

**I. Dissertation**

**Androgen metabolism and reproductive outcome**

submitted by

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## *Declaration*

*I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of “Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz”.*

## *Disclosures*

*Part of this thesis has been published in M Kollmann<sup>1</sup>, P Klaritsch<sup>1</sup>, WP Martins<sup>2</sup>, F Guenther<sup>1</sup>, V Schneider<sup>1</sup>, SA Herzog<sup>3</sup>, L Craciunas<sup>4</sup>, U Lang<sup>1</sup>, B Obermayer-Pietsch<sup>5</sup>, E Lerchbaum<sup>5</sup>, N Raine-Fenning<sup>4</sup>: Maternal and neonatal outcomes in pregnant women with PCOS: Comparison of different diagnostic definitions. Human Reproduction 07/2015; 30(10).*

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*I confirm that all co-authors have explicitly agreed to the use of their data in the thesis and that I have obtained permission to reproduce figures and tables published in Human Reproduction (Maternal and neonatal outcomes in pregnant women with PCOS: comparison of different diagnostic definitions), Fertility and Sterility (Genetic determinants of polycystic ovary syndrome: progress and future directions), and Human Reproduction Update (Pregnancy complications in women with polycystic ovary syndrome and Androgens in pregnancy: roles in parturition).*

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### III. Abbreviations and Definitions

AE-PCOS	Androgen Excess and PCOS Society
AMH	Anti-Müllerian Hormone
ANDR	Androstenedione
AR	Androgen Receptor
ART	Assisted Reproductive Techniques
ASA	Acetylsalicylic Acid
BMI	Body Mass Index
CL	Corpus Luteum
CVD	Cardiovascular Disease
DHEAS	Dehydroepiandrosterone Sulphate
DHT	Dihydrotestosterone
E1	Estrone
E2	Estradiol
E3	Estriol
ESHRE	European Society of Human Reproduction and Endocrinology
FAI	Free Androgen Index
FNPO	Follicle Number per Ovary
FSH	Follicle-Stimulating Hormone
FT	Free Testosterone
G	Grams
GDM	Gestational Diabetes Mellitus
GnRH	Gonadotropin Releasing Hormon
GWAS	Genome-wide Association Studies
HA	Hyperandrogenism
hCG	Human Chorionic Gonadotrophin
ICU	Intensive Care Unit
ICSI	Intracytoplasmic Sperm Injection

IGT	Impaired Glucose Tolerance
IUFD	Intrauterine Fetal Death
IUGR	Intrauterine Growth Restriction
IVF	In-vitro Fertilization
IVM	In-vitro Maturation
LH	Luteinizing Hormone
LGA	Large for Gestational Age
LMWH	Low-Molecular-Weight Heparin
NIH	National Institutes of Health
OA	Oligoanovulation
OD	Ovulatory Dysfunction
oGTT	Oral Glucose Tolerance Test
OHSS	Ovarian Hyperstimulation Syndrome
OR	Odds ratio
P	Progesterone
PCO	Polycystic Ovary
PCOM	Polycystic Ovarian Morphology
PCOS	Polyzystisches Ovar Syndrom/Polycystic Ovary Syndrome
RIA	Radioimmunoassay
RCT	Randomized Controlled Trials
SGA	Small for Gestational Age
SHGB	Sex Hormone Binding Globulin
SNP	Single-Nucleotide Polymorphism
T	Testosterone
TT	Total Testosterone
T2DM	Type 2 Diabetes mellitus

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## VII. Abstract (Deutsch)

Einleitung: Das Polyzystische Ovar Syndrom (PCOS) ist eine heterogene Erkrankung, welche verschiedene Körpersysteme beeinflusst und rund 10% der Frauen im reproduktiven Alter betrifft. Frauen mit einem PCOS leiden häufig an unerfülltem Kinderwunsch und entwickeln während Kinderwunschbehandlungen wie auch während Schwangerschaften häufiger Komplikationen. Das übergeordnete Ziel dieser Arbeit war die Untersuchung der Perinatalperiode von schwangeren Frauen mit PCOS.

Material und Methoden: Drei Studien wurden geplant und an der Medizinischen Universität Graz durchgeführt. In der ersten Studie (1), einer retrospektiven Kohortenstudie, wurde die Prävalenz von mütterlichen und kindlichen Komplikationen bei Frauen mit einem PCOS in Abhängigkeit der derzeit gültigen diagnostischen Kriterien erhoben. Im Rahmen der zweiten Studie (2), einer prospektive Kohortenstudie, wurden bei Frauen mit PCOS die Serumspiegel von Androgenen und Anti-Müller Hormon (AMH) vor, während und nach der Schwangerschaft evaluiert. In der dritten Studie (3) wurde im Rahmen einer prospektiven Kohortenstudie untersucht, ob Kinder von Frauen mit PCOS und Kinder von Frauen ohne PCOS vergleichbare Androgenspiegel haben.

Ergebnis und Zusammenfassung: (1) Unabhängig von den diagnostischen Kriterien weisen rund 60% der Frauen mit einem PCOS und 30% ihrer Kinder perinatale und neonatale Komplikationen auf. Im Vergleich zu Frauen ohne PCOS weisen Frauen mit einem PCOS eine signifikant höhere Rate an mütterlichen Komplikationen auf. (2) Die Serumspiegel von Testosteron, freiem Testosteron, Androstendion (ANDR) und Sexualhormon-Binding-Globulin nehmen mit zunehmendem Gestationsalter zu, während jene von Dehydroepiandrosteron-Sulphat und AMH abnehmen. (3) Die postnatalen Androgenspiegel der Kinder von Frauen mit PCOS und Frauen ohne PCOS unterscheiden sich unabhängig vom Geschlecht nicht signifikant; nur die Serumspiegel von ANDR sind bei Buben von Frauen mit PCOS signifikant höher.

## **VIII. Abstract (English)**

Introduction: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder which occurs in about 10% of women in reproductive age, affects several body systems and leads to reproductive and metabolic complications. Women with PCOS suffer from impaired fertility and higher complication rates during infertility treatment, pregnancy and the perinatal period. The superordinate aim of the project was the investigation of the perinatal period of women with PCOS.

Material and Methods: Three studies were designed and performed at the Medical University of Graz. The first study (1) was a retrospective matched cohort study designed to compare the prevalence of adverse maternal and neonatal outcomes in pregnant women classified with PCOS according to different definitions. The second study (2) was a prospective cohort study investigating serum levels of androgens and anti-Müllerian hormone (AMH) before, during and after pregnancy, and the perinatal outcome of pregnant women with PCOS. The third study (3) was a prospective cohort study evaluating whether androgen and AMH levels in the offspring of women with PCOS are different from those of women without PCOS and whether the perinatal outcome of PCOS and non-PCOS women is different.

Results and Conclusion: (1) Regardless of the PCOS definition, about 60% of women with PCOS and about 30% of their infants were subject to perinatal and neonatal complications. In comparison to healthy controls, the risk for maternal complications was significantly increased in PCOS women, while there was no difference in neonatal complications. (2) Androgen (testosterone, free testosterone, and androstenedione) and sexual hormone binding globulin (SHBG) levels increased throughout pregnancy, while those of dehydroepiandrosterone sulphate (DHEAS) and AMH decreased. (3) Androgen levels in female offspring of PCOS and non-PCOS women did not differ, although maternal hormone levels differed significantly. Postnatal hormone levels were not different in girls and boys of women with PCOS as

compared to those without PCOS women; only androstenedione levels were higher in boys of PCOS women.

## **IX. Introduction**

### **A. History of polycystic ovary syndrome (PCOS)**

PCOS is a heterogeneous endocrine disorder in women which affects several body systems and leads to reproductive and metabolic complications (Fauser et al., 2012; Norman et al., 2007; Wild, 2002). The first description of the association between enlarged ovaries and subfertility was reported in Italy almost 300 years ago, when Antonio Vallisneri described a ‘young peasant woman’ who was ‘moderately plump, infertile, with ovaries larger than normal that, like doves’ eggs, were lumpy, shiny and whitish’ (Vallisneri, 1721). The more common term used to describe such ovaries – ‘polycystic ovaries’ – was not introduced until 1935 (Stein and Leventhal, 1935). Since the mid-1950s the associated health conditions, including subfertility, menstrual disorders and hyperandrogenism (HA) (Norman et al., 2007), have been referred to as ‘polycystic ovary syndrome’ (DAVIS et al., 1956; Keetel et al., 1957).

The prevalence of PCOS varies widely depending on ethnicity, body composition, and the definition used for diagnosis (Alvarez-Blasco et al., 2006; Asunción et al., 2000; Azziz et al., 2009; Group, 2004a; Group, 2004b; Li et al., 2013; Wang and Alvero, 2013). Data, which derives from different countries, show that in women of reproductive age it accounts for 5–10%, but may reach almost 30% in overweight and obese women (Alvarez-Blasco et al., 2006; Apridonidze et al., 2005; Asunción et al., 2000; Li et al., 2013; March et al., 2010). PCOS-like phenotypes have also been detected in monkeys suggesting that PCOS may be an ancient phenotype with a definable pathogenic mechanism (Abbott et al., 2017).

### **B. Current definitions/criteria**

At present, there are three main definitions for PCOS. The National Institutes of Health (NIH) recommended in 1990 to use HA and ovulatory dysfunction (OD) as diagnostic criteria.

The ESHRE/ASRM definition was published in 2004 (Group REA-SPCW, 2004a,b). Accordingly, PCOS is diagnosed when two of the following criteria are present: oligo-/amenorrhea, clinical and/or biochemical signs of HA and polycystic ovarian morphology (PCOM) on ultrasound.

The Androgen Excess and PCOS Society (AE-PCOS) published the most recent definition in 2006 suggesting to involve the presence of HA (clinical and/or biochemical) and ovarian dysfunction [oligoanovulation (OA) and/or polycystic ovaries] (Azziz et al., 2009).

### C. Phenotypes in polycystic ovary syndrome

Different criteria include different phenotypes of PCOS. The NIH criteria describe six different phenotypes. The result of adding PCO as a criterion according to the ESHRE/ASRM definition was the inclusion of four additional phenotypes in the diagnosis of PCOS: women with HA (biochemical and/or clinical) and PCO but normal ovulation, and women with anovulation and PCO but no HA. In total, there are ten different phenotypes. The 2006 AE-PCOS criteria consider HA essential for the diagnosis of PCOS, thereby excluding one of the additional ESHRE/ASRM phenotypes (see table 1) (Azziz et al., 2009; Kollmann et al., 2015).

	PCOS by NIH 1990 (HA + OA + PCO or HA + OA) n = 85						Added using AE-PCOS 2006 (HA + PCO), n = 14			Further added using ESHRE/ASRM 2003 (OA + PCO), n = 78
Group	I	I	I	I	I	I	II	II	II	III
Phenotypes	A	B	C	D	E	F	G	H	I	J
Biochemical HA	+	+	+	+	-	-	+	-	+	-
Clinical HA	+	+	-	-	+	+	+	+	-	-
OA	+	+	+	+	+	+	-	-	-	+
PCO	+	-	+	-	+	-	+	+	+	+
NIH 1990	■	■	■	■	■	■	□	□	□	□
AE-PCOS 2006	■	■	■	■	■	■	■	■	■	□
ESHRE/ASRM 2003	■	■	■	■	■	■	■	■	■	■

HA, Hyperandrogenism; OA, Oligo-anovulation; PCO, Polycystic ovaries;  
 ■, phenotype included in criteria; □, phenotype not included in criteria.

**Table 1 Current possible phenotypes (Kollmann et al., 2015). HA=Hyperandrogenism, OA=Oligo-anovulation, PCO=Polycystic Ovaries. Reproduced from Human Reproduction (Kollmann et al., 2015) with permission of Oxford University Press.**

## 1. ESHRE/ASRM criteria

The ESHRE/ASRM criteria, which were published in 2004, are most commonly used in Europe and will therefore be described in a more detailed way. PCOS is diagnosed when two of the following criteria are present: oligo-/amenorrhea, clinical and/or biochemical signs of HA and polycystic ovarian morphology (PCOM) on ultrasound. Disorders with a similar clinical presentation, such as congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumours, must be excluded (Group, 2004b; group, 2004).

### a. Hyperandrogenism and metabolic markers

The consensus statement suggests that patients can either have a clinical hyperandrogenism, a biochemical hyperandrogenism or both. The clinical hyperandrogenism can be expressed by hirsutism, acne and androgenic alopecia. However, normative data in large populations are still lacking and the assessment of clinical features is relatively subjective (Group, 2004b; group, 2004). The statement suggests to measure biochemical hyperandrogenism, although there are limitations mostly due to the inaccuracy and variability of the laboratory methods of measurement that are often used. They comment on total testosterone (T), free testosterone (FT), free testosterone (free androgen) index (FAI), dehydroepiandrosterone sulphate (DHEAS), and androstenedione (ANDR). Measuring FT or FAI was the more sensitive methods of assessing hyperandrogenemia (Cibula et al., 2000; Imani et al., 2000; Vermeulen et al., 1999). The measurement of T only may not be a very sensitive marker of androgen excess and some PCOS patients may have isolated elevations in DHEAS levels (ESHRE 2004HR/FS). Finally, they state that little data are available on the value of routinely measuring ANDR in hyperandrogenic patients (Laven et al., 2002).

b. Oligo- and/or anovulation

Oligoovulation means infrequent or irregular ovulation and anovulation means absence of ovulation. PCOS patients often present with oligomenorrhea, which means infrequent, often light menstrual periods (intervals exceeding 35 days) or amenorrhea which describes the absence of a menstrual period in a woman of reproductive age. Those symptoms are associated with oligo- and/or anovulation.

c. Polycystic ovary

As previously described it was already 300 years ago that Vallisneri wrote about a 'young peasant woman' who was 'moderately plump, infertile, with ovaries larger than normal that, like doves' eggs, were lumpy, shiny and whitish' (Vallisneri, 1721). However, the more common term used to describe such ovaries – 'polycystic ovaries' – was not introduced until 1935 (Stein and Leventhal, 1935). And it was not until 2003 that PCO became part of diagnostic criteria (Group, 2004b; group, 2004). The 2003 ESHRE/ASRM definition emphasizes the importance of ultrasound as a diagnostic tool (Group REA-SPCW, 2004a,b). Based on one publication by Jonard et al., the statement suggests that the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (>10 mL) should be taken to define a 'polycystic ovary' (Group, 2004b; Jonard et al., 2003). Only one ovary fitting this definition is sufficient to define PCO. This definition does not apply to women taking an oral contraceptive pill and the scan should be repeated during the next cycle if the ovaries contain a dominant follicle (>10 mm) or a corpus luteum (Group, 2004b).

## **D. Recent studies**

Since the publication of the consensus statement in 2004, many studies have been performed looking at PCO diagnosis by ultrasound and further biomarkers.

Regarding the diagnosis of PCOM: The threshold of 12 follicles was based on a study published by Jonard et al. in 2003, which reported that a FNPO of 12 or more offered the best compromise between specificity (99%) and sensitivity (75%) in the detection of hyperandrogenic anovulation (Jonard et al., 2003). However, recent studies, using new ultrasound machines with improved resolution, have shown a high prevalence of ovaries with more than 12 follicles in healthy young women (Duijkers and Klipping, 2010; Johnstone et al., 2010; Jokubkiene et al., 2012). A task force from the Androgen Excess and Polycystic Ovary Syndrome Society (AE-PCOS) reviewed results from recent studies (Dewailly et al., 2011; Duijkers and Klipping, 2010; Johnstone et al., 2010; Kristensen et al., 2012; Lujan et al., 2013) and reported an urgent need to update the diagnostic criteria (Dewailly et al., 2013). They recommend that the threshold should be increased to  $\geq 25$  follicles when using new ultrasound machines and that ovarian volume ( $\geq 10$  mL) should be used in the detection of HA (in the absence of a dominant follicle or a corpus luteum) when a new ultrasound machine is not available (Dewailly et al., 2013). We welcome the call to update the current criteria and hope that these findings will lead to a revision of the present criteria and some long overdue changes (Kollmann et al., 2014a, b; Martins et al., 2014).



**Figure 1** Polycystic ovary (a). Automatic volume calculation (SonoAVC) was used to automatically calculate the volume of the follicle (b)

Anti-Müllerian hormone (AMH), a peptide produced by the granulosa cells (GC) of ovarian follicles is discussed as an additional biomarker in diagnosis and management of PCOS patients (Dewailly et al., 2011; Dewailly et al., 2013; La Marca and Sunkara, 2013). There seems to be a consistent relationship between AMH serum levels and the FNPO seen on

ultrasound (Dewailly et al., 2011; Pigny et al., 2006). Assays have changed over the last years and it seems that the modification of the Beckman-Coulter second-generation enzyme-linked immunosorbent assay protocol improves the reliability of serum AMH measurement (Craciunas et al., 2015). However, only a few studies have assessed specifically whether AMH serum concentrations might be an effective surrogate marker of PCOM (Dewailly et al., 2011; Dewailly et al., 2013; Pigny et al., 2006).

## **E. Etiology**

The etiology of PCOS is not particularly mapped, but a complex interaction of various factors can be assumed. It seems that the disorder arises from interactions between genetic, environmental and intrauterine factors (de Melo et al., 2015).

The first evidence for a genetic basis of PCOS was already reported in 1968 (Cooper et al., 1968). With the performance of genome-wide association studies (GWAS) new insights were brought into the heritability of PCOS (Jones and Goodarzi, 2016; Liu et al., 2016). Currently, six different GWAS studies of PCOS have been published and 16 genes/loci have been identified (Jones and Goodarzi, 2016; Liu et al., 2016) (table 2 (Jones and Goodarzi, 2016)).

PCOS risk loci reported in GWAS to date.					
Locus	Nearest gene	GWAS index SNP	Discovery P value	Discovery population	Replication population(s) <sup>a</sup>
2p16.3	<i>LHCGR</i>	rs13405728	$7.55 \times 10^{-21}$	CHN	SRD, EUR, EGY, IND, ARB, CHN
2p16.3	<i>FSHR</i>	rs2268361	$9.89 \times 10^{-13}$	CHN	EUR, ARB, CHN
		rs2349415	$2.35 \times 10^{-12}$	CHN	
2p21	<i>THADA</i>	rs13429458	$1.73 \times 10^{-23}$	CHN	EUR, CHN
2q34	<i>ERBB4</i>	rs1351592	$1.2 \times 10^{-12}$	EUR	
5q31.1	<i>RAD50</i>	rs13164856	$3.5 \times 10^{-9}$	EUR	
8p32.1	<i>GATA4</i>	rs804279	$8.0 \times 10^{-10}$	EUR	
9q33.3	<i>DENND1A</i>	rs2479106	$8.12 \times 10^{-9}$	CHN	EUR
9q22.32	<i>C9orf3</i>	rs4385527	$5.87 \times 10^{-9}$	CHN	CHN, EUR
11p14.1	<i>FSHB</i>	rs11031006	$1.9 \times 10^{-8}$	EUR	EUR, CHN
11q22.1	<i>YAP1</i>	rs1894116	$1.08 \times 10^{-22}$	CHN	EUR, CHN
		rs11225154	$7.6 \times 10^{-11}$	EUR	CHN
12q14.3	<i>HMGA2</i>	rs2272046	$1.95 \times 10^{-21}$	CHN	EUR
12q13.2	<i>RAB5B/SUOX</i>	rs705702	$8.64 \times 10^{-26}$	CHN	EUR
12q21.2	<i>KRR1</i>	rs1275468	$1.9 \times 10^{-8}$	EUR	
16q12.1	<i>TOX3</i>	rs4784165	$3.64 \times 10^{-11}$	CHN	EUR
19p13.3	<i>INSR</i>	rs2059807	$1.09 \times 10^{-8}$	CHN	EUR
20q13.2	<i>SUMO1P1</i>	rs6022786	$1.83 \times 10^{-9}$	CHN	

Note: ARB = Arabic women in Bahrain; CHN = Han Chinese; EGY = Egyptian; EUR = white of European descent; IND = South Asian Indian; SRD = Sardinian Italian.  
<sup>a</sup> Successful replication was considered to be on a locus-wide basis (not direct replication of the GWAS index SNP) with  $P < .05$ .

**Table 2 PCOS risk loci reported in genome-wide association studies (GWAS). (Jones and Goodarzi, 2016). Reproduced from Fertility and Sterility (Jones and Goodarzi, 2016) with permission of Elsevier.**

More and more studies also discover that epigenetic variations, including gene methylation, histone modification, microRNAs, and RNA binding proteins play a role in determining the PCOS phenotype (Ilie and Georgescu, 2015; Xu et al., 2010; Xu et al., 2011; Yu et al., 2015). However, the heterogeneity of PCOS and the presence of different phenotypes may also suggest further etiological factors.

Environmental determinants of PCOS were summarized in a review by Merkin et al. (Merkin et al., 2016). They main factors they considered included environmental toxins, diet and nutrition, socioeconomic status, and geography. The authors found some evidence that environmental toxins play a role in disrupting reproductive health, but there is limited research as to how these toxins may affect the development of PCOS (Merkin et al., 2016). Studies showed that PCOS symptoms are reduced with certain dietary supplements and with weight loss among obese women. However, additional research is needed to compare various approaches to weight loss (Merkin et al., 2016). Furthermore, an association of low socioeconomic status with certain PCOS phenotypes has been indicated (Merkin et al., 2016).

The third factor which seems to play a crucial role in the development of PCOS is the intrauterine milieu during pregnancy and the early childhood (Barker, 1995; de Melo et al., 2015; Dumesic et al., 2007; Dumesic et al., 2014). Hales and Barker published already in 1992 that a poor fetal and early post-natal nutrition imposes mechanisms of nutritional thrift upon the growing individual which subsequently can lead to an impaired development of the endocrine pancreas (Barker, 1992; Hales and Barker, 1992, 2013). And it has been suggested that prenatal growth restraint followed by catch-up of weight during infancy can lead to PCOS (de Zegher and Ibáñez, 2006). Experimental studies in non-humans and clinical observations in humans support the hypothesis that developmental programming by steroid excess plays a role in the development of PCOS (Abbott et al., 2005; Abbott et al., 2002; Abbott et al., 2013; Melo et al., 2010; Palomba et al., 2012). Sex differences in prenatal androgen levels have been observed and testosterone levels in umbilical cord blood and in amniotic fluid are higher in healthy male babies than in healthy female babies (Maccoby et al., 1979; van de Beek et al., 2004). There are just a few studies reporting on the relation between maternal androgen levels during pregnancy and the respective offspring in PCOS women (Abbott et al., 2005; Barry et al., 2011; Barry et al., 2010; Caanen et al., 2016; Dahlgren et al., 1992; Dunaif et al., 1989; Helseth et al., 2014). Barry showed that umbilical vein (UV) T in PCOS girls was significantly raised, compared with control girls (Barry et al., 2010). Another study did not find any significant differences between girls of PCOS mothers and girls of mothers without PCOS (Caanen et al., 2016).

## **F. Pathophysiology**

As ovarian steroidogenesis requires gonadotropin stimulation, luteinizing hormone (LH) has a key role in hyperandrogenemia of PCOS (Burt Solorzano et al., 2012). The pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus is often disturbed in PCOS patients, which subsequently leads to a LH hypersecretion. PCOS patients often show a

resistance of their GnRH pulse generator to progesterone, which regulates GnRH pulse frequency (Pastor et al., 1998). The resistance to progesterone seems to be mediated by androgen excess (Eagleson et al., 2000). Serum follicle-stimulating hormone (FSH) levels are usually normal. However, ovarian follicles seem to be more resistant to FSH in women with PCOS than in controls, an effect which might be due to increased levels of intra-ovarian AMH (Burt Solorzano et al., 2012; Burt Solorzano et al., 2010; McCartney and Marshall, 2016). Ovaries from PCOS women have an exaggerated steroidogenic response to gonadotropins (Ehrmann, 2005). PCOS is often associated with insulin resistance which leads to hyperinsulinemia, a status which contributes to hyperandrogenemia as well (McCartney and Marshall, 2016). Hyperinsulinemia increases LH-stimulated androgen synthesis by ovarian theca cells, it potentiates corticotropin-mediated adrenal androgen production, and it reduces hepatic production of sex hormone-binding globulin (SHBG), which augments free testosterone levels (McCartney and Marshall, 2016).

## **G. Short-and long-term consequences of polycystic ovary syndrome**

PCOS is associated with different short- and long-term comorbidities. Insulin resistance can be seen in approximately 60–80% of women with PCOS and in 95% of obese women with PCOS (Wild et al., 2010). Studies show that the incidence of metabolic syndrome, gestational diabetes mellitus (GDM), impaired glucose tolerance (IGT) and type-2 diabetes mellitus (T2DM) is increased in premenopausal women with PCOS compared with age-matched and BMI-matched controls (Moran et al., 2010). Hirsutism, acne and androgenic alopecia are clinical signs of hyperandrogenism and more often seen in PCOS patients (Azziz et al., 2009). Even though PCOS occurs in obese and lean women, a recent meta-analysis found that obesity was more prevalent in women with PCOS than in women without PCOS (Lim et al., 2012). Cardiovascular disease (CVD) markers indicate a higher risk of CVD in women with PCOS than in controls (Carmina, 2014): coronary calcification is more prevalent in women with PCOS (Christian et al., 2003; Talbott et al., 2000; Talbott et al., 2004), the intimal layer

thickness of the carotid wall has been reported to be greater in women with PCOS (Lass et al., 2011; Luque-Ramírez et al., 2007), and PCOS patients show an elevated incidence of aortic calcification (Talbot et al., 2004). Mood disturbances, mostly severe depression, are more frequent in PCOS women. A meta-analysis found that abnormal depression scores are higher in women with PCOS compared with those in the control groups (Dokras et al., 2011). Due to the concurrence of hyperestrogenic anovulation and hyperinsulinemia, a higher risk for endometrial cancer was found in women with PCOS (odds ratio [OR] 2.7) (Dumesic and Lobo, 2013). PCOS women may also have a higher risk for ovarian cancer, though the OR for this malignancy is not clear (Dumesic and Lobo, 2013).

## **H. Polycystic ovary syndrome and reproductive consequences**

Women with PCOS have an impaired fertility and significant higher complication rates during infertility treatment, pregnancy and the perinatal period (Azziz et al., 2016; Boomsma et al., 2006; Palomba et al., 2015; Qin et al., 2013). Complications include the occurrence of multiple gestations, ovarian hyperstimulation syndrome (OHSS), and early pregnancy loss (Azziz et al., 2016; McCartney and Marshall, 2016; Norman et al., 2007). When pregnant, these women are susceptible to perinatal complications, including elevated risk of GDM, pregnancy-induced hypertension (PIH), pre-eclampsia, preterm delivery and caesarean section (Boomsma et al., 2006; Kollmann et al., 2015; Palomba et al., 2015; Qin et al., 2013). The offspring of PCOS women is affected by higher intensive care unit (ICU) admission rates and lower birthweight (Qin et al., 2013). The exact aetiology remains unclear, but it seems that the complications are the result of several coinciding factors which have an effect on trophoblast invasion and placentation (Figure 1) (Boomsma et al., 2006; Kollmann et al., 2015; Palomba et al., 2015; Qin et al., 2013). Maternal hyperandrogenism and insulin resistance play a key role (Makieva et al., 2014). The clinical relevance of ovarian dysfunction, defined by polycystic ovarian morphology (PCOM) or anovulation, was discussed controversial (Palomba et al., 2010; Shroff et al., 2007). Therefore, the aim of our first study was to

compare the prevalence of adverse maternal and neonatal outcomes in pregnant women classified with PCOS according to different definitions (Kollmann et al., 2015). Some studies have investigated the prevention and management of pregnancy complications in women with PCOS (Agha et al., 2014; Balsells et al., 2015; Chakraborty et al., 2013a; Chakraborty et al., 2013b; Løvvik et al., 2015; Magnussen et al., 2011; Palomba et al., 2015; Palomba et al., 2014a; Palomba et al., 2014b; Peterson et al., 2015; Ramidi et al., 2009). Many observational findings showed a lower risk of obstetric and neonatal adverse outcomes in normal-weight women compared to overweight/obese women and therefore losing weight before conception up to an optimal body weight is suggested. Pregnant women, who are obese, irrespective of having PCOS, should be informed about the beneficial effects of dietary and/or physical activity during pregnancy on the gestational weight gain (Agha et al., 2014). The American Diabetes Association suggests testing PCOS women for GDM at the first prenatal visit (Association, 2016a, b). In infertile women with PCOS maximum effort should be made to avoid multiple pregnancies (Løvvik et al., 2015).

## **1. Therapeutics**

Different pharmacological measures have been discussed in women with PCOS during pregnancy in order to reduce the obstetric and neonatal risks. The best tested drug in PCOS women is metformin. It seems that metformin is effective and safe for the treatment of GDM, especially in overweight and obese women (Balsells et al., 2015). There might further be beneficial effects of using metformin compared to insulin in GDM. Those are related to maternal weight gain during pregnancy, neonatal outcomes and patient compliance (Lautatzis et al., 2013; Rowan et al., 2008; Sivalingam et al., 2014). However, the beneficial effects of metformin were all seen in non-randomized controlled trials (RCTs). The only RCT on metformin administration during pregnancy in PCOS women did not show a beneficial effect on the development of GDM (Vanky et al., 2010). For the prevention of PIH and PE metformin seems not beneficial (Palomba et al., 2009). Studies regarding pharmacological

measures and spontaneous abortion or recurrent pregnancy loss are scarcer. Two studies showed that the administration of acetylsalicylic acid (ASA) and low-molecular-weight heparin (LMWH), as monotherapy or a combined scheme had a benefit (Chakraborty et al., 2013a; Chakraborty et al., 2013b). Another study found that LMWH alone or in combination with metformin reduced pregnancy loss in PCOS women with a coagulation disorder (Ramidi et al., 2009).

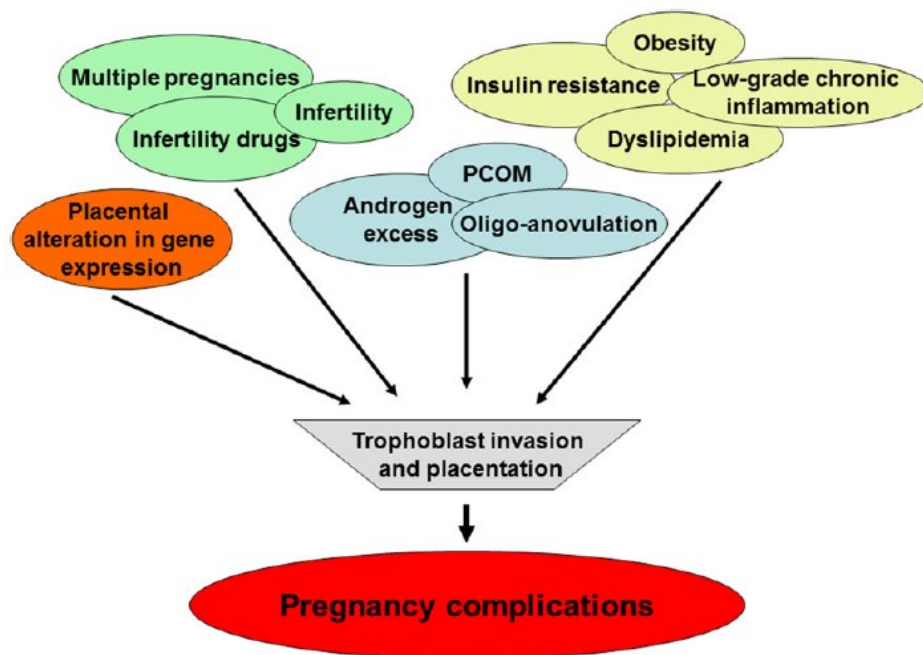


Figure 2 Possible causes of the increased risk of pregnancy complications in PCOS women (Palomba et al., 2015). Reproduced from Human Reproduction Update (Palomba et al., 2015) with permission of Oxford University Press.

## I. Subfertility

According to the World Health Organization, PCOS is the most common cause of anovulatory infertility and eugonadotrophic hypogonadism and 55% to 91% of these women consider having signs and/or symptoms of the disease (Broekmans et al., 2006; Group, 2012). A Birth Cohort study of North Finland showed that women with PCOS are more likely to be subfertile, with 26% struggling to conceive compared with 17% of women without signs of PCOS (Koivunen et al., 2008). However, population studies have revealed that while women

with PCOS may take longer than expected to conceive, their 'lifetime fertility' does not appear to be significantly impaired (Group, 2012; Koivunen et al., 2008).

## **1. Therapeutics**

The first line treatment of PCOS-related infertility includes lifestyle modification (weight loss in overweight/obese) and the use of drugs to induce monofollicular ovulation. Drug treatments normally begin with the use of clomiphene citrate followed by the administration of exogenous gonadotropins, with timed intercourse or intrauterine insemination. Assisted reproductive techniques (ART), particularly in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), represent the third line of treatment (Broekmans et al., 2006). Ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies remain the major complications of ovulation induction and occur despite ultrasound monitoring (Brown et al., 2009; Nastri et al., 2010). PCOS women are typically more difficult to stimulate in a controlled manner, whether the intention is to induce a monofollicular or multifollicular response, are more likely to demonstrate resistance to stimulation and/or an exaggerated response and experience a higher cycle cancellation rate than women without PCOS. Whereas a high number of oocytes may be obtained during ART, there are concerns that the quality and maturity of these oocytes may be impaired (Baumgarten et al., 2013; Coffler et al., 2003a; Coffler et al., 2003b; Doronzo et al., 2004; Jayaprakasan et al., 2012; Kumar et al., 2013; Ocal et al., 2011; Siristatidis et al., 2013). Recent developments have seen the introduction of various actions to reduce the risks of OHSS and cycle cancellation and to improve oocyte quality. These measures include priming with metformin, the use of GnRH antagonist cycles as opposed to the conventional long GnRH agonist protocol, the administration of dopamine agonists and oocyte retrieval without controlled ovarian stimulation through in-vitro maturation (IVM) of oocytes (Al-Inany et al., 2011; Baumgarten et al., 2013; Nardo et al., 2013; Nastri et al., 2010; Ortega-Hrepich et al., 2013; Tang et al., 2012; Tso et al., 2014). To examine the efficacy of all

strategies we performed a systematic review and meta-analysis of randomized controlled trials (RCTs) aiming to improve ART outcomes in women with PCOS (Kollmann et al., 2016).

There is low- to moderate-quality evidence suggesting that antagonist protocols are preferable to agonist ones, because they reduce the incidence of OHSS without interfering with clinical pregnancy and live birth for PCOS women. Moreover, there is low-quality evidence pointing to a benefit of metformin supplementation on clinical pregnancy and live birth; and that ovulation induction and administration of estradiol seem to be equally effective for endometrial preparation before frozen embryo transfer for women with PCOS (Kollmann et al., 2016).

## **J. Hormone levels during pregnancy**

### **1. Androgens in pregnancy**

Androgens are especially important for the development of the male reproductive tract during fetal life and they do act as pro-hormones for biosynthesis of estrogens in both sexes (Macleod et al., 2010; Purohit and Foster, 2012; Rivas et al., 2002; Scott et al., 2007; Traish et al., 2011; Welsh et al., 2008). In women, androgens are normally synthesized by ovaries, the adrenal glands, and also in adipose tissue. Studies show that some androgen levels increase during normal pregnancy and it has been hypothesized that androgens act as substrates for estrogen formation in the placenta (Edman et al., 1981; Makieva et al., 2014; PION et al., 1965; Siiteri and MacDonald, 1966; Smith, 2007). DHEAS, which is produced by the fetal and maternal adrenals, enters the placenta, where it is metabolized to ANDR and T. This gets further metabolized to estrone (E1) and estradiol (E2) (Strauss et al., 1996). E2 can enter the fetal circulation, taken up by the liver and transformed into estriol (E3), which subsequently can pass to maternal circulation (HIRANO, 1961; Schwarzel et al., 1973; Willows, 1966). In women who are not pregnant, 50% of all DHEA is produced by the adrenal glands, 20% by the ovaries, and 30% by peripheral tissue (Abraham, 1974). Regarding ANDR, 50% is synthesized in the adrenal glands and 50% in the ovaries (Longcope, 1986). One half of

testosterone is synthesized in the peripheral tissue, the other half is produced by the ovaries and adrenal glands (25% and 25% respectively) (Piltonen et al., 2002). In pregnant women, the fetus and the placenta are additional sources of androgens (Cantineau et al., 1985; Makieva et al., 2014). As mentioned above, numeral studies have found elevated levels of some circulating androgens during normal pregnancy (Bammann et al., 1980; Buster et al., 1979; Dawood and Saxena, 1977; Mizuno et al., 1968; Rivarola et al., 1968; Saez et al., 1972). Total testosterone (tT) increases from the first trimester of pregnancy and towards term (Bammann et al., 1980; Berger et al., 1984; Saez et al., 1972), free testosterone (fT) levels only at the third trimester (Dawood and Saxena, 1977). ANDR seems to be increased between 37-42 weeks of gestation (Mizuno et al., 1968). On the contrary, DHEAS levels are up to 50% lower in pregnant women than in non-pregnant women (Milewich et al., 1978). Sexual hormone binding globulin (SHBG) levels rise dramatically from the first trimester until term (Wilke and Utley, 1987).

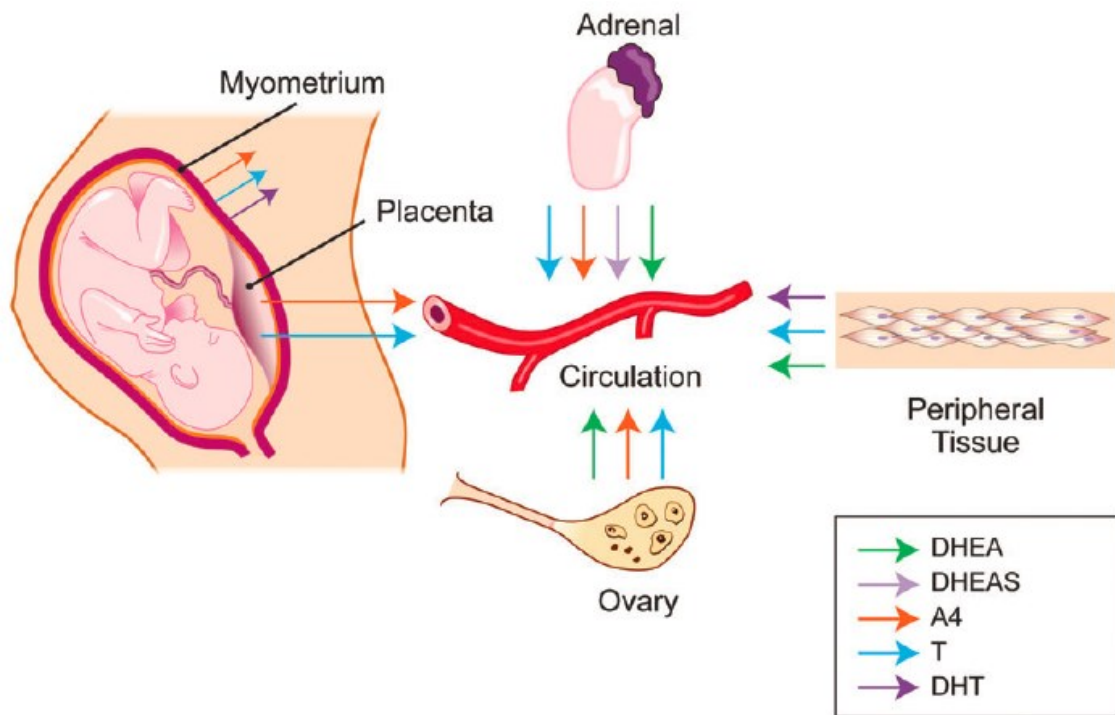
Fetal hormone levels depend on fetal sex and gestation. T levels are higher in male fetuses and increase until the end of first trimester (Rodeck et al., 1985). At 12 weeks of gestation, T levels reach a peak of around 150 ng/dL and fall by around 70% afterward (Rodeck et al., 1985). In female fetuses, T levels are generally lower until the second trimester and they fall further at term (Diez d'Aux and Pearson Murphy, 1974). DHEA concentrations are also higher in male fetus compared to female fetuses (Keelan et al., 2012). On the contrary, ANDR levels are similar in both sexes (Keelan et al., 2012). Interestingly, one study shows that labour is associated with an increase in concentrations of ANDR, DHEA and SHBG, and decrease of tT and fT (Keelan et al., 2012). Rivarola et al. investigated the association between fetal sex and maternal serum levels of any androgen and found none (Rivarola et al., 1968).

As mentioned above, it has been shown that some androgen levels increase during normal pregnancy (Edman et al., 1981; Makieva et al., 2014; PION et al., 1965; Siiteri and MacDonald, 1966; Smith, 2007). Although the origin and cause of the higher levels are not known exactly, it is probable that the production involves the ovary and the placenta (Makieva

et al., 2014). After ovulation, androgens are produced by small luteal cells of the corpus luteum (CL) (Sanders et al., 1996). Within the first trimester, human chorionic gonadotrophin (hCG) stimulates the CL and increases until the end. The increase of hCG could therefore cause the augmentation of T levels (Braunstein et al., 1976; Liu and Hsueh, 1986). On the other hand, we see a steady rise of T after the known peak and further decrease of hCG levels at the end of the first trimester. This suggests either that the androgen production is regulated alternatively or that there is a further source of androgens after the end of first trimester (Braunstein et al., 1976). Maternal adrenal glands are a further important source of androgen production during pregnancy (Makieva et al., 2014). Studies show that the production of DHEA during pregnancy is suppressed by E2 (Albrecht and Pepe, 1995; Tagawa et al., 2004; Umezaki et al., 2001).

Former studies found that the placenta does not have the capacity to synthesize androgens de novo (PION et al., 1965; Siiteri and MacDonald, 1966). However, a recent study found that the placental syncytiotrophoblast has this ability (Escobar et al., 2011). Precisely, the study found that the syncytiotrophoblast expresses the enzyme CYP17, which converts C21 steroids to C19 steroids (Escobar et al., 2011).

A possible role of the myometrium in androgen production has been shown in an animal study. The study found that uteri from non-pregnant and early pregnant pigs can synthesize ANDR and T in vitro (Franczak, 2008). Possible origins of androgens are shown in figure 2.



**Figure 3 Possible sources of androgens during pregnancy; dehydroepiandrosterone (DHEA), testosterone (T), dihydrotestosterone (DHT), dehydroepiandrosterone sulphates (DHEAS), androstenedione (A4) (Makieva et al., 2014). Reproduced from Human Reproduction Update (Makieva et al., 2014) with permission of Oxford University Press.**

As mentioned above, androgen excess is suspected to play a role in the development of pregnancy complications (Palomba et al., 2015). Likewise, we know that there are pregnancy-specific mechanisms to protect both the mother and fetus from pregnancy-induced androgen excess (Crisosto et al., 2012; Hensleigh et al., 1975; Phelan and Conway, 2011). One is the physiological increase of maternal SHBG, which binds elevated androgens (Hammond, 2011). Another mechanism is the increase of progesterone (P), which competes for androgen receptor (AR) binding (Birrell et al., 2007; Slayden et al., 2001). A third mechanism might be that P has an affinity for 5 $\alpha$ -reductase which results in an inhibition of the conversion of T to the more potent dihydrotestosterone (DHT) (Cabeza et al., 1999; Hodgins, 1982).

## 2. AMH in pregnancy

There are some reports on AMH levels during pregnancy (Kuijper et al., 2013; Köninger et al., 2013; Köninger et al., 2015; La Marca et al., 2005; Massé et al., 2011; Nelson et al., 2010;

Plante et al., 2010; Shand et al., 2014; Vanky and Carlsen, 2012). As previously described, we know that AMH is produced in women by the granulosa cells of the ovarian (pre-)antral follicles. Until puberty AMH is very low in females (Kuijper et al., 2013). In male fetuses, AMH is produced by Sertoli cells soon after testicular differentiation and it is essential for regression of the Müllerian ducts (La Marca et al., 2005). The hormone is measurable in the serum of males during their whole life. However, after puberty T suppresses AMH secretion (Bergadá et al., 2006; Lee et al., 1996; Pierik et al., 2009). One study investigated the effect of fetal gender on maternal AMH levels and found no connection (La Marca et al., 2005). The more recent and bigger studies suggest a decline of AMH in maternal serum during gestation (Königer et al., 2013; Königer et al., 2015; La Marca et al., 2005; Li et al., 2010; Nelson et al., 2010). A possible explanation for the decline could be the inactivated menstrual cycle during pregnancy and absence of follicular development (Kuijper et al., 2013).

## **K. Aims**

The superordinate aim of the project was the investigation of the perinatal period of women with PCOS. Therefore, we designed three major studies:

### **1. 1<sup>st</sup> study - Maternal and neonatal outcomes in pregnant women with PCOS: comparison of different diagnostic definitions (Kollmann et al., 2015)**

Studies show that PCOS women are susceptible to perinatal complications including elevated risk of gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, preterm delivery and caesarean section (Boomsma et al., 2006; Palomba et al., 2015; Qin et al., 2013) and that their offspring is affected by higher intensive care unit (ICU) admission rates and lower birth weight (Qin et al., 2013).

At present, there are three main definitions for PCOS: the NIH criteria, the ESHRE/ASRM criteria, and the AE-PCOS criteria (Azziz et al., 2009; Group, 2004b). Thus, there are several different phenotypes of PCOS, which are subject of intense debate (Carmina et al., 2005; Chang et al., 2005; Palomba et al., 2013; Palomba et al., 2010; Shroff et al., 2007).

The aim of this study was to compare the prevalence of adverse maternal and neonatal outcomes in pregnant women classified with PCOS according to different definitions.

## **2. 2<sup>nd</sup> study – Longitudinal study of pregnant PCOS women**

The aim of the longitudinal study of pregnant PCOS women was to evaluate the androgen and AMH levels before, during and after pregnancy and to evaluate the perinatal outcome.

## **3. 3<sup>rd</sup> study – Cross-sectional study of pregnant PCOS women and their offspring compared to non-PCOS women and their offspring**

The first aim of the cross-sectional study of pregnant PCOS and non-PCOS women and their offspring was to investigate whether the offspring from PCOS women already show higher androgen and AMH levels compared to the offspring from non-PCOS women. The second aim was to evaluate the perinatal outcome of PCOS and non-PCOS women.

## **X. 1st study - Maternal and neonatal outcomes in pregnant women with PCOS: comparison of different diagnostic definitions (Kollmann et al., 2015)**

### **A. Material and Methods**

#### Study design

Retrospective matched cohort study

#### Setting

Data of primiparous women with PCOS according to ESHRE/ASRM 2003 criteria and healthy controls giving birth to neonates  $\geq 500\text{g}$  at the Medical University of Graz, Austria, between January 2004 and March 2012 were retrospectively retrieved. Data were extracted from the local perinatal database (PIA, ViewPoint, GE Healthcare, Zipf, Austria) and the medical documentation system or patient files (Kollmann et al., 2015).

#### Ethical Approval

The study was approved by the institutional review board (No: 24-282ex11/12).

#### Participants

PCOS was diagnosed following clinical and sonographic evaluation and a hormonal analysis. Ultrasound examinations are routinely performed by medical doctors specialized in obstetrics and gynaecology. Clinical investigations are performed in cooperation with the Division of Endocrinology and Metabolism at the Department of Internal Medicine at Medical University of Graz, Austria. We report on a population of women that were treated in our specialized unit and were delivered at our institution (Kollmann et al., 2015).

Women were assigned to three groups according to the different definitions for PCOS:

- Group 1: PCOS by NIH criteria = HA + OA + PCO or HA + OA.
- Group 2: Added using AE-PCOS criteria = HA + PCO.
- Group 3: Further added using ESHRE/ASRM criteria = OA + PCO.

The healthy control group comprised women without PCOS, pre-gestational diabetes, or pre-gestational hypertension. For all PCOS case per year four control cases with information on BMI were randomly selected (Kollmann et al., 2015).

### Variables

Primary outcome parameter was the composite complication rate per women and newborns. Secondary outcome parameters were specific maternal and neonatal complications including gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, operative delivery (elective and non-elective caesarean section and operative vaginal delivery), large or small for gestational age infants, preterm birth (< 37 and < 34 weeks of gestation, respectively), acidosis, ICU admission, and pre- and perinatal mortality (Kollmann et al., 2015).

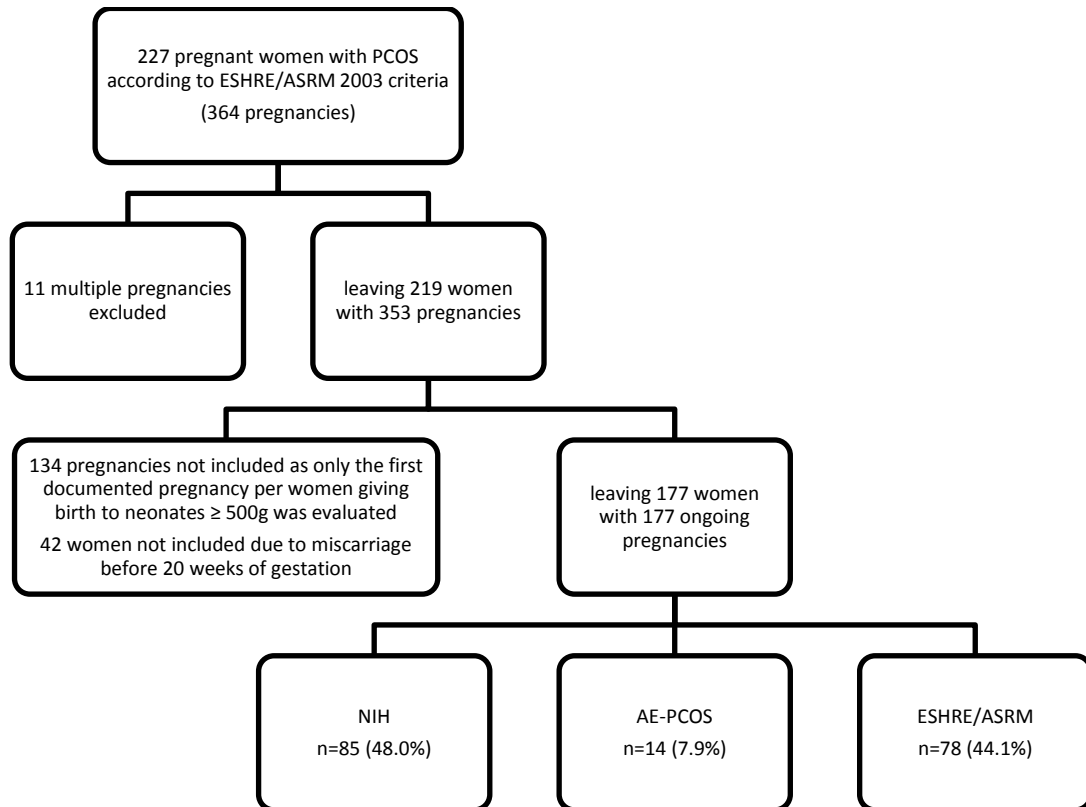
### Data sources/ Measurement/ Quantitative variables

The dataset was investigated on the occurrence of maternal or neonatal complications and categorized as either 'normal' or 'complicated' (Kollmann et al., 2015). The total complication rate per women and newborns is a composite outcome consisting of the cumulative incidence of the following parameters: gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, operative delivery (elective and non-elective caesarean section and operative vaginal delivery), large or small for gestational age infants, preterm birth (< 37 and < 34 weeks of gestation, respectively), acidosis, ICU admission, and pre- and perinatal mortality. At our unit maternal gestational diabetes is diagnosed based on the oral glucose tolerance test (oGTT, HemoCue, Ängelholm, Sweden) which is implemented in routine pregnancy care in Austria as well as on cord blood insulin testing which is routinely performed in all infants born with a weight  $\geq 4000$  g (Kollmann et al., 2015). The normal range of cord blood insulin is 3 - 25 mU/l (ADVIA Centaur, Siemens, Germany) (Chevenne et al., 1999). In the observed period oGTT was performed between 24 – 28 weeks of gestation by capillary blood analysis after 12h fasting and one and two hours following administration of 75g glucose (Kollmann et al., 2015). Glucose cut-off values to diagnose maternal gestational diabetes were 90 / 160 / 140 mg/dl or 5 / 8.9 / 7.8 mmol/l (Metzger et al., 2010). Measurement of blood pressure

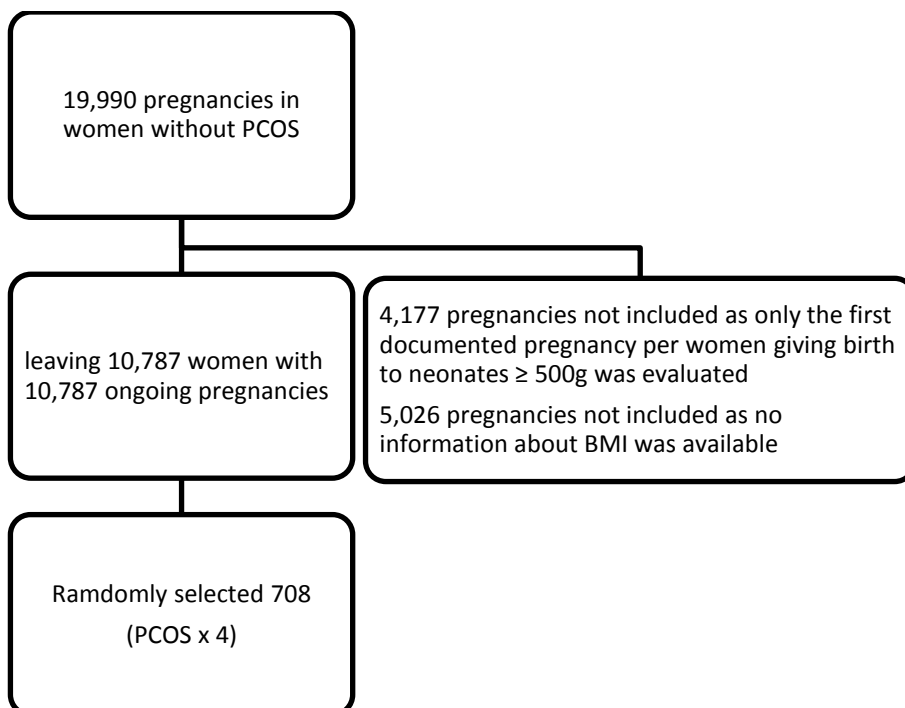
(systolic and diastolic) and analysis of a urine sample were performed at admission. Pregnancy-induced hypertension (PIH) was defined as systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg in 5 out of 21 measurements arising after 20 weeks of gestation. Pre-eclampsia was defined as PIH with proteinuria ( $\geq 300$  mg in 24 hours) (Brown et al., 2001). Operative delivery was defined as birth by forceps, ventouse or caesarean section (Kollmann et al., 2015). Foetal umbilical pH value and neonatal birth weight are determined after delivery. Small for gestational age and large for gestational age was diagnosed, when birth weight was below the 10<sup>th</sup> percentile or above the 90<sup>th</sup> percentile (Voigt et al., 2010). Intrauterine growth restriction was defined as SGA and the occurrence of distinct signs of placental insufficiency such as pathological Doppler waveforms in the umbilical (elevated pulsatility index, absent or reversed end-diastolic flow) or middle cerebral artery (decreased pulsatility index) as well as a cerebroplacental Doppler ratio (middle cerebral artery pulsatility index/umbilical artery pulsatility index) below 1 (Bahado-Singh et al., 1999; Baschat and Gembruch, 2003; Odibo et al., 2005; Oros et al., 2011). For evaluation of foetal acidosis umbilical arterial blood was examined (ABL 800 FLEX analyser, Akandevej, Denmark). Fetal acidosis was defined as an umbilical artery pH  $< 7.10$  at birth (Reif et al., 2014; Reif et al., 2015).

### Study size

Data of primiparous women with PCOS according to ESHRE/ASRM 2003 criteria and healthy controls giving birth to neonates  $\geq 500$ g between January 2004 and March 2012 was included. The study population and control group are shown in Figure 3 and Figure 4. Power calculation was not feasible since data on the rate of the composite maternal and neonatal complications were not available before the study was designed (Kollmann et al., 2015).



**Figure 4** Flow chart shows the study population and distribution (Kollmann et al., 2015). Reproduced from *Human Reproduction* (Kollmann et al., 2015) with permission of Oxford University Press.



**Figure 5** Flow chart shows the control group (Kollmann et al., 2015). Reproduced from *Human Reproduction* (Kollmann et al., 2015) with permission of Oxford University Press.

### Statistical methods

For categorical variables relative and absolute proportions are indicated, continuous variables are expressed as mean  $\pm$  standard deviation or median and range, respectively. We had no missing data for the primary outcomes and no imputation was done for secondary outcomes. For all outcomes, the three PCOS groups were compared with each other. Categorical variables were analysed by using Fisher's exact test or chi-squared test with p-value computed by Monte Carlo simulation, while for continuous outcomes one-way analysis of variance (ANOVA) or Kruskal-Wallis rank sum test followed by Bonferroni correction was applied. No modelling for confounding or interactions was conducted in the comparison between the PCOS groups due to small sample size in one group. The PCOS group was compared to the control group using logistic regression to report odds ratios (ORs) adjusted for BMI and age. All analyses were performed using the statistic software R (version 3.1.1, Vienna, Austria). A p-value  $<0.05$  was considered to be statistically significant. Taking the number of patients into account this is an explorative analysis. (Kollmann et al., 2015)

## **B. Results**

### Participants

A total of 227 pregnant women (364 pregnancies) with PCOS according to ESHRE/ASRM 2003 definition were identified during the study period. Thereafter, 11 cases with multiple pregnancies, another 134 cases with secondary pregnancies and 42 cases with miscarriage before 20 weeks of gestation were excluded. The final study population included 177 primiparous women with singleton pregnancies. Eighty-five women (48.0%) met the NIH 1990 criteria, another 14 (total of 99 = 55.9%) represented the additional phenotypes defined by the AE-PCOS 2006 criteria and 78 (44.1%) were classified as PCOS exclusively by the ESHRE/ASRM 2003 definition (Figure 3).

For the healthy control group, we identified a total of 15,813 women with 19,990 pregnancies. 4,177 pregnancies were not included as only the first documented pregnancy per women  $\geq$  500g was evaluated and 5,026 pregnancies were not included as no information about BMI was present. For all PCOS case per year four control cases were randomly selected from the above population ( $n = 10.787$ ). The final control group consisted of 708 women (Figure 4) (Kollmann et al., 2015).

### Descriptive data

Maternal age ( $p=0.50$ ) was comparable between all groups. BMI ( $p<0.001$ ) and the proportion of overweight women ( $BMI \geq 25$ ;  $p<0.001$ ) were different between PCOS groups and the control group; however there was no difference within the PCOS groups (Kollmann et al., 2015). Nonetheless, the percentage of women with pre-gestational diabetes, impaired glucose tolerance, and hyperinsulinemia did differ statistically between the three groups ( $p=0.007$ ). Regarding those three parameters, women from the ESHRE/ASRM group showed lower rates than women from the NIH group ( $p=0.01$ ). No difference was observed between the ESHRE/ASRM and the AE-PCOS group. Pre-gestational hypertension did not differ ( $p=0.10$ ). Table 3 indicates baseline characteristics and the distribution of PCOS features within the groups.

	PCOS by NIH 1990 (HA + OA + PCO or HA + OA), $n = 85$		Added using AE-PCOS 2006 (HA + PCO), $n = 14$		Further added using ESHRE/ASRM 2003 (OA + PCO), $n = 78$		Control $n = 708$		PCOS versus control	Within PCOS
	Median (n)	IQR (%)	Median (n)	IQR (%)	Median (n)	IQR (%)	Median (n)	IQR (%)	P-value	P-value
Maternal characteristics										
Age (years)	29	26–32	31	26–33	30	27–33	30	25–34	0.50	0.11
BMI	24.3	21.4–29.2	25.5	22.1–31.2	24.2	20.5–29.7	22.5	20.5–25.8	<0.001	0.76
BMI $\geq 25$	38/83	45.8	7/13	53.8	35/75	46.7	204/708	28.8	<0.001	0.92
PD/IGT/hyperinsulinaemia	29/84	34.5	5/14	35.7	11/78	14.1	n.a.	n.a.	n.a.	0.007 <sup>a</sup>
Pregestational hypertension	5/84	6.0	3/14	21.4	4/78	5.1	n.a.	n.a.	n.a.	0.10
PCOS features										
Biochemical HA	59/85	69.4	8/14	57.1	0/78	0				
Clinical HA	55/85	64.7	12/14	85.7	0/78	0				
OA	85/85	100	0/14	0	78/78	100				
PCO	80/85	94.1	14/14	100	78/78	100				

IQR, interquartile range; BMI, body mass index; PD, pregestational diabetes; IGT, impaired glucose tolerance; HA, hyperandrogenism; OA, oligo-anovulation; PCO, polycystic ovaries on ultrasound.  
<sup>a</sup>Statistical difference between NIH and ESHRE ( $P = 0.01$ ).

**Table 3 Comparison of demographics, clinical history, and PCOS features (Kollmann et al., 2015).  
 Reproduced from Human Reproduction (Kollmann et al., 2015) with permission of Oxford University Press.**

### Main results

The composite maternal complication rates were similar in all three groups: 42/85 (49.4%) vs. 9/14 (64.3%) vs. 47/78 (60.3%) ( $p=0.31$ ) (Kollmann et al., 2015).

The composite neonatal complication rates did not differ significantly: 23/85 (27.1%) vs. 5/14 (35.7%) vs. 18/78 (23.1%) ( $p=0.62$ ). In comparison to healthy controls, the odds for maternal complications was increased in PCOS women (OR 2.57 95% confidence interval [CI] 1.82-3.64;  $p<0.001$ ), while there was no significant difference in neonatal complications (OR 0.83 95% CI 0.56-1.21;  $p=0.343$ ) (Kollmann et al., 2015).

### Secondary results

Operative deliveries were more common in PCOS patients than in controls (OR=1.7 [95% CI 1.21-2.42]). Within the PCOS groups the prevalence was different between the NIH and ESHRE group ( $p=0.007$ ). Elective caesarean section, non-elective caesarean section and operative vaginal delivery occurred in 7.2%, 20.5%, and 13.3% (NIH), 21.4%, 21.4%, and 21.4% (AE-PCOS), 22.1%, 32.5%, and 10.4% (ESHRE/ASRM), and 10.9%, 16.7%, and 12.3% (Controls) respectively (Kollmann et al., 2015). The share of women with gestational diabetes ( $p=0.36$ ), pregnancy-induced hypertension ( $p=0.81$ ), and pre-eclampsia ( $p=0.32$ ) were similar within the PCOS groups (Table 4) (Kollmann et al., 2015). In comparison to healthy controls women with PCOS had a higher odds of developing gestational diabetes (OR=10.97 [95% CI 6.02-20.72]) and pregnancy-induced hypertension (OR=8.25 [95% CI 3.60-20.23]); while pre-eclampsia was not significantly different (OR=1.91 [95% CI 0.63-5.20]) (Table 5) (Kollmann et al., 2015).

Preterm birth < 37 weeks of gestation ( $p=0.72$ ), preterm birth < 34 weeks of gestation ( $p=0.32$ ), acidosis ( $p=0.72$ ), and large or small for gestational age newborns ( $p=0.97$ ) emerged with a comparable frequency within the PCOS groups. The ICU admission rate was different between the groups ( $p=0.02$ ). However, when using Bonferroni correction no statistical difference between the individual groups was observed (Kollmann et al., 2015). In comparison

to healthy controls none of those analysed parameters did differ (Table 5). There occurred two intrauterine fetal deaths in the ESHRE/ASRM group (2/77; 2.6%), one neonatal death in the AE group (1/14; 7.1%), and 7 intrauterine fetal deaths in the control group (7/708; 1.0%) (Kollmann et al., 2015).

No evidence was found for an association between pregnancy complications and neonatal complications (p=0.65) (Kollmann et al., 2015).

	NIH 1990 (n = 85)		AE-PCOS 2006 (n = 14)		ESHRE/ASRM 2003 (n = 78)		Within PCOS P-value
	n	%	n	%	n	%	
<b>Maternal complications</b>							
Gestational diabetes	16/85	18.82	2/14	14.3	21/78	26.9	0.36
PIH	8/85	9.4%	2/14	14.3	9/78	11.5	0.81
Pre-eclampsia	4/85	4.7	1/14	7.1	1/78	1.3	0.32
Operative delivery	34/83	41.0	9/14	64.3	50/77	64.9	0.007 <sup>a</sup>
Total complication rate	42/85	49.4	9/14	64.3	47/78	60.3	0.31
<b>Neonatal complications</b>							
Preterm birth <34 + 0	4/83	4.8	1/14	7.1	1/77	1.3	0.32
Preterm birth <37 + 0	11/83	13.3	1/14	7.1	8/77	10.4	0.72
SGA (<10th percentile)	6/81	7.4	1/12	8.3	8/76	10.5	0.97
LGA (>90th percentile)	5/81	6.2	1/12	8.3	4/76	5.3	
Fetal acidosis	3/62	4.8	0/10	0	1/59	1.7	0.72
ICU	6/83	7.2	4/14	28.6	4/75	5.3	0.02 <sup>b</sup>
Pre- and perinatal mortality	0/83	0	1/14	7.1	2/77	2.6	0.06
Total complication rate	23/85	27.1	5/14	35.7	18/78	23.1	0.62

Operative delivery = elective and non-elective Caesarean section and operative vaginal delivery.

Total maternal complication rate = gestational diabetes, PIH, pre-eclampsia, non-elective Caesarean section and operative vaginal delivery.

Total neonatal complication rate = large or small for gestational age, preterm birth, acidosis, ICU admission and pre- and perinatal mortality.

SGA, small for gestational age; ICU, intensive care unit admission.

<sup>a</sup>Statistical difference between NIH and ESHRE (P = 0.008).

<sup>b</sup>Using a Bonferroni correction no statistical significant difference between two groups comparison was observed.

**Table 4 Maternal and neonatal complications in PCOS pregnancies (Kollmann et al., 2015). Reproduced from Human Reproduction (Kollmann et al., 2015) with permission of Oxford University Press.**

	PCOS (n = 177)		Control (n = 708)		PCOS versus Control	
	n <sup>a</sup>	%	n <sup>a</sup>	%	OR (95% CI) <sup>a</sup>	P-value
<b>Maternal complications</b>						
Gestational diabetes	39/171	22.8	18/708	2.5	10.97 (6.02–20.72)	<0.001
PIH	19/171	11.1	9/708	1.3	8.25 (3.60–20.23)	<0.001
Pre-eclampsia	6/171	3.5	11/708	1.6	1.91 (0.63–5.20)	0.221
Operative delivery	89/168	53.0	282/706	39.9	1.70 (1.21–2.42)	0.003
Total complication rate	95/171	55.6	229/708	32.3	2.57 (1.82–3.64)	<0.001
<b>Neonatal complications</b>						
Preterm birth <34 + 0	6/168	3.6	30/708	4.2	0.87 (0.32–2.01)	0.758
Preterm birth <37 + 0	19/168	11.3	66/708	9.3	1.26 (0.71–2.15)	0.408
SGA (<10th percentile)	15/163	9.2	93/701	13.3	0.76 (0.46–1.21)	0.260
LGA (>90th percentile)	10/163	6.1	37/701	5.3		
Fetal acidosis	4/125	3.2	17/668	2.5	1.11 (0.31–3.14)	0.854
ICU	14/166	8.4	39/422	9.2	0.93 (0.47–1.76)	0.827
Pre- and perinatal mortality	3/168	1.8	7/708	1.0	1.88 (0.39–7.04)	0.373
Total complication rate	45/171	26.3	206/708	29.1	0.83 (0.56–1.21)	0.343

Operative delivery = elective and non-elective Caesarean section and operative vaginal delivery.

Total maternal complication rate = gestational diabetes, PIH, pre-eclampsia, non-elective Caesarean section and operative vaginal delivery.

Total neonatal complication rate = large or small for gestational age, preterm birth, acidosis, ICU admission and pre- and perinatal mortality.

SGA, small for gestational age; ICU, intensive care unit admission.

<sup>a</sup>OR are adjusted for BMI and age, the reported number of observations and percentages represent the information used in the logistic regression analysis.

**Table 5 Maternal and neonatal complications: PCOS versus control (Kollmann et al., 2015). Reproduced from Human Reproduction (Kollmann et al., 2015) with permission of Oxford University Press.**

## C. Discussion

Regardless of the PCOS definition, about 60% of women with PCOS and about 30% of their infants are subject to perinatal and neonatal complications. In comparison to healthy controls the risk for maternal complications was increased in PCOS women (OR 2.57 [1.82-3.64]) while there was no difference in neonatal complications (OR 0.83 [0.56-1.21]) (Kollmann et al., 2015).

A limitation of the present study is its retrospective design that increases the likelihood of bias and omits several parameters. We took care to guarantee correct group allocation, however, even in the most careful manner this remains to be a subjective discrimination that is ultimately a simplification of the underlying pathophysiology (Kollmann et al., 2015). To prevent potential confounders, multiple gestations and additional pregnancies of women were excluded and only primiparous women giving birth to neonates  $\geq 500$ g were included. On the one hand this may be advantageous regarding confounders; on the other hand it could induce selection bias. Primiparous women might be different from both nulliparous and multiparous

women with PCOS (Kollmann et al., 2015). A further limitation was the limited number of participants, particularly in the AE-PCOS subgroup (Kollmann et al., 2015).

Our findings confirm previous observations that PCOS women have an increased risk of perinatal complications (Boomsma et al., 2006; Qin et al., 2013). However, on the contrary to the study by Palomba et al., who found that the increased risk varies widely according to the different phenotypes and features of PCOS, we found that all definitions used to define PCOS are associated with a substantial risk for perinatal complications (Palomba et al., 2010).

A study from 2007 proposed that the non-hyperandrogenic phenotype (ESHRE group) may represent a subgroup of PCOS associated with a milder metabolic profile (Shroff et al., 2007). The scientist concluded that women presenting with oligo-/anovulation and polycystic ovaries only, may feature a significantly lower prevalence of metabolic syndrome. As insulin resistance as well as the compensatory hyperinsulinemia look to play a major role in the pathophysiology of metabolic syndrome, it is interesting that there was no decrease in insulin resistance in the respective 'low risk' group (Shroff et al., 2007). When analysing our data we found that the proportion of women with pre-gestational diabetes, impaired glucose tolerance, and hyperinsulinemia did differ statistically between the three groups ( $p=0.007$ ) (Kollmann et al., 2015). Regarding those parameters, women from the ESHRE/ASRM had a lower risk than women from the NIH group ( $p=0.01$  (Kollmann et al., 2015)). Interestingly, the proportion of women with gestational diabetes did not differ within the groups ( $p=0.36$ ) suggesting that the ESHRE group is as representative of risk as the other two groups (Kollmann et al., 2015). Those results highlight the importance of screening in women with PCOS although the timing and nature of this remains controversial.

The proportionately higher number of women with metabolic problems in the ESHRE group before and during pregnancy, may explain the discrepancy in mode of delivery between NIH and ESHRE ( $p=0.008$ ) (Hartling et al., 2014; Kollmann et al., 2015).

The total rate of neonatal complications was, as reported in previous studies, high (27.1%, 35.7% and 23.1% respectively) (Boomsma et al., 2006; Kollmann et al., 2015; Qin et al., 2013). Maternal androgen excess in utero has been proposed to be an important risk factor for impaired fetal growth and might have an effect on fetal outcome (Anderson et al., 2010; Barry et al., 2010). However, in our study we could not find a difference when compared to controls (Kollmann et al., 2015).

Maternal hyperandrogenism and insulin resistance may indeed play an important role in maternal and neonatal outcome. The clinical relevance of ovarian dysfunction, in particular PCOM, stays a matter of debate (Palomba et al., 2010; Shroff et al., 2007). Our data shows that the additional phenotype, introduced as part of the ESHRE/ASRM 2003 criteria, does have clinical significance (Kollmann et al., 2015). However, further studies are needed to investigate the long term outcome of the offspring of PCOS women in general and with ovarian dysfunction only.

The conflicting findings regarding metabolic problems and perinatal outcome of different phenotypes may be caused partly by the impreciseness of defining PCOM. The follicle number per ovary (FNPO  $\geq$  12) was introduced as a diagnostic criterion more than a decade ago (group, 2004, Jonard et al., 2003). The prevalence of ovaries with more than 12 follicles in healthy young women is truly very high when assessed with advanced ultrasound machines, demanding the need to update the diagnostic criteria (Azziz et al., 2009; Duijkers and Klipping, 2010; Johnstone et al., 2010; Jokubkiene et al., 2012; Kollmann et al., 2014a, b; Martins et al., 2014). If the diagnostic criterion for PCO on ultrasound is not altered to take into consideration technological developments, studies are needed to investigate how the prevalence of adverse maternal and neonatal outcome is affected by the use of new ultrasound machines.

The fact that the prevalence of adverse maternal and neonatal outcomes did not differ within the three PCOS groups may be biased by small sample size. However, PCOS women are at increased risk for obstetric complications. Pregnant women with PCOS should be informed

and advised to follow regular checks in units where troubles can be identified early to allow specialized care (Kollmann et al., 2015).

## **XI. 2nd study – Longitudinal study of pregnant PCOS women**

### **A. Material and Methods**

#### Study design

Prospective cohort study

#### Setting

Women with PCOS according to ESHRE/ASRM 2003 criteria, who were treated at the Medical University of Graz, Austria, between March 2013 and December 2015, were prospectively included in the study.

#### Ethical Approval

The study was approved by the institutional review board (No: 24-179ex11/12).

#### Participants

Women, over 18 years with PCOS according to ESHRE/ASRM 2003 criteria, were included. PCOS was diagnosed following clinical and sonographic evaluation and a hormonal analysis. Study examinations were performed in the outpatients department at following gestational weeks: 12-14, 20-22, 24-28, and 34. Ultrasound examinations were performed by medical doctors specialized in obstetrics and gynaecology. Clinical investigations were performed at the Division of Endocrinology and Metabolism at the Department of Internal Medicine at Medical University of Graz, Austria. Patient data were extracted from the local perinatal database (PIA, ViewPoint, GE Healthcare, Zipf, Austria) and the medical documentation system or patient files. We report on a population of women that were treated in our specialized unit and were delivered at our institution.

#### Variables

Following examinations were performed:

At 12-14 weeks of gestation:

- Determination of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)
- Biometry and ultrasound evaluation of the fetus
- Early oral glucose tolerance test (oGTT, HemoCue, Ängelholm, Sweden)
- Evaluation of maternal blood pressure, urine probe (proteins), body weight

At 20-22 weeks of gestation:

- Determination of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)
- Biometry and ultrasound evaluation of the fetus
- Evaluation of maternal blood pressure, urine probe (proteins), body weight

At 24-28 weeks of gestation:

- Determination of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)
- Biometry and ultrasound evaluation of the fetus
- Late oral glucose tolerance test (oGTT, HemoCue, Ängelholm, Sweden)
- Evaluation of maternal blood pressure, urine probe (proteins), body weight

At 34 weeks of gestation:

- Determination of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)
- Biometry and ultrasound evaluation of the fetus
- Evaluation of maternal blood pressure, urine probe, body weight

At delivery:

- Determination of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)
- Biometry and ultrasound evaluation of the fetus

- Evaluation of maternal blood pressure, urine probe, body weight

After ablactation:

- Determination of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)

Primary outcome parameter was the course of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, and AMH) before, during and after pregnancy. Secondary outcome parameters were specific perinatal complications including gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, operative delivery, preterm delivery, intrauterine growth restriction, intrauterine fetal death, small for gestational age, large for gestational age, polyhydramnion, and oligohydramnion.

Data sources/ Measurement/ Quantitative variables

The evaluation of maternal serum hormone levels was performed at the Division of Endocrinology and Metabolism at the Department of Internal Medicine at Medical University of Graz, Austria. Cut-off values for the investigated parameters are listed in table 6.

Hormone	Range	Range	Unit	Additional information
	Women	Men		
Testosterone	0,14 - 0,77	2,41 - 8,3	ng/ml	-
Free Testosterone	0,29 - 3,18	8,69 - 54,69	pg/ml	-
Sexual hormone binding globulin	19 - 117	16 - 76	nmol/L	-
Dehydroepiandrosterone Sulphate	0,46 - 2,75	0,39 - 4,63	µg/ml	-
Androstenedione	0,94 - 3,2	0,6 - 2,7	ng/ml	-
Anti-Müllerian hormone	0,19 - 9,13	0,6 - 13,7	ng/ml	aged 25 -40

**Table 6 Cut-off values for the investigated parameters**

Maternal gestational diabetes was diagnosed based on the oral glucose tolerance test (oGTT, HemoCue, Ängelholm, Sweden). oGTT was performed between 12 – 14 weeks of gestation and 24 – 28 weeks of gestation by venous blood analysis after 12h fasting and one and two hours following administration of 75g glucose. Glucose cut-off values to diagnose maternal gestational diabetes were 92 / 180 / 153 mg/dl (Metzger et al., 2010). Measurement of blood pressure (systolic and diastolic), maternal body weight and analysis of a urine sample were performed at admission. If blood pressure was high patients were advised to perform self-measurements at home. Pregnancy-induced hypertension (PIH) was defined as systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg in 5 out of 21 measurements arising after 20 weeks of gestation. Pre-eclampsia was defined as PIH with proteinuria ( $\geq 300$  mg in 24 hours) (Brown et al., 2001). Small for gestational age and large for gestational age was diagnosed, when birth weight was below the 10<sup>th</sup> percentile or above the 90<sup>th</sup> percentile (Voigt et al., 2010). Intrauterine growth restriction was defined as SGA and the occurrence of distinct signs of placental insufficiency such as pathological Doppler waveforms in the umbilical (elevated pulsatility index, absent or reversed end-diastolic flow) or middle cerebral artery (decreased pulsatility index) as well as a cerebroplacental Doppler ratio (middle cerebral artery pulsatility index/umbilical artery pulsatility index) below 1 (Bahado-Singh et al., 1999; Baschat and Gembruch, 2003; Odibo et al., 2005; Oros et al., 2011).

### Study size

For this study we planned to recruit 30 pregnant PCOS women. As it was not clear how long it would take to recruit that number and if it would be possible at all, we planned a feasibility study.

### Statistical methods

For categorical variables relative and absolute proportions are reported, continuous variables are expressed as mean  $\pm$  standard deviation or median and range, respectively

## **B. Results**

### Demographics

A total of 23 pregnant women were recruited and included for analysis. Mean age was  $30.8 \pm 4.7$  years and body mass index was  $27.0 \pm 6.6$ . 4.3% of women did smoke during pregnancy. Eighteen (78.3%) women presented with hyperandrogenemia, 16 (69.6%) with clinical hyperandrogenism, 20 (87.9%) had polycystic ovaries, and 20 (87.9%) with oligo-, amenorrhoea.

Seven women (30.4%) experienced a previous miscarriage. Seventeen (73.9%) women reported that they did not conceive within one year. Regarding the actual pregnancy, 8 (34.8%) women conceived spontaneously, 12 (52%) got pregnant using metformin, 2 (8.7%) after stimulation and ovulation induction, and one (4.3%) woman was treated with ICSI.

Three (13%) women reported pregestational hypertension and pregestational diabetes, respectively. Thirteen (56.5%) women had a chronic autoimmune thyroiditis with thyroxin supplementation before pregnancy.

Primary outcome

The course of hormonal levels before, during and after pregnancy is shown in figures 5-10.

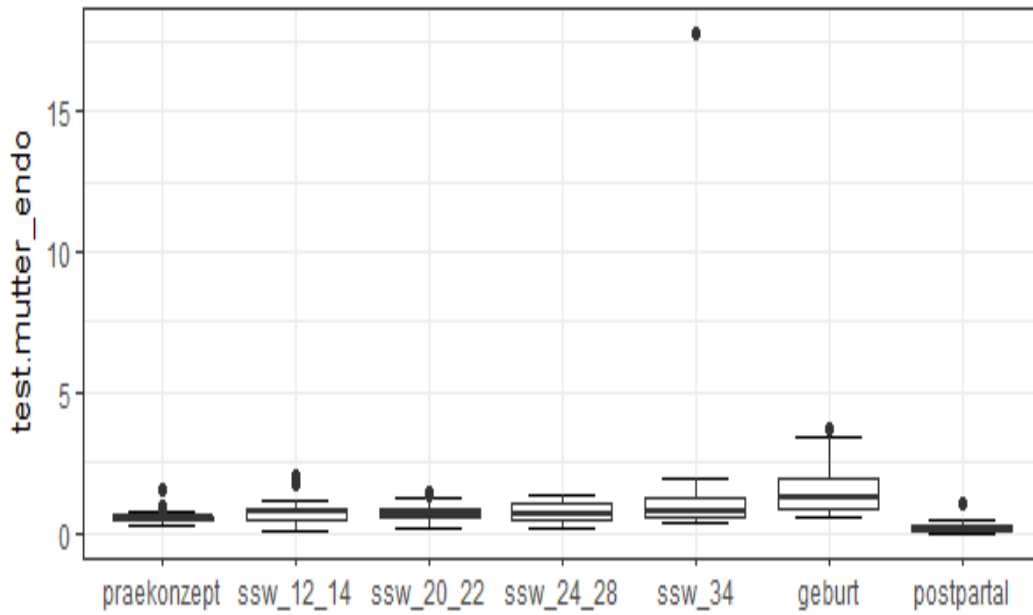
Data regarding available samples and values are presented in table 7.

	<b>Before pregnancy (n)</b>	<b>12-14(n)</b>	<b>20-22(n)</b>	<b>24-28(n)</b>	<b>34(n)</b>	<b>Birth(n)</b>	<b>postpartal(n)</b>
Testosterone (ng/ml)**	0.5 [0.4-0.6] (20)	0.7 [0.5-0.8] (18)	0.6 [0.5-0.8] (23)	0.7 [0.5-1.1] (22)	0.7 [0.5-1.3] (22)	1.2 [0.8-1.9] (16)	0.1 [0.1-0.3] (18)
Free Testosterone (pg/ml) **	2.44 [1.46-3.13] (20)	1.63 [1.33-2.23] (18)	2.41 [2.14-3.33] (23)	3.02 [2.01-3.5] (22)	4.1 [2.88-5.51] (22)	-	-
DHEAS (µg/ml)	1.55 [1.06-2.32] (20)	1.19 [0.93-1.77] (18)	1.18 [0.8-1.51] (23)	1.0 [0.7-1.58] (22)	0.97 [0.59-1.04] (22)	1.1 [0.76-1.35] (17)	0.94 [0.82-1.56] (18)
Androstenedione (ng/ml) **	3.23 [1.99-4.02] (21)	2.62 [1.99-3.82] (18)	2.44 [2.0-3.13] (23)	2.53 [1.91-3.52] (22)	2.76 [1.96-3.52] (22)	3.4 [2.29-5.58] (17)	1.92 [1.38-2.23] (18)
AMH (ng/ml)	6.1 [3.8-8.0] (16)	4.2 [2.7-7.0] (18)	2.6 [1.7-4.7] (23)	2.2 [1.8-4.1] (22)	1.9 [1.2-2.2] (22)	1.4 [0.9-2.0] (17)	3.2 [1.7-5.9] (18)
SHBG (nmol/L)	-	252.98 [208.9-297.8] (10)	280.32 [240.0-291.48] (9)	296.0 [244.0-351.4] (9)	289.62 [2.77.88-303.88] (6)	297.06 [241.0-326.9] (5)	76.31 [57.31-100.01]

**Table 7 Data regarding available samples (n) and values**

Testosterone

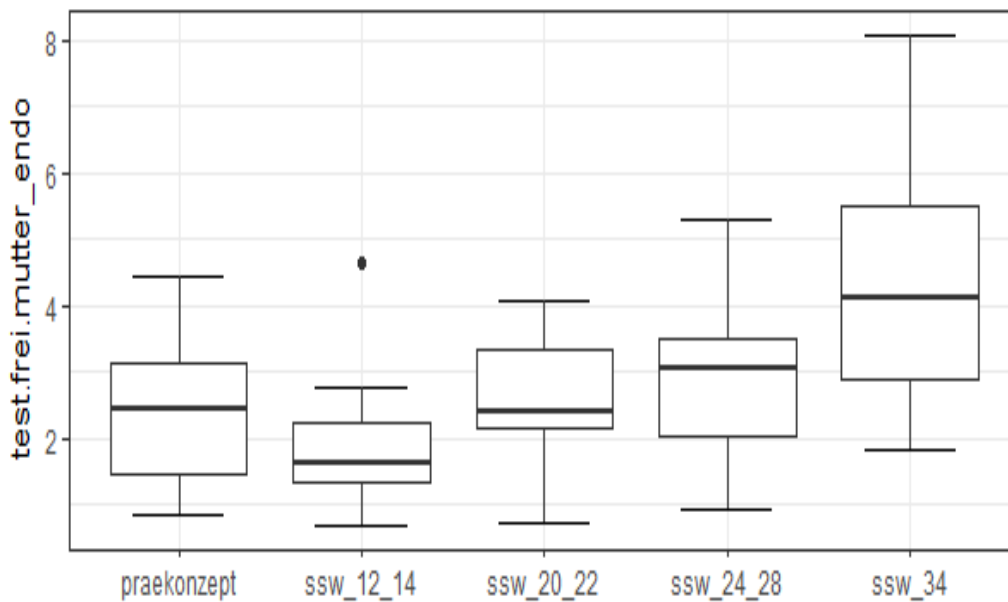
Testosterone was lowest after pregnancy (0.1 ng/ml [0.1-0.3]) and highest at birth (1.2 ng/ml [0.1-0.3]). Levels increased throughout pregnancy irrespective of fetal gender (see table 7 and figure 5).



**Figure 6 Testosterone levels throughout pregnancy**

Free Testosterone

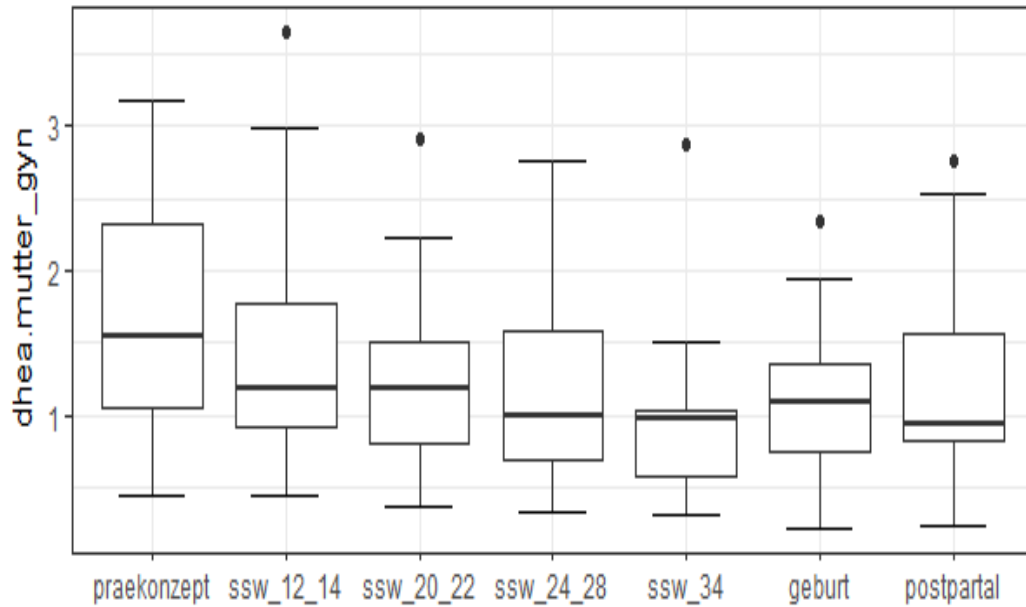
Free Testosterone increased throughout pregnancy irrespective of fetal gender. However, levels decreased at the beginning of pregnancy (see table 7 and figure 6).



**Figure 7 Free Testosterone levels throughout pregnancy**

## DHEAS

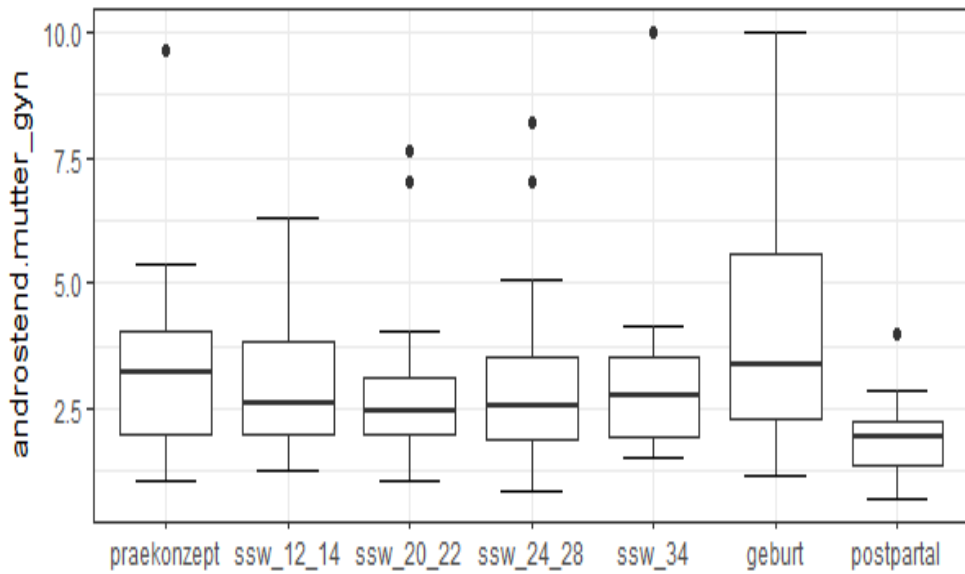
Mean DHEAS levels decreased throughout pregnancy and were even higher before pregnancy (see table 7 and figure 7).



**Figure 8 DHEAS levels throughout pregnancy**

## Androstenedione

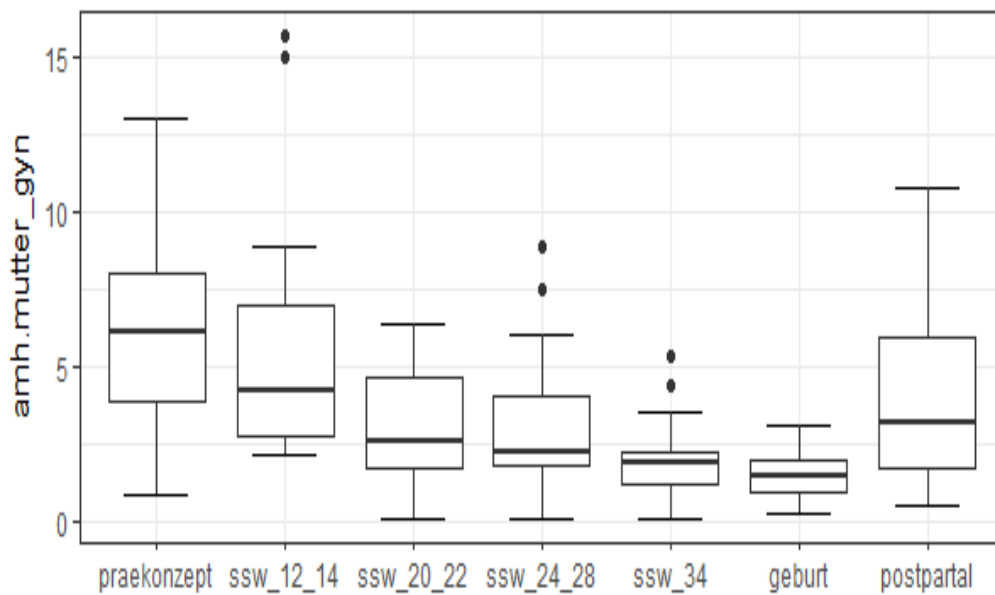
Androstenedione was lowest after pregnancy (1.92 ng/ml [1.38-2.23]) and highest at birth (3.4 ng/ml [2.29-5.58]) (see table 7 and figure 8).



**Figure 9** Androstenedione levels throughout pregnancy

### AMH

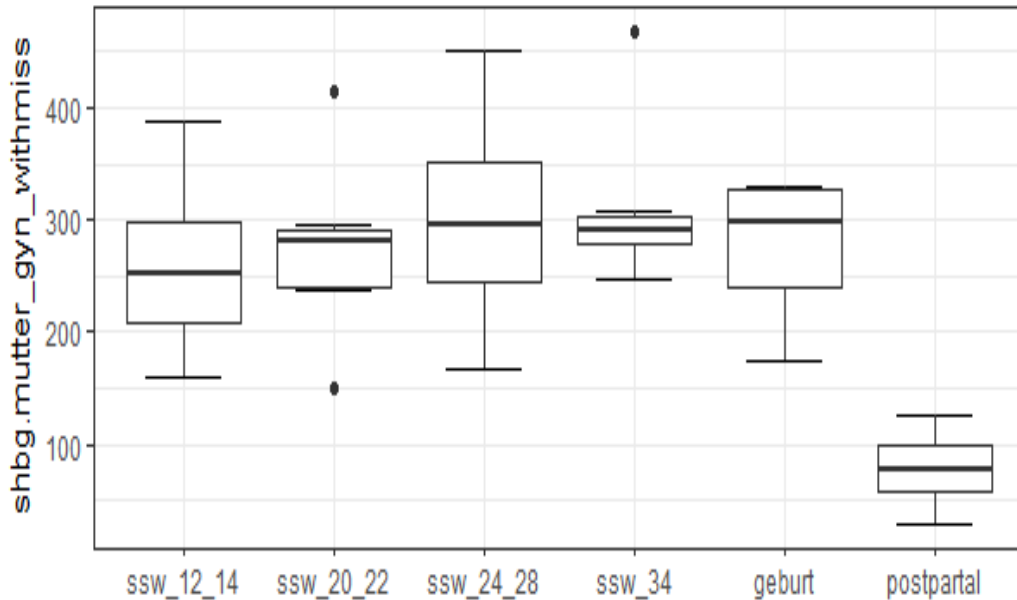
AMH levels decreased throughout pregnancy. Highest levels were measured before pregnancy (6.1 ng/ml [3.8-8.0]). After delivery levels went up again (see table 7 and figure 9).



**Figure 10** AMH levels throughout pregnancy

## SHBG

SHBG levels were high throughout pregnancy and decreased after delivery (see table 7 and figure 10).



**Figure 11 SHBG levels throughout pregnancy**

## Secondary outcomes

Mean gestational age at delivery was  $269.7 \pm 30.9$  days and mean birth weight was  $3131.4 \pm 866.4$  grams. One (4.3%) fetus died in utero (gestational age 152 days). An intrauterine growth restriction was found in 2 (8.7%) fetuses. One (4.3%) newborn was large for gestational age and 3 (13%) were small for gestational age. Three (13%) women presented with a polyhydramnion and 2 (8.7%) women developed gestational diabetes – additionally to the 3 women with pregestational diabetes. Pregnancy-induced hypertension was present in 5 (21.7%) women and 2 (8.7%) women developed pre-eclampsia. Operative delivery, such as elective caesarean section, non-elective caesarean section and operative vaginal delivery was necessary in 14 (60.9%) women.

## C. Discussion

This study found that androgens (testosterone, free testosterone, and androstenedione) and SHBG levels increase throughout pregnancy, while DHEAS and AMH levels decrease. Perinatal complications were frequent in the investigated cohort.

As suspected, only a limited number of PCOS patients could be recruited. However, data dealing with this subject are scarce.

Our results regarding hormone levels throughout pregnancy in PCOS women are in accordance with findings in women without PCOS. As described in the introduction section, numerous studies have found elevated levels of circulating androgens during normal pregnancy (Bammann et al., 1980; Buster et al., 1979; Dawood and Saxena, 1977; Mizuno et al., 1968; Rivarola et al., 1968; Saez et al., 1972). Also in agreement with the findings of a previous study, we found that DHEAS levels are lower throughout pregnancy in PCOS women (Milewich et al., 1978). SHBG levels do rise dramatically in PCOS women from the first trimester until term as described in women without PCOS (Wilke and Utley, 1987). Finally, the decline of AMH levels throughout gestation which is known from controls was also seen in our cohort (Königer et al., 2013; Königer et al., 2015; La Marca et al., 2005; Li et al., 2010; Nelson et al., 2010).

Further studies should compare the hormone levels throughout pregnancy in PCOS women and controls. Although the dynamic is similar, subtle changes in increase and decrease of levels cannot be answered with this study design. Maliqueo et al. aimed to evaluate the placental activities of steroid sulfatase, 3 $\beta$ -hydroxysteroid dehydrogenase type 1 and P450 aromatase in pregnant PCOS women compared to normal pregnant women. Two important enzymes for steroid synthesis, 3 $\beta$ -hydroxysteroid dehydrogenase type 1 and P450 aromatase, showed an altered activity in placental tissue of women with PCOS. There was a higher activity of 3 $\beta$ -hydroxysteroid dehydrogenase type 1 and a lower activity of P450 aromatase.

This finding could explain the increased androgen production during pregnancy (Maliqueo et al., 2013).

As mentioned above, perinatal complications were frequent in the investigated cohort. Gestational diabetes and pregnancy-induced hypertension were present in 21.7% of women, respectively and 8.7% of women developed pre-eclampsia. In comparison to our other two bigger cohorts the rates are higher for pregnancy-induced hypertension and pre-eclampsia (Kollmann et al., 2015). Rates for gestational diabetes are comparable (Kollmann et al., 2015). An explanation for the variable findings might be the relatively small sample size in this cohort. As we know from the other two cohorts, PCOS women develop more perinatal complications and should therefore be informed and advised to follow regular checks in units where troubles can be identified early to allow specialized care (Kollmann et al., 2015).

## **XII. 3rd study – Cross-sectional study of pregnant PCOS women and their offspring compared to non-PCOS women and their offspring**

### **A. Material and Methods**

#### Study design

Prospective cohort study

#### Setting

Women with PCOS according to ESHRE/ASRM 2003 criteria and non-PCOS women, who were treated at the Medical University of Graz, Austria, between March 2013 and December 2015, were prospectively included in the study.

#### Ethical Approval

The study was approved by the institutional review board (No: 24-179ex11/12).

#### Participants

Women, over 18 years, with PCOS according to ESHRE/ASRM 2003 criteria and non PCOS-women were included. PCOS was diagnosed following clinical and sonographic evaluation and a hormonal analysis. The (healthy) control group comprised women without PCOS. Study examinations were performed at birth. Ultrasound examinations were performed by medical doctors specialized in obstetrics and gynaecology. Clinical investigations were performed at the Division of Endocrinology and Metabolism at the Department of Internal Medicine at Medical University of Graz, Austria. Patient data were extracted from the local perinatal database (PIA, ViewPoint, GE Healthcare, Zipf, Austria) and the medical documentation system or patient files. We report on a population of women that were treated in our specialized unit and were delivered at our institution.

#### Variables

Following examinations were performed:

At birth:

- Determination of maternal serum hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)
- Determination of newborn cord blood hormone levels (T, fT, SHBG, DHEAS, ANDR, AMH)
- Documentation and evaluation of perinatal outcome parameters:

Maternal pregnancy complications: gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, eclampsia, polyhydramnios, oligohydramnios, operative delivery (elective and non-elective caesarean section and operative vaginal delivery)

Neonatal complications: preterm birth (< 37 and < 34 weeks of gestation, respectively), intrauterine growth restriction (IUGR), small for gestational age (SGA), large for gestational age (LGA), fetal acidosis, pre- and perinatal mortality, ICU admission rate, intrauterine fetal death (IUFT)

Primary outcome parameters were maternal and newborn hormone levels (T, fT, SHBG, DHEAS, ANDR, and AMH). Secondary outcome parameters were specific perinatal complications.

#### Data sources/ Measurement/ Quantitative variables

The evaluation of maternal serum hormone levels was performed at the Division of Endocrinology and Metabolism at the Department of Internal Medicine at Medical University of Graz, Austria. Cut-off values are listed in table 6.

Maternal gestational diabetes was diagnosed based on the oral glucose tolerance test (oGTT, HemoCue, Ängelholm, Sweden). At our unit maternal gestational diabetes is diagnosed based on the oral glucose tolerance test (oGTT, HemoCue, Ängelholm, Sweden) which is implemented in routine pregnancy care in Austria as well as on cord blood insulin testing which is routinely performed in all infants born with a weight  $\geq 4000$  g. The normal range of

cord blood insulin is 3 - 25 mU/l (ADVIA Centaur, Siemens, Germany) (Chevenne et al., 1999). In the observed period oGTT was performed between 24 – 28 weeks of gestation by venous blood analysis after 12h fasting and one and two hours following administration of 75g glucose. Glucose cut-off values to diagnose maternal gestational diabetes were 92 / 180 / 153 mg/dl (Metzger et al., 2010). Measurement of blood pressure (systolic and diastolic), maternal body weight and analysis of a urine sample were performed at admission. If blood pressure was high patients were advised to perform self-measurements at home. Pregnancy-induced hypertension (PIH) was defined as systolic blood pressure  $\geq$  140 mmHg or diastolic blood pressure  $\geq$  90 mmHg in 5 out of 21 measurements arising after 20 weeks of gestation. Pre-eclampsia was defined as PIH with proteinuria ( $\geq$  300 mg in 24 hours) (Brown et al., 2001). Operative delivery was defined as birth by forceps, ventouse or caesarean section. Neonatal birth weight and foetal umbilical pH value are determined straight after delivery. Small for gestational age and large for gestational age was assumed, when birth weight was below the 10<sup>th</sup> percentile or above the 90<sup>th</sup> percentile (Voigt et al., 2010). Intrauterine growth restriction was defined as SGA and the presence of distinct signs of placental insufficiency such as pathological Doppler waveforms in the umbilical (elevated pulsatility index, absent or reversed end-diastolic flow) or middle cerebral artery (decreased pulsatility index) as well as a cerebroplacental Doppler ratio (middle cerebral artery pulsatility index/umbilical artery pulsatility index) below 1 (Bahado-Singh et al., 1999; Baschat and Gembruch, 2003; Odibo et al., 2005; Oros et al., 2011). For evaluation of fetal acidosis umbilical arterial blood was analysed (ABL 800 FLEX analyser, Akandevj, Denmark). Fetal acidosis was defined as an umbilical artery pH < 7.10 at birth (Reif et al., 2014; Reif et al., 2015).

### Study size

To detect significant differences with an alpha of 0.05 and a power of 80%, we planned to recruit at least 35 patients with PCOS and 350 non-PCOS patients. As we expected some drop outs and difficulties with cord blood analysis, we aimed to include at least 400 patients. An interim analysis revealed that more patients than expected had to be excluded due to

comorbidities in the non-PCOS group and that cord blood analysis was not feasible in some cases due to insufficient material. We therefore aimed to recruit at least 80 PCOS patients and 420 non-PCOS patients.

### Statistical methods

For categorical variables relative and absolute proportions are indicated, continuous variables are expressed as mean  $\pm$  standard deviation or median and range, respectively. Categorical variables were analysed by using Fisher's exact test or chi-squared test with p-value computed by Monte Carlo simulation, while for continuous outcomes one-way analysis of variance (ANOVA) or Kruskal-Wallis rank sum test followed by Bonferroni correction was applied. All analyses were performed using the statistic software R (version 3.1.1, Vienna, Austria). A p-value  $<0.05$  was considered to be statistically significant.

## **B. Results**

### Participants

A total of 499 pregnant women were assessed for eligibility and 433 were finally included for analysis (79 PCOS and 354 Non-PCOS women). Four women did not want to participate, 7 women were not included due to twin pregnancies (4 PCOS, 3 Non-PCOS) [488], and 55 patients (11 PCOS, 44 Non-PCOS) were not included due to severe maternal or fetal comorbidities.

PCOS women gave birth to 36 (45.6%) girls and 43 (54.4%) boys. In the Non-PCOS group 178 (50.3%) girls and 176 (49.7%) boys were born.

### Baseline data

Mean age in PCOS women was  $30.6 \pm 4.6$  years and  $30.3 \pm 5.1$  years in Non-PCOS women. Body mass index was  $29.8 \pm 6.1$  kg/m<sup>2</sup> in the PCOS group and  $28.9 \pm 5.0$  kg/m<sup>2</sup> in the Non-

PCOS group, respectively. 3.8% of PCOS women and 8.8 % of Non-PCOS women did smoke during pregnancy. Mean gestational age at delivery was 279.0±9.6 days in the PCOS and 281.2±6.9 in the control group (see table 8).

PCOS patients showed the following symptoms: 46 (58.2%) women presented with hyperandrogenemia, 67 (84.8%) with clinical hyperandrogenism, 53 (67.1%) had polycystic ovaries, and 68 (86.1%) with oligo-, amenorrhoea. Forty one (51.9%) women in the PCOS group and 27 (7.6%) in the control group reported that they did not conceive within one year. Regarding the actual pregnancy, 67 (84.8%) in the PCOS group versus 346 (97.7%) in the control group conceived spontaneously; 7 (8.9%) versus 1 (0.3%) got pregnant after stimulation and ovulation induction, 5 (6.3%) versus 7 (1.9%) were treated with IVF/ICSI.

	<b>PCOS women</b>		<b>Non-PCOS women</b>		
	<b>n=79</b>		<b>n=354</b>		
	<b>Mean</b>	<b>± SD</b>	<b>Mean</b>	<b>± SD</b>	<b>p-</b>
	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>	<b>value</b>
<b>Maternal characteristics</b>					
Maternal Age (years)	30.6	±4.6	30.3	±5.1	0.660
Body mass index (kg/m2)*	29.8	±6.1	28.9	±5.0	0.241
Smoking	3/79	3.8%	31/354	8.8%	0.169
<b>Neonatal characteristics</b>					
Gestational age (days) at delivery	279	±9.6	281.2	±6.9	0.058

**Table 8 Comparison of demographics**

## Hormone levels

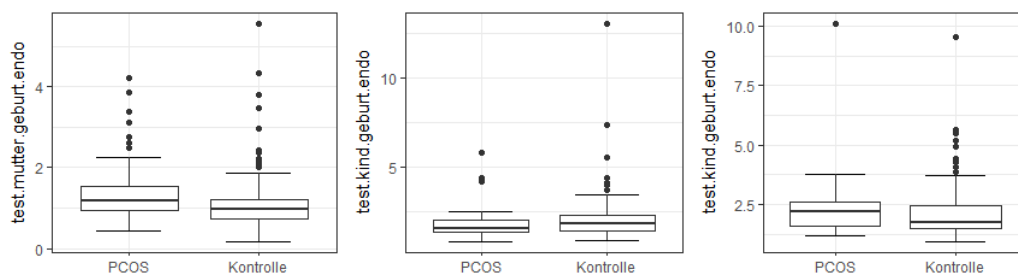
### Testosterone

Testosterone was measurable in 84.81% (67/79) of women with PCOS and in 89.26% (316/354) of Non-PCOS women. Neonatal samples were available in 27 PCOS girls (75%), 151 Non-PCOS girls (84%). 29 PCOS boys (67.4%), and 149 Non-PCOS boys (84.7%).

Mean maternal testosterone was 1.17 ng/ml [range 0.44-4.23] in PCOS women and 0.97 ng/ml [0.18-5.56] in Non-PCOS women. Levels were significantly higher in PCOS women ( $p < 0.001$ ).

Mean testosterone levels from PCOS girls were 1.54 ng/ml [0.84-5.82] and 1.82 ng/ml [0.88-13.05] from Non-PCOS girls. Those levels did not differ significantly ( $p = 0.230$ ).

Mean testosterone levels from PCOS boys (2.17 ng/ml [1.16-10.10]) and Non-PCOS boys (1.76 ng/ml [0.95-9.52]) did not differ either ( $p = 0.120$ ) (figure 11).



**Figure 12 Testosterone levels in mothers, girls and boys**

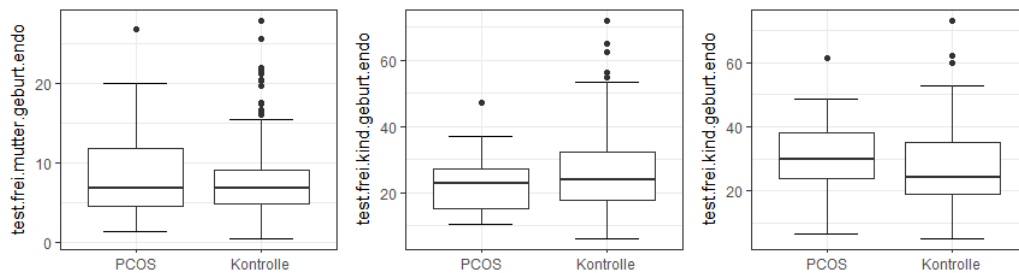
### Free Testosterone

Free Testosterone was measurable in 84.81% (67/79) of women with PCOS and in 89.26% (316/354) of Non-PCOS women. Neonatal samples were available in 26 PCOS girls (72.2%), 150 Non-PCOS girls (84.27%). 29 PCOS boys (67.4%), and 149 Non-PCOS boys (84.7%).

Mean maternal free testosterone was 6.79 pg/ml [1.37-26.80] in PCOS women and 6.72 pg/ml [0.48-27.91] in Non-PCOS women. Levels did not differ significantly ( $p = 0.563$ ).

Mean testosterone levels from PCOS girls were 22.66 pg/ml [10.52-47.0] and 23.89 pg/ml [6.12-72.01] from Non-PCOS girls. Those levels did not differ significantly ( $p = 0.196$ ).

Mean testosterone levels from PCOS boys (29.94 pg/ml [6.39-61.41]) and Non-PCOS boys (24.08 pg/ml [5.23-73.07]) did not differ either ( $p=0.094$ ) (figure 12).



**Figure 13 Free Testosterone levels in mothers, girls and boys**

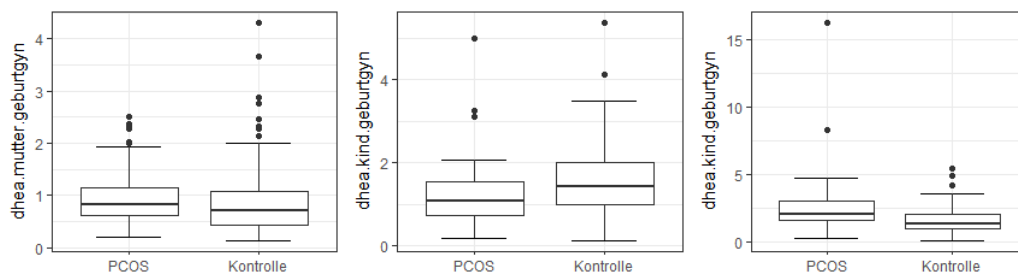
### DHEAS

DHEAS was measurable in 84.81% (66/79) of women with PCOS and in 88.70% (314/354) of Non-PCOS women. Neonatal samples were available in 27 PCOS girls (75%), 149 Non-PCOS girls (83.71%), 28 PCOS boys (65.12%), and 149 Non-PCOS boys (84.66%).

Maternal DHEAS was 0.82  $\mu\text{g/ml}$  [0.21-2.51] in PCOS women and 0.71  $\mu\text{g/ml}$  [0.14-4.31] in Non-PCOS women. Levels did not differ significantly ( $p=0.052$ ).

Mean DHEAS levels from PCOS girls were 1.09  $\mu\text{g/ml}$  [0.18-5.0] and 1.44  $\mu\text{g/ml}$  [0.14-5.38] from Non-PCOS girls. Levels from Non-PCOS girls were significantly higher ( $p=0.014$ ).

Mean DHEAS levels from PCOS boys (2.08  $\mu\text{g/ml}$  [0.29-16.3]) and Non-PCOS boys (1.41  $\mu\text{g/ml}$  [0.17-5.47]) did differ significantly ( $p=0.001$ ) (figure 13).



**Figure 14 DHEAS levels in mothers, girls and boys**

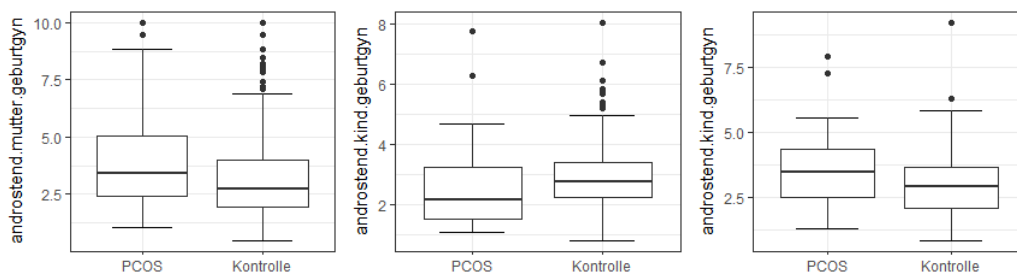
## Androstenedione

Androstenedione was measurable in 83.54% (66/79) of women with PCOS and in 88.14% (312/354) of Non-PCOS women. Neonatal samples were available in 26 PCOS girls (72.22%), 147 Non-PCOS girls (82.58%), 27 PCOS boys (62.79%), and 148 Non-PCOS boys (84.09%).

Maternal androstenedione was 3.44 ng/ml [1.06-10] in PCOS women and 2.74 ng/ml [0.49-10] in Non-PCOS women. Levels did differ significantly ( $p=0.002$ ).

Mean androstenedione levels from PCOS girls were 2.19 ng/ml [1.08-7.77] and 2.78 ng/ml [0.83-8.06] from Non-PCOS girls. Those levels did not differ significantly ( $p=0.113$ ).

Mean androstenedione levels from PCOS boys (3.47 ng/ml [1.26-7.93]) and Non-PCOS boys (2.92 ng/ml [0.83-9.24]) did differ significantly ( $p=0.039$ ) (figure 14).



**Figure 15 Androstenedione levels in mothers, girls and boys**

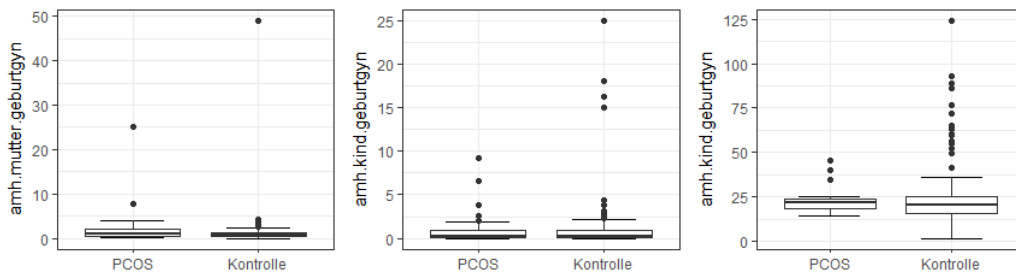
## AMH

AMH was measurable in 84.81% (66/79) of women with PCOS and in 88.42% (313/354) of Non-PCOS women. Neonatal samples were available in 27 PCOS girls (75%), 147 Non-PCOS girls (82.58%), 27 PCOS boys (62.79%), and 148 Non-PCOS boys (84.1%).

Maternal AMH was 1.10 ng/ml [0.10-25.0] in PCOS women and 0.72 ng/ml [0.02-49.0] in Non-PCOS women. Levels did differ significantly ( $p=0.001$ ).

Mean AMH levels from PCOS girls were 0.2 ng/ml [0.0-9.2] and 0.2 ng/ml [0.0-25.0] from Non-PCOS girls. Levels did not differ significantly ( $p=0.975$ ).

Mean AMH levels from PCOS boys (22.0 ng/ml [14.4-45.6]) and Non-PCOS boys (20.01 ng/ml [1.6-124.2]) did not differ significantly ( $p=0.395$ ) (figure 15).



**Figure 16** AMH levels in mothers, girls and boys

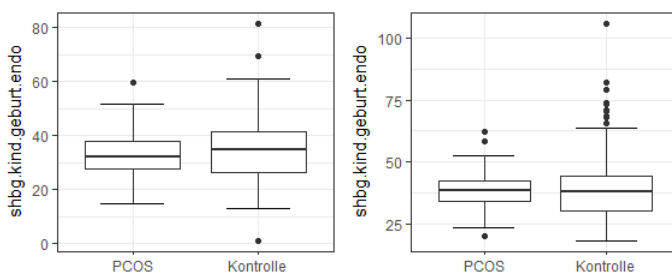
### SHBG

SHBG was measurable in 84.81% (67/79) of women with PCOS and in 89.27% (316/354) of Non-PCOS women. Neonatal samples were available in 27 PCOS girls (75%), 150 Non-PCOS girls (84.27%), 29 PCOS boys (67.44%), and 148 Non-PCOS boys (84.1%).

Maternal SHBG levels were  $>200$  nmol/L in all patients in both groups.

Mean SHBG levels from PCOS girls were 32.0 nmol/L [15.0-35.0] and 35.0 nmol/L [1.20-81.6] from Non-PCOS girls. Levels did not differ significantly ( $p=0.292$ ).

Mean SHBG levels from PCOS boys (38.5 nmol/L [20.1-62.2]) and Non-PCOS boys (37.85 nmol/L [18.3-105.9]) did not differ significantly ( $p=0.919$ ) (figure 16).



**Figure 17** SHBG levels in girls and boys

Comparisons between PCOS women and Non-PCOS women, female offspring of PCOS women and Non-PCOS women, and male offspring of PCOS women and Non-PCOS women are summarized in tables 9-11.

	<b>PCOS women</b>	<b>Non-PCOS women</b>	<b>p</b>
Testosterone (ng/ml)**	1.17 [0.44-4.23]	0.97 [0.18-5.56]	<0.001
Free Testosterone (pg/ml) **	6.79 [1.37-26.8]	6.72 [0.48-27.91]	0.563
Androstenedione (ng/ml) **	3.44 [1.06-10.0]	2.74 [0.49-10.0]	0.002
DHEAS (µg/ml)	0.82 [0.21-2.51]	0.71 [0.14-4.31]	0.052
AMH (ng/ml)	1.10 [0.10-25.0]	0.72 [0.02-49.0]	0.001
SHBG (nmol/L)	-	-	-

**Table 9 Comparison PCOS women and Non-PCOS women; Data presented as median [range] and comparison by U-test.**

	<b>Female offspring of PCOS women</b>	<b>Female offspring of Non-PCOS women</b>	<b>p</b>
Testosterone (ng/ml)**	1.54 [0.84-5.82]	1.82 [0.88-13.05]	0.230
Free Testosterone (pg/ml) **	22.66 [10.52-47.0]	23.89 [6.12-72.01]	0.196
Androstenedione (ng/ml) **	2.19 [1.08-7.77]	2.78 [0.83-8.06]	0.113
DHEAS (µg/ml)	1.09 [0.18-18.5]	1.44 [0.14-5.38]	0.014
AMH (ng/ml)	0.20 [0.00-9.2]	0.20 [0.00-25.0]	0.975
SHBG (nmol/L)	32.0 [20.17-39.0]	35.0 [1.2-81.6]	0.292

**Table 10 Comparison female offspring of PCOS women and Non-PCOS women; Data presented as median [range] and comparison by U-test.**

	<b>Male offspring of PCOS women</b>	<b>Male offspring of Non-PCOS women</b>	<b>p</b>
Testosterone (ng/ml)**	2.17 [1.16-10.10]	1.76 [0.95-9.52]	0.120
Free Testosterone (pg/ml) **	29.94 [6.39-61.41]	24.08 [5.23-73.07]	0.094
Androstenedione (ng/ml) **	3.47 [1.26-7.93]	2.92 [0.83-9.24]	0.039
DHEAS (µg/ml)	2.08 [0.29-16.3]	1.41 [0.17-5.47]	0.001
AMH (ng/ml)	22.0 [14.4-45.6]	20.01 [1.6-124.2]	0.395
SHBG (nmol/L)	38.5 [20.1-62.0]	37.85 [18.3-105.9]	0.919

**Table 11 Comparison male offspring of PCOS women and Non-PCOS women; Data presented as median [range] and comparison by U-test.**

### Pregnancy outcome

Compared to healthy controls, the risk for maternal complications was increased in PCOS women (51/79 [64.56%] versus 171/354 [48.31%]; p=0.009), while there was no significant difference in neonatal complications (17/79 [21.52%] versus 79/354 [22.32%]; p=1.0) (table 12).

	PCOS women n=79		Non-PCOS women n=354		p-value
	n	%	n	%	
<b>Maternal complications</b>					
Gestational diabetes	12/79	15.19	21/354	5.93	0.009
Pregnancy-induced hypertension	8/79	10.13	17/354	4.8	0.103
Pre-eclampsia	3/79	3.8	5/354	1.41	0.164
Operative delivery	41/79	51.89	148/354	41.8	0.259
Total complication rate	51/79	64.56	171/354	48.31	0.009
<b>Neonatal complications</b>					
SGA (< 10 <sup>th</sup> percentile)	9/79	11.39	44/354	12.43	1.0
LGA (> 90 <sup>th</sup> percentile)	3/79	3.8	19/354	5.37	0.779
IUGR	1/79	1.27	3/354	0.85	0.555
Fetal acidosis	2/79	2.53	10/354	2.82	1.0
ICU	1/79	1.27	5/354	1.41	1.0
Total complication rate	17/79	21.52	79/354	22.32	1.0

**Table 12 Maternal and neonatal complications in PCOS pregnancies**

**Operative delivery = elective and non-elective caesarean section and operative vaginal delivery**

**Total maternal complication rate = gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, non-elective caesarean section, and operative vaginal delivery**

**SGA = small for gestational age; ICU = intensive care unit admission, IUGR=intrauterine growth restriction**

**Total neonatal complication rate = large or small for gestational age, IUGR, acidosis, ICU admission, and pre- and perinatal mortality**

Women with PCOS had a higher risk of developing gestational diabetes (12/79 [15.19%] versus 21/354 [5.93%];  $p=0.009$ ) when compared to healthy controls. No significant difference was found for pregnancy-induced hypertension (8/79 [10.13%] versus 17/354 [4.8%];  $p=0.103$ ), pre-eclampsia (3/79 [3.8%] versus 5/354 [1.41%];  $p=0.164$ ), and operative delivery (41/79 [51.89%] versus 148/354 [41.8%];  $p=0.259$ ).

Large ( $p=0.779$ ) or small ( $p=1.0$ ) for gestational age newborns, intrauterine growth restriction ( $p=0.555$ ), fetal acidosis ( $p=1.0$ ), and ICU admission rate ( $p=1.0$ ) occurred with a comparable frequency in both groups (table 12).

### **C. Discussion**

Our main findings are that androgen levels in female offspring of PCOS and non-PCOS women do not differ although maternal hormone levels differ significantly. Boys of PCOS women show similar hormone levels compared to boys of non-PCOS women; only androstenedione levels were higher in boys of PCOS women. Compared to healthy controls, the risk for maternal complications was increased in PCOS women, while there was no significant difference in neonatal complications.

Our findings do not support the hypothesis that elevated androgen concentrations in PCOS mothers lead to elevated levels in the offspring. This hypothesis has been strongly encouraged by Abbott et al., who performed studies in rhesus monkeys, which were prenatally exposed to androgens (Abbott and Bacha, 2013; Abbott et al., 2005; Abbott et al., 2008a; Abbott et al., 2002; Abbott et al., 2013; Abbott et al., 2009; Abbott et al., 2008b; Dumesic et al., 1997). However, there are not many available human studies dealing with this subject. The most recent study was published in 2016 (Caanen et al., 2016). In the latter study researchers investigated androgen levels in 20 PCOS women and 83 controls at 20 weeks of gestation and at delivery. They also looked at the umbilical cord blood of the respective offspring. Analysed parameters were testosterone, androstenedione, dehydroepiandrosterone, estrone, estradiol,

and estriol. Hormones were measured using the liquid chromatography tandem mass spectrometry methods. The results were similar to ours. In spite of elevated maternal androgen concentrations during pregnancy in PCOS women, offspring showed no signs of elevated androgen concentrations in cord blood at birth (Caanen et al., 2016). Other available studies show assimilable results (Anderson et al., 2010; Boutzios et al., 2013; Hickey et al., 2009; Maliqueo et al., 2013). Anderson et al included 39 PCOS patients and 31 controls. They investigated mixed cord blood testosterone, androstenedione, dehydroepiandrosterone, 17-hydroxyprogesterone, estradiol, and dihydrotestosterone levels. Testosterone and dehydroepiandrosterone did not differ significantly in the offspring (Anderson et al., 2010). Interestingly, the study found, equal to further two studies, lower levels of androstenedione in cord blood of female offspring of PCOS women in comparison to female offspring of non-PCOS women (Anderson et al., 2010; Caanen et al., 2016; Maliqueo et al., 2013). Cord blood estradiol levels were also significantly lower in PCOS women, without any difference in the testosterone to estradiol ratio. Authors suggest a decreased fetal or placental production of steroids (Anderson et al., 2010). Boutzios et al. performed a study on PCOS patients (n=41), patients with a gestational diabetes (n=54), controls (n=56) and the respective offspring (Boutzios et al., 2013). Testosterone levels did differ in mothers, but did not show any difference in the offspring (Boutzios et al., 2013). Maliqueo et al. examined 20 pregnant PCOS women and 30 controls and determined progesterone, DHEAS, DHEA, androstenedione, testosterone, estrone, estradiol and total estriol (Maliqueo et al., 2013). They further investigated the activities of steroid sulfatase, 3 $\beta$ -hydroxysteroid dehydrogenase type 1, and P450 aromatase in placental tissue (Maliqueo et al., 2013). Findings regarding hormonal levels were similar to ours. Despite of elevated maternal androgen concentrations in PCOS women they did not find a difference in the offspring (Maliqueo et al., 2013). But as mentioned above this group found lower levels of androstenedione and higher estriol concentrations in female newborns of women with PCOS compared to daughters of control women (Maliqueo et al., 2013). In comparison to control women, placental tissue from women with PCOS showed higher 3 $\beta$ -hydroxysteroid dehydrogenase type 1 and lower P450

aromatase activities which could increase androgen production during pregnancy (Maliqueo et al., 2013). There is only one study which showed elevated testosterone levels in cord blood in female offspring of PCOS mothers compared to controls (Barry et al., 2010). Overall, it seems that the hypothesis that maternal androgen excess contributes to elevated androgen concentrations in the offspring and therefore lead to the development of PCOS cannot be supported. A large prospective cohort study, which evaluated the relationship between prenatal androgen exposure and PCOS in adolescence in normal pregnancy, did not find a statistically significant relationship too (Hickey et al., 2009).

Reasons for this finding may be protective mechanisms which work throughout pregnancy. As mentioned in the introduction section we are aware of the fact that there are pregnancy-specific mechanisms to protect both the mother and fetus from pregnancy-induced androgen excess (Crisosto et al., 2012; Hensleigh et al., 1975; Phelan and Conway, 2011). First of all the placenta forms an effective barrier by producing placental aromatase (Kragie, 2002). The aromatase quickly catalyses the conversion of androstenedione to estrone, 16-hydroxytestosterone to estriol and testosterone to estradiol (Kragie, 2002). Another mechanism is the increase of progesterone, which competes for androgen receptor binding (Birrell et al., 2007; Slayden et al., 2001) and has an affinity for 5 $\alpha$ -reductase which further results in an inhibition of the conversion of T to the more potent dihydrotestosterone (DHT) (Cabeza et al., 1999; Hodgins, 1982). A further protective factor is the physiological increase of maternal SHBG which was found in both groups and which leads to higher share of bounded and biologically inactive sex steroids.

Although we did not find higher androgen levels in the offspring, a negative effect of the higher androgen concentrations in PCOS mothers during pregnancy cannot be ruled out. Altered hormone levels might have their effect on PCOS offspring in a more indirect way such as fetal programming. A study on zebrafish embryos which were exposed to androgens (testosterone and dihydrotestosterone) showed altered global methylation levels in the ovary and elevated postprandial glucose levels (Xu et al., 2014). Similar results were found by

Zhang et al and Xu et al who examined the effect of a hyperandrogenic milieu in utero on rats (Zhang et al., 2014) and on rhesus monkeys (Xu et al., 2011). Data on humans are scarce. The first pilot study on PCOS patients and controls did not find a significant difference in global methylation (6.7% for PCOS women and 7.1% for controls) (Xu et al., 2010), whereas a more recent study report a significant difference (Yu et al., 2015). The latter study also looked at seven potentially interesting gene loci and found a hypermethylation in the promotor region of *SLC2A8*, *NRIP1*, *IGF2BP2*, *AMHR2* and a hypomethylation of *INSR* and *AMH* (Yu et al., 2015).

The effect of different PCOS phenotypes on perinatal outcome has been studied in our first cohort (Kollmann et al., 2015). While a study by Shroff et al. suggests that the non-hyperandrogenic phenotype may represent a subgroup of PCOS associated with a milder metabolic profile we did not find a difference regarding perinatal outcome (Kollmann et al., 2015; Shroff et al., 2007). Regarding this cohort we have to bear in mind that not all mothers had biochemically proven hyperandrogenism. However, 58.2% of women presented with hyperandrogenemia and 84.8% with clinical hyperandrogenism. Moreover, PCOS women had significant higher androgen levels when compared to non-PCOS women. Nevertheless, further studies should investigate how different maternal PCOS phenotypes influence the hormonal levels of the offspring.

The ideal way to examine hormone exposure during prenatal life would be the measurement of circulating fetal hormone levels at repeated time points during pregnancy. However, this is not without significant risk to the fetus and hence surrogate markers of fetal hormone levels must be applied. A relatively simple way is the collection of umbilical cord blood at birth (Hollier et al., 2014). Alternatively, amniotic fluid samples could be investigated. However, this approach is also associated with a higher complication rate and can therefore not be used in study settings. Furthermore, the exact correlation between hormone levels in fetal blood and amniotic fluid is not exactly known (Hollier et al., 2014).

Fetal blood, which is loaded with placental steroid metabolites and some maternal steroids leaves the placenta via the umbilical vein and returns from the fetus to the placenta via the umbilical artery (Ishimoto and Jaffe, 2011). A review published in 2014 examined the accuracy and biological interpretation of the measurement of androgens and estrogens in cord blood (Hollier et al., 2014). Usually venous and arterial blood samples are taken at once and the exact proportion of each component is not known precisely. None the less, in spite of different steroid concentrations in umbilical artery and vein, Pašková et al. showed that concentrations strongly correlate (Hollier et al., 2014; Pašková et al., 2014). However, one has to be cautious when interpreting hormone levels which were measured in umbilical cord blood. Various factors, such as obstetric and maternal ones can influence the concentrations, although the amount of this influence is not exactly known (Hollier et al., 2014). On the one hand it is known that gestational age and labour have an effect on fetal adrenal steroid production and on the other hand we know that levels of steroid metabolizing enzymes in the placenta are modulated by factors connected to labour and delivery such as glucocorticoids, pro-inflammatory cytokines, and exposure to reactive oxygen species (Albrecht and Pepe, 1990; Hollier et al., 2014). Recently published studies showed that gestational age and delivery significantly influence androgen and estrogen concentrations in cord blood (Hickey et al., 2014; Keelan et al., 2012). Labour was associated with significant lower median cord blood testosterone and free testosterone levels and higher SHBG, androstenedione and dehydroepiandrosterone levels. Gestational age at delivery was significantly negatively correlated with testosterone and free testosterone and significantly positive correlated with SHBG, androstenedione, and dehydroepiandrosterone. They further investigated the effect of an antenatal glucocorticoid administration and also found a significant effect (Keelan et al., 2012). Looking at our cohort we report no significant difference for those factors when we compared PCOS women and non-PCOS women. However, we found a significant higher rate of gestational diabetes and total maternal complication rate in PCOS women and a possible association should be investigated in a further step.

Radioimmunoassay (RIA) was used as the detection methodology for hormonal levels in our study and we are aware of issues related to this method. A main limitation of RIA is the measurement of low concentrations of sex steroids (Demers, 2010; Hollier et al., 2014; Rosner et al., 2007; Stanczyk et al., 2003). Particularly for testosterone we know that levels derived from mass spectrometry are consistently lower than those derived by RIA (Demers, 2010; Krogh et al., 2011; Soldin et al., 2005; Stanczyk and Clarke, 2010; Vicente et al., 2006). Our levels did not show a significant difference and we do not expect testosterone levels to be significantly different in the investigated groups when using mass spectrometry.

Regarding our secondary outcome we found that the risk for maternal complications was increased in PCOS women when compared to healthy controls, while there was no significant difference in neonatal complications. This finding is identical to the results from our first study and in accordance to published data (Boomsma et al., 2006; Kollmann et al., 2015; Palomba et al., 2015; Qin et al., 2013).

### **XIII. Conclusion**

Regardless of the PCOS definition, about 60% of women with PCOS and about 30% of their infants were subject to perinatal and neonatal complications. In comparison to healthy controls the risk for maternal complications was increased in PCOS women (OR 2.57 [1.82-3.64]) while there was no difference in neonatal complications (OR 0.83 [0.56-1.21]).

Serum levels of androgens (testosterone, free testosterone, and androstenedione) and SHBG increased throughout pregnancy, while those of DHEAS and AMH decreased. Postnatal Androgen levels in female offspring of PCOS and non-PCOS women did not differ, although maternal hormone levels differed significantly. Boys of PCOS women showed similar hormone levels postpartum compared to boys of non-PCOS women; only androstenedione levels were higher in boys of PCOS women.

#### **XIV. References**

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## **XV. Appendix**

### **A. Curriculum Vitae**

#### **PERSONAL INFORMATION**

	Martina Brigitte Kollmann, MD, MSc
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Nationality	Austria
Telephone	0043 650 5104828
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#### **Medical Practice**

2011-2017	Residency in Obstetrics and Gynecology, Medical University of Graz (MUG)
2013-2014	Clinical Research Fellowship in Reproductive Medicine, Nurture, Nottingham, UK
12/2010 – 6/2011	Internship (Turnus) Klinikum Wels - Grieskirchen
09/2010 – 10/2010	Hospitation at the Department of Obstetrics and Gynecology, Medical University of Graz
08/2010	Hospitation at the Department of Obstetrics and Gynecology, Hospital Civil de Guadalajara, Mexico

#### **Education and Training**

2015-2017	Master ‚Human Genetics‘, MUG
2013-2014	BFS Certificates (‘Assisted Conception’, ‘Embryo Transfer and IUI’, ‘Management of male fertility’, ‘Management of the Infertile Couple’, ‘Pelvic ultrasound’)
Since 2011	PhD, Medical University of Graz „Androgen metabolism and reproductive outcome“
2004 - 2010	Study of Medicine MUG

### **Training abroad**

08/2013 – 09/2014	Nurture, Nottingham University, Nottingham, UK
08/2010	Obstetrics and Gynecology, Hospital Civil de Guadalajara, Mexico
07/2009	Ophthalmology, Nepal Eye Hospital, Nepal
02/2009	Traditional Chinese Medicine, BUCM, China
02/2008 – 05/2008	„Erasmus“ Brussels, Belgium Gynecology, Hôpital Erasme, Brussels, Belgium
07/2007	Traditional Chinese Medicine, Taipei, Taiwan
07/2006	Internal Medicine (Nephrology), Beirut, Lebanon
08/2003 – 08/2004	Universidad Autónoma del Carmen, Mexico

### **School education**

20/06/2003	General qualification for university entrance
09/1995 – 06/2003	Austrian secondary education school type with emphasis on science, Ried im Innkreis
09/1991 – 07/1995	Elementary school, Taufkirchen an der Trattnach

### **Thesis adviser**

2012	Placenta praevia: Incidence, risk factors and outcome (cand.med. Gaulhofer)
2012	PCOS and Subfertility (cand.med. Schneider)
2012	PCOS and pregnancy outcome (cand.med. Günther)
2013	Acupuncture before and during labour (cand.med. Aldrian)

2013 TORCH infections during pregnancy and outcome of affected infants (cand.med. Pratl)

### **Further qualifications and awards**

2017 Scientific Award (Österreichischen Gesellschaft für Reproduktionsmedizin und Endokrinologie und der Österreichischen IVF Gesellschaft for Androgenspiegel weiblicher Neugeborener von Frauen mit und ohne PCOS')

2016 Scientific Award (Österreichischen Gesellschaft für Reproduktionsmedizin und Endokrinologie und der Österreichischen IVF Gesellschaft for Interventionen zur Verbesserung des Outcomes der assistierten Reproduktion bei Frauen mit PCOS: Systematischer Review und Metaanalyse')

2016 ÖGGG Award for 'Früher Haut zu Haut Kontakt nach Kaiserschnitt – eine randomisierte klinische Pilotstudie'

2015 Scientific Award (Österreichischen Gesellschaft für Reproduktionsmedizin und Endokrinologie und der Österreichischen IVF Gesellschaft for 'Polyzystisches Ovar Syndrom und perinatales Outcome: Vergleich unterschiedlicher diagnostischer Kriterien')

2014 Oral Communication Award (Do the different PCOS criteria predict pregnancy and neonatal outcome?) - 24th World Congress on Ultrasound in Obstetrics and Gynecology in Barcelona, Spain; 14 - 17 September 2014

01/2012 Marietta Blau – fellowship from the Austrian Ministry for Science and Research (BMWF) for 1 year at the Nottingham University / Prof. Raine-Fenning

09/2011 Foreign exchange fellowship, MUG (GENDER:UNIT)

2009/2010 Performance-based scholarship, MUG

2006-2010 Foreign exchange scholarships, MUG

2009 Performance-based scholarship, MUG for diploma thesis

ÖÄK-certificates Acupuncture, Genetics

Languages German, English, Spanish, French

### **Memberships**

Since 2013 Brithish Fertility Society

Since 2013 ESHRE

Since 2012 International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)

Since 2012 ÖGUM – Österreichische Gesellschaft für Ultraschall in der Medizin

Since 2011 Österreichische Gesellschaft für Gynäkologie und Geburtshilfe (ÖGGG)

**Publications**

- M. Kollmann, W.P. Martins, M.L.S. Lima, L. Craciunas, C.O. Nastri, A. Richardson, N. Raine-Fenning: *Strategies to improve the outcomes of assisted reproduction in women with polycystic ovarian syndrome: a systematic review and meta-analysis*. Ultrasound in Obstetrics and Gynecology 02/2016; DOI:10.1002/uog.15898
- M Kollmann, P Klaritsch, W.P. Martins, F Guenther, V Schneider, S.A. Herzog, L Craciunas, U Lang, B Obermayer-Pietsch, E Lerchbaum, N Raine-Fenning: *Maternal and neonatal outcomes in pregnant women with PCOS: Comparison of different diagnostic definitions*. Human Reproduction 07/2015; 30(10). DOI:10.1093/humrep/dev187
- Laurentiu Craciunas, Nikolaos Tsampras, Martina Kollmann, Nick Raine-Fenning: *Recombinant versus bovine hyaluronidase for oocyte denudation before intracytoplasmic sperm injection: a systematic review and meta-analysis*. BJOG An International Journal of Obstetrics & Gynaecology 12/2015; 122.
- Laurentiu Craciunas, Nikolaos Tsampras, Martina Kollmann, Nick Raine-Fenning: *The use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection: a systematic review and meta-analysis*. BJOG An International Journal of Obstetrics & Gynaecology 12/2015; 122. DOI:10.1111/1471-0528.13810
- M Kollmann, A Scheuchenegger, L Aldrian, S Herzog, E Mautner, U Lang, B Urlesberger, B Obermayer-Pietsch, RB Raggam, P Klaritsch: *Früher Haut zu Haut Kontakt nach Kaiserschnitt – eine randomisierte klinische Pilotstudie*. Geburtshilfe und Frauenheilkunde 04/2016; 76(04). DOI:10.1055/s-0036-1582188
- M Kollmann, WP Martins, MLS Lima, L Craciunas, CO Nastri, N Raine-Fenning: *Interventionen zur Verbesserung des Outcomes der assistierten Reproduktion bei Frauen mit PCOS: Systematischer Review und Metaanalyse*. Geburtshilfe und Frauenheilkunde 04/2016; 76(04). DOI:10.1055/s-0036-1582167
- MM Wölfler, A Rutzinger, M Ballon, V Bjelic-Radisic, M Kollmann, U Lang, W Schöll: *Kryokonservierung von Ovarialgewebe zur Fertilitätsprotektion – erste Ergebnisse*. Geburtshilfe und Frauenheilkunde 04/2016; 76(04). DOI:10.1055/s-0036-1582168

- M Kollmann, P Klaritsch, W P Martins, L Craciunas, N Raine-Fenning: *Reply: Maternal and neonatal outcomes in women diagnosed with PCOS by different definitions*. Human Reproduction 11/2015; 31(1). DOI:10.1093/humrep/dev285
- Laurentiu Craciunas, Nikolaos Tsampras, Martina Kollmann, Laura Stirbu, Nicholas John Raine-Fenning: *Use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection: A systematic review and meta-analysis*. 11/2015; 4(4). DOI:10.5317/wjog.v4.i4.113
- M Kollmann, A Scheuchenegger, L Aldrian, S Herzog, E Mautner, U Lang, B Urlesberger, B Obermayer-Pietsch, P Klaritsch: *Pilotstudie: „Sectio Bonding/Early Skin-To-Skin Contact (SSC) after Caesarean Section“*. Geburtshilfe und Frauenheilkunde 07/2015; 75(06). DOI:10.1055/s-0035-1555044
- M Kollmann, F Günther, V Schneider, SA Herzog, WP Martins, N Raine-Fenning, B Obermayer-Pietsch, U Lang, P Klaritsch: *Polyzystisches Ovar Syndrom und perinatales Outcome: Vergleich der unterschiedlichen diagnostischen Kriterien*. Geburtshilfe und Frauenheilkunde 07/2015; 75(06). DOI:10.1055/s-0035-1555037
- M. Kollmann, F. Gunther, V. Schneider, H. Sereina, W. P. Martins, N. Raine-Fenning, P. Klaritsch: *Do the different PCOS criteria predict pregnancy and neonatal outcome?*. Ultrasound in Obstetrics and Gynecology 09/2014; 44(Suppl. 1). DOI:10.1002/uog.13611
- Tim Van Mieghem, Roel De Heus, Liesbeth Lewi, Philipp Klaritsch, Martina Kollmann, David Baud, Yvan Vial, Prakesh S Shah, Angela C Ranzini, Lauren Mason, Luigi Raio, Regine Lachat, Jon Barrett, Vesal Khorsand, Rory Windrim, Greg Ryan: *Prenatal Management of Monoamniotic Twin Pregnancies*. Obstetrics and Gynecology 09/2014; 124(3).
- W. P. Martins, M. Kollmann, N. Raine-Fenning: *Counting ovarian follicles: Updated threshold for diagnosis of hyperandrogenic anovulation*. Ultrasound in Obstetrics and Gynecology 08/2014; 44(2). DOI:10.1002/uog.13402
- M Kollmann, J Voetsch, C Koidl, E Schest, M Haeusler, U Lang, P Klaritsch: *Etiology and Perinatal Outcome of Polyhydramnios*. Ultraschall in der Medizin 04/2014; 35(4). DOI:10.1055/s-0034-1366115
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- H Hubmann, C Sommer, S Kaddu, P Kroisel, M Kollmann, M Haeusler, B Csapo, B Urlesberger, P Klaritsch: *Discordant Schimmelpenning-Feuerstein-Mims syndrome in monozygotic twins after successful laser coagulation for twin-to-twin transfusion syndrome*. Ultraschall in der Medizin 10/2013; 34(S 01). DOI:10.1055/s-0033-1355012

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- M Kollmann, J Gaulhofer, U Lang, P Klaritsch: *Placenta praevia: Evaluation von Inzidenz, Risikofaktoren und Outcome*. *Geburtshilfe und Frauenheilkunde* 06/2013; 73(05). DOI:10.1055/s-0033-1347811
- M Kollmann, F Günther, U Lang, P Klaritsch: *Polyzystisches Ovar Syndrom: Schwangerschaftskomplikationen und reproduktives Outcome*. *Geburtshilfe und Frauenheilkunde* 06/2013; 73(05). DOI:10.1055/s-0033-1347778
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- M. Kollmann, J. Walch, C. Rotky-Fast, M. Haeusler, B. Csapo, U. Lang, P. Klaritsch: *OP30.08: Neurodevelopmental long-term outcome of preterm monochorionic and dichorionic twins*. *Ultrasound in Obstetrics and Gynecology* 09/2012; 40(S1). DOI:10.1002/uog.11687
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- M. Kollmann, M. Glawitsch, B. Csapo, M. Haeusler, U. Lang, P. Klaritsch: *P23.01: Monochorionic twin pregnancies: outcome and management*. *Ultrasound in Obstetrics and Gynecology* 09/2012; 40(S1). DOI:10.1002/uog.12062
- M Kollmann, M Haeusler, J Haas, B Csapo, U Lang, P Klaritsch: *Procedure-Related Complications after Genetic Amniocentesis and Chorionic Villus Sampling*. *Ultraschall in der Medizin* 06/2012; 34(4). DOI:10.1055/s-0032-1312939
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- Laurentiu Craciunas, Nikolaos Tsampras, Martina Kollmann, Nicholas Raine-Fenning: *The use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection*:

*a systematic review and meta-analysis*. British Fertility Society (BFS) Annual Meeting 2015, Birmingham, UK; 01/2015