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Placental steroidogenesis in pregnant women with polycystic ovary syndrome

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ABSTRACT

Objective: To evaluate the placental activity of steroid sulfatase (STS), 3β-hydroxysteroid dehydrogenase type 1 (3β-HSD-1) and P450 aromatase (P450arom) in polycystic ovarian syndrome (PCOS) compared to normal pregnant women.

Design: Twenty pregnant women with PCOS and 30 control pregnant women who delivered at term were studied. Samples of placental tissue and cord blood were obtained after delivery. A maternal blood sample was obtained during the 34th week of gestation. In placental tissue, the activities of STS, 3β -HSD-1 and P450arom were evaluated. In the blood samples, progesterone, DHEAS, DHEA, androstenedione, testosterone, estrone, estradiol and total estriol were determined.

Result: In placental tissue from women with PCOS, higher 3 β -HSD-1 and lower P450 aromatase activities were observed compared to control women. Moreover, women with PCOS showed higher androstenedione and testosterone concentrations compared to normal pregnant women (p = 0.016 and p = 0.025, respectively). In cord blood, female newborns of women with PCOS exhibited lower androstenedione and higher estriol concentrations compared to daughters of control women (p = 0.038; p = 0.031, respectively).

Conclusion: These data suggest that placental tissue from women with PCOS shows changes in the activities of two important enzymes for steroid synthesis, higher 3β -HSD-1 and lower P450, which could increase androgen production during pregnancy.

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1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinemetabolic disorder, affecting approximately 5–10% of women at reproductive age. It is characterized by ovulatory/menstrual irregularity, polycystic ovaries and hyperandrogenism [1]. Thus, it has deleterious consequences for fertility; and PCOS pregnancy has a high prevalence of complications and adverse outcome [2]. Moreover, women with PCOS exhibit a significant increase in androgen concentrations during pregnancy [3,4]. Interestingly, elevated androgen levels have been reported in women with preeclampsia and gestational diabetes [5,6].

During pregnancy the placenta is the most important organ for steroid production, but the possible role of placental steroidogenesis in the modifications of the androgen profile of women with PCOS has not been studied. The human placenta utilizes fetal and

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maternal adrenal-derived C19 androgens, mainly dehydroepiandrosterone sulfate (DHEAS). In placental tissue, the sulfate group is cleaved by steroid sulfatase (STS). The unconjugated steroids are converted by the activity of 3 β -hydroxysteroid dehydrogenase type 1 (3 β -HSD-1) into androstenedione, which is subsequently transformed into testosterone by 17 β -hydroxysteroid dehydrogenase. These C19 androgens are then aromatized to estrone and estradiol, respectively, by P450 aromatase (P450arom) [7]. Moreover, in the fetal adrenal DHEAS can also undergo 16 α hydroxylation leading to the formation of 16 α -hydroxy-DHEAsulfate (16 α -OH-DHEAS), which is the androgen precursor for the synthesis of estriol. Therefore, modifications in the STS, 3 β -HSD-1 and/or P450 aromatase activities in the placenta of women with PCOS could lead to an increase of androgen concentrations in maternal or fetal circulation.

The aim of the present study was to evaluate the placental activities of STS, 3β -HSD-1 and P450 aromatase in women with PCOS during pregnancy, compared to normal pregnant women. In order to understand the possible functional meaning of the changes in the enzymatic activities, the sex steroid serum concentrations in maternal blood and cord blood were also determined.

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2. Material and methods

2.1. Subjects

A case–control study design was used. Twenty pregnant women with PCOS were studied: samples of placental tissue and maternal and cord blood were obtained. As a control group, 30 pregnant women with regular menses and without hyperandrogenism were included.

All PCOS mothers were consecutively recruited from patients attending the Unit of Endocrinology and Reproductive Medicine, University of Chile. The diagnosis of PCOS was made according to the criteria of the NIH consensus [8] and the Rotterdam ESHRE/ ASRM-sponsored PCOS consensus workshop group [9]. PCOS mothers were evaluated before pregnancy, and all of them exhibited chronic oligomenorrhea or amenorrhea, hirsutism (Ferriman–Gallwey score ≥ 8), serum testosterone concentration >0.6 ng/ml and/or free androgen index (FAI) > 5.0, androstenedione concentration >3.0 ng/ml and a characteristic ovarian morphology on ultrasound, based on the criteria described by Adams et al. [10]. All women had been anovulatory as indicated by progesterone measurements and ultrasound examinations. We excluded patients with hyperprolactinemia, androgen-secreting neoplasm, Cushing's syndrome and late-onset 21-hydroxylase deficiency, as well as thyroid disease.

As part of their initial evaluation, all patients underwent a lifestyle assessment and were placed on a diet and exercise treatment program as previously described [3]. In addition, most of them received 500–2000 mg of metformin in standard formulation based on their weight, medication tolerance and insulin levels. Women with PCOS stopped metformin when they had a positive pregnancy test. No medications to induce ovulation, such as clomiphene citrate or exogenous gonado-tropins, were used.

As control mothers, we selected 30 women of similar socioeconomic level as the PCOS patients. The control women had a history of regular 28- to 32-day menstrual cycles, absence of hirsutism and other manifestations of hyperandrogenism, and absence of galactorrhea and thyroid dysfunction. All were healthy and were not receiving any drug therapy. These women were recruited from the antenatal care unit of our hospital from the 12th week of gestation during the same time period.

Only non-smoking and non-alcohol or drug abusing PCOS and control pregnant women were included in the study. Women with preterm delivery in the present pregnancy were not included. This investigation was approved by the Institutional review board of University of Chile and an informed written consent was obtained from all women.

2.2. Study protocol

2.2.1. Blood samples

In all women during the 34th week of pregnancy, a blood sample was obtained. Cord blood samples were collected as umbilical mixed arterial-venous cord blood samples at delivery. The samples were immediately centrifuged and the serum was frozen at -80 °C until further analyses.

2.2.2. Placental tissue

Placentas from full-term pregnancies (37–40 weeks of gestation) were collected immediately after delivery or cesarean section. Placental samples were rapidly processed as described by Wyatt et al. [11]. Briefly, each placenta was sectioned transversely using a sterile scalpel near the cord insertion site (approximately 5 cm), divided into three horizontal segments from the chorionic surface toward the basal plate and codified as S1: near to chorionic plate; S2: zone between chorionic plate and basal plate; and S3: near to basal plate.

2.3. Assays

2.3.1. Enzymatic assay

A microsomal fraction of placental tissue (≈ 1 g) was obtained according to Jimenez et al. [12]. STS activity was assayed according to previous reports [13]. Briefly, the enzyme solution (100 µg protein) was mixed with cold DHEAS (Sigma) at 40 µM and [1,2,6,7-³H]DHEAS (20 nM) (Perkin-Elmer NEN, specific activity 94.5 Ci/mmol). The reaction mixture was incubated at 37 °C for 30 min. The enzyme reaction was ended by adding 2.0 ml of toluene, vortexing for 1 min and centrifuged at 600 × g for 5 min to separate DHEAS (aqueous phase) from DHEA (organic phase). The toluene layer was collected, and measured in a liquid scintillation counter (Packard Instrument Co., IL, USA).

Placental 3 β -HSD activity was measured according to the method of Talalay [14]. 100 μ g of protein were mixed with 200 μ M of sodium pyrophosphate buffer (pH 8.9) and 100 μ M of DHEA (Sigma) in 40 μ l of methanol, making the incubation mixture a total of 1 ml. The enzyme activity was measured after addition of 4.0 μ mol of NAD (Sigma) in a 4010 photometer (Roche Diagnostics GmbH, Mannheim, Germany) at 340 nm against a blank (without NAD). One unit of enzyme activity is the amount causing a change in absorbance of 0.001 min⁻¹ at 340 nm.

The aromatase activity was quantified by tritiated waterrelease assay [15]. 100 µg of protein from the microsomal fraction were mixed with 50 nmol of $[1\beta^{-3}H]$ -androst-4-ene-3,17-dione (Perkin-Elmer NEN, specific activity 23.5 Ci/mmol) and 1.2 mM of NADPH (MP Biomedicals, Aurora, OH, USA) in 0.5 ml of phosphate buffer (pH 7.4). The reaction mixture was incubated at 37 °C for 30 min in a shaking water bath. The reaction was stopped with 1 ml of 20% trichloroacetic acid and centrifuged at 12,000 × g for 10 min. The supernatant was mixed with 4.0 ml of chloroform, vortexed for 3 min and centrifuged at $600 \times g$ for 10 min. A five hundred microliter aliquot of the aqueous phase was mixed with 0.5 ml of 5% charcoal suspension, incubated for 30 min and centrifuged for 15 min at 12,000 × g. The supernatant was then measured using a liquid scintillation counter.

2.3.2. Steroid assay

Progesterone, DHEAS, DHEA, androstenedione, testosterone, estrone, estradiol and estriol were measured in serum from maternal blood and cord blood. All assays were performed in the same batch for cases and controls. Progesterone and total estriol were assayed by ELISA (DRG Instruments, GmbH, Germany). DHEAS (Diagnostic Products Corp. LA, USA), DHEA, androstenedione, testosterone, estrone (Diagnostic System Labs, TX, USA) and estradiol (Diagnostic Products Corp) were assayed by RIA. The assay sensitivities were 0.3 ng/ml, 2.0 ng/ml, 1.0 μ g/dl, 0.1 ng/ml, 0.1 ng/ml, 5.0 pg/ml and 8.0 pg/ml, respectively, and the intra- and inter-assay coefficients of variation were 4.0 and 7.0% for progesterone; 4.8 and 8.8% for estriol; 5.1 and 11.0% for DHEAS; 3.8 and 8.6% for DHEA; 5.0 and 5.0% for androstenedione; 4.2 and 5.2% for testosterone; 4.3 and 6.0% for estrone and 4.0 and 4.2% for estradiol.

2.4. Statistical analysis

The results in the tables are expressed as mean \pm SD or median and (25th–75th interquartile range) if skewed. Normal distribution was assessed by the Kolmogorov–Smirnov test. Categorical data were analyzed using chi-square (χ^2) or Fisher's exact test. Comparisons between groups were performed by Student's *t* test when data were normally distributed or Mann–Whitney test for data not normally

100

25

0

S1

distributed. The comparison between the three areas of placental tissue sampled was performed by one-way ANOVA. The significance level was set at 5%.

Sample size was calculated based in a previous study from our group that reported elevated androgen concentrations in maternal serum from women with PCOS. In that study, we analyzed samples from 20 pregnant women and 26 pregnant control women [3].

3. Results

3.1. Clinical characteristics

Table 1 shows the clinical characteristics of the groups of pregnant women and their newborns. By design, there were no significant differences between groups in maternal characteristics such as age, initial weight, initial BMI and height. However, pregnant women with PCOS showed higher weight at the end of pregnancy compared to control women (p = 0.020). Weight gain during pregnancy was not significantly different between groups. Gestational diabetes was observed in three PCOS women. On the other hand, percentages of cesarean section were comparable between groups (control: 60.0% and PCOS: 70%). Forty seven percent of women in the control group and 60% of PCOS women were primiparous.

Newborns of the two groups were comparable in gestational age, weight, length, weight standard deviation scores (SDS) and length SDS. Moreover, placental weight was comparable between groups. The proportion of female fetuses was comparable between control and PCOS groups (60% and 50%, p = 0.112).

3.2. Enzymatic activities

STS activity was similar between control and PCOS groups in the three areas of placental tissue studied. Fig. 1 shows 3β-HSD-1 and P450 aromatase activities in placental tissue of control and PCOS. Women with PCOS showed higher 3β-HSD-1 activity in S1 $(40 \pm 4.3 \text{ vs } 56 \pm 4.4 \text{ U/min} \times \text{mg protein}; p = 0.014)$, S2 $(63 \pm 5.2 \text{ mm})$ vs 83 ± 6.8 U/min \times mg protein; p = 0.020) and S3 (42 ± 6.4 vs $63 \pm 6.2 \text{ U/min} \times \text{mg}$ protein; *p* = 0.05). Moreover, in PCOS the 3β-HSD-1 activity was higher in S2 compared to S1 and S3 (p = 0.002 and 0.030, respectively). On the other hand, the P450arom activity was lower in PCOS compared to control in the three areas studied (S1: 1.25 ± 0.12 vs 0.92 ± 0.06 pmol/min × mg protein; *p* = 0.036; S2: $1.18 \pm 0.09 \text{ vs } 0.88 \pm 0.08 \text{ pmol/min} \times \text{mg protein}; p = 0.042 \text{ and } S3:$ 1.35 ± 0.14 vs 0.81 ± 0.21 pmol/min × mg protein; *p* = 0.025). There

Table 1

Clinical characteristics of the mothers and the newborns.

3β-HSD-1 activity (U/min/ mg protein) 75 50

3B-HSD-1



S2

Fig. 1. Enzymatic activities of 3β-hydroxysteroid dehydrogenase type 1 (3β-HSD-1) and P450 aromatase (P450arom) in samples of placental tissue of control women and women with PCOS. The assays were performed in duplicate. The values for samples from different zones of the placenta are shown. S1: near to chorionic plate; S2: zone between chorionic plate and basal plate; and S3: near to basal plate. Values are mean \pm SEM, *p < 0.05 between control and PCOS.

	Control (<i>n</i> = 30)	PCOS (<i>n</i> = 20)	<i>p</i> -Values
Mothers			
Age (years)	26.5 ± 6.9	28.0 ± 5.8	0.430
Height (m)	1.6 (1.5–1.6)	1.6 (1.6–1.7)	0.371
Initial weight (kg)	64.0 ± 8.2	69.0 ± 12.1	0.090
Initial body mass index (kg/m ²)	25.0 ± 5.5	27.0 ± 6.2	0.240
Weight at term of pregnancy (kg)	71.0 ± 12.3	$82.3 \pm 14.5^{*}$	0.020
BMI at term of pregnancy (kg/m ²)	29.8 ± 6.0	32.6 ± 7.2	0.142
Weight gain during pregnancy	13.0 ± 4.2	13.3 ± 4.6	0.541
Newborns			
Gestational Age (weeks)	38.0 (38.0-39.5)	38.0 (38.0-40.0)	0.238
Birth weight (kg)	3.4 ± 0.5	3.6 ± 0.8	0.781
Weight SDS	0.2 ± 0.7	0.1 ± 1.0	0.686
Length (cm)	49.3 ± 1.9	49.8 ± 2.0	0.425
Length SDS	-0.4 ± 0.9	-0.1 ± 0.9	0.276
Placental weight (g)	560 ± 108.0	615 ± 166.0	0.161

Values are expressed as mean ± SD or median (25th-75th interquartile ranges). BMI = body mass index; SDS = standard deviation score. p < 0.05 between control and PCOS.

□Control

■PCOS

S3

Table 2

Sex steroid concentrations in maternal blood of control and PCOS.

	Control (n=30)	PCOS (<i>n</i> =20)	p-Values
Progesterone (ng/ml)	268.4 (229.0-384.2)	247.6 (117.1-574.1)	0.564
DHEAS (µg/dl)	93.0 (56.7-104.6)	93.5 (68.9-110.9)	0.331
DHEA (ng/ml)	2.7 (1.9-4.4)	4.0 (1.9-5.9)	0.453
Androstenedione (ng/ml)	2.4 (1.9-3.3)	3.7 (2.9–5.8)*	0.016
Testosterone (ng/ml)	1.2 (0.4–1.8)	1.8 (1.1–3.1)*	0.025
Estrone (ng/ml)	4.5 (0.9–11.9)	1.0 (0.9-4.7)	0.111
Estradiol (ng/ml)	15.4 (9.8-21.8)	14.8 (10.6-25.9)	0.726
Estriol (ng/ml)	123.0 (58.6–168.0)	118.5 (111.4–188.0)	0.301

Values are expressed as median (25th-75th interquartile ranges).

 * *p* < 0.05 between control and PCOS.

Table 3

Sex steroid concentrations in cord blood of control and PCOS newborn.

	Control	PCOS	p-Values
Females (n)	15	12	
Progesterone (ng/ml)	492.0 (385.5-658.9)	405.9 (122.6-593.9)	0.731
DHEAS (µg/dl)	200.1 ± 93.3	160.4 ± 85.6	0.267
DHEA (ng/ml)	20.6 (17.3-23.1)	20.4 (18.5-24.5)	0.799
Androstenedione (ng/ml)	9.2 ± 4.1	$6.2\pm3.7^{*}$	0.038
Testosterone (ng/ml)	0.9 (0.3-1.1)	0.8 (0.7-1.5)	0.829
Estrone (ng/ml)	7.8 (4.8-9.9)	4.1 (2.2-6.8)	0.061
Estradiol (ng/ml)	6.8 (5.2-10.8)	4.2 (2.8-5.8)	0.170
Estriol (ng/ml)	484.5 (400.9–616.9)	657.0 (531.0–711.0) [*]	0.031
Males (n)	15	8	
Progesterone (ng/ml)	572.1 (407.2-701.4)	462.8 (445.7-519.0)	0.523
DHEAS (µg/dl)	195.8 ± 69.7	246.2 ± 80.6	0.093
DHEA (ng/ml)	16.2 (14.5–16.6)	19.0 (17.2–19.5)	0.399
Androstenedione (ng/ml)	8.5 (5.6–11.1)	6.0 (4.9–7.4)	0.054
Testosterone (ng/ml)	1.3 (0.9–1.5)	1.1 (0.9–1.4)	0.865
Estrone (ng/ml)	7.4 (3.6–9.5)	2.2 (1.7-7.6)	0.086
Estradiol (ng/ml)	6.2 (3.2-7.8)	3.5 (2.9-6.5)	0.326
Estriol (ng/ml)	598.6 (536.8-931.0)	648.3 (558.7-805.0)	0.521

Values are expressed as mean \pm SD or median (25th–75th interquartile ranges).

p < 0.05 between control and PCOS.

were no differences in P450arom activity between the three areas studied, neither in control nor in PCOS women.

3.3. Steroid concentrations

Table 2 shows the sex steroid concentrations in maternal blood at week 34 of gestation in PCOS and control women. Women with PCOS exhibited higher androstenedione and testosterone concentrations compared to the control group. No major differences according to gender of the newborns were observed.

The steroid concentrations in cord blood are shown in Table 3. Female newborns of pregnant women with PCOS exhibited lower androstenedione and higher estriol concentrations compared to female newborns of control women. Moreover, estrone concentrations tended to be lower in newborns of PCOS compared to control newborns (p = 0.06). In male newborns, the androstenedione levels tended to be lower in PCOS compared to controls (p = 0.054).

4. Comments

The present study increases knowledge about pregnancy in women with PCOS and provides new information about the role of placental steroidogenesis in modifications of the endocrine profile in women with PCOS during gestation.

Elevated serum androgen concentrations during week 34 of pregnancy were observed in women with PCOS, which is in agreement with previous observations during the second and third trimesters of pregnancy [3,4]. As stated above, 3β -HSD-1

catalyzes the conversion of $\Delta 5$ -3 β -hydroxysteroids to $\Delta 4$ -3-ketosteroids [7]. Therefore, an increase in the activity of 3 β -HSD-1 in placental tissue could lead to a greater synthesis of androgens in women with PCOS. These are rapidly aromatized by P450 aromatase, leading to the synthesis of estrogens. In this regard, it would be expected that the decrease in P450 activity in placental tissue could induce an accumulation of androgenic substrate. Thus, the high androgen levels observed in the present study in maternal blood could be attributed, in part, to these abnormalities in placental steroidogenesis.

The regulators of 3β -HSD-1 and P450 aromatase activities in placental tissue are poorly understood. In human cytotrophoblasts, it has been demonstrated that insulin and insulin growth factors inhibit P450 aromatase activity [16] and stimulate 3β -HSD-1 activity [17]. This is an interesting point, since women with PCOS exhibit an exacerbated insulin resistance and increased levels of insulin from early to late gestation [18]. In turn, in other conditions related to exacerbated insulin resistance and hyperinsulinemia, such as gestational diabetes and preeclampsia, a decrease in the activity of placental P450 aromatase has been described [19,20]. Therefore, the metabolic abnormalities observed in women with PCOS during pregnancy could explain the changes in placental 3β -HSD-1 and P450 aromatase activities.

The implications for the fetus of the changes in the activities of 3β -HSD-1 and P450 aromatase in placental tissue from women with PCOS are uncertain. It has been described that placental aromatase has a high capacity to convert androgen to estrogen, such that less than 1% of the enzyme activity prevents the virilization of the fetus by increased maternal androgens [21]. In

this regard, we did not find any signs of virilization in this cohort of female newborns. However, we cannot exclude that abnormal enzymatic activities in placentas from PCOS women can lead to subtle effects on ovarian or metabolic function, as daughters of women with PCOS show an altered follicular mass and insulin resistance at early age [22,23]. Supporting this concept, animal models of prenatal androgen exposure have demonstrated that female fetuses develop a PCOS phenotype in adulthood [24].

To the best of our knowledge, only two studies have explored the steroid concentrations in cord blood of fetuses from women with PCOS. Barry et al. demonstrated higher testosterone concentrations in the umbilical vein of newborn females of women with PCOS [25], which would indicate a possible placental source of androgens. We did not observe any differences in testosterone concentrations. This could be attributed to the fact that we measured the steroid concentration in mixed cord blood. Anderson et al. [26] observed lower androstenedione and estradiol serum concentrations in mixed cord blood of newborn females of women with PCOS. We found a decrease of androstenedione and an increase in estriol levels in cord blood of female PCOS newborns. This is an important point, since estriol is a product of the aromatization of 16α -OH-androstenedione. On the other hand, the main site for 16α -hydroxylation of androgens in the fetus is the liver, possibly through an enzymatic activity that has been attributed to CYP3A7 [27]. Also, it has been demonstrated that the fetal adrenal may have the capacity to metabolize androgens by 16α -hydroxylation, as was demonstrated for testosterone [28]. Thus, this increase in estriol concentrations in cord blood can have two possible explanations. First, there may be an increased 16α hydroxylation of DHEAS produced by the fetal adrenal. Second. androstenedione synthesized by the placenta may pass to the fetus where it could be metabolized to 16α -OH-androstenedione by the fetal liver or adrenal and, then, aromatized to estriol by placental tissue. Estriol has been useful as marker of the activity of the fetal adrenal gland and fetal wellbeing. Low estriol concentrations in maternal serum during the second trimester of pregnancy have been related to Down syndrome, 18 trisomy, fetal metabolic diseases such as steroid sulfatase deficiency and fetal death [29]. Elevated estriol in cord blood has not been associated with adverse pregnancy outcomes [30]. More studies are needed, however, to evaluate the long-term effect of these alterations.

In summary, our data suggest that placental tissue of women with PCOS could have a potential increased capacity for producing androgens, which could be preferentially cleared to the maternal circulation. The decreased levels of androstenedione and increased levels of estriol found in cord blood of female newborns could reflect the modifications of steroid metabolism by the fetus.

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References

- Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nature Reviews Endocrinology 2011;7:219–31.
- [2] Kjerulff LE, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. American Journal of Obstetrics and Gynecology 2011;204(558):e1–6.
- [3] Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Perez-Bravo F, Recabarren SE. Maternal serum androgens in pregnant women with polycystic ovary syn-

drome: possible implications in prenatal androgenization. Human Reproduction 2002;17:2573-9.

- [4] Falbo A, Rocca M, Russo T, et al. Changes in androgens and insulin sensitivity indexes throughout pregnancy in women with polycystic ovary syndrome (PCOS): relationships with adverse outcomes. Journal of Ovarian Research 2010;3:23.
- [5] Salamalekis E, Bakas P, Vitoratos N, Eleptheriadis M, Creatsas G. Androgen levels in the third trimester of pregnancy in patients with preeclampsia. European Journal of Obstetrics Gynecology and Reproductive Biology 2006;126:16–9.
- [6] Uzelac P, Lee J, Zhang X, Paulson R, Miller D, Stanczyk F. Gestational diabetes augments hyperandrogenemia in the third trimester of pregnancy. Journal of the Society for Gynecologic Investigation 2005;12(Suppl. 2):620.
- [7] Pasqualini JR. Enzymes involved in the formation and transformation of steroid hormones in the fetal and placental compartments. Journal of Steroid Biochemistry and Molecular Biology 2005;97:401–15.
- [8] Zawadzky JK, Dunaif A. Diagnosis criteria: towards a rational approach. In: Hershmann JM, editor. Current issues in endocrinology and metabolism. Boston: Blackwell Scientific Publications; 1992. p. 377–84.
- [9] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. In: Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Human Reproduction 2004;19:41–7.
- [10] Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. British Medical Journal 1986;293:355–9.
- [11] Wyatt SM, Kraus FT, Roh CR, Elchalal U, Nelson DM, Sadovsky Y. The correlation between sampling site and gene expression in the term human placenta. Placenta 2005;26:372–9.
- [12] Jimenez V, Henriquez M, Llanos P, Riquelme G. Isolation and purification of human placental plasma membranes from normal and pre-eclamptic pregnancies. A comparative study. Placenta 2004;25:422–37.
- [13] Suzuki T, Miki Y, Fukuda T, Nakata T, Moriya T, Sasano H. Analysis for localization of steroid sulfatase in human tissues. Methods in Enzymology 2005;400:303-16.
- [14] Talalay P, Marcus P. Specificity, kinetics, and inhibition of alpha- and betahydroxysteroid dehydrogenases. Journal of Biological Chemistry 1956;218:675.
- [15] Sanderson JT. Placental and fetal steroidogenesis. Methods in Molecular Biology 2009;550:127-36.
- [16] Nestler JE. Regulation of the aromatase activity of human placental cytotrophoblasts by insulin, insulin-like growth factor-I, and -II. Journal of Steroid Biochemistry and Molecular Biology 1993;44:449–57.
- [17] Nestler JE. Insulin and insulin-like growth factor I stimulate the 3β-hydroxisteroid dehydrogenase activity of human placental cytotrophoblast. Endocrinology 1989;125:2127–33.
- [18] Sir-Petermann T, Echiburú B, Maliqueo M, et al. Serum adiponectin and lipid concentrations in pregnant women with polycystic ovary syndrome. Human Reproduction 2007;22:1830–6.
- [19] Uzelac PS, Li X, Lin J, et al. Dysregulation of leptin and testosterone production and their receptor expression in the human placenta with gestational diabetes mellitus. Placenta 2010;31:581–8.
- [20] Hahnel ME, Martin JD, Michael CA, Hahnel R. Metabolism of androstenedione by placental microsomes in pregnancy hypertension. Clinica Chimica Acta 1989;181:103–8.
- [21] McClamrock AD, Adashi EY. Gestational hyperandrogenism. Fertility and Sterility 1992;57:257–74.
- [22] Crisosto N, Echiburú B, Maliqueo M, et al. Improvement of hyperandrogenism and hyperinsulinemia during pregnancy in women with polycystic ovary syndrome: possible effect in the ovarian follicular mass of their daughters. Fertility and Sterility 2012;97:218–24.
- [23] Sir-Petermann T, Codner E, Pérez V, et al. Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism 2009;94:1923–30.
- [24] Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome. Human Reproduction Update 2005;11:357–74.
- [25] Barry JA, Kay AR, Navaratnarajah R, et al. Umbilical vein testosterone in female infants born to mothers with polycystic ovary syndrome is elevated to male levels. Journal of Obstetrics and Gynaecology 2010;30:444–6.
- [26] Anderson H, Fogel N, Grebe SK, Singh RJ, Taylor RL, Dunaif A. Infants of women with polycystic ovary syndrome have lower cord blood androstenedione and estradiol levels. Journal of Clinical Endocrinology and Metabolism 2010;95:2180–6.
- [27] Kitada M, Kamataki T, Itahashi K, Rikihisa T, Kanakubo Y. P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16-hydroxylase of dehydroepiandrosterone 3-sulfate. Journal of Biological Chemistry 1987;262:13534–37.
- [28] Wang H, Xu D, Peng RX, Yue J. Testosterone-metabolizing capacity and characteristics of adrenal microsomes in human fetus in vitro. Journal of Pediatric Endocrinology and Metabolism 2010;23:143–52.
- [29] Craig WY, Palomaki G, Roberson M, Haddow JE. Further insights into implications of undetectable or very low unconjugated estriol in maternal serum during the second trimester. Prenatal Diagnosis 2011;31:616–8.
- [30] Gagnon A, Wilson RD, Audibert F, et al. Obstetrical complications associated with abnormal maternal serum markers analytes. Journal of Obstetrics and Gynaecology Canada 2008;30:918–49.