

Polycystic ovary syndrome
- Metformin treatment in pregnancy -

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Contents

I. Acknowledgements	7
II. List of papers	9
III. Abbreviations	11
IV. The history of polycystic ovary syndrome	13
V. Polycystic ovaries	15
a. Definition	15
b. Prevalence	15
VI. Polycystic ovary syndrome	15
a. Definition	15
b. Prevalence	17
VII. Pathogenesis	17
a. Genetic predisposition	18
b. Prenatal factors	18
c. Environmental factors	19
d. Insulin resistance	19
e. Anthropologic reflections	20
VIII. Clinical presentations of PCOS	21
a. Menstrual disturbances	21
b. Obesity	21
c. Hirsutism	21
d. Androgenic alopecia	22
e. Acanthosis nigricans	22
IX. PCO, PCOS and early pregnancy complications	22
a. PCO and early pregnancy loss	22
b. PCOS and early pregnancy loss	23
1. Hypersecretion of LH	23
2. Hyperandrogenism	24
3. Insulin resistance	24
X. PCOS and pregnancy complications in the second and third trimester	26
a. PCO, PCOS and gestational diabetes mellitus	26
b. PCOS and pre-eclampsia	27

c. PCOS and preterm delivery	28
XI. Androgen and estradiol levels in normal pregnancy	28
XII. Androgen excess, pregnancy complications and fetal programming	29
a. Placental dysfunction	30
b. Pregnancy complications and excess androgens	31
1. Pre-eclampsia	31
2. PCOS	31
3. Gestational diabetes mellitus	32
XIII. Metformin	33
a. History	33
b. Absorption	33
c. Metabolism and elimination	33
d. Metformin action	34
e. Adverse effects	34
XIV. Metformin treatment in non-pregnant PCOS women	35
a. Effect on menstruation	35
b. Ovulation induction	35
c. Pregnancy and live birth rate	36
d. Effects on weight	36
e. Effects on hirsutism	36
f. Effects on acne	37
g. Effects on androgen levels	37
XV. Safety aspects of metformin use in pregnancy	37
a. Animal studies	37
b. Studies in diabetic women	38
c. Studies in PCOS women	38
XVI. Background of the study	40
XVII. Aims of the study	41
a. Study I	41
b. Study II	41
XVIII. Material and methods	42
Paper I	42
Paper II	42
Statistical analyses	44

Paper I-II	44
Paper III-IV	44
XIX. Main results	45
Study I	45
Study II	45
XX. Discussion and interpretation of the results	46
Paper I	46
Paper II	46
Paper III	48
Paper IV	48
XXI. Conclusions	49
XXII. Final reflections and future aspects	49
XXIII. Tables	51
XXIV. References	68
XXV. Paper I-IV	85

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II List of papers

Vanky E, Kjotrød S, Salvesen KÅ, Romundstad P, Moen MH, Carlsen SM.
Clinical, biochemical and ultrasonographic characteristics of Scandinavian women with PCOS.
Acta Obstet Gynecol Scand 2004;83: 482-6.

Vanky E, Salvesen KÅ, Heimstad R, Fougner K, Romundstad P, Carlsen SM.
Metformin reduces pregnancy complications without affecting androgen levels in pregnant PCOS women: results of a randomized study.
Hum Reprod 2004; 19:1734-40.

Vanky E, Zahlse K, Spigset O, Carlsen SM.
Placental passage of metformin in women with polycystic ovary syndrome.
Fertil Steril 2005; 83:1575-8.

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The beneficial effect of metformin on pregnancy outcome in PCOS women is not associated with major changes in CRP or indices of coagulation.
(Submitted)

III Abbreviations

AMP	Adenosine Monophosphate
AGA	Appropriate for Gestational Age
AR	Assisted Reproduction
ASRM	American Society of Reproductive Medicine
BMI	Body Mass Index
CAH	Congenital Adrenal Hyperplasia
CC	Clomiphene Citrate
DHEAS	Dehydroepiandrosterone
ELISA	Enzyme Linked Immunosorbent Assay
EPL	Early Pregnancy Loss
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicle Stimulating Hormone
FT	Free Testosterone
FTI	Free Testosterone Index
GDM	Gestational Diabetes Mellitus
GnRH	Gonadotropine Releasing Hormone
HCG	Human Chorionic Gonadotropin
HOMA	Homeostasis Model Assessment
ID/GC-MS	Isotope-Dilution Gas Chromatography-Mass Spectrometry
IGT	Impaired Glucose Tolerance
IR	Insulin Resistance
IVF	In Vitro Fertilization
LBR	Live Birth Rate
LH	Luteinizing hormone
NIH	National Institute of Health
NIR	Non –Insulin Resistant
OGTT	Oral Glucose Tolerance Test
PCO	Polycystic Ovary
PCOS	Polycystic Ovary Syndrome
PE	Pre-eclampsia
PIH	Pregnancy Induced Hypertension

PM	Perinatal Mortality
RCT	Randomized Clinical Trial
RIA	Radioimmunoassay
RPL	Recurrent Pregnancy Loss
SGA	Small for Gestational Age
SHBG	Sex Hormone Binding Globulin
W/H ratio	Waist / Hip ratio

IV The history of the polycystic ovary syndrome

“Giovane rustica, maritata, modicamente pingue, et infeconda, con due ovaie più grandi del normale, come uova di colomba, bernoccolute, lucenti et biancastre...” (Young peasant woman, married, moderately lump and infertile, with ovaries larger than normal, like doves’ eggs, lumpy, shiny and whitish...) This description from 1721 by the Italian scientist Antonio Vallisneri is probably the first text of polycystic ovary syndrome (PCOS) ¹.



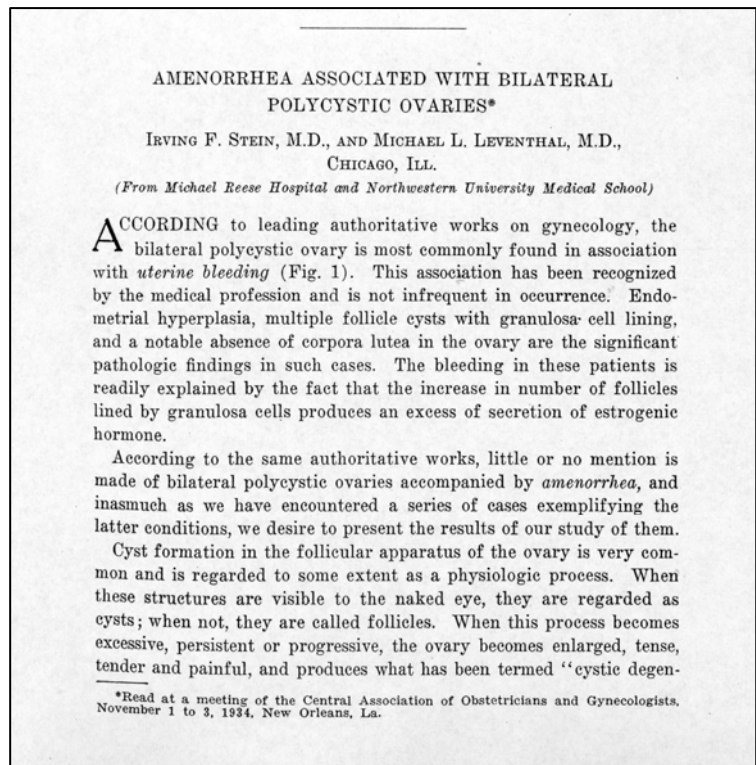
Antonio Vallisneri (1661-1730) Italian physician, biologist, botanist, geologist veterinarian, linguist and hydrologist.

In 1844 Chereau described sclerotic changes in the ovary ². The association between hyperandrogenism and diabetes was first described by Achard and Thiers in 1921, in the paper “Le virilism pileaire et son association à l’insuffisance glycolytique” ³.

In 1935, the two American gynecologists, Stein and Leventhal published a classic paper on a series of seven patients ⁴. They described bilaterally enlarged polycystic ovaries, “two to four times the normal size, sometimes distinctly globular”, “tunica thickened, though, and fibrotic”, “follicle cysts near the cortex and almost entirely confined to the cortex”. “The colour of the ovary was oyster gray with bluish areas where the cysts were superficial and appeared on the surface as sago-like bodies”. Other characteristics included oligo-amenorrhea, hirsutism and infertility.

According to Stein and Leventhal the diagnosis was based on the clinical appearance: hirsutism, amenorrhea, infertility and histological specimen of polycystic ovaries with prominent theca, fibrotic thickening of the tunica albuginea and multiple cystic follicles ⁴.

In the 1960s it became evident that the “Stein-Leventhal syndrome” represented a variety of clinical manifestations. In the early 1970s, the scientific community focused on the changed function in the hypothalamic – pituitary – ovarian axis, increased serum levels of LH, and elevated LH/FSH ratio ⁵.



In the late 1970s the concept of PCOS developed. Burghen et al. were first to point out a link between PCOS and insulin resistance. They demonstrated that hyperandrogenism correlate with hyperinsulinemia in obese PCOS women ⁶. This was an important milestone.

Later, ultrasound became central in visualizing polycystic ovaries (PCO) and diagnosing PCOS. Swanson et al. were the first to describe the typical ultrasonographic appearance of polycystic ovaries in 1981 ⁷, and Adams et al. refined the criteria for the ultrasonographic diagnosis of PCO ⁸.

The first treatment for PCOS was bilateral wedge resection of the ovaries, suggested by Stein and Leventhal. They reported regain of regular menstruations in seven patients, and pregnancy in two women, after wedge resection. Half a century later Gjønnaess, a Norwegian gynecologist, introduced laparoscopic ovarian drilling, as a more conservative method with fewer problems with adhesions ⁹.

V Polycystic ovaries

a) Definition

PCO reflects a description of ovarian morphology, from histological visualization or ultrasound imaging. The first definition suggested by Adams et al. was 10 or more follicles arranged peripherally ⁸.

The definition for PCO was revised in 2003 ¹⁰. Balen and co-workers suggested:

1. 12 or more follicles in each ovary, each follicle measuring 2-9 mm in diameter and/or
2. Ovarian volume >10ml

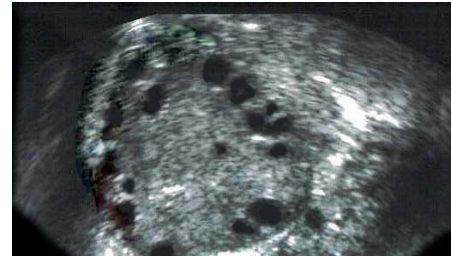
Stromal density and distribution of the follicles are not included in the revised definition of PCO. Transabdominal and transvaginal ultrasound examination methods correlate well, and both are acceptable tools for the diagnosis ¹¹. The criteria for polycystic ovaries are not applicable for adolescent girls ¹², women using contraceptive pills ¹³, and postmenopausal women ¹⁴. In menstruating women, examination should be performed on the third to the fifth day of the menstrual cycle ¹⁰.

b) Prevalence

The prevalence of PCO in the general population is reported to be between 17-22 % when ovaries are assessed by transabdominal ultrasound ^{11,15-17}.

With transvaginal ultrasound the prevalence of PCO is reported slightly higher (21-28 %) ^{11,18}.

Younger women seem to have PCO more often than those above 35 years of age ¹⁸.



*Ultrasound image of a polycystic ovary.
Photo: Vidar v. Düring*

VI Polycystic ovary syndrome

a) Definition

Until 2003 there was no international consensus on the definition of PCOS. In the United States, the National Institute of Health (NIH) Conference on PCOS 1990 recommended that diagnostic criteria should include evidence of hyperandrogenism (clinical or

biochemical) and ovulatory dysfunction in the absence of non-classic congenital adrenal hyperplasia (CAH). Polycystic ovarian morphology was not considered essential. A clear definition of ovulatory dysfunction, hirsutism or hyperandrogenism was, however, not given¹⁹. PCOS according to this definition was recognized in three principal phenotypes:

- 1) women with hirsutism, hyperandrogenemia and oligo-ovulation,
- 2) women with hirsutism and oligo-ovulation and
- 3) women with hyperandrogenemia and oligo-ovulation.

In Europe, the definition of PCOS was restricted to a condition with polycystic ovaries, identified by ultrasonography and one or more of the following; oligoamenorrhea, hyperandrogenism, obesity, elevated serum testosterone and / or elevated LH concentrations¹⁹. Ovulatory dysfunction was not mandatory^{20,21}.

The need for a universal agreement on the definition of PCOS was obvious. In Rotterdam in 2003, the European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) achieved a new consensus²². The new definition reflects the awareness that PCOS represents a multitude of clinical expressions and emphasizes the importance of realising it.

According to the Rotterdam 2003 consensus, two of the following three criteria must be fulfilled for the diagnosis:

1. Polycystic ovaries; 12 or more follicles in each ovary, each follicle measuring 2-9 mm in diameter and/or ovarian volume >10ml. One polycystic ovary is sufficient for the diagnosis
2. Oligo-/anovulation; clinically diagnosed as oligo-/ amenorrhea, i.e. menstrual cycles longer than 35 days or less than 10 menstruations per year
3. Hyperandrogenism; clinical or biochemical

The clinical definition of hyperandrogenism includes: hirsutism, acne and androgen alopecia, but the evaluation of hirsutism is difficult, because of racial differences^{23,24}.

Cosmetic treatment abolishes the expression and although there exists standardised scoring, the Ferriman-Gallwey score is seldom used²⁵. The Rotterdam consensus on the definition of PCOS has not defined clinical hyperandrogenism. The definition of biochemical hyperandrogenism is not without problems;

1. Modern immunoassay methods in routine clinical practice have recently been shown to be inaccurate for measuring testosterone in women²⁶.
2. Normative ranges are not established, and adjustment for age and BMI should be recommended.

3. Other androgens than testosterone should also be considered, especially DHEAS and androstenedione.

The diagnosis of PCOS should not be based on one single criterion, and it can be argued that PCOS is a diagnosis of exclusions. Congenital adrenal hyperplasia, non-classic congenital adrenal hyperplasia, Cushing syndrome, acromegaly and androgen secreting tumours should be ruled out.

It is important to realise that the Rotterdam criteria has expanded the definition of PCOS compared with the NIH criteria, and created two new phenotypes i.e. 1) women with PCO, hirsutism / hyperandrogenemia and regular ovulations and 2) women with PCO, oligo-ovulations and normal androgens ²⁷.

b) Prevalence

Although PCOS occurs universally, the prevalence, the time of onset of the clinical symptoms, and the severity of clinical presentations differ between ethnic and racial groups ^{18,28-31}. It is also possible that the occurrence of PCOS varies with time within a population, due to the association with insulin resistance and circulating insulin levels (see below).

The reported prevalence of PCOS varies from 3 % to 11 % depending on diagnostic criteria used and the population studied ^{29,32-35}. Prevalence studies according to the Rotterdam criteria are not yet available. Probably, the prevalence will be higher because the Rotterdam criteria have a wider definition of PCOS than the previously used definitions.

VII Pathogenesis

PCOS is a common, but complex disorder. Its etiology is poorly understood, and different hypotheses have been proposed. There is some evidence for a genetic predisposition ^{36,37}. Environmental factors seem to play an important role in the clinical presentation of the disorder.

a) Genetic predisposition

There is a reported familial clustering of PCOS, which may suggest a genetic component of the disorder³⁸⁻⁴¹. Hyperandrogenism is one of the important inheritable characteristics⁴². Confounding environmental factors, limited number of family members due to sub-fertility, heterogeneous phenotypes, and lack of a uniform definition of PCOS have made genetic studies difficult.

Insulin resistance and early male pattern baldness in men seems to be a male phenotype of PCOS⁴³. There is evidence for an autosomal dominant genetic model with low penetrance⁴⁴. Genes involved in the regulation of steroid hormone synthesis and androgen receptor modulation have been suggested as an explanation for the genetic basis of PCOS^{45,46}. These genes are CYP17A gene which encodes for the P450c 17 α enzyme, and CYP11A gene encoding for the P450 side-chain cleavage enzyme⁴⁷. The insulin gene, variable number tandem repeat (VNTR) has also been associated with the disturbances of the carbohydrate metabolism in PCOS⁴⁶. More than one gene seems to be involved. Hence, polygenic inheritance seems to be responsible in the majority of cases. Results are, however, not conclusive and larger studies are needed⁴⁸.

In one study, Hispanic American, Japanese, and Italian women with PCOS were investigated⁴⁹. They had similar androgen levels, insulin resistance and prevalence of polycystic ovaries, but the Japanese PCOS women were less hirsute and less obese than the others. This demonstrates that clinical expression might differ according to genetic differences in ethnic populations.

b) Prenatal factors

One hypothesis for the pathogenesis of PCOS, is that the developmental process in utero or in early postnatal life is influenced by genetic and environmental factors^{50,51}.

Female rhesus monkeys exposed prenatally to increased levels of testosterone, (their mothers were treated with testosterone during pregnancy), exhibit abnormal LH secretion, abnormal insulin secretion, central obesity and hyper-androgenic anovulation as adult animals, and their menarche was delayed by six months⁵². Similar observations have been made in sheep⁵³. In both species, the ovaries were enlarged with multiple antral follicles. The clinical picture was equivalent to PCOS in humans.

Androgens are potent gene transcription factors. They can interact with their own receptors and potentiate gene expression through serine phosphorylation of cAMP⁵⁴.

Hence, there is a possibility that androgen excess in human fetuses results in imprinting, which later in life can result in the PCOS phenotype.

Ewes exposed to testosterone during pregnancy gave birth to small newborn lambs⁵⁵. This supports the concept that fetal growth restriction and postnatal catch-up growth, can be programmed by prenatal exposure to sex steroids.

Other hypotheses suggest that a hyper-androgenic fetal ovary⁵⁶ or a hyper-androgenic adrenal cortex⁵⁷ are sources for prenatal androgen excess. Androgen exposure in utero may diminish hormonal negative feedback on the hypothalamic-pituitary axis leading to increased LH amplitude and frequency. LH is an important stimulator of ovarian steroid hormone synthesis, and this may result in increased stimulation of androgen production and secretion from the ovaries later in life. The ovarian response to HCG and GnRH is exaggerated in women with PCOS. Both in vitro and in vivo studies have shown increased androgen production from the theca cells in PCOS women^{58,59}.

c) Environmental factors

Diet and physical activity have major influence on the severity and expression of PCOS. Calorie restrictions, leading to at least 5% weight loss, resulted in increased insulin sensitivity, decreased circulating insulin levels and ovulation^{60,61}.

A diet rich in carbohydrates and monosaturated fatty acids may adversely influence the metabolic manifestation of the syndrome. In Great Britain PCOS women from the Indian subcontinent have more pronounced insulin resistance, hirsutism, acne, and sub-fertility than Caucasian counterparts with similar BMI. Clinical and biochemical presentations seem to be influenced by diet and lifestyle factors³¹.

d) Insulin resistance

It is reported on association between obesity, insulin resistance and impaired fertility in PCOS women^{60,62,63}. Although insulin resistance is not a criterion for the PCOS diagnosis, it is a central feature of the syndrome.

The definition of insulin resistance is based on the effect of insulin on glucose homeostasis. Insulin resistance may be defined as increased insulin demand to maintain blood glucose levels normal. Among the most frequently used methods to measure insulin resistance in large scale studies are: fasting insulin, insulin c-peptide, insulin levels during an oral glucose tolerance test (OGTT), and homeostasis assessment model (HOMA) and

hyperinsulinemic clamp. More sophisticated methods also exist, but they are not feasible in routine clinical use or large scale studies.

Anovulatory PCOS women are more insulin resistant and, thus, hyperinsulinemic compared to weight matched women with normal ovulations. Insulin resistance seems to be selective for fibroblasts, muscle cells and adipose tissue in PCOS ⁶⁴.

Insulin stimulates androgen synthesis in both the ovaries and adrenals ^{65,66}. Ovarian stroma, theca cells and the adrenals are not resistant to the effect of insulin on androgen synthesis. Further, insulin acts synergistically with LH and augments steroid synthesis in the ovaries, resulting in follicle arrest and anovulation ⁶⁷.

Insulin also inhibits the synthesis of sex hormone binding globulin (SHBG) in the liver, resulting in higher levels of free testosterone ⁶⁸.

Rhesus monkeys exposed to androgens in utero show impairment of insulin secretion and insulin action ⁶⁹. Abott et al. hypothesized that hyperandrogenemia during fetal development has an effect on body fat distribution, leading to centralized fat distribution, which in turn predisposes to insulin resistance ⁵⁰.

The timing of fetal exposure to hyperandrogenism seems important, as maternal injection of testosterone in the early gestation leads to impaired beta-cell function and diminished ability of pancreatic beta-cells to respond to hyperglycemic episodes. Testosterone exposure in late gestation seems to result in increased visceral adiposity and increased insulin resistance in monkeys ⁵⁰.

e) Anthropologic reflections

Until recently it may have been advantageous for a woman to be hyper-androgenic. With increased physical strength she had increased working capacity, in hunter, gatherer and agricultural societies, and, hence a better chance for survival. Probably, she could also better defend herself and her offspring. Further, insulin resistance enhanced her ability to store energy as subcutaneous fat when food was abundant. This helped her through periods of starvation. Food was limited in most societies and the physical demands of everyday life so hard, that she did not develop a full-blown PCOS. Hence, her fertility would not be significantly reduced.

VIII Clinical presentations of PCOS

a) Menstrual disturbances

Menstrual disturbances are common among PCOS women. The typical pattern is infrequent menstruations and occasional spottings, frequently seen as a result of anovulation. The three largest studies estimate that 80-90 % of the PCOS women have oligo-amenorrhea^{19,70,71}. Neither of these studies was based on the Rotterdam 2003 criteria.

A regular menstrual pattern is influenced by weight loss and weight gain, and obesity augments anovulation⁷².

b) Obesity

The prevalence of obesity varies largely among PCOS women, depending on the population studied and the diagnostic criteria used. It is estimated that up to half of the PCOS patients are overweight (BMI > 25 kg/m²) or obese (BMI > 30 kg/m²)^{61,73}.

PCOS women usually have the so called “male pattern obesity” or “central obesity”, which is associated with insulin resistance, increased risk of cardiovascular disease and type 2 diabetes^{74,75}.

It is a clinical experience that PCOS women date their debut of symptoms to a period of increase in body weight, which often coincidence with a time of decreased physical activity.

c) Hirsutism

The prevalence of hirsutism varies with the population studied. Hirsutism in women is male pattern of body hair growth and is a clinical sign of hyperandrogenemia. Androgens stimulate hair follicles to thicker and darker hair growth.

Typically, hirsutism is seen as excess facial hair and /or hair on the chest and lower abdomen. The Ferriman-Gallwey score can be used to objectively classify hirsutism²⁵. In PCOS women, the incidence of hirsutism is as high as 65-70 %^{19,70,71}. The prevalence and extent of hirsutism depend on the ethnicity of the group studied. Hirsutism in Japanese women with PCOS is less common⁴⁹.

d) Androgenic alopecia

Androgenic alopecia is a male type of hair loss from the terminal hair of the scalp. From clinical experience, we believe that alopecia is under-diagnosed in Scandinavian women with PCOS. Balen and Franks estimated the prevalence of alopecia to 6% and 3 %, respectively, in PCOS women ^{19,70}. Interestingly, one study reported that of 89 women with androgenic alopecia, 67% had polycystic ovaries ⁷⁶.

e) Acantosis nigricans

Acantosis nigricans manifests as skin pigmentation and papillomatosis. It is associated with insulin resistance and increased insulin secretion. It has been suggested that the skin lesion is due to increased insulin stimulated melanin synthesis in the melanocytes of the dermis. Acantosis nigricans is typically seen in areas with increased mechanical exposure, such as the neck, the armpits and the groin. It has been reported to be present in 3 % of women with PCOS and is more frequent in dark-skinned populations. ¹⁹.

IX PCO, PCOS and early pregnancy complications

Infertility and sub-fertility is one of the main reasons for PCOS women to consult the health service. Increased risk for pregnancy complications has, however, not been acknowledged by clinicians, and this problem has not been a major topic in textbooks of obstetrics and gynecology.

The notion that PCOS patients had increased risk for early pregnancy loss (EPL) became evident by studies carried out on patients with recurrent pregnancy loss (RPL) and IVF treatment.

a) PCO and early pregnancy loss

In a pioneer work from 1988, Sagle and colleagues reported that 82% of 56 spontaneously ovulating women with recurrent pregnancy loss (RPL) had polycystic ovaries (PCO) compared to 18% among parous controls ⁷⁷. Later, four prospective studies confirmed a high prevalence of PCO (36-81 %) in women with RPL ⁷⁸⁻⁸¹. Sub-fertility and obesity are increased among women with RPL ^{78,80}. A retrospective study on 1060 IVF treated

women demonstrated that those with PCO had 36% spontaneous abortions compared to 24% among those with normal ovaries ⁸² (Table 1).

However, one prospective study on lean, regularly menstruating women suffering from RPL, found that PCO did not predict poor pregnancy outcome ⁸¹ (Table 1). Probably this can be explained by that, only a few women in this group of lean patients with regular menstruations and PCO had PCOS.

b) PCOS and early pregnancy loss

Homburg found, in a prospective study, that 11 of 27 PCOS women who became pregnant after pulsatile LH releasing hormone treatment experienced EPL. Two retrospective studies reported 42% and 62% EPL in PCOS women ^{83,84} (Table 2). Clinically recognized EPL in a general population in Norway was estimated to be about 8-9% ⁸⁵. A prospective study on healthy women found 16 % EPL ⁸⁶.

There is increasing evidence that women diagnosed with PCOS have an increased risk for EPL. There seems to be a 2-4 fold increased risk, depending on the population studied and diagnostic criteria used.

The pathogenesis for early pregnancy loss in PCOS women is unknown. Hypersecretion of LH, hyperandrogenism and insulin resistance has been suggested as possible etiological factors.

1) Hypersecretion of LH

Elevated or disturbed LH secretion during the follicular phase may play a negative role for pregnancy outcome. Homburg et al. reported that PCOS women with EPL had high concentrations of LH during the follicular phase compared with PCOS women who continued the pregnancy ⁸⁷. High pre-pregnant LH levels predicted increased risk for early pregnancy loss in women suffering from RPL. Sixty five percent of the women who had LH levels above 10 IU/L in pre-pregnancy follicular phase experienced EPL ⁸⁸. In a prospective study among women with RPL, 81% had abnormalities in the LH secretion ⁸⁰. Study results are, however, somewhat diverging dependent on the LH measurement methods and the populations studied. Rai et al. reported that in spontaneously conceiving, normal weight women with RPL, LH or testosterone levels were not associated with the miscarriage rate ⁸⁹. Serial measurements or urinary samples are necessary, because of the pulsatile nature of LH secretion (Table 3).

Suggested mechanisms of action for abnormal LH secretion are premature oocyte maturation, follicle atresia and an androgen-dominated milieu in the endometrium.

Abnormal LH secretion in the follicular phase seems to have a negative impact on early pregnancy outcome.

2) Hyperandrogenism

Obesity and the accompanying insulin resistance will increase hyperandrogenemia in PCOS, by inhibiting the hepatic synthesis of sex hormone binding globulin (SHBG). Tulppala reported that women with RPL with a new miscarriage in the index pregnancy had higher free testosterone and DHEAS levels than those who completed the pregnancy⁷⁹. Women with RPL have higher testosterone levels than normal fertile controls^{80,90}. Androgens may act as antagonists to estrogens in the endometrium. Okon et al. postulated that high levels of androgens might have a detrimental effect on the endometrial function resulting in miscarriages⁹⁰. Elevated androgen levels and increased number of androgen receptors in the endometrium might be, partly, responsible for the poor reproductive performance⁹¹. Increased androgen levels in PCOS women may originate from the ovaries or the adrenals. Elevated androgen levels may directly or indirectly influence early pregnancy outcome (Table 4).

3) Insulin resistance

Women suffering from recurrent pregnancy loss are more insulin resistant (IR) than age, weight and race matched controls⁹². Anovulatory PCOS women are more IR than weight matched ovulatory PCOS women⁶⁰. The majority of PCOS patients are obese.

Exercise and weight loss, will reduce insulin resistance and result in better reproductive health. Weight loss (mean 10.2 kg) in 67 obese infertile women, of whom 75 % had PCOS, resulted in live born babies in 67% of the women⁶².

Hyperinsulinaemia might affect pregnancy outcome through enhanced androgen production both in the ovaries⁵⁷ and the adrenals⁶⁶. The androgen milieu interferes with normal endometrial function and development⁹⁰. Insulin stimulates PAI-1 synthesis⁹³ and increased PAI-1 activity has been demonstrated in PCOS women with miscarriages⁹⁴.

Both obese and lean PCOS women are more IR than weight matched controls⁷⁵, and obesity worsens IR. IR has a negative influence on fertility and seems to result in low conception rate⁶³. In a study of 383 IVF/ICSI treated women, EPL was higher in obese

women than in lean ones. Obesity seems to be an independent risk factor for EPL ⁹⁵.

Wang and colleagues claim that a high risk of spontaneous abortion in PCOS is related to a high prevalence of obesity ⁹⁶ (Table 5).

Glycodelin is a glycoprotein produced by the secretory glands of the decidualized endometrium. Decreased serum concentration of glycodelin is associated with early pregnancy loss and recurrent spontaneous abortions ^{97,98}. PCOS women have low glycodelin concentrations, in particular when they experience EPL. ⁹⁹. Hyperinsulinemia seems to suppress endometrial secretion of glycodelin ⁹⁹. This could explain the deleterious effect of hyperinsulinemia on pregnancy.

In conclusion, increased insulin resistance and elevated insulin levels may be a metabolic mediator for reduced fecundity both in PCOS women and in obese women generally. Improving insulin sensitivity and reducing circulating insulin levels seem to improve fertility.

X PCOS and pregnancy complications in the second and third trimester

When evaluating pregnancy outcome data for pregnancies achieved by assisted fertilization, clinicians became aware that pregnancies in PCOS women comprised a special group, different from other groups receiving assisted fertilization.

The incidences of pre-eclampsia and gestational diabetes mellitus (GDM) among PCOS women have been studied, but the results were contradictory. This may be due to classification difficulties, since the criteria used for diagnosing PCOS were not uniform, and the criteria for diagnosing GDM also differ between countries. It may also be due to the fact that the PCOS populations studied differ with respect to body mass index, parity, race and single vs. multiple gestations. The control groups, with whom the PCOS populations were compared, were also heterogenic (i.e., normal controls, or non-PCOS patients who received fertility treatment). With a few exceptions, the studies were retrospective. A review on studies dealing with the association between PCOS pregnancies and GDM, pre-eclampsia and prematurity follows in Table 6 to Table 9.

a) PCO, PCOS and gestational diabetes mellitus

The relationship between PCO and GDM in a prior pregnancy has been studied in Sweden, Finland and the United Kingdom ¹⁰⁰⁻¹⁰³ (Table 6). The control groups consisted of women with previous normal pregnancies. The studies were performed 6-54 months post partum. In all, 189 women with previous GDM were included. The prevalence of PCO ranged from 40% to 50 % in the GDM groups and from 3% to 27% in the control groups. Holte et al. reported that women with previous GDM were more obese, hirsute and had more irregular menstrual bleedings than the control group ¹⁰¹. Kousta et al. found that women with previous GDM were more obese and more insulin resistant than normoglycemic controls ¹⁰³. No differences were observed in androgen levels. Koivunen et al., however, reported that former GDM patients were not only more insulin resistant, but also had higher adrenal androgen levels than age and parity matched controls ¹⁰².

These data indicate that as many as 30-50% of women with a history of GDM would meet the diagnostic criteria of PCOS, and that there is an association between GDM and PCOS. This association could, however, be explained by obesity.

In most studies, where the PCOS population had similar BMI as the control group, PCOS women did not have an increased risk for GDM ¹⁰⁴⁻¹⁰⁷. Radon et al., however reports an increased prevalence of GDM among PCOS women compared to age and weight matched normal controls ¹⁰⁸. Mikola et al. found that PCOS was an independent predictor for GDM in singleton pregnancies ¹⁰⁹. The reported incidences for GDM in PCOS women vary from 7.5% to 41% ¹⁰⁷⁻¹¹¹ (Table 7). The reported incidence for normal controls in the same studies vary from zero to 9% ¹⁰⁷⁻¹¹¹ (Table 7).

There seems to be a higher incidence of GDM in PCOS women, despite the observation that these patients increase their insulin secretion during pregnancy more than non-PCOS patients ¹¹². The increment of insulin secretion seems to be similar in PCOS women, who develop GDM, and those who do not. This is in accordance with the hypothesis that insulin resistance is more important than the capacity for insulin secretion in the pathogenesis of GDM in PCOS women.

Women with PCOS should be routinely screened for GDM in pregnancy according to the recommendations from The Royal College of Obstetrics and Gynecology ¹¹³.

There is a need for standardized, prospective studies in which age, BMI and multiple gestations are accounted for to estimate the prevalence of GDM in PCOS women.

b) PCOS and pre-eclampsia

Diamant and colleagues were the first to study the prevalence of pre-eclampsia (PE) in PCOS patients ¹¹⁴. In a retrospective study, they found that PCOS women compared to normal primipara had a three fold higher risk for PE. De Vries et al. found similar results in a study of PCOS women and age and parity matched controls ¹¹⁵. Mikola et al., however, found that the increased risk of PE was confined to nulliparous women ¹⁰⁹. Gjønnaess concluded that the high prevalence of PE (13 %) was related to high BMI ¹¹⁶. One other Norwegian study reported a 22% incidence of PE among insulin resistant PCOS patients ¹¹⁷. The only prospective study found a high rate of PE in PCOS women compared to normal controls ¹¹¹. Diverging results make the conclusion difficult. It seems, however, that the symptoms of pre-eclampsia commence relatively late in gestation and is of a mild form in most PCOS pregnancies (Table 8).

c) PCOS and preterm delivery

There has been little focus on the possible association between PCOS pregnancies and preterm delivery. Studies dealing with pregnancy outcome in PCOS women sporadically present figures for preterm delivery, but somehow, there have been no focus on this topic. In five central studies on pregnancy outcome in PCOS women with singleton pregnancies the preterm delivery rate is remarkably high^{84,105,109,116,117}. Gjønnaess reported 10% preterm delivery rate before 32 gestational weeks in PCOS women previously treated with ovarian wedge resection¹¹⁶. Bjercke et al. observed 23% preterm birth rate (<37 weeks) among PCOS women after infertility treatment¹¹⁷. These two studies represent a Norwegian population with good socioeconomic standards, but they were from two different hospitals and 14 years apart. The prevalence of preterm deliveries (< 37 weeks) in Norway has remained stable around 6-7 % during this period of time. Hence, in Norwegian PCOS women the preterm delivery rate appears to be three fold increased. The preterm delivery rate in Venezuela among PCOS women was also surprisingly high (33% <37 weeks)⁸⁴.

In contrast, two retrospective studies from the Czech Republic and Finland observed no increased risk for preterm delivery in PCOS patients^{105,109} (Table 9).

XI Androgen and estradiol levels in normal pregnancy

Steroid hormone measurements in pregnancy were used for fetal surveillance and monitoring of fetal wellbeing in the 1960s and 1970s, before ultrasonography and other more informative tools became the method of choice.

Placenta plays a major role in steroid hormone synthesis and elimination. Fetal and maternal DHEAS is metabolized to estrogens via androstenedione and testosterone. The process converting androgens to estrogens is called aromatization.

DHEAS: DHEAS in maternal serum is reduced in pregnancy as a result of a 6-10 fold increased clearance¹¹⁸. At delivery, however, maternal DHEAS levels are increased compared with earlier in pregnancy¹¹⁹. This might reflect both increased production in the adrenals as a result of stress and / or decreased clearance in connection with delivery. DHEAS concentration is lower in the umbilical vein compared to maternal serum.

Androstenedione: Androstenedione levels increase slightly throughout pregnancy and reaches a maximum at delivery ¹¹⁸. Androstenedione levels in the umbilical vein are about 1/3 of the concentration in maternal serum. At birth, androstenedione levels in umbilical vessels are higher in boys than in girls ¹²⁰.

Total testosterone: The total testosterone concentration increases about 30% during pregnancy and is at its peak at delivery ¹¹⁸⁻¹²⁰. Fetal sex does not affect testosterone levels in either maternal or umbilical cord serum ¹¹⁸⁻¹²⁰. Ninety-eight percent of the total testosterone is bound to SHBG and albumin and only 2 % is free and assumed to be biologically active ¹²¹.

SHBG: Estrogens stimulate the synthesis of SHBG in the liver, and there is a 3-4-fold increase of SHBG in the second half of the pregnancy ¹¹⁸.

Free testosterone levels: FT is consistently low throughout pregnancy. Fetal FT is higher than maternal levels at delivery ¹²².

Estradiol: Estradiol is increased throughout the pregnancy, and in particular between week 20 and 35 because of enhanced synthesis of DHEAS in the increasing mass of placental tissue ¹¹⁸. At the end of pregnancy, the placental capacity for aromatization decreases as a sign of placental ageing. Possibly, this explains the increased maternal levels of testosterone and androstenedione at the end of pregnancy.

XII Androgen excess, pregnancy complications and fetal programming

It has been proposed that fetal growth restriction resulting from placental dysfunction, maternal stress or malnutrition increases the risk for cardiovascular disease, diabetes mellitus type-2 and hypertension in adult life ¹²³⁻¹²⁶.

Imprinting is when expressions of genes are permanently altered, so that the same genotype is expressed as different phenotypes.

The effect of excess androgens in pregnancy has been studied in animal models. It has been speculated on a possible mechanism, of excess androgens, on pregnancy complications and on fetal programming. This opens the possibility that increased fetal androgen exposure is the common pathogenic pathway to cardiovascular disease, diabetes mellitus type-2 and PCOS in adult life.

Eisner et al. demonstrated that female offspring of rhesus monkeys exposed to androgen excess *early* in pregnancy had impaired beta-cell function as adult animals with decreased ability to respond to hyperglycemic stimulation. Monkeys treated with testosterone in *late* pregnancy gave birth to offspring who developed high BMI, increased insulin resistance and excess visceral fat ⁶⁹. By adulthood, prenatally androgen exposed rhesus monkeys demonstrated increased LH secretion, anovulation, hyperandrogenism and polycystic ovaries, and this condition was very similar to PCOS in man ¹²⁷. These studies suggest that excess prenatal androgen exposure can be responsible for fetal programming of impaired insulin secretion and action.

Prenatal testosterone excess in sheep caused fetal growth restriction and postnatal catch-up growth ⁵⁵. Increased testosterone levels in ewes were associated with increased levels of IGFBP-1 and IGFBP-2 in the fetus post natally. Both IGFBP 1 and IGFBP 2 are inversely associated with fetal growth. Decreased IGF-1 in humans are early markers of future adult diseases, such as type 2 diabetes and cardio-vascular diseases ¹²⁸.

The intriguing question is, if excess androgen exposure in utero is a possible common pathway, where do these androgens come from? Finally, what are the mechanisms by which increased prenatal fetal androgen exposure leads to growth restriction?

a) Placental dysfunction

Placental dysfunction, leads to reduced utero-placental blood flow and fetal growth restriction. In baboons, reduced utero-placental blood flow resulted in an increase in fetal androstenedione, a precursor for testosterone ¹²⁹.

In an in vitro study, using three placentas from mothers with small for gestational age (SGA) infants and three placentas from mothers with appropriate for gestational age (AGA) infants, the placentas were perfused with DHEAS ¹³⁰. There was an accumulation of androstenedione and testosterone and low estrone and estradiol levels in the placental fluid from the SGA infant placentas. In placentas from AGA pregnancies, no accumulation of androgens occurred. In an in vivo study, DHEAS was administered to pregnant women with suspected SGA infants ¹³¹. Serial measurements of androgens and estrogens were performed for four hours. Mothers, who subsequently gave birth to SGA infants, had higher levels of androstenedione and testosterone measured in venous blood following DHEAS administration compared to those with AGA infants. This finding

indicates that dysfunctional, damaged or sub optimally functioning placentas have reduced capacity to aromatize androstenedione and testosterone to estrogens.

In humans, elevated androgen levels in utero is seen in fetuses with congenital adrenal hyperplasia (CAH) ¹³². In adult women with CAH, 80% have PCO ¹³³. There is also evidence that exposure to excess androgen in utero programs the hypothalamic-pituitary axis to hypersecretion of LH. Elevated LH levels and disturbed pulsatory pattern of LH is seen in anovulatory PCOS women ⁵⁶.

In conclusion, there is evidence that dysfunctional placentas can create androgen excess in utero, which in turn has a potential to modulate beta-cell function and insulin sensitivity, and to alter the hypothalamic-pituitary axis function concerning LH-secretion. In addition, excess androgens without placental dysfunction can result in growth restriction of the animal fetus. These alterations might result in adult diseases connected to the metabolic syndrome and PCOS.

b) Pregnancy complications and excess androgens in humans

Androgen excess has been investigated and described in different pregnant populations with different pregnancy complications. Most studies have focused on PE, PCOS pregnancies and GDM.

1) Pre-eclampsia

In searching for the etiology of pre-eclampsia, several studies have investigated the steroid hormone levels in pre-eclamptic women ¹³⁴⁻¹⁴¹. Most, but not all studies, ^{137,139} report elevated maternal androstenedione and testosterone in pre-eclamptic pregnancies in the third trimester or at delivery ^{134-136,138,140,141}. It has been hypothesized that elevated testosterone levels are involved in the pathogenesis of PE. Jiricek et al. demonstrated that androstenedione levels correlated positively to blood pressure and uric acid levels in PE women ¹³⁸. The more severe the PE was, the higher were the androstenedione levels. Carlsen and colleagues found that women who eventually developed PE, had elevated androstenedione and testosterone levels already in the early second trimester ¹³⁶. It is not known whether the elevated androgens are pathogenic factors for the development of PE or a result of a dysfunctional placenta. One important question is if maternal serum levels of androgens reflect the fetal environment.

2) Polycystic ovary syndrome

Hyper-androgenemia is one of the cardinal characteristics of non-pregnant PCOS women. The androgen status in pregnant PCOS women is, however, scarcely studied. Sir-Peterman and colleagues examined 20 pregnant PCOS women and 26 normal pregnant women at gestational weeks 10-16 and later at gestational weeks 22-28 ¹¹¹. DHEAS, androstenedione, testosterone and FTI tended to be higher in early pregnancy and were significantly higher in later pregnancy in PCOS women.

3) Gestational diabetes mellitus

In in vitro studies, insulin and IGF-1 regulate steroidogenesis in the human placenta ^{137,142,143}. Insulin stimulates 3-beta-hydroxysteroid dehydrogenase leading to increased synthesis of progesterone from pregnenolone ¹⁴². Progesterone is further converted to 17-OH-progesterone and androstenedione. The same enzyme 3-beta-hydroxysteroid dehydrogenase facilitates the conversion of DHEA to androstenedione. Insulin also inhibits the aromatization of androstenedione to estradiol ¹⁴³. Theoretically, insulin acting by all three mechanisms, could lead to elevated androstenedione and testosterone levels in pregnant women. Dokras and colleagues demonstrated that high insulin levels during OGTT in pregnant women correlated to high total testosterone and high free testosterone levels ¹⁴⁴. Amniotic fluid from insulin treated diabetic mothers had higher testosterone levels than amniotic fluid from normal pregnant women ⁵⁷.

Insulin resistance and GDM are frequent in PCOS pregnancies, and insulin levels are higher in pregnant PCOS women than in normal pregnant women ¹¹¹. Hence, elevated insulin levels could explain the elevated androgen levels in pregnant PCOS women.

XIII Metformin

a) History

Galega officinalis or French lilac is a perennial herb that blooms in July and August in most of Europe. It can be grown as far north as Trondheim. The extract of *Galega officinalis* contains isoamyline guanidine, a hypoglycemic compound, which was used to treat diabetes mellitus in medieval Europe^{145,146}. The biguanide metformin was discovered in the 1950s to have hypoglycemic effect. Two guanine molecules that are dimethylated make up metformin. In 1957 metformin was introduced as an agent for treatment of diabetes mellitus type-2. The use of metformin was restricted during the next decades because of reports of deaths associated to lactic acidosis during metformin treatment.

The drug had a renaissance in the 1990s, when metformin was proven to be particularly useful in the treatment of diabetes mellitus type 2.

Other manifestations of metabolic syndrome could also be treated with metformin.



Linnean herbarium. Swedish Museum of Natural History.

b) Absorption

Metformin is mainly absorbed from the jejunum^{147,148}. Absorption of an oral dose is incomplete, about 20-30 % of metformin passes out with feces. Oral bioavailability of 500-1500 g metformin is estimated to be 50-60 %. Absorption is complete within 6 hours of administration¹⁴⁷⁻¹⁴⁹. Peak plasma concentrations in healthy volunteers are reached within 2 hours^{147,148} and estimated half-life is 2.8 hours¹⁴⁹. Co-administration of food slightly decreases the rate and extent of metformin absorption¹⁵⁰.

c) Metabolism and elimination

Metformin is water-soluble and is rapidly eliminated by renal excretion due to active tubular secretion. Renal clearance of metformin correlates well to creatinine clearance

both in healthy volunteers and patients with kidney disease. Thirty to forty percent is recovered in urine after oral administration. Metabolites of metformin have not been detected, and metformin does not bind to plasma proteins ^{147,149,151}.

d) Metformin action

In non-diabetic patients, metformin does not influence blood glucose levels ¹⁵²⁻¹⁵⁴.

In patients with diabetes mellitus type-2, metformin lowers fasting blood glucose and improves glucose tolerance. The anti hyperglycaemic activity of metformin is achieved by stimulation of peripheral glucose uptake, reduced hepatic gluco-neogenesis and to a minor degree delayed intestinal absorption ¹⁵⁵. Metformin has a major effect on hepatic gluco-neogenesis. In non-diabetic subjects metformin has been demonstrated to lower cholesterol and LDL/HDL cholesterol ratio ^{150,156}.

A recent study suggests that the mechanisms of action of metformin might be through its activation of AMP- activated protein kinase ¹⁵⁷.

Long-term metformin therapy moderately impair intestinal absorption of vitamin B₁₂ in 30-46 % of the patients ¹⁵⁸. The reduced absorption of vitamin B₁₂ and folate, leads to increased homocysteine levels ¹⁵⁹. Homocysteine is the only cardiovascular risk factor adversely affected by metformin treatment. Megaloblastic anemia is rare in long-term metformin therapy ^{160,161}, and peripheral neuropathy has not been reported. In some, but not all studies, reduced levels of serum folate has been demonstrated after metformin treatment ^{159,162}.

e) Adverse effect

Gastrointestinal side effects occur in about 20 % of patients. Adverse effects are usually transient and resolve within one month of treatment, but 5 % of the patients cannot tolerate the drug. Gastrointestinal side effects, such as bloatedness, nausea, vomiting, diarrhea, constipation and metallic taste are most frequent ¹⁶³. Increasing the dose slowly and taking the drug together with food, can diminish side effects ¹⁵⁵. A severe and feared adverse effect is the development of lactic acidosis. This occurs in less than 1 in 10 000 patient years, and it has never been observed when contraindications to metformin administration is adhered to ¹⁵⁵. The contraindications to metformin are impaired renal and hepatic function, alcohol abuse, and serious cardiovascular and pulmonary diseases ¹⁵⁵. These are all conditions that predisposes to lactic acidosis per se.

XIV Metformin treatment in non-pregnant PCOS women

Since the first report on successful treatment of PCOS women with metformin for a decade ago ¹⁶⁴, many trials have been performed, and metformin treatment of PCOS women has become widespread. However, large well-designed studies are scarce, and few studies have lasted longer than six months. Many questions are unanswered. Should all PCOS patients be treated with metformin? If not, how shall we select the women who will benefit from treatment? Which symptoms should or could be treated and for how long time? What are the long-term effects of metformin in PCOS? Most review articles conclude that, large randomized controlled trials are needed. One review from the UK's National Institute of Clinical Excellence states that metformin can be used as adjuvant treatment to general lifestyle improvements, or in combination with clomiphene in women who have failed to ovulate on clomiphene alone ¹⁶⁵.

The metformin dosage used in PCOS treatment varies between studies. Most studies have used doses of 500 mg three times daily or 850 mg twice daily.

a) Effect on menstruation

Uncontrolled observational studies report restored regular menses in approximately 62% of PCOS women with irregular cycles ¹⁶⁶. Five RCTs reported significant improvement in menstrual cycles ¹⁶⁷⁻¹⁷¹. However, one trial found minimal or no difference in menstrual patterns ¹⁷².

b) Ovulation induction

Nine studies have evaluated the effects of metformin on ovulation ^{66,173-180}. Four of these were RCTs ^{173,176-178}. The combined data from all nine studies indicate that around 60% of previously anovulatory and mainly obese PCOS patients ovulated on metformin treatment. The restoration of regular menses and ovulations in these patients were seen within 3 to 6 months ¹⁶⁶. Harborne et al. reviewed seven RCTs with respect to ovulation induction ^{167,168,173,178,181-183}. She concluded that one additional ovulation was attained in every 5 month interval with metformin compared to placebo ¹⁸⁴. Lord et al. reviewed seven placebo controlled trials and found that the odds ratio for metformin treated PCOS

women to ovulate was 3.88 compared to placebo treated women ¹¹³. Metformin also seems to induce ovulation in normo-androgenic anovulatory women ¹⁸⁵.

Nestler and colleagues evaluated the effect of metformin combined with CC. They concluded that metformin in addition to CC enhanced ovulation in obese PCOS women ¹⁷⁸. The odds ratio for ovulation was 4.41 in studies where metformin was combined with ovulation induction agent vs. ovulation induction agent alone. ¹¹³. Non-obese PCOS women seemed to respond better to metformin treatment than obese PCOS women ¹⁷¹.

c) Pregnancy and live birth rate

There are no available data supporting improved pregnancy rate for metformin treatment alone ^{113,166,186}. However, in overweight CC resistant PCOS women, six-months metformin treatment resulted in 32 live births among 54 women while 20 live births were achieved in 55 women treated with ovarian diathermy ^{185,187}. Metformin combined with CC increased ovulation and pregnancy rate compared with CC alone ¹⁶⁶. Among normal weight PCOS women, pre-treatment with metformin prior to conventional IVF/ICSI tends to improve pregnancy rates ¹⁸⁸.

The impact of metformin on live-birth rate has not been proved.

d) Effects on weight reduction

Out of seven RCTs, which had weight as endpoint, six demonstrated reduction of body weight with metformin treatment compared with placebo ¹⁸⁴. The studies lasted from one to six months and the average reduction in BMI was 4%. However, in a systematic review and meta-analysis of RCTs, Lord et al. concluded that there was no evidence that metformin caused weight reduction in PCOS women ¹¹³. The most recent prospective randomized study, which had BMI as a main outcome measure, reported that metformin significantly reduced body weight during an eight-months period of treatment. Weight reduction seems to be dose-related in obese PCOS women ¹⁸⁹.

e) Effects on hirsutism

Six trials have examined the effect of metformin on hirsutism ^{113,167,168,190-193}. Three trials demonstrated a statistically significant, but small reduction in the Ferriman-Gallwey score. The other three trials did not find any effect. The studies were small and had a short follow up time. Recently, a long-term RCT evaluated hirsutism as primary endpoint. Both

Ferriman-Gallwey score, mean hair diameter and patient perception showed that metformin had an effect on moderate to severe hirsutism in a 12-months perspective ¹⁸⁴.

f) Effects on acne

There is essentially no data available.

g) Effects on androgen levels

In seven RCT the average reduction of androgen hormone levels (free testosterone, free androgen index and total testosterone) were around 20%, with wide variations, in the metformin groups ^{167,168,173,178,181-183}. The data on SHBG are inconclusive ¹⁸⁴.

XV. Safety aspects of metformin use in pregnancy

The present knowledge about the effect of metformin on the fetus is mainly based on animal studies, mice embryo experiments, case reports and clinical observations. Some reports are almost 45 years old, and the quality of the studies varies. The American Food and Drug Administration classifies metformin as a Category B drug, which is defined as: *“Either animal-reproduction studies have not demonstrated a fetal risk but there are no controlled studies in pregnant women or animal reproduction studies have shown an adverse effect (other than a decreased fertility), that was not confirmed in controlled studies in women in the first trimester (and there is no evidence of risk in later trimesters)* ¹⁹⁴.

a) Animal studies

Four studies in rat and mouse embryos have been published. Two studies from 1961 report that metformin passes the placenta ¹⁹⁵, but it was not teratogenic when administered in high doses to pregnant rats ¹⁹⁶ (Table 10). Two more recent studies on whole mouse embryo cultures, showed no teratogenic or toxic effect on embryo development ^{197,198}, although Denno et al. reported delayed closure of the neural tube ¹⁹⁸ (Table 10).

b) Studies on diabetic women

Placental passage: The first report on metformin passage through the placenta was based on detection of metformin in the amniotic fluid from two women who had used metformin in pregnancy¹⁹⁹. Metformin was not found in fetal cord blood. A laboratory study of placentas from eight healthy women, reported that metformin did not influence glucose transport or uptake²⁰⁰. Hague et al. found metformin in cord blood from seven diabetic mothers²⁰¹.

Possible teratogenic effects: Any reported teratogenic effect of metformin should be evaluated with care. Most studies have been retrospective, descriptive and the prospective ones are not randomized. Until recently, the use of metformin in pregnancy was restricted to patients with diabetes mellitus type-2. Thus, the study populations consist of women with high risk for pregnancy complications and congenital fetal malformations. Diabetic mothers have higher incidence of fetal anomalies, possibly due to hyperglycemia in early pregnancy. The treatment period (pregnancy weeks) and the dosage used vary between different studies. Some of the studies were conducted in developing countries with considerable socioeconomic problems^{202,203} (Table11).

There is no evidence of teratogenic effects of metformin on the human fetus (Table11). When compared with other diabetic women, congenital malformations were similar or fewer in the offspring of metformin treated women^{199,202-204}.

Perinatal morbidity and mortality: Except for one study²⁰⁵, perinatal mortality and morbidity seem to be similar or lower in metformin treated diabetics compared with diet or insulin treated diabetics²⁰²⁻²⁰⁴. Hellmuth et al. reported increased prevalence of pre-eclampsia and perinatal mortality in metformin treated versus insulin or sulfonylurea treated diabetic women²⁰⁶. This study was retrospective and lasted for decades, and women in the metformin group were significantly more obese than women treated with sulphonylureas or insulin.

c) Studies on PCOS women.

Glueck and colleagues reported on a large number of PCOS patients treated with metformin in pregnancy²⁰⁷. Until April 2004, they had prospectively followed 126 PCOS women with 151 fetuses. All the women used metformin at the time of conception. The offspring of PCOS women were compared to gender specific data from the general American population, and among the 126 live born babies, they reported only two congenital birth defects; one sacrococcygeal teratoma and one tethered spinal cord.

Metformin did not influence birth length and weight, growth or motor-social development in the first 18 months of life.

In conclusion, so far, there is insufficient evidence to decide whether or not metformin has teratogenic effects (Table 10 to Table 12).

XVI Background of the studies

In 1998, I heard a very inspiring lecture about PCOS, by Dr Sven M. Carlsen. The endocrinological approach to PCOS was a new aspect for me, and many of my colleagues at the Department of Obstetrics and Gynecology. After several creative discussions we agreed to initiate a small study involving 45 non-pregnant PCOS women, followed up and treated for 14 weeks²⁰⁸. This successful co-operation was continued by a six-months study on 50 non-pregnant PCOS women²⁰⁹. The first paper in this thesis is based on these two studies.

Being an obstetrician and gynecologist, it was natural to focus on PCOS and pregnancy. In 2000, when we planned the study, the general scientific focus was on problems connected to infertility treatment. The reports of Velazquez and Glueck on metformin treatment of pregnant PCOS women were unpublished^{84,210}. The few studies investigating pregnancy complications in PCOS women indicated an elevated risk for gestational diabetes and pre-eclampsia. We suspected that hyperandrogenemia was involved in the pathogenesis. The idea emerged to treat PCOS women with metformin throughout pregnancy, hypothesizing that it would reduce maternal androgen levels, and thus, influence pregnancy outcome.

XVII Aims of the studies

Study I:

- 1) To investigate the clinical, biochemical and ultrasonographic characteristics of non-pregnant, Scandinavian PCOS women and to evaluate possible differences between eumenorrhoeic and oligoamenorrhoeic PCOS women
(Paper I)

Study II:

- 1) To investigate possible effect of metformin on androgen levels in pregnant PCOS women (Paper II)
- 2) To investigate a possible effect of metformin on pregnancy outcome in PCOS women
(Paper II)
- 3) To investigate the trans-placental passage of metformin in PCOS women
(Paper III)
- 4) To analyze if metformin influenced the acid-base status of the newborn
(Paper III)
- 5) To investigate a possible effect of metformin on C-reactive protein levels and indices of coagulation factors and fibrinolysis in pregnant women with PCOS
(Paper IV)

XVIII Material and methods

Paper I

In two prospective studies^{208,209}, we used identical inclusion and exclusion criteria and definition for PCOS. Clinical, biochemical and ultrasonographic data from screening consultations in these two studies are the underlying material for the present paper.

Inclusion criteria were polycystic ovaries (defined as ≥ 9 sub-capsular follicles with a diameter of 3-8 mm), identified by transvaginal ultrasonography, and age between 18 and 40 years. In addition, at least one of the following criteria had to be fulfilled; testosterone > 2.5 nmol/l, SHBG < 30 nmol/l, fasting C-peptide > 1.0 nmol/l, oligo-amenorrhea, or hirsutism, judged clinically as male pattern excessive growth of body hair.

Exclusion criteria included pregnancy, breast-feeding, known liver disease, alanine aminotransferase > 60 IU/l, creatinine > 130 μ mol/l, known alcohol abuse, diabetes mellitus and treatment with oral glucocorticoids. Congenital adrenal hyperplasia was excluded by 17-hydroxyprogesterone measurements.

We collected data from 95 PCOS women and we excluded 15 patients from the analyses. Six women were excluded because of hormonal contraception use at the time of screening evaluation, and six because of BMI < 25 kg/m². In one woman ovarian insufficiency has been overlooked and two women was not fasting when blood samples were drawn.

The remaining 80 women were recruited between December 1999 and June 2001 from the gynecological or endocrinological outpatient clinics at Trondheim University Hospital or by advertisement in the local newspaper. All participants presented one or more of the following clinical problems; anovulatory infertility, oligoamenorrhea or hirsutism.

Retrospectively, we evaluated the participating patients according to the “Rotterdam Consensus” criteria from 2003 and found that all women met the criteria for PCOS according to the new guidelines.

Paper II – IV

These three papers are based on a study population of 40 pregnant PCOS women with singleton pregnancies included in a RCT. Two women withdrew within two weeks after inclusion, because of motivation failure. Thirty-eight women completed the study. The

participants were recruited from October 2000 to March 2003 from the gynecological and infertility outpatient clinic. Two of the 38 women, also participated in study I (Paper 1) described above.

The diagnosis of PCOS was based on the criteria described in Study 1. All the patients met the Rotterdam criteria for PCOS, when this was assessed retrospectively. Inclusion criteria were PCOS diagnosis, singleton viable fetus between gestational week 5 and 12 and maternal age of 18 – 40 years at inclusion. We chose to include a broad spectrum of PCOS patients with no restrictions regarding mode of conception and metformin pretreatment.

To avoid clustering of participants in the trial, a pharmacist performed stratification according to metformin use at conception and the patients were block randomized. Blood samples were drawn at inclusion and at gestational weeks 19, 32 and 36. Oral glucose tolerance test were performed at inclusion and at gestational weeks 19 and 32. When pregnancy complications occurred, all patients were examined, followed up and treated according to clinical routines in Norway.

Blood samples

Testosterone and androstenedione analyses:

For testosterone analysis, we initially used the commercially available Elecsis 2010 method, which was the routine method used at the Department of Clinical Chemistry, The University Hospital of Trondheim. Elecsis 2010 (Roche-Boehringer-Mannheim) is a competitive ELISA method. This method is automated, quick and relatively inexpensive. Unfortunately, we were not aware of the report by Tayeb and colleagues²⁶. She found that none of the ten most used, commercially available immunoassay methods were sufficiently reliable for the low testosterone concentrations seen in women. Most methods overestimate low concentrations of testosterone. Elecsis 2010, however, underestimate testosterone values in women.

In addition, we suspected that androstenedione values measured by Immulite 2000, an automated immunoassay, were imprecise. According to advice from reviewers and specialists in clinical chemistry, we reanalyzed our material. We chose Diagnostic Products Corporation (DPC) Coat-A-Count (Los Angeles, CA). This method is a RIA method, which turned out to slightly overestimate the low testosterone values compared to isotope-dilution gas chromatography-mass spectrometry (ID/GC-MS) in the study of Tayeb. This overestimation is assumed to be an effect of interference with other androgen metabolites. Torjesen reported that RIAs agreed well with the ID/GC-MS, although

interferences happen²¹¹. To solve this problem an extraction was performed before testing.

Analysis of metformin concentration in serum

Immediately after, and independently of the mode of delivery, blood samples were drawn from the antecubital vein from the mother, and samples both from the umbilical artery and vein. Information, about the last capsules of metformin taken before delivery, was registered. Metformin in serum was analyzed by liquid-chromatography-mass spectrometry method.

Statistical analyses

Paper I and II

Values are reported in means and standard deviations for continuous normally distributed variables. Categorical variables are presented as numbers and percents. Differences between the study groups were compared with two tailed t-tests for independent samples. Non-parametric tests, Mann-Whitney, for independent sample were used where normality could not be assured. Pearson's correlation coefficient was used for correlation analyses. Simple linear regression analyses were used to investigate linear trends. Fisher's exact test was used for evaluation of discrete data. Data were analyzed according to the intention to treat principle (Paper II).

Paper III and IV

Values are reported as medians and inter quartile ranges (IQR). For correlation analyses Spearman's rank test were used. In Paper IV values are log transformed to overcome uneven distribution. General linear model was used for repeated measures (Paper IV).

XIX Main results

Study I

“Clinical, biochemical and ultrasonographic characteristics of Scandinavian women with PCOS” (Paper I)

- 1) Except for thicker endometrium and smaller ovarian volume in regularly menstruating women, there were no clinical or biochemical differences between eumenorrhoeic and oligoamenorrhoeic PCOS women. There was a positive linear trend for increasing BMI with increasing number of diagnostic criteria met.

Study II

“Metformin reduces pregnancy complications without affecting androgen levels in pregnant PCOS women: results of a randomized study” (Paper II)

- 1) Metformin did not influence androgen levels in pregnant PCOS women.
- 2) None of the women in the metformin group experienced severe pregnancy complications. Seven women experienced severe pregnancy complications in the placebo group.

“Placental passage of metformin in women with polycystic ovary syndrome” (Paper III)

- 2) Metformin passes freely the placenta. At the time of birth, the fetus is exposed to therapeutic metformin concentrations.
- 3) Metformin does not seem to influence pH levels in the umbilical artery.

“The beneficial effect of metformin on pregnancy outcome in PCOS women is not associated with major changes in CRP or indices of coagulation” (Paper IV)

- 4) Metformin treatment in pregnancy did not result in major changes in C-reactive protein, D-dimer, antithrombin III, activated protein C resistance and activated partial thromboplastin time. Protein C increased slightly in the metformin group.

XX Discussion and interpretation of the results

Paper I

In 1999 to 2001, when this study was planned and performed, there was no general agreement on the definition of PCOS. We discussed whether or not we should include “normal weight” ($\text{BMI} < 25 \text{ kg/m}^2$) PCOS women. There was a widespread opinion, that slim PCOS women differed from obese, not only in the pathogenesis of the disease, but also in clinical features and prognosis. They might not only represent a subgroup, but perhaps a separate entity. Therefore, we chose to include those with BMI above 25 kg/m^2 . Today, however, we would have used the Rotterdam criteria, without selection according to BMI.

After Carmina and Lobo presented their study results suggesting that almost 75% of hyper-androgenic, normally menstruating women had evidence of PCOS²¹², we decided to assess, if eumenorrhoic and oligomenorrhoic patients differed in clinical or biochemical characteristics. Our study confirmed that normally menstruating Scandinavian women can have PCOS and this is important to know in everyday clinical practice.

There has been, and still are, an ongoing discussion about the implications of PCOS on the present and future health of these women. Are these possible implications due to PCOS per se, or are they related to other endocrine and metabolic changes secondary to overweight and adiposity? The present study seems to strengthen the view that obesity is involved in the pathogenesis of PCOS. The severity of PCOS was positively correlated with increasing obesity.

Paper II

This paper was a pilot study with a limited number of participants. The primary aim of the study was to investigate possible differences in androgen levels between metformin and placebo treated pregnant PCOS women. Power calculations were made from change assumptions in testosterone levels. Metformin effects on the pregnancy outcomes were secondary outcomes. We assumed that there might be minor differences in clinical outcomes. We were very surprised by the results. The pilot study was meant to be a

feasibility study, and to form the basis for power calculations of a full-scale study on pregnancy outcome.

Among women, who had been pregnant before, almost half of the previous pregnancies had ended as spontaneous abortions. This is well in line with other retrospective reports on pregnancy outcome in PCOS women (Table 2).

The pregnancy related complications were unexpectedly high among placebo treated women. This is so, even if we take in to account that prospective follow-up studies are more “accurate” in identifying pathology. We believe that the women included in this study had severe PCOS. They sought help for their condition, and they were motivated to participate in a study.

We believe that pregnancies in PCOS women should be regarded as high-risk pregnancies.

We included both normal weight and overweight women independent of mode of conception. Women, who conceived on metformin treatment, were also included. As metformin treatment has become widespread among Norwegian PCOS women, this reflects the real world in clinical practice. Pre-study metformin treatment could theoretically have influenced our results. Indeed, nineteen women used metformin at conception. Thus, we did a stratified block randomization to control for pre-randomization use of metformin.

Eleven of 22 women in the placebo group used metformin at inclusion, and 8 of 18 women in the metformin group used metformin at inclusion. Even if pre-study metformin could influence pregnancy complications, which we do not know, this influence would be equally distributed between the two randomized groups.

The minimum two days “wash-out” period may be insufficient to abolish any metformin effects, although $t_{1/2}$ for metformin is 1.5-4.5 hours in non-pregnant women. Only seven women had a “wash-out” period shorter than two weeks. Exploratory analyses with exclusion of these seven patients did not change the results materially.

Metformin was surprisingly well tolerated in pregnancy. None of the participant stopped medication, and only six women used reduced dosage. Three of these six women received metformin and three received placebo.

Testosterone and androstenedione measurements were a problem. Isotope-dilution gas chromatography-mass spectrometry (ID/GC-MS) is considered as the gold standard. This method does not suffer from cross reactivity and matrix effects. It is, however, expensive

and laborious, and it was not available for us at the time of the study. In Norway, it is still not available for androgen analyses in serum.

Steroid hormones, i.e. androgens are commonly analyzed by commercially available automated immunoassays. Immunoassays use either radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) method for testosterone measurements. Traditionally, testosterone immunoassays involve extraction of structurally related molecules, which can interfere with the results.

Testosterone and androstenedione values are presented according to Elecsis 2010 and RIA methods after extraction (Table 12). Testosterone and androstenedione values differed in the two analyzes, but the conclusion is unchanged; metformin does not influence androgen levels in pregnant PCOS women.

Paper III

There is, in the present study, no evidence that metformin has any teratogenic or adverse effects on the mother or the fetus. The present study demonstrates that metformin passes the placenta to the fetus, and that some hours after ingestion, concentrations tended to be higher in the umbilical artery than maternal serum. In adults, metformin is eliminated by renal tubular secretion. Hence, one may speculate that metformin is excreted to the amniotic fluid and reabsorbed to the fetal circulation by swallowing. This could explain the higher concentrations found in the umbilical artery.

Possible long-term effects of metformin in the offspring are unknown. Theoretically, metformin can alter or modulate metabolic pathways in the fetus. It is of outmost importance to follow up children exposed to metformin in utero in RTCs.

Paper IV

Elevated CRP levels in early pregnancy have been associated with pregnancy complications such as GDM, pre-eclampsia and fetal growth restriction in non-PCOS women. Metformin has been reported to decrease CRP levels in non-pregnant PCOS women. Impaired fibrinolytic capacity has been demonstrated in PCOS women. It was a logical step to investigate the possible effect of metformin on CRP levels and coagulation indices. However, we found no major effect of metformin on the measured parameters that could explain the effect of metformin on pregnancy complications.

XXI Conclusions

We found no major differences in oligoamenorrhoeic and eumenorrhoeic PCOS women with respect to clinical and biochemical parameters, except for thicker endometrium and smaller ovarian volume. Obesity seemed to worsen the clinical presentation of PCOS.

PCOS is associated with an increased risk for pregnancy related complications. The results indicate that PCOS pregnancies should be regarded as high-risk pregnancies. Metformin is well tolerated in pregnancy. It seems to prevent pregnancy complications in the second and third trimester in PCOS women. Metformin passes the placenta, and the fetus is exposed to therapeutic levels of metformin. However, metformin have no major effects on androgens, CRP or coagulation factors. How metformin exerts its possible effect on pregnancy outcome is unrevealed.

XXII Final reflections and future aspects

One great challenge for future research is to motivate women to participate in randomized, placebo-controlled trials. Many women seem to be convinced of the positive effects of metformin on pregnancy, by the information they receive from the web, and they are not willing to “risk” to end up with placebo in a trial. To communicate and interpret scientific results in a sober and nuanced way to a general population is a major task and a great challenge. Also doctors should understand that it is unwise to advocate metformin treatment in pregnancy before solid, reliable data are available.

The present data are encouraging, but before routine use of metformin can be recommended to pregnant PCOS patients, our results must be confirmed by at least one well-designed large-scale study.

If the results are confirmed by a larger randomized controlled trial, there are still many questions to answer:

- What is the mechanism of action, by which metformin exerts its effect on pregnancy complications? What are the pregnancy complications that metformin can prevent?

- Which patients should be recommended to use metformin in pregnancy?
All PCOS women, only those with earlier complications or only obese PCOS women? Or just those who conceive on metformin?
- When should one start and stop the treatment and what is the optimal dose that should be used? Should there be given additional folic acid or vitamin B₁₂?
- Are there any negative consequences of stopping metformin use in pregnancy?
- How does metformin influence the offspring on short or long-term basis?
As long as we do not know the long-term effects of antenatal metformin exposure, follow-up of the offspring from clinical trials should be compulsory.

As a continuum of my thesis, a prospective randomized, double blind, multi-centre study initiated from our department was started up in February 2005. We aim to include 300 - 350 pregnant PCOS patients, treat them with metformin or placebo and follow them throughout pregnancy. The offspring will be followed at least to the age of 18 years. This will hopefully provide some of the answers, - and most certainly raise new questions.

Tables

Table 1. Early pregnancy loss and PCO

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Sagle M 1988	Retrospective	56 women with RPL 11 parous women	RPL ⇒ 82% PCO Controls ⇒ 18% PCO	High prevalence of PCO among women with RPL
Balen AH 1993	Retrospective	1060 IVF pregnancies	27 % EPL 5 % ectopic pregnancies PCO ⇒ 36% EPL Normal ovaries ⇒ 24% EPL	Higher EPL rate among women with PCO compared to women with normal ovaries
Watson 1993	Prospective	21 women with RPL 10 multiparous controls	RPL ⇒ 81% PCO Controls ⇒ 10% PCO High LH ⇒ 81% EPL	High LH among women with RPL High testosterone in the RPL group
Tulppala M 1993	Prospective	50 women with RPL 20 healthy controls	RPL ⇒ 44% PCO Controls ⇒ 12% PCO	High prevalence of PCO among women with RPL
Clifford K 1994	Prospective	500 women with RPL	56 % PCO 31 % obesity 32 % sub-fertility	High prevalence of PCO among women with RPL
Liddell HS 1997	Prospective	73 women with RPL Mean BMI 24 kg/m ²	36 % PCO 64 % normal ovaries	PCO in RPL did not predict poor pregnancy outcome
Rai R 2000	Prospective cohort	486 RPL	RPL ⇒ 41% PCO	High LH was not associated with high miscarriage rate PCO morphology did not predict pregnancy loss in RPL women conceiving spontaneously

RPL = Recurrent Pregnancy Loss

EPL = Early Pregnancy Loss

LH = Luteinizing Hormone

Table 2. Early pregnancy loss and PCOS

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Homburg R 1988	Prospective	54 PCOS women treated with pulsatile LH releasing hormone	41 PCOS women ovulated 27 PCOS women became pregnant 11 PCOS women experienced EPL	High LH levels associated negatively with conception and pregnancy outcome High rate of EPL in PCOS women
Glueck CJ 2002	Retrospective	40 PCOS women	100 pregnancies in 40 PCOS women 62 % of the pregnancies ended as EPL	High rate of EPL in PCOS women
Jakubowicz DJ 2002	Retrospective	31 PCOS women	42% EPL	High rate of EPL in PCOS women

EPL = Early Pregnancy Loss

LH = Luteinizing Hormone

Table 3. Early pregnancy loss and LH

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Homburg R 1988	Prospective	54 PCOS women	LH levels lower in those who conceived vs. not conceived LH levels higher in those who miscarried vs. continued pregnancy	Low LH levels associated both with conception and continued pregnancy
Regan L 1990	Prospective	197 women with spontaneous ovulations	LH < 10U I/L 88 % pregnant 12 % EPL LH > 10 UI/L 67 % pregnant 65% EPL	High pre-pregnancy follicular phase LH is negative for conception and pregnancy outcome
Watson H 1993	Prospective	21 women with RPL 10 multi-parous controls	RPL ⇒ 81% PCO Controls ⇒ 10 % PCO High LH ⇒ 81% EPL	High LH among women with RPL
Clifford K 1996	Prospective, Randomised, controlled	106 RPL	LH suppression ⇒ 52% LBR No LH suppression ⇒ 63% LBR	Pre-pregnancy LH suppression did not improve life birth rate
Rai R 2000	Prospective cohort	486 RPL	RPL ⇒ 41% PCO	High LH was not associated with high miscarriage rate PCO morphology did not predict pregnancy loss in RPL women conceiving spontaneously

LBR = Life Birth Rate

RPL = Recurrent Pregnancy Loss

LH = Luteinizing Hormone

Table 4. Early pregnancy loss and androgens

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Tulppala M 1993	Prospective	50 women with a history of RPL 20 healthy controls	Before index pregnancy: ↑ total testosterone and DHEAS levels in women with EPL vs. successful pregnancies	↑ androgens in women with EPL
Watson H 1993	Prospective	21 women with RPL 10 multiparous controls	↑ testosterone in RPL compared to controls	↑ testosterone in the RPL group
Okon MA 1997	Retrospective	43 regularly menstruating women with RPL 18 fertile controls	↑ testosterone, ↑ DHEAS, ↑ androstenedione, ↑ FTI and ↓ SHBG in the RPL group vs. controls	RPL women, with or without PCOS, had higher androgen levels than normal controls. High androgen concentrations may result in abnormal secretory endometrium

RPL = Recurrant Pregnancy Loss
 DHEAS = Dehydroepiandrosterone Sulphate
 EPL = Early Pregnancy Loss
 FTI = Free Testosterone Index
 SHBG = Sex Hormone Binding Globuline

Table 5. Early pregnancy loss, obesity and insulin resistance

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Dale PO 1998	Prospective	42 IVF treated PCOS women	17 IR PCOS 25 NIR BMI higher in IR PCOS Higher FTI and lower SHBG in the IR-PCOS	IR PCOS had higher cancellation of IVF treatment, and lower conception rate than NIR PCOS
Clark AM 1998	Prospective	67 obese infertile women completing diet and life style program 20 obese infertile drop outs (75% of the participants had PCOS)	Completing study: ↓ BMI (3.7) and 18% vs. earlier 75% EPL 67% LBR Drop outs: ↓ BMI 0.4 No pregnancies	Weight loss in obese PCOS women improves fertility
Huber-Buchholz M 1999	Prospective	18 anovulatory PCOS 10 ovulatory PCOS Weight matched	Decreased insulin sensitivity and increased insulin levels in anovulatory PCOS women	Exercise and sensible eating with minimal weight loss improve reproductive health in obese PCOS women
Fedorcsak P 2000	Retrospective	383 IVF/ICSI patients Lean (BMI < 25) vs. Obese (BMI ≥ 25)	12 % EPL in the lean group 22 % EPL in the obese group	Obesity is an independent risk factor for early pregnancy loss
Wang JX 2001	Retrospective	376 PCOS women of 1018 infertile women	25 % EPL in PCOS	High risk of EPL in PCOS could be due to obesity
Craig LB 2002	Prospective	74 RPL vs. 74 controls Age, race, BMI matched	Fasting insulin and HOMA-IR higher in the RPL group	Increased prevalence of IR in RPL patients

IR = Insulin resistance
NIR = Non-Insulin Resistant
HOMA-IR = Homeostasis Model Assessment – Insulin Resistance
RPL = Recurrent Pregnancy Loss

Table 6. Gestational diabetes mellitus in previous pregnancy and polycystic ovaries

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Holte J 1998	Retrospective / prospective	34 former GDM vs. 36 earlier normal pregnancies 4.5 years after index pregnancy	GDM: 41% PCO Controls: 3% PCO	High prevalence of PCO among former GDM patients GDM + PCO: 50% PIH, Higher BMI, higher androgens
Anttila L 1998	Retrospective	31 former GDM vs. 31 earlier normal pregnant women age and weight matched 6 -16 months after delivery	GDM: 45% PCO Controls: 7% PCO	Women with PCO had high risk for GDM
Kousta E 2000	Postpartum follow up	91 former GDM 73 normo-glycemic controls Twins included	20 and 29 months post partum GDM: 52% PCO vs. 27% controls ↑ BMI + W/H ratio ↑ Fasting insulin	High incidence of PCO among GDM patients
Koivunen R 2001	Case control	33 former GDM 48 age and parity matched controls	GDM: 40% PCO Abnormal OGTT Higher DHEAS Controls: 17% PCO	Women with previous GDM have often PCOS and are more insulin resistant

BMI = Body Mass Index
 GDM = Gestational Diabetes Mellitus
 PIH = Pregnancy Induced Hypertension
 OGTT = Oral Glucose Tolerance test

Table 7. PCOS and gestational diabetes mellitus

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Wortsman J 1991	Retrospective	53 PCOS women 2306 general pregnancies Maternal age and ponderal index similar	PCOS: 7.5% GDM Controls: 6.6% GDM Macrosomia similar	No increased risk for GDM in PCOS women
Lanzone A 1995	Prospective	12 PCOS 12 healthy controls	Increased insulin secretion during pregnancy in PCOS	Increased risk for GDM in PCOS women
Urman B 1997	Retrospective	47 PCOS 100 healthy controls Slim population	PCOS: 13% GDM Controls: 2% GDM	Increased risk of GDM and hypertensive disorder in pregnant PCOS women
Radon PA 1999	Retrospective cohort	22 PCOS 66 age and weight matched normal controls. Not matched for parity	PCOS: 41% GDM OR:22 ↑ pre-eclampsia OR:15 ↑ compl. deliv. OR:5.5 Controls: 3% GDM	Women with PCOS have increased risk for GDM, pre-eclampsia and delivery complications
Fridström M 1999	Case-control	33 PCOS 66 Fertility treated women Similar weight. Twin pregnancies included		No difference in blood glucose levels
Vollenhoven B 2000	Case-control	60 PCOS 17 hypogonadism 12 unexplained infertility 60 BMI, age and race matched controls	GDM similar in PCOS, controls and other infertile women	No difference in prevalence of GDM in PCOS vs. age, BMI and race matched controls
Mikola M 2001	Retrospective	80 PCOS women with 99 pregnancies 737 controls	PCOS: 20% GDM Controls 9% GDM	Increased risk of GDM in PCOS women (Explained largely by increased BMI)
Bjercke S 2002		29 NIR PCOS 23 IR PCOS compared to 355 women who received AR	PCOS: 7.7% GDM AR: 0.6% GDM	GDM is increased in PCOS pregnancies
Glueck CJ 2002	Retrospective	72 PCOS pregnancies (obese women) No controls	31% GDM	High rate of GDM in PCOS women
Sir-Peterman T 2002	Prospective	20 PCOS 26 Controls with singleton pregnancies	PCOS: 15% GDM Controls: 0%	High rate of GDM in PCOS women
Türhan NÖ 2003	Retrospective	38 PCOS 136 Non-PCOS (random selection)	Main predictor for GDM is BMI >25 kg/m ²	High IGT among PCOS women is related to obesity

Table 8. PCOS and pre-eclampsia

Name of first author and publication year	Study design	Participants	Results	Conclusions
Diamant YZ 1982	Retrospective	33 PCOS with 70 pregnancies 39 anovulatory women with 71 pregnancies 1000 normal pregnant primipara	PCOS 28.5% PE Anovulatory 16.5% PE Controls 11% PE	Higher prevalence of PE in PCOS
Gjønnæss H 1989	Retrospective	69 PCOS after LOD No controls	Perinatal mortality: 9% PE: 13% GDM: 8% Preterm (< 32 weeks): 10%	The high prevalence of PE and GDM is related to high BMI
Urman B 1997	Retrospective	47 PCOS 100 healthy slim controls	PCOS: 25% PIH Controls: 8% PIH	Increased risk of PIH in PCOS women Difference between lean PCOS and lean controls
De Vries 1998	Retrospective	81 PCOS pregnancies 81 age and parity matched controls	PCOS: 26% PIH, 14% PE Controls: 25% PIH, 2% PE Similar BMI	PCOS women are at higher risk of PE
Fridström M 1999	Retrospective	33 PCOS 66 No-PCOS Twins included	↑ risk of hypertension in the III. trimester	Increased risk of PIH in PCOS women
Kashyap S 2000	Retrospective	22 PCOS 27 infertile controls Singletons	PCOS: 32% PIH Controls: 3.7% PIH Age, BMI, parity similar	Increased risk of PIH in PCOS
Mikola M 2001	Retrospective	80 PCOS with 99 pregnancies 737 controls	PCOS: 4% PE Controls: 2% PE	PE and prematurity not associated with PCOS
Bjercke S 2002		29 NIR PCOS 23 IR PCOS singleton pregnancies 355 AR women	PCOS: 13% PE (IR; 22%, and NIR; 7%) AR-controls: 7% PE	Higher incidence of PE in IR-PCOS compared to NIR-PCOS and controls
Sir-Peterman T 2002	Prospective	20 PCOS 26 Singleton controls	PCOS: 10% PE Controls: 0% PE	High rate of PE in PCOS
Haakova L 2003	Retrospective	66 PCOS 66 age and weight matched controls Singletons and multiple gestations mixed	No difference between the groups	PCOS not associated with high risk of PE

LOD = Laparoscopic ovarian drilling
PIH = Pregnancy Induced Hypertention

IR = Insulin Resistant
NIR = Non Insulin Resistant

PE = Pre-eclampsia
AR = Assisted Reproduction

Table 9. PCOS and preterm delivery

Name of first author and year of publication	Study design	Participants	Results	Conclusions
Gjønnæss H 1989	Retrospective No controls	69 PCOS after LOD	Preterm: < 32 weeks: 10 % Perinatal mortality: >16 weeks: 7.3%	High prevalence of preterm delivery.
Mikola M 2001	Retrospective Singletons and twins	80 PCOS women with 99 pregnancies 737 controls	PCOS: 10% preterm Controls: 6% preterm In singleton pregnancies	No significant difference in preterm
Jakubowicz DJ 2002	Retrospective	18 PCOS no metformin 62 PCOS + metformin	PCOS no metformin: 33% preterm PCOS + metformin: 15% preterm	High incidence of preterm delivery (< 37 weeks) in PCOS
Bjercke S 2002	Retrospective?	29 NIR PCOS 23 IR PCOS singleton 355 AR women	PCOS: 23% preterm AR-controls: 15% preterm	High incidence of preterm delivery (<37 weeks) in PCOS women
Haakova L 2003	Retrospective Singletons and twins	66 PCOS 66 age and weight matched controls	Same gestational age in PCOS and controls if only singletons were studied	No difference in length of gestation in singleton pregnancies
Glueck CJ 2004	Prospective	90 PCOS women with 97 pregnancies. Metformin treatment in pregnancy. 252 Healthy controls	PCOS: 20% preterm Controls: 10% preterm	Increased risk for preterm delivery (<37 weeks) in PCOS

LOD = Laparoscopic Ovarian Drilling

AR = Assisted Reproduction

NIR = Non Insulin Resistant

IR = Insulin Resistant

Table 10. Animal and in vitro studies on metformin effects in pregnancy

Name of the first author and year of publication	Study design	Subjects	Metformin dose and treatment period	Results	Conclusions
Cohen Y 1961	Laboratory study	Mouse Radio-labeled metformin	Not available	No visible radioactivity in the fetus after 1 and 4 hours. The placenta was radioactively marked	Metformin passes to the placenta, but not to the fetus
Tuchman-Duplessis H Mercier-Parot L 1961	Laboratory study	Rat	500 – 1000 mg/kg (10-20 x human therapeutic dose)	Less than 0.5 % major malformations	Metformin is not teratogenic
Denno KM 1994	Laboratory study In vitro	Mouse embryo culture	0.15-1.8 mg/ml metformin solution 30 hours of culture	No alterations of embryonic growth, no major malformations. Delayed closure of neural tube	Metformin is not teratogenic
Bedaiwy MA 2001	Laboratory study In vitro	Two-cell mouse embryos	5-100 µg/ml, 72 hours of culture	Normal embryonic development at 5 folds higher doses than the maximum clinical dose recommended for humans	Metformin is not teratogenic. No direct toxic effect on mouse embryo development.

Table 11. Studies on diabetic women treated with metformin in pregnancy

Name of first author and year of publication	Study design	Participants	Metformin dose and treatment period	Results	Conclusions
Cited in Stowers JM and Clark TM 1975	Analysis method: Pignard 1962	Amniotic fluid from two women	850 mg daily and 500 mg daily	75µg/100 mL and 90µg/100 mL metformin	Metformin passes the placenta
Stowers JM and Sutherland HW 1975	Retrospective	5 women with diabetes	1000 – 3000 mg daily Mean 40 days before delivery	All babies survived	Metformin is not teratogenic
Pedersen J Mølstedt-Pedersen L 1975	Retrospective	15 women with GDM	1500 mg daily Entire pregnancy Mostly in III. trimester	Perinatal mortality in the metformin treated group similar to the total diabetic population	Metformin is not teratogenic No evidence of adverse effect of metformin in mother or child
Coetzee EJ 1979	Retrospective	33 women with diabetes type-2 and GDM	1500 – 3000 mg daily in the II. and III. trimester	2 perinatal deaths (one malformation and one prematurity)	Lower mortality and morbidity in the metformin treated group compared to no treatment or insulin treatment groups
Coetzee EJ 1984	Retrospective	21 women with diabetes type-2	1000 – 3000 mg daily in the I. trimester	1 minor congenital abnormality	Metformin is not teratogenic No difference in perinatal mortality and morbidity in diabetic women who received metformin during the first trimester and those who did not
Piacquadio K 1991	Observational	One woman with diabetes type-2	Dose unknown I. trimester	Healthy baby	Metformin is not teratogenic
Hellmuth E 1993	Retrospective	7 women with diabetes type-2	1000 - 2000 mg daily 5-39 weeks treatment	No congenital malformations	Metformin is not teratogenic
Sarlis NJ 1998	Case report	One hyper-androgenic patient	1000 mg daily From weeks 14 - 30	Decreased maternal total testosterone level	Metformin is not teratogenic No malformations or other adverse effects
Hellmuth E 2000	Retrospective Not randomized	50 women with diabetes type-2 or GDM Compared with 42 insulin treated women	Accumulated dose: 1,5 – 507 g. Gestational weeks 13-40	Metformin group: 31% PE 12% PM Insulin group: 10% PE 1.3% PM	Prevalence of pre-eclampsia and perinatal mortality higher in the metformin group

PE = Pre-eclampsia

PM = Perinatal Mortality

GDM = Gestational Diabetes Mellitus

Table 12. Studies on PCOS women according to metformin use in pregnancy

Name of first author and year of publicaion	Study design	Participants	Metformin dose and treatment period	Results	Conclusions
Jakubowicz DJ 2002	Retrospective	65 PCOS women 68 pregnancies 32 PCOS women - no treatment	1000 - 2000 mg daily Before conception and throughout pregnancy	1 achondrodysplasia (inherited disorder)	Metformin is not teratogenic
Glüeck CJ 2004	Prospective	126 PCOS women 151 pregnancies	1500 – 2550 mg daily Before conception and throughout pregnancy	1 sacrococcygeal teratoma 1 tethered spinal cord	Metformin is not teratogenic. No negative effect on birth size or psychomotor development during the first 18 month of life

Table 13. Changes in maternal androgen levels in PCOS women during pregnancy, according to treatment by metformin or placebo. Measured by two different methods.

		Inclusion				Change to week 19		Change to week 32		Change to week 36			
Group		N	Mean (SD)		N	Mean (SD)		N	Mean (SD)		N	Mean (SD)	
			Elecsys 2010	Extraction + RIA		Elecsys 2010	Extraction + RIA		Elecsys 2010	Extraction + RIA		Elecsys 2010	Extraction + RIA
Androstenedione (nmol/L)	Placebo	21	23.4 (16.6)	18.2 (12.1)	21	-0.5 (16.6)	-2.1 (12.5)	18	8.9 (27.5)	4.6 (20.2)	17	16.8 (28.5)	6.2 (17.5)
	Metformin	17	20.0 (5.8)	14.3 (6.2)	17	1.6 (5.7)	-1.9 (4.5)	17	3.7 (4.1)	1.0 (4.3)	17	13.1 (10.6)	5.2 (4.3)
Testosterone (nmol/L)	Placebo	21	4.2 (2.6)	4.4 (2.9)	21	-1.0 (2.4)	-0.1 (2.9)	18	-0.0 (5.1)	1.7 (6.7)	17	0.4 (5.0)	2.5 (6.9)
	Metformin	17	3.7 (1.5)	3.5 (1.8)	17	-0.8 (1.1)	0.3 (1.8)	17	-0.9 (1.1)	0.4 (1.8)	17	-0.3 (1.3)	1.6 (2.6)
Free Testosterone Index	Placebo	21	4.3 (3.8)	4.0 (2.8)	21	-3.0 (3.4)	-2.3 (2.3)	18	-3.2 (3.9)	-2.2 (3.2)	17	-3.4 (3.9)	-2.4 (2.9)
	Metformin	17	3.7 (2.6)	3.2 (2.2)	17	-2.7 (2.5)	-1.8 (2.2)	17	-2.8 (2.4)	-1.9 (2.0)	17	-2.7 (2.4)	-1.7(2.1)

XXIV References

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Paper I - IV

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Placental passage of metformin in women with polycystic ovary syndrome

Metformin passes the placenta. Fetal serum levels are comparable with maternal values. (Fertil Steril® 2005; 83:1575–8. ©2005 by American Society for Reproductive Medicine.)

The biguanide derivative metformin is an oral hypoglycemic drug used in type 2 diabetes. It improves glucose tolerance mainly by decreasing hepatic glucose production and by increasing insulin sensitivity. Metformin is a highly water-soluble drug. It is not metabolized and is eliminated through the kidneys by tubular secretion (1–3). The recognition that metformin induces ovulation in many women with polycystic ovary syndrome (PCOS) (4, 5) has resulted in more or less controlled use of metformin in the first trimester, although it is not licensed for use in pregnancy.

Recent studies indicate that metformin might reduce pregnancy complications such as gestational diabetes and premature delivery in women with PCOS (6–8). As the incidence of type 2 diabetes increases also in the younger age groups, metformin will probably be more extensively used in pregnant women in the future. The effect of metformin on the fetus is essentially unknown. However, teratogenic effects have not been reported in humans (8–10). Data on the passage of metformin across the placenta are few and contradictory. A study in rodents suggested no placental passage of metformin (11); in humans, placental glucose uptake seems unaffected by metformin (12).

In a recent report, metformin was detected in umbilical cord blood at delivery (13). However, in that small study, arterial and venous umbilical cord blood were not analyzed separately and it is unclear whether the maternal venous blood samples were obtained at delivery or earlier during pregnancy. Moreover, as the maternal venous and the cord blood samples were not obtained at the same time interval relative to the last dose intake of metformin, the study design is inappropriate to provide a precise quantitative estimate of the transplacental passage of the drug.

The aim of our study was to investigate to what extent metformin passes to the fetus in women with PCOS. Because metformin may induce lactic acidosis, we also investigated if metformin affected the acid–base status of the newborn.

The study population has been described in detail elsewhere (8). In brief, 18 pregnant women with PCOS were treated with metformin, 850 mg twice daily. The women started treatment between gestational weeks 5 and 12 (on average, at gestational week 8) and continued the medication until delivery. One woman withdrew within 2 weeks after inclusion because of motivation failure. The remaining 17 women completed the study. One woman gave birth in an ambulance without any possibilities for blood sampling, and blood samples from another one were missing for administrative reasons. Thus, the results are presented for a total of 15 women.

All participants received written and individual-verbal diet and lifestyle counseling at their inclusion to the study in early pregnancy. Thereafter, treatment with metformin (metformin, 425-mg capsules; Weifa AS, Oslo, Norway) was initiated. All participants used 850 mg, once daily, during the first week and 850 mg, twice daily (1700 mg/day), for the rest of the study period. Three of the 15 women used a reduced dosage (850 mg/day) because of gastrointestinal side effects. Written informed consent was obtained from each patient before inclusion in the study, and the declaration of Helsinki was followed throughout the study. The Committee for Medical Research Ethics of Health Region IV, Norway, and The Norwegian Medicines Agency approved the study.

Blood samples from an antecubital vein of the mother and the umbilical artery and umbilical vein were separately drawn within 1 hour after delivery. Blood samples were centrifuged at room temperature within 30 minutes, and the serum was stored (8 to 36 months) at -70°C until analysis. Immediately after delivery, the pH, base excess (BE), Po_2 , and Pco_2 were measured in the umbilical artery blood using a Rapidlab 248pH/Blood Gas Analyzer (Bayer Corp., Tarrytown, NY) with reagents and calibrators supplied by the manufacturer.

Metformin in serum was analyzed by a liquid chromatography–mass spectrometry method. In brief, metformin was precipitated from serum according to the procedure of Zhang et al. (14) with the modifications of Chen et al. (15). The mass spectrometric analysis was performed on an Agilent 1100 LC-MSD single quadrupole instrument (Agilent Technologies, Palo Alto, CA). Metformin and the

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TABLE 1

Metformin concentrations in maternal and umbilical cord blood in women with polycystic ovary syndrome treated with metformin (850 mg, twice daily).

Patient No.	Last drug intake before sampling (hours)	Maternal serum concentration ($\mu\text{mol/L}$)	Umbilical vein serum concentration ($\mu\text{mol/L}$)	Umbilical artery serum concentration ($\mu\text{mol/L}$)	pH in umbilical artery blood	Base excess in umbilical artery blood (mmol/L)	Po ₂ in umbilical artery blood (kPa)	Pco ₂ in umbilical artery blood (kPa)	APGAR score at 1, 5, and 10 minutes
1	4	13.73	12.54	11.94	7.04	−7.5	1.09	11.72	7, 7, 8
2	12	0.68	2.31	2.81	7.25	−4.2	2.37	7.10	9, 9, 10
3	14	2.80	3.96	5.02	7.25	−4.5	2.79	7.02	9, 9, 10
4	16	1.59	1.96	2.06	missing	missing	missing	missing	9, 9, 10
5 ^a	17	1.90	2.92	3.25	7.34	−0.1	1.45	6.52	9, 10, 10
6	20	0.86	2.81	3.21	7.24	−5.7	2.46	6.95	8, 10, 10
7	20	2.02	3.24	3.16	7.30	−0.2	2.49	7.24	9, 10, 10
8 ^a	32	0.08	0.21	0.26	7.33	−3.2	2.94	5.89	9, 9, 10
9	unknown	3.60	5.92	6.47	7.26	−8.7	3.61	5.54	9, 9, 10
10	unknown	6.29	5.30	4.76	missing	missing	missing	missing	10, 10, 10
11	unknown	0.64	1.83	1.36	7.28	−9.8	2.35	4.88	9, 10, 10
12	unknown	4.32	7.84	6.81	missing	missing	missing	missing	4, 7, 8
13	unknown	0.05	1.25	1.55	7.27	missing	missing	missing	9, 10, 10
14	>48	0.00 ^b	0.00 ^b	0.00 ^b	7.28	0.3	0.63	8.03	9, 10, 10
15 ^a	>48	0.00 ^b	0.00 ^b	0.00 ^b	7.27	−13.7	3.93	3.56	9, 9, 10
Median (IQR) ^e		1.50 (0.50; 3.60)	2.81 (1.25; 5.30) ^c	3.16 (1.36; 5.02) ^d	7.27 (7.25; 7.30)	−4.5 (−8.7; −0.2)	2.46 (1.45; 2.94)	6.95 (5.54; 7.24)	

^a Metformin dose: 850 mg daily.

^b Below the limit of detection of the method.

^c Metformin concentration in umbilical vein versus maternal serum: $P=.017$.

^d Metformin concentration in umbilical artery versus maternal serum: $P=.033$.

^e Interquartile range.

Vanky. Placental passage of metformin in women with PCOS. *Fertil Steril* 2005.

internal standard diphenhydramine were monitored at m/z 130.1 and 256.2, respectively, using electrospray positive ionization. Unknown samples were analyzed together with quality control samples and calibrators covering a range from 0.05 (limit of detection) to 50 $\mu\text{mol/L}$.

All statistical procedures were performed using the SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL). For comparative statistics, Wilcoxon's test and Spearman's rank correlation test were used, as the data were not normally distributed. Consequently, data are presented as medians and interquartile ranges. $P < .05$ was considered statistically significant.

Metformin concentrations were detectable in 13 of the 15 maternal serum samples, ranging from 13.78 $\mu\text{mol/L}$ 4 hours after drug intake, to 0.08 $\mu\text{mol/L}$ 32 hours after last drug intake. Except for two patients (patients 1 and 10), metformin was detected in both umbilical vein and umbilical artery in higher concentrations than in maternal serum (Table 1). Metformin concentrations in both the umbilical vein ($P = .017$) and the artery ($P = .033$) were higher than metformin levels in maternal venous blood. Maternal metformin concentrations were significantly correlated to metformin levels in the umbilical vein ($R^2 = 0.96$, $P < .001$) and umbilical artery ($R^2 = 0.90$, $P < .001$). Concentrations in the umbilical artery and umbilical vein were close to equal (see Table 1). The pH, BE, Po_2 , and PCO_2 in umbilical artery blood did not statistically significantly correlate to the metformin concentration in maternal serum or umbilical artery or umbilical vein serum.

The present finding, that a significant amount of metformin passes to the fetus, supports the results of Hague et al. (13). However, contrary to that study, we found that the metformin concentrations in both umbilical artery and vein serum were roughly the same as in maternal serum. The reason for the discrepancy between these studies is that in the study by Hague et al. (13) the maternal venous blood samples were obtained 4.5 hours after the intake of the last metformin dose whereas umbilical cord blood was obtained 10 hours after intake of the last metformin dose. In contrast, in our study, all blood samples were obtained simultaneously. Given the short elimination half-life of metformin (1.5 to 4.5 hours), a delay in umbilical blood sampling relative to maternal venous blood sampling will erroneously underestimate the transplacental passage of metformin.

Our results indicate that metformin passes freely through the placenta and that the fetus is exposed to levels of metformin comparable with therapeutic concentrations in adults. Our findings also indicate that, except for the first hours after metformin intake, the fetus is exposed to higher concentrations of metformin than the mother. The knowledge on metformin metabolism in the fetus is scarce. In adults, metformin is eliminated by renal tubular secretion. Hence, one may speculate that in the fetus some of the

metformin is excreted to the amniotic fluid (16) and reabsorbed to the fetal circulation by swallowing. Metformin is then eliminated from the fetus by passage through the placenta into the maternal circulation.

This would explain our findings of higher fetal metformin concentrations in all but two fetuses as compared with the maternal levels. The two latter cases represent the two mothers with the highest metformin concentrations, most likely due to a short time interval from metformin intake. The fact that the fetus seems to be exposed to higher concentrations than the mother for a substantial part of a dose interval is noteworthy. It indicates that longitudinal studies on the effect of metformin exposure in pregnancy should be conducted in the offspring into adult age. Also, the incidence of metabolic syndrome should be investigated in these individuals.

In conclusion, the results from the present study indicate that metformin freely passes the placenta and that the fetuses are exposed to therapeutic concentrations. Although no teratogenic effects are reported for metformin, and metformin does not seem to influence pH levels in umbilical artery blood, we nevertheless consider long-term follow-up to be mandatory in these infants.

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Running title:

Metformin effect on CRP in PCOS pregnancies.

The beneficial effect of metformin on pregnancy outcome in PCOS women is not associated with major changes in CRP or indices of coagulation.

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Capsule:

The preventive effect of metformin on pregnancy complications in PCOS women seems not to be associated with major changes of CRP levels or indices of coagulation factors and fibrinolysis.

Abstract

Objective: To investigate a possible effect of metformin on C-reactive protein levels and indices of coagulation factors and fibrinolysis in pregnant women with polycystic ovary syndrome.

Design: Randomised double blind placebo-controlled study.

Setting: University hospital.

Patient(s): Forty pregnant women with polycystic ovary syndrome followed through pregnancy.

Intervention(s): All patients received diet and lifestyle counselling and were randomised to either metformin 850 mg twice daily or placebo. Outcome measures were serum levels of C-reactive protein and indices of coagulation factors and fibrinolysis.

Result(s): In women with polycystic ovary syndrome, C-reactive protein levels as well as D-dimer, antithrombin III, activated protein C resistance and activated partial thromboplastin time were unaffected by metformin treatment throughout pregnancy. Protein C levels increased slightly in the metformin group compared to the placebo group.

Conclusions: Metformin treatment of pregnant women with polycystic ovary syndrome seems to prevent pregnancy complications but this effect is unlikely to be associated with major changes of CRP levels or indices of coagulation or fibrinolysis.

Key words: coagulation factors / C-reactive protein / metformin / PCOS

Women with polycystic ovary syndrome (PCOS) have increased risk for spontaneous abortion (1-6) and pregnancy complications, such as gestational diabetes mellitus (7-10), preterm delivery (3;9;11;12) and preeclampsia (11;13;14). Recently, we reported that metformin treatment throughout pregnancy prevented pregnancy complications in PCOS women. However, the preventive effect of metformin was not explained by changes in androgen levels as we hypothesized (4).

Polycystic ovary syndrome (PCOS) has been associated with an increased risk for cardiovascular disease (15-17) and a 3 fold increased risk of postmenopausal hypertension (18). As many as forty percent of women with PCOS may have impaired glucose tolerance or type 2 diabetes (19;20). C-reactive protein (CRP) is a sensitive indicator of systemic inflammation (21). Studies show that chronic low-grade inflammation, as measured by CRP, is strongly predictive for the development of cardiovascular disease and type-2 diabetes (22-26). Several studies demonstrate elevated CRP levels in PCOS women compared to healthy controls (27-30), although also one negative study has been published (31). Morin-Papunen found that metformin reduced CRP levels in non-pregnant PCOS women (32).

First trimester CRP levels are increased among women who subsequently develop gestational diabetes mellitus, preeclampsia and fetal growth restriction (33-37). The effect of metformin on CRP levels in pregnant PCOS women has not been reported.

PCOS women have impaired fibrinolytic capacity, which may partially explain an increased risk for deep venous thrombosis (38). Thrombosis of the placenta is sometimes the cause of placental insufficiency and fetal demise (39). Velazquez et al. found that metformin treatment reduced plasminogen activator inhibitor-1 (PAI-1) in non-pregnant women with

PCOS (40). Thus, a possible effect of metformin on indices of coagulation or fibrinolysis could help to explain the preventive effect of metformin against pregnancy complications.

We hypothesized that metformin could influence CRP levels and /or indices of coagulation and fibrinolysis. Serum from a previously reported prospective randomized trial was used to investigate if coagulation and fibrinolysis indices could hint possible mechanisms of action of metformin in PCOS pregnancies (4).

MATERIALS AND METHODS

From October 2000 to March 2003, 40 pregnant women with PCOS were recruited from the gynaecological and the infertility outpatient clinic at The University Hospital of Trondheim. The women were included in a prospective, randomised, placebo controlled trial with the aim to study the effect of metformin treatment on androgen levels and pregnancy complications PCOS women (4). Inclusion criteria in the trial were: 1) diagnosis of PCOS before the actual pregnancy, 2) age 18 to 40 years, 3) gestational age between five and twelve weeks, and 4) a singleton viable fetus judged by ultrasonography. The diagnosis of PCOS was based on the presence of polycystic ovaries (nine or more sub-capsular follicles with a diameter of 3-8 mm) verified by transvaginal ultrasonography. Furthermore, at least one of the following five criteria had to be present: testosterone > 2.5 nmol/l, sex hormone binding globulin (SHBG) < 30 nmol/l, fasting insulin C-peptide > 1.0 nmol/l, oligo-amenorrhea (length of menstrual cycle > 35 days or less than ten periods per year), or hirsutism (judged clinically as male pattern growth of body hair). The study population have been described in detail previously (4). All women in the study fulfilled the “Revised 2003 consensus” on diagnostic criteria for PCOS (41).

All participants signed a written informed consent before inclusion. The Regional Committee for Medical Research Ethics and The Norwegian Medicines Agency approved the study. The declaration of Helsinki was followed throughout the study.

At the time of inclusion (\leq gestational week 12) and later at gestational weeks 19, 32 and 36, venous blood samples were drawn from an antecubital vein, between 8:00 and 11:00 a.m. after an overnight fast. Blood samples for CRP analysis, were centrifuged at room temperature within 30 minutes and stored at -70°C until analysis (6-28 months). Coagulation factors were analysed subsequently on the day of sampling.

Study protocol

All participants received individual, verbal and written diet and lifestyle counselling at inclusion. Eighteen women were randomised to metformin medication and twenty-two to placebo. Randomisation was performed in blocks of ten and women were stratified according to whether or not they used metformin at conception.

At conception eleven women in the placebo group and eight in the metformin group used metformin. Seven of the women who used metformin at conception (five in the placebo group and two in the metformin group), continued to use it until a few days before inclusion. All women had a “wash out” period of at least two days before inclusion. The randomised groups were balanced with respect to maternal age, body mass index (BMI), and gestational age at the time of inclusion, apart from diastolic blood pressure which was higher in the metformin group(4).

The women in the study group received two capsules of metformin 425 mg (metformin hydrochloride, Metformin[®], Weifa AS, Oslo, Norway) twice daily, and the control group received identical placebo capsules. All participants used two capsules once daily during the first week and two capsules twice daily for the rest of the study period. In addition, they all received 1 mg of folate daily, and one daily multivitamin tablet. Two women, one in each group, withdrew within two weeks after inclusion. The remaining 38 women completed the study.

Assays

CRP was analysed on a Roche/Hitachi 902 analyzer using a Tina-quant CRP high sensitive assay (Mannheim, Germany). D-dimer and antithrombin III were analysed on a Hitachi 917 instrument by the Tina-quant (Roche Diagnostics) and Coamatic 400 reagents (Chromogenics AB, Mölndal, Sweden), respectively. Activated partial thromboplastin time (APTT), activated protein C resistance (APC resistance) and protein C were analysed on a Trombolyzer

instrument from Behnk Elektronik using reagents from Nycomed Pharma AS (Oslo, Norway), Coatest APC-resistance V-S reagents (Chromogenics) and Coamatic Protein C reagents (Chromogenics), respectively. Protein S was analysed by enzyme-linked immunosorbent assay (ELISA) method with Coaliza reagents (Chromogenics).

Statistical Analysis

All statistical procedures were performed using the SPSS version 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Values are reported as medians and inter quartile ranges (IQR). To evaluate the difference between the study groups, we used general linear model for repeated measures on log transformed values to overcome the effect of uneven distribution. For patient characteristics mean values and standard deviations are given and two-tailed T-test for independent samples were used. For correlation analyses we used Spaerman's rho.

RESULTS

At inclusion, the placebo group and metformin group were equal regarding age (28.3 ± 3.7 years vs. 28.9 ± 4.8 years, $p = 0.7$), BMI ($29.3 \pm 8.0 \text{ kg/m}^2$ vs. $32.1 \pm 6.1 \text{ kg/m}^2$, $p = 0.2$), gestational age (55 ± 17 days vs. 59 ± 14 days, $p = 0.4$), and systolic blood pressure (117 ± 16 mmHg vs. 120 ± 14 mmHg, $p = 0.5$). However, diastolic blood pressure was lower in the placebo group (72 ± 10 mmHg vs. 78 ± 9 mmHg, $p = 0.05$). At conception 50% of the women in the placebo group and 44% in the metformin group used metformin ($p = 0.8$).

At inclusion, and throughout the study, CRP levels were equal in the two groups (Table 1). General linear model for repeated measures of CRP through pregnancy did not show tendency to differences between the groups ($p = 0.51$). CRP correlated to BMI at inclusion ($R^2 = 0.41$; $p < 0.001$), at gestational week 19 ($R^2 = 0.36$; $p = 0.001$), at gestational week 32 ($R^2 = 0.22$; $p = 0.005$) and at gestational week 36 ($R^2 = 0.15$; $p = 0.02$).

Compared to the placebo group, protein C increased slightly in the metformin group ($p = 0.04$). Metformin did not affect other measured coagulation and fibrinolytic factors during pregnancy.

DISCUSSION

In a previous report, from the same study population we, reported that metformin reduced pregnancy complications among PCOS women, while androgens, SHBG and body mass index were unaffected through pregnancy (4). The present study is of limited size, but it is performed on a well-characterized population in a prospective, controlled design. To our best knowledge this is the first study to report on CRP levels and coagulation parameters through pregnancy in metformin treated PCOS patients.

CRP is a surrogate marker for inflammation and is associated with poor outcome in both cardiovascular diseases and a variety of obstetric conditions (24;25;33;37;42;43). Furthermore, CRP is elevated in PCOS and other aspects of the metabolic syndrome (27-29). We therefore hypothesized that metformin might reduce CRP levels in pregnant PCOS women, but we found no such effect in the present study. This is surprising since Morin-Papunen found that metformin decreased CRP levels in non-pregnant PCOS women (32). Metformin treatment in that study was however compared to ethinyl estradiol (35 µg) cyproterone acetate (2 mg) and not placebo, and the study participants were not pregnant. In PCOS women elevated CRP levels associated with adiposity, and weight loss resulted in reduced CRP in non-pregnant PCOS women (32). Thus, one possible explanation for the lack of association between CRP levels and metformin in the present study might be that metformin influence pregnant and non-pregnant women differently. There is also a possibility for type 2 statistical errors in studies with small numbers of participants. However, the present study indicates that prevention of pregnancy complications in PCOS women by metformin is not mediated by a major effect on inflammation, as measured by CRP.

Decreased fibrinolysis has been observed in non-pregnant PCOS women (38). In the present study, we observed an effect of metformin on protein C. Increased Protein C levels would, at least theoretically, indicate a positive effect of metformin on coagulation. We do,

however, not think that this is an important factor in the action of metformin, since the size of the difference is relatively small and protein C levels were within the normal range in the both groups.

We also studied thrombophilic factors and in keeping with the study by Tsanadis we found no increased incidence of thrombophilia (44). This is in contrast to a study by Velazquez among non-pregnant women with PCOS (45). She found an effect of metformin treatment on fibrinolysis measured by PAI-1, indicating that metformin increases fibrinolysis. More sensitive assessments of fibrinolytic capacity were not performed in our study. Measurements of D-dimer are however, sensitive enough to detect improved fibrinolysis in non-pregnant PCOS women on low-calorie diet (46).

There is an increasing body of evidence that metformin may have a preventive effect on pregnancy complications. Its possible mechanism of action is, however, still not understood. Neither inflammation, as measured by CRP, nor disturbances in coagulation or fibrinolysis seem to explain the beneficial effect of metformin. Other, still unveiled, mechanisms are probably involved. Well-designed, large-scale studies are needed both to confirm the positive effect of metformin on pregnancy complications and to clarify the mechanisms that might be involved.

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Table 1. CRP and indices of coagulation factors and fibrinolysis in pregnant PCOS women according to treatment group.

	Group	Inclusion			Week 19			Week 32			Week 36			P- ^a value
		N	Median	IQR	N	Median	IQR	N	Median	IQR	N	Median	IQR	
CRP mg/L	Placebo	21	5.7	2.4 – 9.4	21	7.6	3.4 – 12.3	18	5.8	1.8 – 10.0	17	7.4	2.6 – 9.1	0.51
	Metformin	16	4.1	1.1 – 10.2	16	7.2	2.9 – 17.0	16	5.4	2.2 – 14.1	16	4.3	2.1 – 7.6	
APTT (seconds)	Placebo	19	25	23 - 27	18	25	24 - 27	15	26	24 - 26	15	25	23 - 26	0.88
	Metformin	17	25	23 - 28	16	25	23 - 28	16	24	22 - 27	15	25	23 - 26	
D-dimer (mg/L)	Placebo	20	0.30	0.22 – 0.48	19	0.40	0.40 – 0.70	16	0.80	0.60 – 1.00	16	1.05	0.70 – 1.40	0.12
	Metformin	17	0.20	0.20 - 0.30	16	0.40	0.30 – 0.50	16	0.60	0.45 – 1.00	15	0.95	0.55 – 1.38	
Antitrombine III (%)	Placebo	20	102	90 - 112	19	98	90 - 106	15	106	94 - 111	16	102	99 - 110	0.48
	Metformin	17	102	89 - 108	16	104	90 - 107	16	105	100 - 114	15	104	99 - 112	
APC resistance	Placebo	20	1.01	0.97 - 1.06	19	0.98	0.94 – 1.01	16	0.98	0.92 – 1.03	16	1.00	0.90 – 1.05	0.16
	Metformin	17	1.03	1.00 – 1.04	16	1.01	0.97 – 1.09	15	1.00	0.97 – 1.04	15	0.99	0.95 – 1.03	
Protein C (%)	Placebo	22	106	96 - 117	20	117	98 - 130	17	106	99 - 121	17	102	86 - 116	0.04
	Metformin	18	108	102 – 130	16	128	108 - 130	17	117	104 - 130	15	124	102 - 130	
Protein S (%)	Placebo	22	76	50 - 96	20	61	55 - 71	17	45	44 - 56	16	46	39 - 52	0.28
	Metformin	17	87	65 - 112	14	60	56 - 72	16	53	44 - 66	14	53	48 - 56	

^a p value for general linear model for repeated measures on log transformed values