Macroscopic and microscopic findings of the placenta in women with polycystic ovary syndrome

S. Palomba¹,*, T. Russo¹, A. Falbo¹, A. Di Cello², A. Tolino³, L. Tucci⁴, G.B. La Sala¹, and F. Zullo²

¹Obstetrics and Gynecology Unit, University of Modena and Reggio Emilia, Azienda ASMN, IRCCS, Viale Risorgimento 80, 42123 Reggio Emilia, Italy ²Department of Obstetrics and Gynecology, University “Magna Graecia” of Catanzaro, Viale Europa, 88100 Catanzaro, Italy ³Department of Obstetrics and Gynecology, University “Federico II” of Naples, Via Pansini 5, 80131 Naples, Italy ⁴Unit of Pathology, “Pugliese-Ciaccio” Hospital, Via Pio X, 88100 Catanzaro, Italy

*Correspondence address: Academic Department of Obstetrics and Gynecology, Arcispedale “Santa Maria Nuova”, IRCSS, University of Modena and Reggio Emilia, Viale Risorgimento 80, 42123 Reggio Emilia, Italy. Tel: +39-3475880503; E-mail: stefanopalomba@tin.it, stefano.palomba@asmn.re.it.

Submitted on March 29, 2013; resubmitted on March 29, 2013; accepted on April 22, 2013

STUDY QUESTION: Do patients with polycystic ovary syndrome (PCOS) have macroscopic and/or microscopic placental alterations?

SUMMARY ANSWER: The placental structure in patients with PCOS, even in those with uncomplicated pregnancy, is altered.

WHAT IS KNOWN ALREADY: The spectrum of pregnancy complications seems to have a common denominator: a defective trophoblast invasion and placentation. In women with PCOS, alterations in endovascular trophoblast invasion related to insulin resistance and hyperandrogenism have been observed.

STUDY DESIGN, SIZE, DURATION: For this prospective case–control study, 30 pregnant patients with PCOS (cases) and 60 healthy pregnant women without PCOS features (controls) were enrolled and studied until delivery. Clinical, biochemical, ultrasonographic and obstetric data were recorded. The baseline clinical and biochemical data for screening for PCOS and for inclusion/exclusion were obtained before the seventh week of gestation. At delivery, placentas were collected and detailed macroscopic and microscopic analyses were performed.

PARTICIPANTS, SETTING, METHODS: Cases and controls were matched for age and BMI (all < 30 kg/m²). The matching procedure was one-to-two. Only subjects with spontaneous conception and uncomplicated pregnancies were included in the final analysis.

MAIN RESULTS AND THE ROLE OF CHANCE: Placental weight (P = 0.04), thickness (P = 0.02), density (P = 0.02) and volume (P = 0.01) were significantly inferior in women with, compared with those without PCOS. The placentas from patients with PCOS more frequently had an irregular shape (P = 0.03) and a higher cord coiling index (P = 0.02). Differences between cases and controls also concerned the extent of villous (P = 0.04) and intervillous (P = 0.01) spaces, the extent of fibrosis (P = 0.03), endovascular trophoblast (depth, extension and morphology) (P < 0.05) and mitotic activity (P = 0.01). The percentage of patients with lesions [22/30 (73.3%) versus 25/60 (41.7%), respectively; P = 0.01] and the mean number of placental lesions (3.5 ± 2.1 versus 1.4 ± 1.1, respectively; P = 0.02) were higher in the PCOS than the control group. The odds ratio for placental alterations, adjusted for weight gain, was 2.8 (95% confidence interval 1.3–9.9).

LIMITATIONS, REASONS FOR CAUTION: The main limitation of the study was the selection of a specific PCOS sample, which is probably not representative of the PCOS phenotype as a whole. In fact, we excluded patients with PCOS who were obese and who achieved a pregnancy following the use of ovulation inductors or assisted reproduction techniques.

WIDER IMPLICATIONS OF THE FINDINGS: The present study is the first to demonstrate that the morphology and microscopic structure of placenta in patients with PCOS with an uncomplicated pregnancy are altered. Further studies are needed to assess a correlation of these changes with the increased risk of obstetric complications observed in some pregnancies of women with PCOS.

STUDY FUNDING/COMPETING INTEREST(S): The authors declare no conflict of interest and no financial support for the research.

TRIAL REGISTRATION NUMBER: N/A.

Key words: complications / PCOS / polycystic ovary syndrome / placenta / pregnancy

© The Author 2013. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved.

For Permissions, please email: journals.permissions@oup.com
Introduction

Polycystic ovary syndrome (PCOS) is an endocrine heterogeneous syndrome related to various short- and long-term consequences (Fauser et al., 2012).

The pregnancy outcome in women with PCOS has received great scientific attention over the last few years as an integral aspect of reproductive health (Boomsma et al., 2006; Kjerulf et al., 2011). In their meta-analysis, Boomsma et al. (2006) were the first to demonstrate that pregnant women with PCOS have an increased risk of pregnancy and neonatal complications. More recently, the meta-analysis by Kjerulf et al. (2011) confirmed in a large population a strict association between PCOS and gestational diabetes mellitus, pregnancy-induced hypertension, pre-eclampsia, preterm delivery and small-for-gestational-age infants.

The spectrum of pregnancy complications seems to have a common denominator: defective trophoblast invasion and placentation (Jindal et al., 2007; Longtine and Nelson, 2011).

Recent experimental data have demonstrated insulin resistance and androgen-related alterations in the endovascular trophoblast invasion in patients with PCOS undergoing legal pregnancy termination, assessed through surrogate parameters and direct histological analysis (Palomba et al., 2012). However, at present, it is unknown whether the impaired trophoblast invasion observed in patients with PCOS results in an abnormal placenta, or if compensatory mechanisms arise. In fact, to the best of our knowledge, no specific and direct data on the characteristics of the placenta in women with PCOS are currently available in the literature. Based on these considerations, the current experimental study was performed to test the hypothesis that pregnant patients with PCOS have abnormal macroscopic and microscopic findings of the placenta.

Materials and Methods

The present cohort study protocol followed the STrengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (http://www.strobe-statement.org/).

Ethics

The study was approved by the local Institutional Review Board. The procedures used were in accordance with the Helsinki Declaration on human experimentation guidelines. The purpose of the protocol was carefully explained to each subject at study entry, and a written informed consent was obtained.

Population

Between September 2009 and June 2011, pregnant women affected by PCOS at the time of their first pregnancy were consecutively screened at the Academic Department of Obstetrics & Gynaecology of the ‘Pugliese-Ciaccio’ Hospital of Catanzaro (Italy) and enrolled in the current study protocol (PCOS group).

PCOS was diagnosed before pregnancy according to the criteria specified by the European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Specifically, the diagnosis of PCOS was based on the presence of at least two of the following three criteria: oligo-anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovary (PCO) on ultrasound, after the exclusion of other pathologies with a similar clinical presentation (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

Sixty age- and body mass index (BMI)-matched healthy primigravidae were consecutively enrolled as controls (control group). The matching procedure was one-to-two, and women were defined as age- and BMI-matched when the differences between them were <1 year for age and <1 kg/m² for BMI. The health of the controls was determined by their medical history, a physical and pelvic examination and a complete blood chemistry panel. In addition, all control women had previous regular menstrual cycles, no phenotypic signs of clinical hyperandrogenism, normal range of serum androgens levels and no PCO morphology on transvaginal ultrasound.

In both PCOS and control groups, only subjects who conceived spontaneously were included.

The baseline clinical and biochemical data for the screening process and for the inclusion/exclusion in the study protocol were obtained before the seventh week of gestation, which was considered the study entry point.

For the PCOS and control groups, the exclusion criteria were as follows: age over 35 years; obesity (defined as BMI > 30 kg/m²); multiple pregnancies; premalignancies or malignancies; any major medical conditions; cigarette smoking; drug/alcohol use; uterine malformations; high-altitude residents; women noncompliant with the study protocol; current or previous residents; women noncompliant with the study protocol; current or previous (within the preceding 3 months) use of any hormonal and/or anti-diabetic drugs; pregnancy achieved with the use of assisted reproduction techniques (ART).

Only subjects with normal obstetric outcomes were included in the final analysis. A normal outcome was defined as the coexistence of the following conditions: (i) absence of complications throughout pregnancy; (ii) normal term of gestation (from 38th to 42nd week); (iii) giving birth to a healthy baby with appropriate size for gestational age.

Protocol

At study entry and throughout the study period, all women received folic acid (0.4 mg daily) and were instructed to follow their usual diet and physical activity.

In each subject, serial clinical, biochemical and ultrasonographic assessments for the monitoring of mother and/or fetal wellbeing were performed during the pregnancy according to our schedule (Palomba et al., 2010b).

At delivery, the placentas were collected for macroscopic and microscopic evaluation. These analyses were performed by clinicians and pathologists blinded to the patients’ data, pregnancy course and outcomes.

Clinical assessments

Clinic visits took place at study entry, every 2 weeks during the first trimester, and then every 4 weeks until delivery.

Visits included an evaluation of daily diet and physical activity (Palomba et al., 2010a,b,c), obstetric examination, anthropometric measurements, Ferriman–Gallwey score calculation and heart rate and blood pressure assessment.

Daily diet and caloric intake were assessed by an experienced clinical dietician using a self-administered semiquantitative validated food-frequency questionnaire and software designed to analyse food habits and to estimate nutrient and caloric intake (WinFood, release 1.5; Medimatica, Martinsicuro, Teramo, Italy); physical activity was evaluated by means of a leisure-time physical activity questionnaire and a calculation of the weekly energy expenditure score (total leisure-time physical activity level) in metabolic equivalents per hour per week (Palomba et al., 2010a).

Anthropometric measurements included height, weight, BMI and waist-to-hip ratio (WHR). Specifically, BMI was calculated as the ratio between the weight and the square of the height, and WHR as the ratio...
between the waist (considered to be the smallest circumference of torso between the 12th rib and the iliac crest) and the circumference of the hip (considered as the maximal extension of the buttocks). All measurements were performed when the patients were in a standing position with relaxed abdomen, arms at their sides and feet together.

The Ferriman–Gallwey score was calculated by the standard method. Specifically, nine body areas were visually scored on a scale from zero to four; a score of zero indicated no terminal hair growth, while a score of four indicated full female pattern terminal hair growth. A score of eight or more indicated the presence of androgen excess.

Blood pressure was measured in the right arm using a mercury sphygmomanometer and with the subject in a relaxed sitting position. The average of six measurements (three taken by each of two examiners) was calculated.

Biochemical assessments
Serial blood samples were drawn from each subject. Specifically, blood samples were taken at study entry, and at the 12th, the 20th and 32nd week of gestation (Falbo et al., 2010). Samples were obtained between 08:00 h and 09:00 h a.m. after at least an 8-h overnight fast and bed rest. All blood samples for each woman were immediately centrifuged, and the serum was then stored at −80°C until analysis in duplicate.

A complete hormonal and metabolic profile was only assessed at study entry, whereas total serum testosterone, androstenedione, dehydroepiandrosterone sulphate (DHEAS), and sex hormone-binding globulin (SHBG) were assayed serially (Falbo et al., 2010).

Fasting glucose and insulin concentrations were assessed at study entry and serially during gestation, and after a 75 g oral glucose tolerance test, at study entry and at 26 weeks to screen for gestational diabetes mellitus. According to the trapezoidal method, glucose and insulin responses were calculated as area under the curve (AUC glucose and AUC insulin, respectively). The AUC glucose/AUC insulin was also obtained for each subject.

All plasma hormone concentrations were measured by specific radioimmunoassay, whereas SHBG levels were determined using an immunoradiometric assay.

The free androgen index (FAI) [testosterone (nmol/l)/SHBG × 100] and the homeostasis model assessment of insulin resistance (HOMA-IR) [fasting glucose (mmol/l) × fasting insulin (µU/ml)/22.5] were also calculated.

Ultrasonographic and obstetric evaluations
Each subject received ultrasonographic and obstetrics examinations. All scans were performed transvaginally by the same experienced operator, blind as per the study protocol and clinical data, until the 12th week of gestation; thereafter, scans were performed transabdominally with the patient placed in a lithotomy position and with an empty bladder. Just before ultrasound examination, systolic and diastolic blood pressure and heart rate were measured; in the presence of abnormal values, the examination was delayed until normalization of parameters.

During the first trimester, the embryonic heartbeat was recorded and the crown–rump length (CRL) was measured. Gestational age was calculated from the last menstrual period and confirmed by first-trimester ultrasound (CRL measurement). Thereafter, fetal growth, placental location and degree, amniotic fluid index and velocimetry of the umbilical vessels (when required for high-risk pregnancy) were monitored.

A careful examination for fetal malformations was also performed at the 20th week of gestation. Obstetric and neonatal outcomes for each woman were carefully recorded.

Uterine artery velocimetry was assessed serially during the first (at 8, 10 and 12 weeks of gestation) and second (at 20 and 24 weeks of gestation) trimesters, according to standardized procedures (Palomba et al., 2010c). For each examination and for each side, the presence or absence of an early diastolic notch (defined as a definitive upward change in velocity after the initial deceleration slope of the primary wave) and the pulsatility (PI) and resistance indexes (automatically generated using at least three clear and similar consecutive waveforms) were noted (Palomba et al., 2010c).

Morphological examination of the placenta
Placentas were collected, prepared and examined according to a standard protocol. All placentas were placed in a double bag and labelled within 15 min of delivery. The bag was kept in a dry, clean plastic container with an airtight lid in a fridge with the temperature maintained at 4–6°C.

The placentas were then examined macroscopically within 12 h from delivery. Based on gross examination, a portion of placenta (with and/or without macroscopic alterations) was submitted for microscopic examination.

Macroscopic and microscopic evaluations were performed by two different expert researchers [one gynaecologist (ADC) and one pathologist (LT) for each kind of evaluation] in order to minimize inter-observer bias. Each operator was blinded with regard to the study protocol and clinical data.

Macroscopic examination
The preparation of the placentas for macroscopic examination consisted of excision of the cord from the point of insertion into the chorionic plate, the draining of the blood and the removal of all adherent blood clots from the maternal surface and the trimming of all extraplacental membranes from the chorionic plate (Langston et al., 1997; Fox and Sebire, 2007).

The macroscopic analysis (Langston et al., 1997; Fox and Sebire, 2007) of the placentas included the systematic evaluation of: general characteristics (odour, colour, shape), membranes (completeness, membrane rupture site measure), weight, fetal surface (colour and appearance, surface and subchorionic region, fetal surface vessels), umbilical cord (length and diameter, spiralling, insertion, knots and umbilical vessels) and placental disk measured in three dimensions, i.e. largest and smallest dimensions, and thickness (at the centre of the placental tissue by piercing it with a calibrated knitting needle).

Any macroscopic lesion of the maternal and/or fetal surface was sought, identified, described and then characterized microscopically (Langston et al., 1997; Fox and Sebire, 2007).

Based on direct measures, other derived measures for each subject, i.e. fetal–placental weight ratio, placental surface areas, placental volume and placental density, were calculated using standardized mathematical formulas (Salafia et al., 2007; Barker et al., 2010).

Three further morphological parameters were calculated (Pathak et al., 2010, 2011): the cord centrality index (CCc), the eccentricity index (EI) and the cord coiling index (CCoI). The CCel was used to define how close to or far from the point of umbilical cord insertion the centre of the placenta was (calculated as the ratio between distance of umbilical cord insertion from centre and half of the longest diameter of the placenta; range 0–1, with 0 representing a central insertion and 1 a marginal insertion). The EI was used to identify the shape of the placenta and, in particular, how deviant from circular it was, and was calculated as the ratio of the distance between the foci and the length of the major axis of the placenta (range 0–1, 0 representing a completely circular shape). The CCoI was calculated using an established method by dividing the number of coils by the length (cm) of the umbilical cord.

For each case, two samples of umbilical cord, two samples of extraplacental membranes (one from the rupture site and one from the placental margin) and four full-thickness samples of grossly normal chorionic villi were collected. Additionally, samples were taken for each gross lesion.

Microscopic examination
All collected tissues were immediately processed by separating chorionic villous from placental tissue with repeated washes with Hanks’ balanced salts solution I. Each placenta was fixed in 4% paraformaldehyde for at
least 24 h and subsequently embedded in paraffin. Five-micrometer-thick tissue horizontal sections of the middle region of the placenta were prepared and placed on slides.

For each histological examination, the standard haematoxylin and eosin stain was used. A morphometric evaluation of the normal placental areas, as well as any pathologic features, was performed.

For each sample, the depth of the endovascular trophoblast, the density of placental villus arrangement, the percentage of stem villus area occupied by stem vessel lumina, the relative thickness of stem arterial walls, the extent of fibrosis and the mitotic index were evaluated.

Specifically, to measure the density of placental villus arrangement, the four quadrants of each slide were analysed, with the area occupied by placental vilii measured and expressed as a percentage of the total field area. The average of the measurements for the four quadrants was determined for each slide (Ducray et al., 2011). The volume and surface of vilii and volume of intervillous space were also calculated for each sample.

The percentage of stem villus area occupied by stem vessel lumina was calculated utilizing the features of stem vilii. Specifically, two stem villi were selected in each section on the basis of clarity of morphology regardless of the levels of branching (and therefore of calibres). For each stem vilus, the sum of all the vessel luminal areas was expressed as a percentage of total villus areas.

Using the same micrographs, the relative thickness of stem arterial walls was assessed. In particular, the main arterial vessel was selected, its area was defined using the outer adventitial boundary and the lumen area was subtracted from the first in order to ascertain the area occupied by arterial wall. In order to compensate for differing calibres of stem vilii, relative thickness of stem arterial walls was expressed as a percentage of total vessel area (Ducray et al., 2011).

The extent of fibrosis was calculated utilizing the 10 × objective; the area of greatest fibrosis was selected in each quadrant. The sum of all areas of fibrosis in the field was expressed as a percentage of total field area (Ducray et al., 2011).

Finally, the mitotic index was evaluated by randomly examining 500 nuclei per section (∼1000) and exploring at least 30 fields for each placenta (10 fields from each section). Mitosis was defined by the absence of nuclear membrane and was expressed as percentage of pixels (see below) indicating the number of positive nuclei out of the total number of nuclei studied in the trophoblastic compartment.

Pathological definitions were based on the guidelines published by the College of American Pathologists (Langston et al., 1997) and categorized as follows: (i) utero–placental vascular lesion [utero–placental vessel thrombosis (occlusive or non-occlusive lesions), haemorrhagic endovascularitis, perivillous fibrin deposition, villous ischaemia and haemorrhage, and chorangiitis, vessel with fibrinoid necrosis and atherosis]; (ii) secondary effects of utero–placental vascular lesions (including villous infarction and decidual necrosis); (iii) chronic villitis and intervillositis; (iv) abnormal villus maturity (delayed, advanced, variable and dysmaturity); (v) absence of physiologic change of spiral vessels; (vi) histological evidence of placental abruption (frank or consistent); (vii) meconium staining and (viii) others (including villous fibrosis or hypovascularity, increased syncytiotrophoblast knotting, proliferation of X cells, villous oedema, intervillous thrombosis, amnion nodosum and congested vilii).

Pathological lesions were characterized and noted for each subject. In cases where there was more than one histological lesion, each lesion was counted separately.

The morphometric evaluations of the placental characteristics (density of placental villus arrangement, percentage of stem villus area occupied by stem vessel lumina, relative thickness of stem arterial walls and extent of fibrosis) and of the extent of each placental lesion were quantified by using a computerized analysis of the image (Palomba et al., 2012). Specifically, the images were acquired directly by optic microscopy at ×200 enlargement using an advanced digital camera; after optimization of the optic quality by modifying the brightness and the contrast, images were digitally and successively processed. All areas of interest were automatically calculated and expressed as pixels, with the average of pixels calculated for each tissue sample.

Statistical analysis
At study start, the sample size was defined in an arbitrary fashion in consideration of the experimental design of the study and the lack of published data on the best macroscopic and microscopic placental feature to use as primary end-point.

The normal distribution of continuous variable data was evaluated with the Kolmogrov–Smirnov test. Thus, our data were expressed as the mean ± standard deviation (SD) and analysed using an unpaired Student’s t-test.

Categorical variables were compared using the Pearson’s chi-squared test, whereas the Fisher’s exact test was used for the frequency tables when >20% of the expected values were <5.

Forward stepwise multivariate logistic regression analysis was performed to identify independent predictors of placental alterations, including as dependent variables hyperandrogenism, insulin resistance, uterine artery velocimetry and weight gain during pregnancy.

Statistical significance was set at P < 0.05, whereas a statistical trend was arbitrarily established for P-values between 0.05 and 0.07.

Confounder-adjusted estimates [adjusted odds ratio (OR) with 95% confidence intervals (CI)] of the risk difference for placental alterations using propensity score-based weighting were calculated.

The Statistical Package for Social Sciences (SPSS 14.0.1, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results
Figure 1 illustrates the flow-chart diagram of the study.

One hundred and twenty-four pregnant women with PCOS and 93 healthy pregnant women were consecutively screened and a total of 90 pregnant women—30 subjects in the PCOS and 60 in the control group—were included in the final analysis (Fig. 1).

Clinical and biochemical hyperandrogenism, and also PCO, were reassessed at study entry before the seventh week of gestation. Neither in cases and controls, these PCOS features disappeared or appeared, respectively.

Clinical, biochemical and ultrasonographic data
No significant difference in gestational age at study entry was observed between the groups (week of pregnancy: 5.3 ± 2.1 versus 5.5 ± 1.8 for PCOS and control group, respectively).

Clinical, biochemical and obstetric data are summarized in Table I. PCOS and control groups were well matched for age and BMI. Significant differences between groups were observed in WHR and Ferriman–Galway score, in serum testosterone, SHBG and fasting insulin levels, and in the FAL, the HOMA-IR and the AUC_{glucose}/AUC_{insulin}.

At the 26th week in women with PCOS and controls, respectively, the AUC_{glucose} (1010.5 ± 123.0 and 1012.7 ± 144.3), the AUC_{insulin} (8881.4 ± 1015.4 and 4681.6 ± 1205.6) and the AUC_{glucose} / AUC_{insulin} (0.09 ± 0.05 and 0.20 ± 0.10) were significantly different from baseline in both groups, and significantly different between groups.

Weight gain (kg) during pregnancy was significantly higher in the PCOS than in the control group (14.5 ± 6.8 versus 12.3 ± 5.4, respectively;
lesions identified macroscopically (1.1

The features of membranes, maternal and fetal surface and/or umbilical

were detected regarding the general characteristics of the placentas,

(28.3%) (Table III).

Macroscopic features of placentas from PCOS and control groups are

was significantly reduced in women with PCOS (data not shown).

A statistical trend towards a lower neonatal weight (g) was observed in

versus 0.16 ± 0.08, respectively, P = 0.02)

At the general microscopic evaluation, mean villous surface (cm²) and

versus 0.16 versus 9.7 ± 1.3; P = 0.72) and 10 (9.7 ± 1.2 versus 9.8 ± 1.3; P = 0.87) minutes.

Serial ultrasonographic data for uterine artery velocimetry in PCOS

No difference was found between women with PCOS and controls,

respectively, in operative delivery (10.0 versus 8.3%; P > 0.99), gesta-
tional age at delivery (weeks, 39.3 ± 1.5 versus 39.0 ± 1.6; P = 0.77)

and Apgar score at I (9.0 ± 1.2 versus 9.2 ± 1.3; P = 0.68), 5 (9.5 ± 1.1 versus 9.7 ± 1.3; P = 0.72) and 10 (9.7 ± 1.2 versus 9.8 ± 1.3; P = 0.87) minutes.

Morphological findings

Macroscopic features of placentas from PCOS and control groups are

Sixteen out of 30 women in the PCOS group (53.3%) had an irregular

placental shape, compared with 17 out of 60 women in the control group

(28.3%) (P = 0.03). No other significant differences between groups

were detected regarding the general characteristics of the placentas,

the features of membranes, maternal and fetal surface and/or umbilical
cord. The two groups were also similar in the mean number of placental
lesions identified macroscopically (1.1 ± 0.2 versus 1.0 ± 0.2, for PCOS
and control group, respectively; P = 0.62).

Placental weight, thickness, density and volume were significantly

(0.19 versus 0.49; P = 0.07) towards a lower pla-
cental surface area was detected in PCOS group versus the control
group (Table III).

No significant differences between groups were observed in placenta

CCei and EI (0.34 ± 0.20 versus 0.35 ± 0.23, and 0.49 ± 0.19 versus

0.5 ± 0.2, for PCOS and control group, respectively), whereas CCei
was significantly higher in PCOS than in control group (0.22 ± 0.10
versus 0.16 ± 0.08, respectively, P = 0.02)

At the general microscopic evaluation, mean villous surface (cm²) and

villous volume (cm³) were significantly lower in PCOS than in control
group [98.1 ± 9.2 versus 116.3 ± 8.6 (P = 0.04) and 211.4 ± 25.5
versus 257.6 ± 20.2 (P = 0.01), respectively], whereas the volume of
intervillos space (cm³) was significantly higher (244.6 ± 38.5 versus
183.3 ± 27.4, respectively, P = 0.01). The percentage of fields occupied
by placental villi was significantly higher in PCOS than in control group
(61.5 ± 3.6 versus 49.7 ± 2.2, respectively; P = 0.02). Stem villi morph-
ometry in the PCOS versus control group had a significantly lower total
luminal area (% pixels) in relation to the villus area (15.3 ± 8.1 versus
7.6 ± 4.5, respectively; P = 0.01), and a significant increase in the thick-
ness (% pixels) of stem villi arterial walls (69.0 ± 6.9 versus 86.9 ± 6.8,
respectively; P = 0.04) (Fig. 2A and B).

The extent (mm) of fibrosis was significantly higher in PCOS than in
control group (1.2 ± 0.4 versus 0.6 ± 0.1, respectively; P = 0.03)
(Fig. 2C and D), whereas the mitotic activity (% pixels) in endovascular
trophoblast was significantly lower in PCOS than in control group
(23.9 ± 1.2 versus 36.1 ± 1.3, respectively; P = 0.01).

At microscopic analysis, a total of 77 and 36 placental lesions
were identified in the PCOS and control group, respectively. The percentage
of patients with placental lesions [22/30 (73.3%) versus 25/60 (41.7%),
respectively; P = 0.01] and the mean number of placental lesions (3.5 ±
2.1 versus 1.4 ± 1.1, respectively; P = 0.02) were significantly higher
in PCOS than in control group.

The frequency and the extent of each placental lesion are detailed in
Table IV. The rate of chronic villitis/intervillitis was significantly
higher in PCOS than in control samples [35/77 (45.5%) versus 9/36
(25.0%), respectively; P = 0.04] (Fig. 3A and B). No other significant
differences in the frequency of other specific placental lesions were
observed between groups. The extent of utero–placental vascular
lesions, chronic villitis and intervillitis, abnormal villus maturity and
absence of physiologic change of spiral vessels were significantly
(P < 0.05) different between groups (Table IV).

In the PCOS group, the cumulative extent of the placental alterations
was significantly influenced by FAI (P = 0.01), HOMA-IR (P < 0.01),
AUC glucose/AUC insulin at 26 weeks of gestation (P = 0.01), PI values at
24 weeks of gestation \( P = 0.02 \) and weight change \( P = 0.03 \). On the other hand, in the control group, the cumulative extent of the placental alterations was significantly influenced by PI values at 24 weeks of gestation \( P = 0.04 \) and weight change \( P = 0.04 \).

The OR for placental alteration in PCOS patients adjusted for weight gain was 2.8 (95% CI 1.3–9.9).

### Discussion

To the best of our knowledge, this is the first experimental study designed to analyse macroscopic and microscopic findings of the placenta in pregnant patients with PCOS.

Macroscopic analysis of the placentas of women with PCOS showed no increase in placental alterations versus controls. Moreover, a significant reduction in the placental weight, thickness, density and volume was observed in patients with PCOS. The fetal–placental weight ratio and the CCoI were also higher in the PCOS population than in healthy controls. In addition, an irregular placental shape was more frequent in PCOS patients.

Although the clinical significance of these macroscopic placental parameters in low-risk pregnancies, such as those in our study, is unclear, they have been associated previously with adverse maternal and perinatal outcomes (de laat et al., 2005; Shehata et al., 2011; Hutcheon et al., 2012; Salafia et al., 2012). In particular, a high fetal–placental weight ratio is associated with increased rates of admission to the neonatal intensive care unit, of Apgar scores <7 at 5 min, of breech presentation and of Cesarean section (Shehata et al., 2011), and placental weight...
adjusted for gestational age is an independent risk factor for adverse perinatal outcomes (Hutcheon et al., 2012). In addition, an increase or decrease in cord spirals is also related to adverse pregnancy outcomes (de Laat et al., 2005) and, specifically, subjects with hypercoiled cords, as observed in the PCOS population, have an increased incidence of fetal growth restriction, premature delivery, vascular thrombosis and/or cord stenosis (de Laat et al., 2005). Finally, an irregular placental shape could be the expression of abnormal early patterns of trophoblast invasion and placentation (Salafia et al., 2012), as confirmed in patients with PCOS (Palomba et al., 2012).

The macroscopic findings of placentas from patients with PCOS could be interpreted as an epiphenomenon of microscopic placental changes. In fact, the microscopic analysis suggested that women with PCOS, when compared with age- and BMI-matched healthy controls, had not only a higher frequency, extent and number of placental lesions, but also differences in the histology of normal areas of placenta.

Both the proportion of subjects with microscopic placental lesions and the mean number of microscopic placental lesions were higher in the PCOS group than in controls. The extent of utero-placental vascular lesions, chronic villitis and intervillitis, abnormal villus maturity and absence of physiologic change of spiral vessels were greater in patients with PCOS; the incidence of chronic villitis/intervillitis was also higher. Of note, microscopic placental lesions were significantly influenced by basal FAI, HOMA-IR and AUC_glucose/AUC_insulin at 26 weeks of gestation, suggesting a potential role played by either hyperandrogenism or insulin resistance in their pathogenesis, even if the specific mechanisms through which they act are still unknown.

The absence of physiologic change and/or remodelling of spiral vessels can be attributed to the reduced depth of endovascular trophoblast, as demonstrated in patients with PCOS during early pregnancy (Palomba et al., 2012). In fact, a reduced villous surface and volume were observed here also at term in women with PCOS. Moreover, precocious markers of impaired decidual trophoblast invasion, such as abnormal blood flow in the uterine artery, were here found in PCOS patients.

Our data are particularly interesting in light of the fact that only non-complicated pregnancies were included in the analysis and studied. Moreover, we could hypothesize that compensatory mechanisms could be involved (Murray, 2012).

When there is a reduction in oxygen supply, placental and fetal tissues respond in order to optimize the allocation of oxygen; the placenta remodels its metabolism by decreasing oxygen consumption and increasing adenosine-5′-triphosphate production via anaerobic glycolysis due to activation of hypoxia-inducible factor 1 (Murray, 2012). In this regard, we can rule out only the decrease in oxygen consumption in our PCOS population because all newborns were appropriate size for gestational age. However, an activation of anaerobic glycolysis cannot be ruled out.

However, a higher density of placental villi and volume of intervillous space, along with a significantly lower total luminal area and a significant increase in the thickness of stem villi arterial walls, were observed in women with PCOS. This morphological variant can be considered as a compensatory morphometric adaption to unfavourable conditions whose aim is to improve the materno-fetal oxygen and nutrient transfer. In this regard, a placental plasticity has been demonstrated in animal and human models (Mayhew, 1996; Coan et al., 2010).
Two main mechanisms, alone or combined, may be responsible for the changes in placental anatomy and histology observed in PCOS.

First, it is possible that the abnormal pattern of several markers of low-grade chronic inflammation, as demonstrated in non-pregnant women with PCOS (Orio et al., 2005), induces abnormal immune regulation during pregnancy (Samy et al., 2009) with an increase in the frequency and the extent of immune-mediated placental pathologies, such as chronic villitis and intervillositis. The immune system dysregulation in PCOS could induce a reduction in maternal immunological permissiveness to trophoblastic invasion and placentation throughout pregnancy. In this regard, an increased incidence in pro-inflammatory lesions in pre-eclamptic pregnancy has been demonstrated (Moffett and Loke, 2004), and pre-eclampsia and pregnancy-induced hypertension is about four times higher in the PCOS population (Kjerulff et al., 2011).

Second, a subclinical impairment of vascular structure and function has been demonstrated clearly in non-pregnant women with PCOS (Orio et al., 2004; Randeva et al., 2012). Similarly, here we observed an increased thickness of stem villi arterial walls that may reflect subclinical endovascular damage, and a close relationship between placental alterations and PI values that may be potential causative factors for the higher incidence of hypertensive disorders in PCOS (Boomsma et al., 2006; Kjerulff et al., 2011).

The strength of the current study (although perhaps also a limitation) was that only uncomplicated pregnancies were selected, and only non-obese patients with PCOS who had a spontaneous pregnancy were enrolled in the study protocol. Our decision was related to the hypothesis that the placental lesions in pathological pregnancies were characteristic of the pathology itself, as occurs in pre-eclampsia and diabetes (Higging et al., 2011; Naeye, 1978; Stevens et al., 2012). In addition, in order to avoid any potential bias affecting the placental structure in the current study we excluded patients with PCOS with a BMI > 30 kg/m² and/or those who received hormonal drugs, ovulation induction agents or achieved a pregnancy with the use of ART. Thus, our population of patients, although well selected, was probably not representative of the PCOS phenotype, including 50% of patients with PCOS with an ovulatory phenotype and no obese patients, which are both commonly considered characteristics related to metabolic and hormonal alterations. However, even if the current data cannot be formally extended to women with PCOS in general, it is reasonable to assume that the exclusion of such confounders does not limit the importance of our findings and to hypothesize that obese and/or anovulatory patients with PCOS could be affected even more by placental alterations.

In conclusion, the present novel experimental study demonstrated that the morphology and microscopic structure of placenta in patients with PCOS with uncomplicated pregnancy are altered. Further studies evaluating the potential clinical impact of these subclinical placental alterations in increasing the risk of obstetric complications in PCOS are needed.

### Table IV Frequency and extent of the placental lesions in PCOS and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>PCOS</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utero-placental vascular lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>8 (10.4)</td>
<td>4 (11.1)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>57.4 ± 4.0</td>
<td>40.7 ± 4.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Secondary effects of utero-placental vascular lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>6 (7.8)</td>
<td>4 (11.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>24.0 ± 6.2</td>
<td>19.9 ± 6.8</td>
<td>0.10</td>
</tr>
<tr>
<td>Chronic villitis and intervillositis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>35 (45.5)</td>
<td>9 (25.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>50.3 ± 4.5</td>
<td>18.7 ± 2.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Abnormal villus maturity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>7 (9.1)</td>
<td>4 (11.1)</td>
<td>0.74</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>40.6 ± 5.2</td>
<td>25.7 ± 4.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Absence of physiologic change of spiral vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>10 (13.0)</td>
<td>7 (19.4)</td>
<td>0.40</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>36.4 ± 4.7</td>
<td>23.8 ± 6.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Histological evidence of placental abruption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>5 (6.5)</td>
<td>3 (8.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>18.2 ± 3.9</td>
<td>17.7 ± 1.2</td>
<td>0.91</td>
</tr>
<tr>
<td>Meconium staining</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>4 (5.2)</td>
<td>3 (8.3)</td>
<td>0.68</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>30.6 ± 5.5</td>
<td>28.8 ± 3.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [n (%)]</td>
<td>2 (2.6)</td>
<td>2 (5.6)</td>
<td>0.59</td>
</tr>
<tr>
<td>Extension (% pixels)</td>
<td>32.8 ± 5.5</td>
<td>29.9 ± 2.5</td>
<td>0.65</td>
</tr>
</tbody>
</table>

**Figure 3** Images of villitis (A) and intervillositis (B) in placenta samples from women with PCOS (× 600 magnification. Haematoxylin and eosin stain).
Acknowledgements

The authors would like to thank doctors Lucía Mangone and Jacqueline Costa who give their support in the language revision.

Authors’ roles

S.P., study design, manuscript preparation and manuscript revision; T.R., data collection; A.F., data collection, data analysis and manuscript preparation; A.D.C., data collection; A.T., manuscript revision; L.T., data collection; G.B.L.S., study design and manuscript revision; F.Z., manuscript revision.

Funding

The authors declare no financial support for the research.

Conflict of interest

None declared.

References


