS traits in the offspring. One follow-up study regarding the association between cord blood androgen levels (testosterone, androstenedione, DHEAS) in normal pregnancies and the development of PCOS in adolescence has been performed, which reported no statistically significant association between either feature (39).

In conclusion, the results from our investigation suggest that androstenedione concentrations are increased in the cord blood of male and female offspring of mothers with PCOS, although this requires confirmation. This finding would support the hypothesis that a maternal hyperandrogenic environment during pregnancy in women with PCOS may predispose the offspring to fetal hyperandrogenism. The potential associations between fetal hyperandrogenism and long-term health effects remain to be elucidated.

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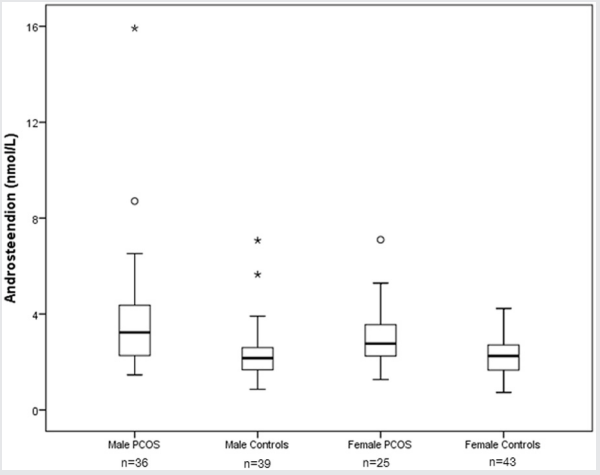
SUPPLEMENTAL FIGURE 1



Box plots reflecting androstenedione concentrations within each study group. The circles represent outliers of >1.5 times the interquartile range, and asterisks represent outliers of >3 times the interquartile range. One asterisk in the female controls is not displayed (29.05 nmol/L).

Daan. Cord blood characteristics with PCOS. Fertil Steril 2016.

SUPPLEMENTAL FIGURE 2

Male PCOS offspring

	AD	DHEAS	SHBG	E2	INS	CRP	TRIG	CHOL	LDL	HDL	T	FAI	Adipo	Leptin
AD	1	-,08	-,16	,07	,10	-,13	-,01	,08	,11	,08	,29	,31	-,01	,21
DHEAS	-,08	1	-,18	,18	,13	-0,34	-0,39	-0,45	-0,44*	-,09	,09	,17	,26	-,25
SHBG	-,16	-,18	1	-,18	-,29	0,69*	0,48*	0,33*	,19	,25	-,13	-0,70*	,18	-,01
E2	,07	,18	-,18	1	-,09	-,15	,17	-,17	-,15	-,19	-,10	,04	-,03	-,03
INS	,10	,13	-,29	-,09	1	-0,40*	-,23	-,30	-,32	-,02	,11	,26	,27	-,06
CRP	-,13	-0,34*	0,69*	-,15	-0,40*	1	0,57*	0,61*	0,53*	,26	-,10	-0,48*	,15	,11
TRIG	-,01	-0,39*	0,48*	,17	-,23	0,57*	1	,28	,23	-,14	-,05	-,32	,13	,09
CHOL	,08	-0,45*	0,33*	-,17	-,30	0,61*	,28	1	0,94*	0,68*	-,02	-,21	-,23	,02
LDL	,11	-0,44*	,19	-,15	-,32	0,53*	,23	0,94*	1	0,47*	,07	-,06	-,26	,06
HDL	,08	-,09	,25	-,19	-,02	,26	-,14	0,68*	0,47*	1	-,14	-,25	-,04	-,00
T	,29	,09	-,13	-,10	,11	-,10	-,05	-,02	,07	-,14	1	0,80*	-,03	,00
FAI	,31	,17	-0,70*	,04	,26	-0,48*	-,32	-,21	-,06	-,25	0,80*	1	-,13	,01
Adipo	-,01	,26	,18	-,03	,27	,15	,13	-,23	-,26	-,04	-,03	-,13	1	0,40*
Leptin	,21	-,25	-,01	-,03	-,06	,11	,09	,02	,06	-,00	,00	,01	0,40*	1

Male Control offspring

	AD	DHEAS	SHBG	E2	INS	CRP	TRIG	CHOL	LDL	HDL	T	FAI	Adipo	Leptin
AD	1	,27	,02	0,56*	-,09	,02	0,39*	,05	-,07	,04	,17	,18	0,32*	,18
DHEAS	,27	1	-,04	,024	-,05	,19	,04	,03	-,02	,03	,20	,23	0,33*	,15
SHBG	,02	-,04	1	,01	-,25	-,25	,28	,31	,19	,19	0,40*	,01	,17	-,09
E2	0,56*	,02	,01	1	-,23	-,24	,31	,07	-,08	,09	-,31	-0,34*	,00	-,14
INS	-,09	-,05	-,25	-,23	1	,09	-,09	-0,33	-0,34	,01	-,17	-,07	-,10	0,44*
CRP	,02	,19	-,25	-,24	,09	1	,03	-,02	-,01	-,01	-,08	,02	,04	0,33*
TRIG	0,39*	,04	,28	,31	-,09	,03	1	,11	-,03	-,25	-,02	-,15	,02	,07
CHOL	,05	,03	,31	,07	-0,33*	-,02	,11	1	0,83*	0,66*	,12	,00	,31	,01
LDL	-,07	-,02	,19	-,08	-0,34*	-,01	-,03	0,83*	1	,30	,20	,14	0,35*	,07
HDL	,04	,03	,19	,09	,01	-,01	-,25	0,66*	,30	1	-,00	-,08	,22	,04
T	,17	,20	0,40*	-,31	-,17	-,08	-,02	,12	,20	-,00	1	0,92*	0,41*	,11
FAI	,18	,23	,01	-0,34*	-,07	,02	-,15	,00	,14	-,08	0,92	1	0,38*	,16
Adipo	0,32*	0,33*	,17	,00	-,10	,04	,02	,31	0,35*	,22	0,41*	0,38*	1	,20
Leptin	,18	,15	-,09	-,14	0,44*	0,33*	,07	,01	,07	,04	,11	,16	,20	1

Pearson's correlation coefficients between log-transformed cord blood parameters within male offspring. *$P<.05$, statistically significant difference. AD = androstenedione; Adipo = adiponectin; CHOL = total cholesterol; CRP = c-reactive protein; DHEAS = dehydroepiandrosterone sulfate; E_2 = estradiol; FAI = free androgen index (T/SHBG × 100); HDL = high-density lipoprotein cholesterol; INS = insulin; LDL = low-density lipoprotein cholesterol; PCOS = polycystic ovary syndrome; SHBG = sex hormone-binding globulin; T = testosterone; TRIG = triglycerides.

Daan. Cord blood characteristics with PCOS. Fertil Steril 2016.

SUPPLEMENTAL FIGURE 3

Female PCOS offspring

	AD	DHEAS	SHBG	E2	INS	CRP	TRIG	CHOL	LDL	HDL	T	FAI	Adipo	Leptin
AD	1	,00	-,20	,07	,05	,18	-,19	,22	,12	,34	0,51*	0,53*	,15	,02
DHEAS	,00	1	-,15	,14	-,02	-,23	,34	,12	-,20	,01	-,10	,02	,14	,16
SHBG	-,20	-,15	1	0,44*	-0,42*	-,24	0,40	-,09	-,15	-,09	,02	-0,64*	,36	,09
E2	,07	,14	0,44*	1	-0,64*	-,22	,27	,13	,02	,04	,01	-,28	-,08	-,19
INS	,05	-,02	-0,42*	-0,64*	1	,08	-0,50*	-,04	,22	,08	-,08	,21	-,08	-,04
CRP	,18	-,23	-,24	-,22	,08	1	-,09	,28	,16	,35	-,04	,13	-0,40*	-,18
TRIG	-,19	,34	0,40	,27	-0,50*	-,09	1	-,11	-0,65*	-,23	-,12	-,36	,32	,02
CHOL	,22	,12	-,09	,13	-,04	,28	-,11	1	0,56*	0,86*	-,09	-,01	,11	-,37
LDL	,12	-,20	-,15	,02	,22	,16	-0,65*	0,56	1	,38	-,06	,05	-,11	-,25
HDL	,34	,01	-,09	,04	,08	,35	-,23	0,86	,38	1	-,04	,03	,09	-,36
T	0,51*	-,10	,02	,01	-,08	-,04	-,12	-,09	-,06	-,04	1	0,76*	,20	-,03
FAI	0,53*	,02	-0,64*	-,28	,21	,13	-,36	-,01	,05	,03	0,76*	1	-,09	-,08
Adipo	,15	,14	,36	-,08	-,08	-0,40*	,32	,11	-,11	,09	,20	-,09	1	-,02
Leptin	,02	,16	,09	-,19	-,04	-,18	,02	-,37	-,25	-,36	-,03	-,08	-,02	1

Female Control offspring

	AD	DHEAS	SHBG	E2	INS	CRP	TRIG	CHOL	LDL	HDL	T	FAI	Adipo	Leptin
AD	1	0,38*	0,54*	,18	-,26	0,53*	0,36*	,22	,26	-,01	0,47*	,21	,24	,10
DHEAS	0,38*	1	,05	0,45*	-,03	-,04	,12	-,05	-,04	-,08	-,02	-,06	,03	-,10
SHBG	0,54*	,05	1	,12	-0,62*	,29	0,56*	,17	,13	-,10	0,55*	-,01	0,40*	-,02
E2	,18	0,45*	,12	1	-,25	-,17	,28	,22	,15	,20	,25	,21	,16	-0,37*
INS	-,26	-,03	-0,62*	-,25	1	-,22	-0,49*	-,29	-,23	-,10	-0,57*	-,27	-,29	,15
CRP	0,53*	-,04	,29	-,17	-,22	1	0,38*	0,32*	0,35*	,08	,28	,14	,10	,25
TRIG	0,36*	,12	0,56*	,28	-0,49*	0,38*	1	,26	,23	-,17	,28	-,03	,20	,07
CHOL	,22	-,05	,17	,22	-,29	0,32*	,26	1	0,96*	0,81*	,23	,17	-,09	-,14
LDL	,26	-,04	,13	,15	-,23	0,35*	,23	0,96*	1	0,67*	,27	,23	-,07	-,04
HDL	-,01	-,08	-,10	,20	-,10	,08	-,17	0,81*	0,67*	1	,05	,12	-,16	-,26
T	0,47*	-,02	0,55*	,25	-0,57*	,28	,28	,23	,27	,05	1	0,83	0,43*	,07
FAI	,21	-,06	-,01	,21	-,27	,14	-,03	,17	,23	,12	0,83*	1	,25	,10
Adipo	,24	,03	0,40*	,16	-,29	,10	,20	-,09	-,07	-,16	0,43*	,25	1	,27
Leptin	,10	-,10	-,02	-0,37*	,15	,25	,07	-,14	-,04	-,26	,07	,10	,27	1

Pearson's correlation coefficients between log-transformed cord blood parameters within female offspring. *$P<.05$, statistically significant difference. AD = androstenedione; Adipo = adiponectin; CHOL = total cholesterol; CRP = c-reactive protein; DHEAS = dehydroepiandrosterone sulfate; E_2 = estradiol; FAI = free androgen index (T/SHBG × 100); HDL = high-density lipoprotein cholesterol; INS = insulin; LDL = low-density lipoprotein cholesterol; PCOS = polycystic ovary syndrome; SHBG = sex hormone-binding globulin; T = testosterone; TRIG = triglycerides.

Daan. Cord blood characteristics with PCOS. Fertil Steril 2016.