Polycystic ovary syndrome and pregnancy complications

Focus on hyperandrogenism and comorbidity

RAGNHEIÐUR VALDIMARSDÓTTIR
Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women, affecting their lives in many ways. PCOS is characterised by ovulatory dysfunction, polycystic ovary morphology and hyperandrogenism, either clinical or biochemical. Women with PCOS face a higher risk of obstetric complications than women without PCOS. There are many factors that contribute to these complications, such as metabolic disturbances, insulin resistance, chronic inflammation, hyperandrogenism and factors related to infertility.

The overall aim of the research presented in this thesis was to study factors that might affect the association between PCOS and pregnancy complications. The thesis consists of matched cohort studies based on data from the Uppsala Biobank of Pregnant Women (Papers I and II) and national register-based cohort studies (Papers III and IV). In the first two studies, we included women with PCOS (n = 159) and BMI-matched controls (n = 320), and the aim was to study the effect of high anti-Müllerian hormone (AMH) and testosterone on pregnancy complications. The third study (n = 138 219) explored whether the association between PCOS and preeclampsia depends on treated clinical hyperandrogenism and whether PCOS is associated with different subtypes of preeclampsia. In the fourth study (n = 281 806), the aim was to explore association and risk estimates for pregnancy outcomes in women with either or both PCOS and gestational diabetes mellitus (GDM).

The main results were that women with PCOS have higher levels of AMH and testosterone and a higher free androgen index during second trimester pregnancy than non-PCOS controls. High AMH levels were not associated with adverse pregnancy outcome or birthweight. PCOS women with the highest testosterone levels had the highest risk for preeclampsia. Compared to non-PCOS controls, women with PCOS have increased risk of preeclampsia, especially the more severe subtypes of preeclampsia, early onset or with a birth of an infant born small for gestational age. With available data, we were unable to determine whether hyperandrogenism affects the risk of preeclampsia. The combination of PCOS and GDM exacerbates the risk of adverse pregnancy outcomes for both mother and infant compared with women with either PCOS or GDM alone.

In conclusion, the research presented in this thesis adds important information about the association of PCOS and the more severe subtypes of preeclampsia and underpins the importance of an increased awareness of PCOS in antenatal care along with early screening for diabetes and hypertensive disorders.

Keywords: Polycystic ovary syndrome, PCOS, epidemiology, pregnancy complications, anti-Müllerian hormone, AMH, testosterone, preeclampsia, gestational diabetes mellitus, GDM

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To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


*Joint first authors, both authors contributed equally to this work.

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## Abbreviations

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<th>Description</th>
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<tr>
<td>AE-PCOS</td>
<td>The Androgen Excess and PCOS Society</td>
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<tr>
<td>AMH</td>
<td>Anti-Müllerian hormone</td>
</tr>
<tr>
<td>aOR</td>
<td>Adjusted odds ratio</td>
</tr>
<tr>
<td>ART</td>
<td>Assisted reproductive technology</td>
</tr>
<tr>
<td>ATC</td>
<td>Anatomic Therapeutic Chemical</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>FAI</td>
<td>Free androgen index</td>
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<td>MBR</td>
<td>Medical Birth Register</td>
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<tr>
<td>NICE</td>
<td>National Institute for Health and Clinical Excellence</td>
</tr>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
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<tr>
<td>IVF</td>
<td>In vitro fertilisation</td>
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<td>LGA</td>
<td>Large for gestational age</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PCO</td>
<td>Polycystic ovary</td>
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<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
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<tr>
<td>PCOM</td>
<td>Polycystic ovarian morphology</td>
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<tr>
<td>SGA</td>
<td>Small for gestational age</td>
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<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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Introduction

Polycystic ovary syndrome

Definition

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among reproductive aged women with a prevalence of 10 to 13%, according to the Rotterdam criteria, and many remain undiagnosed. PCOS is characterised by ovulatory dysfunction and polycystic ovary morphology and hyperandrogenism, either biochemical or clinical (1).

This condition was described as early as 1721 by an Italian medical scientist, physician and naturalist, and more reports followed during the 18th and 19th centuries (2). In 1935, Stein and Leventhal presented a group of women with menstruation disturbances, hirsutism and enlarged ovaries with the presence of many small follicles. They assumed that these symptoms resulted from abnormalities in hormonal stimulation; subsequently this clinical condition was named after them, Stein-Leventhal syndrome (2).

A diagnosis of PCOS is made by combining clinical symptoms, blood tests and clinical examination with gynaecological ultrasound. No single test can be used. The disease is characterised by a spectrum of signs and symptoms that vary between women, but anovulation is a prominent symptom in most. Other endocrinopathies must be excluded and screening is recommended for thyroid disease, hyperprolactinaemia and non-classic congenital adrenal hyperplasia as a cause of anovulation. Furthermore, evaluation is needed in women with amenorrhoea and more severe clinical symptoms of androgen excess, suspected androgen-secreting tumour, Cushing disease or severe insulin resistance syndrome (1, 3).

Three slightly different diagnostic criteria have been used through the years. The National Institutes of Health (NIH) defined PCOS in 1990 based on the presence of both hyperandrogenism and menstrual dysfunction (4). In 2003, the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) established the Rotterdam criteria, which were adopted early across the Nordic countries. According to the Rotterdam criteria, the diagnosis of PCOS is based on two of the following three signs/symptoms (4): oligo- and/or anovulation, hyperandrogenism and polycystic ovaries. The Androgen Excess and PCOS
Society (AE-PCOS) proposed their own criteria in 2006, stating that the diagnosis of PCOS requires the presence of hyperandrogenism and ovarian dysfunction, the latter manifested as either oligo-anovulation or polycystic ovaries (PCO) (5). In this thesis, PCOS is defined by the Rotterdam criteria from 2003, as described above.

**Oligo-anovulation**
Irregular menstrual cycles reflect ovulatory dysfunction. A normal cycle length is 21–35 days. An irregular menstrual cycle is defined as a cycle longer than 90 days more than a year post menarche and 3 years after menarche as cycles shorter than 21 days or longer than 35 days or less than 8 cycles per year. In adolescents, irregular cycles are normal in the first year post menarche, due to the pubertal transition, and the cycles can be longer, up to 45 days, in the first 3 years after menarche (1).

**Hyperandrogenism**
Hyperandrogenism, as a criterion for diagnosing PCOS, can either be identified clinically or biochemically. The most important clinical symptom is hirsutism, expressed as increased male-pattern hair growth on the face and body and is defined as a modified Ferriman-Gallway score of $\geq 4$–$6$. Other symptoms are acne and androgenic alopecia (female-pattern hair loss) (6). Biochemical hyperandrogenism is defined as an elevated free androgen index (FAI). FAI is calculated as the ratio between total testosterone divided by sex hormone binding globulin (SHBG) x 100. FAI $> 5$ is considered elevated and indicative of hyperandrogenism if the reference interval for total testosterone is 0.5 to 3 nmol/L and 35 to 150 nmol/L for SHBG (7).

**Polycystic ovaries**
Ovarian morphology is assessed by transvaginal ultrasound. During the period in which the research presented and discussed in this thesis was performed, the definition of polycystic ovarian morphology (PCOM) was that at least one of the ovaries had either 12 or more follicles measuring 2–9 mm in diameter or increased ovarian volume (>10 ml) (4). Nowadays, improved ultrasound technology using a frequency bandwidth including 8 MHz, the threshold is 20 or more follicles and/or an ovarian volume $\geq 10$ mL, ensuring no corpora lutea, cysts or dominant follicles being present (1, 8). Again, there is controversy as to which PCOM criteria to adhere to. The AE-PCOS has suggested the minimal number of follicles should be 25 (3).
Phenotypes

Discrepancies in diagnostic criteria used globally have raised issues regarding comparability between studies. This has led to the establishment of PCOS phenotypes. In 2012, the NIH consensus panel recommended four different phenotypes classified A–D based on the Rotterdam criteria (Table 1) (5).

Table 1. PCOS phenotypes.

<table>
<thead>
<tr>
<th>PCOS Phenotype groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
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<tbody>
<tr>
<td>Hyperandrogenism</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ovulatory dysfunction</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Polycystic ovarian morphology</td>
<td>+</td>
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</table>

+ = present

The phenotypes with hyperandrogenism are considered more severe and seem to have higher metabolic risk (9-11). Among these, phenotype A and B are the classic phenotypes, entailing the most severe metabolic disturbances. These phenotypes are associated with insulin resistance, risk for metabolic syndrome and obesity. Phenotype C is the ovulatory phenotype, and phenotype D is normoandrogenic with a metabolic profile similar to non-PCOS women (5, 12, 13). In an unselected PCOS population, approximately 40–45% have either phenotype A or B, 35% have phenotype C and 20% have phenotype D (11). This phenotyping is currently not reflected in the International Classification of Diseases, version 10 (ICD-10). Ethnic variation in prevalence and severity of clinical presentations, such as obesity and metabolic disturbances, results in ethnic differences within each phenotype (14).

Clinical features

The clinical features of PCOS can vary between women with PCOS. Apart from the diagnostic criteria irregular cycles and clinical hyperandrogenism, the typical clinical features are reproductive, metabolic and psychological.

PCOS is a leading cause of infertility in women due to a hormonal imbalance that disrupts the ovulation process, leading to greater frequency of anovulatory cycles or anovulation. PCOS accounts for approximately 80–95% of infertility caused by anovulation and can often be treated with ovulation induction (1, 15, 16). Women with PCOS have a good prognosis for live birth, and pregnancy can be achieved naturally or with assistance such as lifestyle changes, medical treatment or assisted reproductive technology (ART) (1). Longitudinal studies suggest a high overall fecundity (17, 18).

Insulin resistance is the major factor in the increased metabolic risk for women with PCOS, even when the body mass index (BMI) is normal (1, 10).
Obesity is more prevalent in women with PCOS; approximately half of the PCOS population are obese compared to 20% among women in the general population in the Nordic countries (6).

The psychological impacts of PCOS are depression, anxiety, negative body image and decreased quality of life. Women with PCOS also have an increased risk of eating disorders (1, 3, 12).

Aetiology

Figure 1 represents the complex, multifactorial pathogenesis of PCOS, involving endocrine, metabolic, genetic and epigenetic factors and maternal-fetal environmental factors (3, 6, 19, 20). Familial clustering of PCOS is well documented in twin studies, and daughters of women with PCOS have a fivefold increased risk of developing PCOS. The exact mechanism of inheritance for PCOS remains unclear. More than 20 PCOS-associated genes have been identified, but these account for only 10% of the estimated 70% heritability (11, 21). The main candidates are involved in the synthesis, transport, regulation and effects of androgens and gonadotropins, while others are involved in metabolic dysfunction (12, 19, 20). An association has also been found between proinflammatory genotypes and PCOS (19). Epigenetic theories suggest that PCOS is caused by a reprogramming of genes regulating reproduction and metabolism due to hyperandrogenism in utero (19).
Figure 1. Pathophysiology of PCOS. The gonadotropin-releasing hormone (GnRH) pulse generator in women with PCOS is resistant to the negative feedback effects of ovarian steroids, which are probably mediated by androgen excess. The resulting high GnRH pulse frequency results in hypersecretion of luteinising hormone (LH), which stimulates theca cell hyperandrogenism and is thus a key factor in the hyperandrogenaemia of women with PCOS. Women with PCOS also have hyperinsulinaemia independent of obesity, which further stimulates theca cells to produce testosterone, exacerbates LH hypersecretion and lowers the production of sex hormone-binding globulin (SHBG) in the liver, thereby further increasing hyperandrogenaemia. The diminished secretion of follicle-stimulating hormone (FSH) inhibits the expansion of follicular size and maturation. Therefore, women with PCOS also have excessive production of anti-Müllerian hormone (AMH) as a result of the large number of preantral and small antral follicles. AMH inhibits expression of CYP19A1, the gene encoding aromatase, thereby preventing the conversion of androgens to oestrogens and contributing to elevated androgen levels. Elevated AMH levels also increase the activity of GnRH neurons and directly stimulate the GnRH-dependent secretion of LH, which probably further stimulates ovarian hyperandrogenism. Women with PCOS have an increased risk of obesity, which not only worsens all symptoms of this syndrome but also causes PCOS. ↓, decreased; ↑, increased; ↔, unchanged. Reprinted from Stener-Victorin, E., Deng, Q. Epigenetic inheritance of polycystic ovary syndrome — challenges and opportunities for treatment. Nature Reviews Endocrinology. 2021; 17, 521–533, with permission from Springer Nature.

Long-term health
The impact of PCOS on health differs across the lifespan. Associated morbidities are obesity, insulin resistance and hyperinsulinemia, as well as long-term increased risk of type 2 diabetes mellitus, dyslipidaemia, hypertension and cardiovascular disease (1, 3, 12, 19, 22, 23). Women with PCOS have a 3–7 times higher risk of developing type 2 diabetes, and the diagnosis is made at
a younger age than women with non-PCOS. (4, 24). Also, hyperandrogenic women with PCOS have higher risk of cardiovascular disease than normoandrogenic women and potentially increased cardiovascular mortality as well (1, 23, 25). Screening for cardiovascular disease should be initiated at the time of diagnosis. Furthermore, adverse pregnancy outcomes in women with PCOS have been associated with an increased risk of cardiovascular disease later in life (25). Women of reproductive age with PCOS have increased lipid levels and blood pressure regardless of BMI (23). Factors that affect the risk for these long-term health outcomes include obesity, lack of physical exercise and a family history of diabetes or cardiovascular disease. Most women with PCOS, or at least those with either obesity or hyperandrogenism, have some components of the metabolic syndrome (12). The overall prevalence of insulin resistance among women with PCOS is between 50% and 75%, greater in obese than in lean women (3). Obesity, hyperandrogenism and insulin resistance are associated with obstructive sleep apnoea, and women with PCOS may also experience disordered sleep (12). Women with PCOS have increased risk of endometrial hyperplasia and endometrial cancer compared with women without PCOS due to chronic anovulation, obesity and hyperinsulinaemia (1, 3, 26, 27). Studies on the association between PCOS and the risk of other cancers are inconsistent (26, 27), but a recent review suggests an increased risk of both ovarian and breast cancer (28). Women with PCOS have twice as many hospital admissions compared to the general population (5).

Treatment
There is no cure for PCOS. The treatment includes lifestyle changes with weight loss and exercise as well as pharmacological treatment for improvement of individual symptoms or clinical problems. First-line therapy for both irregular menstruations and hirsutism is combined oral contraceptives. Metformin, an insulin sensitising agent, has been used in the treatment of women with PCOS for many years and has several beneficial effects, such as reducing circulating androgen levels by inhibiting ovarian androgen production, improving ovulatory function and reducing weight. In PCOS women with high metabolic risk, metformin in combination with lifestyle changes is recommended. The glucagon-like peptide-1 receptor agonist can be considered as a treatment for obesity in women with PCOS in addition to an active lifestyle. Some women need additional antiandrogen treatment because of clinical hyperandrogenism. First-line pharmacological treatment of anovulatory infertility is ovulation induction by the aromatase inhibitor letrozole, second-line is gonadotropin stimulation and then lastly in vitro fertilisation (IVF) (1, 29).
PCOS and hormones

Anti-Müllerian hormone

AMH is a member of the transforming growth factor-β (TGF-β) superfamily. During fetal development, AMH is important for male sex differentiation. In fetal males, it is produced by the Sertoli cells of the testis and signals the regression of the Müllerian ducts. In the absence of AMH, the Müllerian ducts differentiate into the upper vagina, uterus and oviduct. In females, AMH is produced by the granulosa cells of preantral and small antral follicles in the ovaries (30, 31). Levels are low during prepubertal development, increase during early puberty and reach a plateau around 20–25 years of age, thereafter gradually decreasing until they become undetectable around menopause (31). AMH is involved in the regulation of follicle growth initiation and inhibits the recruitment of primordial follicles from the resting oocyte pool and may suppress FSH action, thereby contributing to ovulatory disturbances (32, 33).

AMH The serum level of AMH is strongly correlated with antral follicle counts and is an endocrine marker for ovarian reserve. Measurement of AMH as ovarian reserve is relevant in different clinical situations, such as diagnosing ovarian dysfunction, predicting age at menopause and for individualizing infertility treatment (30-33).

Women with PCOS have two- to threefold higher levels of AMH compared with women without PCOS (31, 33). It has been discussed whether AMH could be used as a diagnostic marker for PCOS, or at least a substitute for the transvaginal ultrasound examination, as there is a strong correlation between AMH and antral follicle count (34). However, the current literature is limited, and a universally agreed cut-off has not been established. Cut-offs also need to be age-adjusted because AMH decreases with age (12, 35-38). According to the newly updated PCOS guidelines, AMH should not be used as a single diagnostic test; however, it could be used to define PCOM in adults as an alternative to pelvic ultrasound (1). Also, AMH assays lack an international standard and values are method-dependent (32, 33). Furthermore, AMH is not increased only in women with PCOS, but is also increased in women with functional hypogonadotropic hypogonadism (39).

During pregnancy AMH levels decrease by approximately 50% but normalise within a few days postpartum (40-42). Maternal adiposity is negatively associated with AMH during pregnancy (41, 43, 44).

High levels of AMH during pregnancy in a mouse model of PCOS resulted in PCOS-like phenotypic traits in offspring, suggesting a possible causal role in development of the disorder through in-utero exposure. Also, prenatal AMH exposure leads to increased maternal and offspring testosterone levels (44).

The role of AMH in the development of obstetric and perinatal complications has, thus far, been assessed in a relatively limited number of studies, and
during the planning phase of the research presented in this thesis, current knowledge regarding AMH, PCOS and birth complications was limited and inconclusive. Studies reported either that pregnant women with gestational hypertension and preeclampsia had lower levels of AMH than controls (45, 46), or that AMH levels had no effect on these disorders (47). Meanwhile, other studies report an association between elevated AMH levels and preterm birth in women with PCOS (48). These inconclusive findings and scarcity of studies that focus on pregnancy complications in women with PCOS warranted further studies on this topic.

Testosterone

Testosterone is a steroid sex hormone, and one of the androgens present in the human body. In non-pregnant females, 50% of testosterone production arises from peripheral conversion of androstenedione and a small amount from the conversion of dehydroepiandrosterone, 25% is secreted by the adrenal gland, and 25% by the ovary. While circulating in the blood, testosterone is mainly bound to SHBG, 30% is bound to albumin, a very small percentage to corticosteroid-binding globulin, and only about 1% circulates free (3). Testosterone is converted by P450 aromatase to oestradiol (49).

During pregnancy, the placenta becomes the predominant source of maternal steroids, and testosterone levels increase. However, FAI decreases during pregnancy because of about a sixfold increase in SHBG concentration due to increased synthesis in the liver caused by high oestrogen levels in pregnant women (50). Women with PCOS have higher levels of testosterone than women without PCOS, both pregnant and non-pregnant (49, 51, 52).

The specific role of testosterone in the development of obstetric and neonatal complications has been assessed in a relatively limited number of studies, but rarely in PCOS populations. Prior findings consistently suggest that high testosterone and high placental expression of androgen receptors are features of manifest pre-eclampsia (53-58), potentially due to a dysregulation of placental aromatase levels (58). However, it is unknown whether high testosterone causes pre-eclampsia or if it is a result of manifest pre-eclampsia, as the predictive potential of testosterone levels in early pregnancy in relation to later development of pre-eclampsia has not been ascertained (59, 60). In utero exposure to high levels of testosterone has also been suggested to be involved in the pathogenesis of PCOS (61).

Findings are less conclusive regarding the role of hyperandrogenism in the obstetric complications that women with PCOS may encounter (62). Some studies suggest that a combination of hyperandrogenism and insulin resistance and/or hyperinsulinaemia may result in an increased risk for an adverse obstetric outcome (63, 64). Palomba et al. assert that their study in 2010 was the first study with the aim of identifying risks among different PCOS phenotypes, and concluded that the non-hyperandrogenic and ovulatory phenotypes had a
lower risk for adverse obstetric outcomes (65). Studies from Denmark have yielded contradicting results; a study from Odense did not find difference in pregnancy outcome between the PCOS phenotypes (66), another study from Copenhagen found that hyperandrogenic women have increased risk for preeclampsia and preterm birth (67). Many experts argue for further well-powered studies based on phenotypes (65, 68). In addition, most previous studies in this field have used immunoassays, which are no longer considered to be the gold standard in steroid hormone analysis (69, 70).

### PCOS and pregnancy complications

Once pregnant, women with PCOS face a higher risk of adverse pregnancy outcomes than controls (1, 68, 71, 72), independent of diagnostic criteria (73) and even after adjustments for confounding factors (74). Pregnant women with PCOS have increased risk of higher gestational weight gain, miscarriage, gestational diabetes mellitus (GDM), gestational hypertension, pre-eclampsia, preterm birth and caesarean section (1, 62, 68, 71, 72, 75). The risk increase is independent of obesity, even though obesity on its own is a major risk factor for these complications (74). Despite the known increased risk, which has been researched to a certain degree, there is a lack of PCOS awareness during antenatal care and pre-pregnancy management. International PCOS guidelines emphasise that PCOS status should be identified and recommendations in the guidelines need to be implemented to give this group of women appropriate monitoring and support during pregnancy (1).

There are many factors that contribute to these complications, such as metabolic disturbances, insulin resistance, chronic inflammation, hyperandrogenism and factors related to infertility (62). All of these factors may affect trophoblast invasion and placentation and cause fetal-maternal complications (12). Even though PCOS is a heterogeneous condition, the majority of previous studies have not taken the variety of PCOS phenotypes into account. The results of studies that did stratify for phenotypes or PCOS features have been inconsistent; some have concluded that there is an increased risk for hyperandrogenic phenotypes (65, 76), while others have not found any difference at all (66). Palomba et al. reported that ovarian dysfunction and biochemical hyperandrogenism increased pregnancy complications significantly; conversely no significant effect was found for clinical hyperandrogenism and PCOM (65). De Wilde et al. reported that hyperandrogenic phenotype had higher risk than normoandrogenic women with PCOS (76), whereas Mumm et al. did not find a difference in outcomes between the PCOS phenotypes (66). Most of these studies had small study populations and therefore relatively few participants in each PCOS phenotype which could explain the divergent results. The PCOS guidelines from 2018 emphasise the importance of explicitly reporting specific phenotypes in PCOS research (8).
Preeclampsia

Preeclampsia is a serious hypertensive disorder during pregnancy and a leading cause of maternal and perinatal mortality and morbidity (77). Preeclampsia is a severe pregnancy complication with a global incidence of 3–5% (78).

In this thesis, we use the British National Institute for Health and Clinical Excellence (NICE) definition of preeclampsia from 2000, which defines preeclampsia as new onset of hypertension (systolic blood pressure $\geq 140$ mmHg and/or diastolic blood pressure $\geq 90$ mmHg) and proteinuria (≥ 300 mg/24) after 20 weeks of pregnancy (79). Preeclampsia of early onset and preeclampsia in women with a small for gestational age (SGA) infant are regarded as subtypes of preeclampsia that have a more evident placental component than late-onset preeclampsia and preeclampsia in women without an SGA infant (80, 81).

The aetiology and pathophysiology involve several factors and are not perfectly understood. Hypotheses include defective placentation and maternal factors, and different clinical presentations can be explained by different pathological mechanisms (77, 81). Nulliparous women and women with previous preeclampsia have increased risk for preeclampsia compared to women with previous uncomplicated pregnancies (81).

Women with PCOS have a three to four times higher risk for preeclampsia (12, 68, 71, 72), even after adjustments for BMI and ART (82). Most studies in this field have not been not stratified by phenotypes of PCOS or specific subgroups of preeclampsia. A Danish study examined the role of hyperandrogenaemia in the risk of adverse pregnancy outcome, and concluded that only hyperandrogenic women with PCOS had increased risk for preeclampsia (67).

Gestational diabetes mellitus

GDM is defined as impaired glucose tolerance first diagnosed during pregnancy (83). The prevalence of GDM is increasing due to increased obesity and is estimated globally to 14%, varying between 1% and 28% depending on ethnicity, screening methods and diagnostic criteria (84, 85). GDM is associated with adverse pregnancy outcomes for both the mother and the infant, such as preeclampsia, preterm birth, shoulder dystocia, macrosomia, large for gestational age (LGA) infant and neonatal hypoglycaemia (83, 84, 86).

The pathophysiology of GDM is usually the result of pancreatic $\beta$-cell dysfunction on a background of insulin resistance during pregnancy. The progressive increase in insulin resistance during pregnancy is predominantly due to increased placental hormones. Insulin resistance results in higher maternal postprandial glucose levels and free fatty acids, which leads to greater availability of glucose for fetal growth (83).
GDM is the most frequent pregnancy complication in women with PCOS, with a two- to threefold risk compared to non-PCOS women, independent of BMI (1, 12, 87, 88).

Results from Nordic studies on women diagnosed with both PCOS and GDM have been discrepant. A recent population-based study from Finland suggested a higher risk of preterm birth, both spontaneous and overall (89). In contrast, a randomised trial from Norway found no increased risk for pregnancy complications other than late miscarriage for women with both PCOS and GDM (90). Further studies are warranted on the combination of these common conditions.

Neonatal outcome

Infants born to mothers with PCOS have increased risk for Apgar score <7 at 5 minutes, meconium aspiration, admission to a neonatal intensive care unit and higher perinatal mortality (62, 71, 72, 82). Results have been divergent regarding risk of intrauterine growth restriction and birthweight (68). A summary of the newly updated PCOS guidelines emphasises a higher risk in infants of PCOS mothers for intrauterine growth restriction, low birthweight and SGA, but no increased risk for macrosomia and an LGA infant (6).

Elevated maternal testosterone levels at gestational weeks 17 and 33 have been associated with lower birthweight of the infant in one study (91), while other studies did not find an association between testosterone levels and adverse neonatal outcome (92, 93). Altogether, there is limited knowledge about the relationship between high levels of AMH and testosterone and neonatal outcome.

The literature is limited regarding neonatal outcome in women with the combination of PCOS and GDM. Recent studies have focused on the birthweight, and, to our knowledge, the effects of this combination on rare severe outcomes have not been reported (1).
Aims

The overall aim of this work was to increase knowledge and understanding about PCOS and pregnancy complications to improve maternal care for women with PCOS, thereby reducing their risk for adverse pregnancy outcomes.

The specific aims of the individual studies were:

I  To evaluate whether AMH levels are associated with common PCOS complications in pregnancy, including preeclampsia, gestational hypertension, gestational diabetes mellitus, preterm birth and birthweight.

II To investigate whether women with PCOS have increased testosterone levels during pregnancy compared with non-PCOS women. Furthermore, to elicit the role of high testosterone levels in women with PCOS with respect to the development of gestational diabetes mellitus, gestational hypertension and pre-eclampsia and to determine whether high testosterone levels in women with PCOS are associated with preterm birth and birthweight abnormalities.

III To explore whether the association between PCOS and preeclampsia depends on treated clinical hyperandrogenism and whether PCOS is associated with different subtypes of preeclampsia.

IV To explore the association and risk estimates for maternal and neonatal outcomes in women with both PCOS and GDM.
Material and methods

Overview of the studies

This thesis consists of the four studies described in Table 2.

Table 2. Overview of the studies.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Study design</th>
<th>Study population</th>
<th>Exposure</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>I</td>
<td>Matched cohort study</td>
<td>479 pregnant women in Uppsala County 2007–2015</td>
<td>AMH and PCOS</td>
<td>Testosterone and SHBG levels, pregnancy complications and birthweight</td>
</tr>
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<td>II</td>
<td>Matched cohort study</td>
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<td>Testosterone and PCOS</td>
<td>Obstetric complications, maternal metabolic factors and birthweight</td>
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<td>IV</td>
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<td>281,806 pregnancies in Sweden 1997–2015</td>
<td>PCOS and GDM</td>
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</table>

AMH, anti-Müllerian hormone; PCOS, polycystic ovary syndrome; SHBG, sex hormone binding globulin; GDM, gestational diabetes mellitus.

Ethics

Papers I and II

The Biobank and the specific substudies were approved by the Regional Ethical Review Board in Uppsala, diary number 2007/181 date of approval 07 August 2007 and diary number 2017/029 date of approval 22 March 2017. Written informed consent was obtained from all women upon acceptance to participate in the Biobank.
Papers III and IV
Approved by the Regional Ethical Review Board in Uppsala, diary number 2017/309 date of approval 9 August 2017. The need for oral or written informed consent was waived since all data received from the Swedish registries were anonymised.

Data sources

Uppsala Biobank of Pregnant Women
The Uppsala Biobank of Pregnant Women collected blood samples from pregnant women from 31 May 2007 until 1 October 2022. All Swedish-speaking pregnant women, aged 18 years and older, without blood-borne diseases, attending the second trimester routine ultrasound scan were invited to participate in the Biobank. The Biobank is considered population-based because 97% of the pregnant population participates in the routine ultrasound examination, and as all routine ultrasound examinations in Uppsala County are performed at Uppsala University Hospital. However, participation in the Biobank was dependent upon a research nurse being available. It is estimated that the Biobank covers approximately half of the pregnant population of Uppsala County (94). Upon inclusion, brief demographic data are collected, including previous and ongoing chronic disorders, ongoing medication and smoking in early pregnancy. Uppsala University Hospital is the only available delivery ward within the county, leading to excellent follow-up of the participants. The blood samples were collected in EDTA-containing tubes and centrifuged at 1500 g for 10 minutes. Plasma and buffy coat were separated within 2 hours and stored at \(-70^\circ C\). Information on obstetric outcomes was extracted from medical records according to diagnoses at discharge from the hospital after birth.

Swedish National Registers
The Swedish National Board of Health and Welfare provided data from the National Patient Register, the Swedish Medical Birth Register (MBR) and the Swedish Prescribed Drug Register. Statistics Sweden provided data from the Education Register and the Total Population Register. All individuals in Sweden are assigned an individual unique personal registration number, which enables linkage between registers (95).

The National Patient Register includes nationwide information on hospital admissions and discharge diagnoses since 1964, with complete coverage from 1987. Since 1997, diagnoses are classified according to ICD-10. From 2001, the register also includes specialised out-patient visits such as those to gynaecologists and fertility clinics (96).
The MBR was established in 1973 and contains information on 98% of all births in Sweden. The MBR includes prospectively recorded demographic and clinical data, information on health, medication use and reproductive history, smoking habits, cohabitation status and self-reported height and measured weight, which allows calculation of BMI. At discharge from hospital after birth, information regarding delivery and neonatal data is registered. The overall quality of the register is very high (97).

The Swedish Prescribed Drug Register contains information since 2005 on Anatomic Therapeutic Chemical (ATC) classification codes for prescribed drugs, doses and filled prescription of drugs (98). The Education Register contains information on education level in years and educational orientation of the Swedish population from 1985 with annual updates. The Total Population Register provides information from 1968 about the country of birth and the municipality of residence (99).

Study populations and study designs

Papers I and II

Retrospective matched cohort study including participants from the population-based Uppsala Biobank of Pregnant Women. Figure 1 presents an overview of the study population, exposures and outcomes. All women diagnosed with an ICD-10 diagnosis of PCOS (E282), between 2003 and 2015, were identified in electronic medical records at Uppsala University Hospital. During these years, PCOS was diagnosed according to the Rotterdam criteria (4). All women with PCOS had normal prolactin levels and thyroid function tests, as this is a prerequisite for PCOS diagnosis. The medical records of all women with PCOS were scrutinised to ensure a correct diagnosis was established and to obtain information on obstetric and perinatal outcomes.

By September 2015, 174 pregnant women with PCOS had participated in the Biobank. Fifteen women with PCOS were excluded: four women due to twin pregnancies, one due to late miscarriage, one due to stillbirth, seven due to deliveries outside Uppsala County, and two women misdiagnosed as having PCOS, leaving 159 women with PCOS available for hormonal analyses. The majority of women ($n = 99$) had received the PCOS diagnosis as part of an infertility work-up, whereas the remaining women had consulted a physician for PCOS symptoms, such as menstrual disturbances, hirsutism and bleeding problems. Unfortunately, the diagnostic evaluation for infertility at the time did not include analyses of testosterone or androstenedione serum concentrations.
For each pregnant woman with PCOS, two BMI-matched non-PCOS controls with singleton pregnancies were selected. Each non-PCOS control had donated a blood sample to the Biobank during the same week as the respective PCOS woman and was otherwise healthy according to the self-report collected in conjunction with the blood sample. The medical records of the women in the control group who became pregnant following ART were reviewed to ensure that none of them had been previously been diagnosed with PCOS or had an anovulation-related infertility factor. The two controls selected for the woman with PCOS who was excluded due to stillbirth were kept in the study population. The total number of non-PCOS women was 320 (Figure 2).

Figure 2. Study population, exposure and outcome in Papers I and II. PCOS, polycystic ovary syndrome; AMH, anti-Müllerian hormone; SHBG, sex hormone binding globulin; FAI, free androgen index.

In Paper I, based on measurements from the initial pre-pregnancy diagnostic work-up, each woman with PCOS was further classified as having hyperandrogenic PCOS (with signs of biochemical and/or clinical hyperandrogenism) or normoandrogenic PCOS. The women were also categorised as having phenotypes A–D as previously described. The majority of the women with PCOS were categorised as having normoandrogenic phenotype D (59.1%). The next largest groups had phenotype A (39.6%), only two women had phenotype C (1.3%), and none had phenotype B.

In Paper II, the women with PCOS were grouped into testosterone tertiles, based on testosterone analyses obtained during the early second trimester,
with the following cut-offs < 1.44 (low), 1.44–2.36 (medium), > 2.36 (high) nmol/L.

Papers III and IV
Retrospective nationwide register-based cohort studies. The study population consisted of primiparous women born between 1950 and 1999, having a singleton birth, at 22 completed gestational weeks or later, in the years 1997 to 2015. For each woman with PCOS, five non-PCOS women, matched for age and geographic area at the time of PCOS diagnosis, were randomly selected as controls.

The PCOS group was defined as women diagnosed with the following ICD-10 codes: PCOS (E282), androgen excess (E281) and anovulatory infertility (N970). The diagnosis of anovulatory infertility was included because PCOS accounts for the majority of infertility cases due to anovulation. As PCOS affects women throughout their entire life span, women were included as exposed regardless of whether the diagnosis was made before, during or after the pregnancy.

Exclusions from the exposed group were women with both anovulatory infertility and either hyperprolactinaemia or primary ovarian insufficiency as well as women with congenital adrenal hyperplasia. Non-PCOS controls who became pregnant after ovulation stimulation were excluded to minimise the risk of misclassification.

A flowchart of the study population in Paper III is presented in Figure 3. Women with PCOS were grouped according to treatment for clinical hyper-androgenism.
Figure 3. Flowchart of study population in Paper III. PCOS, polycystic ovary syndrome; GDM, gestational diabetes mellitus.
In Paper IV, women with pregestational diabetes mellitus were also excluded. Figure 4 presents the study population in Paper IV. The women with PCOS and GDM were divided into the following four groups: PCOS-only, PCOS and GDM, GDM-only and controls without PCOS or GDM.

![Flowchart of study population in Paper IV](image)

**PCOS**
Women born in 1950–1999 having a singleton birth in 1997–2015 according to the Medical Birth Register

**Non-PCOS**
Controls matched by age and geographic area to PCOS women 1:5 by linkage to the Swedish Total population Register and having a singleton birth in 1997–2015 according to the Medical Birth Register

Figure 4. Flowchart of study population in Paper IV. PCOS, polycystic ovary syndrome; GDM, gestational diabetes mellitus.

## Exposures

### Paper I

The main exposure was AMH levels and PCOS diagnosis. AMH values were available for 476 of 479 women. Total testosterone, SHBG and FAI were also included in the analysis in Paper I.

Plasma AMH levels were measured using the fully automated DxI Access sandwich immunoassay (B13127, Beckman Coulter). This assay measures the AMH hormone precursor and the cleaved AMH$_{N,C}$ complex and uses recombinant human AMH as a calibrator. The limit of quantification of the assay is 0.57 pmol/L, with intra- and inter-assay imprecision less than 5%.

### Paper II

Testosterone levels were the main exposure, using both total testosterone levels and FAI. We had 472 testosterone values available for analysis ($n = 156$ in the PCOS group).

Testosterone was assayed with Supercritical Fluid Chromatography (Waters ACQUITY® UPC$^2$TM, Milford, MA, USA) coupled with tandem mass
spectrometry (XEVO® TQ-S, Milford, MA, USA). Mass spectrometer (MS) data were collected using two separate scan functions. All data collected in centroid mode were obtained using Masslynx NT4.1 software (Waters Corp., Milford, MA, USA). Duplicate analyses of each sample were carried out, and the average values were reported (CV <3%). The linearity of the method was evaluated over a range of concentrations (0.1–50 nmol/L) and correlation coefficients (R²) were 0.998. The limit of quantification was 0.2 nmol/L.

Immuno-assay kits for SHBG analyses in plasma were obtained from R&D Systems (Minneapolis, MN, USA) with the catalogue number DSHBG0B. The assay was of the sandwich-type using a pre-coated 96- well plate and a supply of enzyme-labelled secondary antibody as well as washing buffer, assay buffer, substrates and standard. The assays were performed according to the manufacturer’s instructions. The resulting absorbance was read in a Bio-Rad Model 680 Microplate Reader at 450 nm with 595 nm as background. The standards were plotted in Excel (Microsoft), and several variants of curve fitting were tested. Often a couple of high concentration samples were present, and the whole standard range, not just the linear part, had to be used. A polynomial (up to x²) fit was therefore applied to the standard curve as a default using the in-built trend fitting function in Excel. Goodness of fit was checked with R² values. Repeatability of the assay was checked, and the median repeatability standard deviation RSD for SHBG was 13.5%. The accuracy is presented by the manufacturer using an in-house preparation calibrated against NIBSC/WHO International Standard 08/266.

Paper III

Exposures were PCOS diagnosis and treatment for clinical hyperandrogenism. PCOS was defined as previously described according to the following ICD-10 codes: PCOS (E28), the related diagnoses androgen excess from the ovary (E28.1) and anovulatory infertility (N97.0).

Treatment for clinical hyperandrogenism was used as a proxy for the hyperandrogenic phenotype of PCOS. Using linkage to the Prescribed Drug Register, we identified women who had filled at least two prescriptions during the years 2005–2017 for anti-androgenic drugs identified with the following ATC codes: C03DA01 (spironolactone), D11AX10 (finasteride), D11AX16 (eflornithine), G03HB01 (combined oral contraceptives with ethinyl oestradiol and cyproterone acetate), G04CB (finasteride and dutasteride), L02BB (bicalutamide) and L02BB01 (flutamide). Anti-androgenic drug treatment was either before or after pregnancy.
Paper IV

PCOS and GDM were the exposures. PCOS was defined as described in Paper III. GDM was identified by the ICD diagnosis O24.4 given at discharge from the hospital after delivery.

During the study period, the decision to screen for GDM in most regions in Sweden was based on known risk factors and a random capillary blood glucose $\geq 8$ mmol/L or plasma glucose $\geq 9$ mmol/L. Diagnosis of GDM was based on the result of a 75 g oral glucose tolerance test (OGTT) among those screened. No national consensus regarding screening for and diagnosing GDM existed in Sweden during the study period, resulting in varying cut-off values between regions within the country. The main diagnostic criteria for GDM during the study period were fasting venous plasma $\geq 7.0$ mmol/L and a 2-hour cut-off value after OGTT $\geq 10.0$ mmol/L (100, 101).

Definition of outcomes

Papers I and II

Pregnancy complications of interest were identified by the ICD-10 codes preeclampsia (O14), gestational hypertension (O13) and GDM (O244). Preterm birth was defined as delivery prior to 37+0 gestational weeks. Spontaneous preterm birth included all pregnancies with preterm birth after spontaneous onset of labour and/or preterm rupture of the membranes. Birthweight was expressed as standardised birthweight scores, i.e. standard deviation in relation to gestational length and offspring sex (102). SGA and LGA were defined as having a birthweight of more than two standard deviations below or above, respectively, the mean birthweight for gestational age according to the reference curve (102).

Information on obstetric outcome and birthweight was derived from the standardised antenatal, obstetric and paediatric medical records.

Additionally, in Paper II, metabolic variables during pregnancy were blood pressure at first and last antenatal visit, non-fasting glucose levels and gestational weight gain.

Paper III

The main outcome was preeclampsia registered in the MBR with the ICD-10 codes preeclampsia (O14), eclampsia (O15) and preeclampsia superimposed on chronic hypertension (O11).

During the study period, preeclampsia was defined as new onset hypertension (systolic blood pressure $\geq 140$ mmHg and/or diastolic blood pressure $\geq 90$ mmHg measured on two subsequent occasions at least 4 hours apart) and proteinuria ($\geq 300$ mg/24 hour) after 20 weeks of gestation.
Preeclampsia was further categorised by subtype into early (delivery before 34 gestational weeks) or late (delivery at 34 gestational weeks or later) preeclampsia and preeclampsia with or without the birth of an SGA infant. SGA was defined as a birthweight two standard deviations (−2SD) or more below the mean weight for gestational age and sex according to the Swedish intrauterine growth reference range (102).

Paper IV

Maternal outcomes assessed were gestational hypertension, preeclampsia, postpartum haemorrhage and obstetric anal sphincter injury. Hypertensive disorders were defined as in Paper III. Postpartum haemorrhage was defined as bleeding exceeding 1000 mL. Obstetrical anal sphincter injury included third- and fourth-degree perineal lacerations.

Neonatal outcomes explored were preterm birth, shoulder dystocia, stillbirth, SGA, LGA, macrosomia, Apgar score <7 at 5 minutes, respiratory distress, meconium aspiration syndrome, neonatal hypoglycaemia, birth trauma and cerebral impact of the infant. Preterm birth was defined as the birth of an infant before 37 completed gestational weeks. Spontaneous preterm birth was defined as a preterm birth with a spontaneous onset of labour. Stillbirth was defined as fetal death before or during labour (before 28 completed weeks prior to July 2008 or 22 weeks onwards). SGA and LGA were defined as infants as a birthweight above or below two standard deviations (+/−2SD) from the mean weight for gestational age and sex according to the Swedish intrauterine growth reference range (102). Macrosomia was defined as birthweight ≥4500 g. Apgar score is registered at 1, 5 and 10 minutes after birth, and we chose to report Apgar score <7 at 5 minutes. Neonatal hypoglycaemia was defined as blood glucose <2.6 mmol/L at least 3 hours after birth and identified by ICD-10 codes. Infant birth trauma was defined as injury to the skeleton, peripheral and central nervous systems and retinal haemorrhage. Cerebral impact of the infant included any of the following diagnoses: intracranial lacerations and haemorrhage, intrauterine hypoxia, convulsions, other disturbances of cerebral status and hypoxic ischemic encephalopathy.

Statistical methods

All statistical analyses were performed using IBM SPSS statistics version 24.0, 26.0 and 28.0, with level of significance set at $p < 0.05$.

Paper I

Clinical characteristics and obstetric outcomes were compared between PCOS and non-PCOS controls using an independent t-test or chi-squared test.
Comparisons of hormone levels between controls and normo- and hyper-androgenic women with PCOS were performed using one-way analysis of variance with the post-hoc Tukey HSD test or the Kruskal-Wallis test followed by the Mann-Whitney U-test, depending on whether the distribution was normal or abnormal.

Correlations between AMH and testosterone, FAI, age and BMI were performed by using Spearman rank correlations, because of skewed distribution.

In the primary analyses, all women were studied, but in a second step, analyses were stratified by PCOS diagnosis. These analyses were adjusted for age, parity, BMI, smoking, maternal country of birth, gestational length at blood sampling and year of delivery.

Multivariable regression models were used to analyse the associations between AMH levels, obstetric outcomes and birthweight. Adjustments were made for age, parity, BMI, smoking, maternal country of birth, gestational length at blood sampling and year of delivery, and in the birthweight analysis, maternal height was also included. The co-variables were chosen based on findings in bivariate and correlational analyses, but also on information from the literature.

Paper II

Comparison of hormone levels between the groups was performed with the Mann-Whitney U-test. Obstetric and neonatal outcomes were compared between groups using logistic regression, after adjusting for age, parity, maternal country of birth and smoking.

To evaluate the effect of high testosterone on birthweight, we used multivariable linear regression. In this analysis, women with pre-pregnancy diabetes \( (n = 4) \), GDM \( (n = 6) \) and preeclampsia \( (n = 17) \) were excluded. The analysis was adjusted for age, maternal BMI, maternal height, parity, maternal country of birth and smoking.

Co-variables were chosen based on differences between women with PCOS and controls, findings in bivariate and correlational analyses, and also on information from the literature (62, 103-106). We did not adjust for ART because we considered it a mediator, not a confounder (Figure 5).

Paper III

Conditional logistic regression analysis was used to estimate the association between maternal PCOS and preeclampsia. Crude and adjusted odds ratios (OR and aOR) with 95% confidence intervals (CIs) were calculated, and non-PCOS controls were used as the reference. Since associations are of interest both for increased pathophysiological knowledge and potential improved prediction of preeclampsia, we performed adjustments in two separate models, a
confounding-adjusted model for causal analysis and a predictor-adjusted model.

For the causal approach, a directed acyclic graph (DAG) was constructed to obtain a systemic representation of a possible causal relationship between PCOS exposure and preeclampsia (107). Figure 5 presents the DAG we constructed to identify true confounders: Maternal age, educational level, country of birth and year of delivery.

![DAG Diagram](image)

Figure 5. Directed Acyclic Graph (DAG). PCOS, polycystic ovary syndrome; BMI, body mass index; ART, assisted reproductive technology.

In the prediction approach, we added all high and moderate risk factors for preeclampsia according to the NICE preeclampsia guidelines (108), except a family history of preeclampsia, which was not available. Predictors were maternal age, educational level, country of birth, year of delivery, type 1 or 2 diabetes mellitus, chronic hypertension, chronic kidney disease, systemic lupus erythematosus and antiphospholipid syndrome. Covariates were included as continuous variables where possible.

In Model 1, we adjusted for confounders and predictors separately and added BMI in Model 2 to evaluate the impact of high BMI on the association between PCOS and preeclampsia. Due to non-random missing values for BMI and educational level in the first two models, Model 3 was enriched with multiple imputed values using the same adjustments as those in Model 2.

Women with PCOS were stratified into treated and non-treated clinical hyperandrogenism, and all analyses repeated. Finally, we calculated the association between PCOS and preeclampsia stratified by subtype: early and late preeclampsia and preeclampsia with and without an SGA infant.
Paper IV

Multiple logistic regression analysis was used to estimate the association between exposure and maternal and neonatal outcomes. Crude and aOR with 95% CIs were calculated using the generalised estimation equation method because observations were not independent in women giving birth more than once during the study period. Adjustments were calculated in two steps, first with the following confounders: maternal age, parity, educational level and country of birth (Model 1), and in a second step, BMI was added (Model 2). Covariates were included as continuous variables where possible. Pregnancies with missing data on the chosen covariates were excluded from the multivariable analyses. A sensitivity analysis was performed by excluding all pregnancies conceived through ART treatment to estimate whether the ART treatment affected the associations.
Results

Demographic data

Papers I–II

The same study population was used in Papers I and II, and demographic data regarding this population are presented in Table 3.

Table 3. Demographic information on study population, outcomes and hormone levels in Papers I and II.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Non-PCOS, n = 320</th>
<th>PCOS, n = 159</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.2±5.1</td>
<td>31.7±4.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.1±5.5</td>
<td>26.2±5.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Nordic</td>
<td>304 (95.0)</td>
<td>148 (93.1)</td>
<td>0.3</td>
</tr>
<tr>
<td>Primipara</td>
<td>141 (44.1)</td>
<td>90 (56.6)</td>
<td>0.010</td>
</tr>
<tr>
<td>Previous miscarriage*</td>
<td>48 (19.4)</td>
<td>38 (37.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ART</td>
<td>11 (3.4)</td>
<td>78 (49.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>19 (5.9)</td>
<td>5 (3.1)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Pregnancy outcome

| Gestational diabetes mellitus | 3 (0.9) | 4 (2.5) | 0.2 |
| Gestational hypertension     | 15 (4.7) | 7 (4.4) | 0.9 |
| Preeclampsia                 | 6 (1.9)  | 11 (6.9) | 0.005|
| Preterm birth                | 12 (3.8) | 7 (4.4)  | 0.7  |

Neonatal outcome

| Birthweight (g)             | 3596±543 | 3606±548 | 0.9 |
| Small for gestational age   | 5 (1.6)  | 0 (0)    | 0.1  |
| Large for gestational age   | 21 (6.6) | 7 (4.4)  | 0.3  |

Hormone levels

| AMH (pmol/L)                | 12.2 (7.4–19.7) | 20.4 (11.9–32.9) | <0.001|
| Testosterone (nmol/L)       | 1.41 (0.89–1.97) | 1.94 (1.21–2.64) | <0.001|
| SHBG (nmol/L)               | 782 (584–1012)  | 789 (604–975)    | 0.98 |
| Free androgen index         | 0.18 (0.11–0.28) | 0.25 (0.15–0.36) | <0.001|

Hormone levels expressed as median and interquartile range. Age, BMI and birthweight as mean ± standard deviation.

PCOS, polycystic ovary syndrome; ART, assisted reproductive technology; AMH, anti-Müllerian hormone; SHBG, sex hormone binding globulin.

*Information on previous miscarriage was available for 350 women.

Women with PCOS were older, more often primipara and had more often conceived through ART, compared with non-PCOS. BMI was similar between
the groups due to BMI matching. Individuals of Nordic origin were the majority in both groups. No differences in smoking habits were seen between the groups.

Women with PCOS more often had a history of miscarriage and developed preeclampsia more commonly than controls. No difference was observed between women with PCOS and non-PCOS controls for other known PCOS pregnancy complications, such as gestational GDM, gestational hypertension and preterm birth.

Birthweight was similar among the groups, and there was no difference in the number of LGA infants. No case of SGA was encountered in the PCOS group.

**Papers III–IV**

The study populations in Papers III and IV were from the same database.

In Paper III, only primiparas were included, and women with PCOS were grouped according to anti-androgenic treatment. Among women with PCOS, 10% had filled two prescriptions for anti-androgenic drugs and were classified as having PCOS with treated clinical hyperandrogenism. Women with PCOS were older and had a higher mean BMI than non-PCOS controls. Women with PCOS reported more often involuntary childlessness (≥1 year) and were more likely to conceive by ART treatment. Women with PCOS had a higher rate of both gestational and pre-gestational diabetes mellitus than non-PCOS controls.

In Paper IV, age and BMI were higher in the exposed groups than in the control group; women with both PCOS and GDM were oldest and had the highest mean BMI and the highest proportion of obesity (BMI ≥ 30 kg/m²). Women with PCOS (with or without GDM) were more often nulliparous, faced more often involuntary childlessness and used ART more frequently. Metformin use before or in the first trimester of pregnancy was most prevalent among women with both PCOS and GDM (8.5%), followed by women with PCOS only (2.9%), and was lower in the GDM-only and control groups.

Detailed demographic information is found in the respective papers.

**Paper I**

Women with PCOS had higher levels of total testosterone, AMH and FAI than did the non-PCOS controls during the early second trimester of pregnancy (Figure 6). No differences were observed in total testosterone, FAI and AMH levels between the normoandrogenic and hyperandrogenic women with PCOS or between the different PCOS phenotypes.
Figure 6. Second trimester hormone levels in women with PCOS and non-PCOS controls. Boxplots display median values, interquartile range and 5th and 95th percentiles. PCOS, polycystic ovary syndrome; AMH, anti-Müllerian hormone; SHBG, sex hormone binding globulin.

***p < 0.001

The relationship between AMH and testosterone, FAI, age and pre-pregnancy BMI in both women with and without PCOS is presented in Figure 7. In women with PCOS, AMH levels were positively correlated with total testosterone levels (Spearman’s rho = 0.17; p = 0.031) and FAI (Spearman’s rho = 0.16, p = 0.045), whereas no relation with age or pre-pregnancy BMI was found. In the control group, AMH was positively correlated with total testosterone levels (Spearman’s rho = 0.26; p < 0.001) and FAI (Spearman’s rho = 0.23; p < 0.001), whereas there was a negative correlation with age (Spearman’s rho = −0.25; p < 0.001) and no correlation with pre-pregnancy BMI.
Figure 7. Correlations between AMH and total testosterone, free androgen index, age and BMI in women with PCOS and controls. AMH, anti-Müllerian hormone; PCOS, polycystic ovary syndrome.

In the entire study population, higher AMH levels were not associated with increased risk of gestational hypertension, preeclampsia, gestational diabetes, preterm birth or birthweight (Table 4).
### Table 4. AMH levels and pregnancy outcomes in the study population in Paper I.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>AMH (pmol/L)</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>aOR* (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>15.1 (8.8–23.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12.8 (5.6–19.7)</td>
<td>0.57 (0.37–0.88)</td>
<td>0.01</td>
<td>0.55 (0.34–0.87)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Preeclampsia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14.8 (8.6–23.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17.3 (8.4–23.5)</td>
<td>0.94 (0.53–1.65)</td>
<td>0.8</td>
<td>1.01 (0.56–1.82)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Gestational diabetes mellitus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14.9 (8.7–23.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9.2 (7.5–19.1)</td>
<td>0.86 (0.36–2.06)</td>
<td>0.7</td>
<td>1.05 (0.36–3.09)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Preterm birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14.8 (8.6–23.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14.7 (11.1–22.6)</td>
<td>1.11 (0.62–1.98)</td>
<td>0.7</td>
<td>1.20 (0.68–2.12)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Small for gestational age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14.9 (8.6–23.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10.1 (7.4–13.4)</td>
<td>0.64 (0.25–1.64)</td>
<td>0.4</td>
<td>0.54 (0.18–1.64)</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Large for gestational age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14.8 (8.6–23.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15.2 (8.6–19.9)</td>
<td>1.00 (0.62–1.62)</td>
<td>0.99</td>
<td>1.16 (0.67–2.00)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

AMH levels displayed as median (interquartile range). Statistical analyses by multivariable logistic regression. AMH was modelled as a continuous variable. AMH, anti-Müllerian hormone; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval.

*Adjusted for age, parity, maternal country of birth, BMI, smoking, gestational length at blood sampling and year of delivery.

**Paper II**

Women with PCOS had higher early second trimester total testosterone and FAI than non-PCOS women (Table 3). SHBG levels were similar in women with and without PCOS (Figure 6).

No differences in age, BMI, gestational weight gain or blood pressure at first or last antenatal visits were demonstrated between the women with PCOS who had low, medium or high testosterone levels, data not shown.

Women with highest levels of testosterone within the PCOS group had an increased risk for preeclampsia, even after adjustments for age, parity, country of birth and smoking. No association was found between high testosterone levels in women with PCOS and gestational diabetes, gestational hypertension, preterm birth or LGA (Table 5).
Table 5. Risk for obstetric and neonatal complications in women with PCOS grouped according to testosterone tertiles.

<table>
<thead>
<tr>
<th>Testosterone tertile</th>
<th>n (%)</th>
<th>OR (95% CI)</th>
<th>aOR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-PCOS</td>
<td>3 (0.9)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>PCOS Low</td>
<td>1 (1.9)</td>
<td>2.05 (0.21–20.05)</td>
<td>1.42 (0.13–15.63)</td>
</tr>
<tr>
<td>Medium</td>
<td>1 (1.9)</td>
<td>2.05 (0.21–20.05)</td>
<td>2.83 (0.25–32.45)</td>
</tr>
<tr>
<td>High</td>
<td>2 (3.8)</td>
<td>4.17 (0.68–25.60)</td>
<td>6.44 (0.84–49.62)</td>
</tr>
<tr>
<td>Non-PCOS</td>
<td>15 (4.7)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS Low</td>
<td>(0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium</td>
<td>4 (7.7)</td>
<td>1.67 (0.53–5.25)</td>
<td>1.34 (0.40–4.42)</td>
</tr>
<tr>
<td>High</td>
<td>3 (5.8)</td>
<td>1.23 (0.34–4.40)</td>
<td>1.12 (0.31–4.13)</td>
</tr>
<tr>
<td>Non-PCOS</td>
<td>6 (1.9)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>PCOS Low</td>
<td>3 (5.8)</td>
<td>3.16 (0.77–13.06)</td>
<td>2.79 (0.66–11.80)</td>
</tr>
<tr>
<td>Medium</td>
<td>2 (3.8)</td>
<td>2.07 (0.41–10.53)</td>
<td>1.53 (0.29–8.12)</td>
</tr>
<tr>
<td>High</td>
<td>6 (11.5)</td>
<td>6.74 (2.09–21.78)</td>
<td>6.16 (1.82–20.91)</td>
</tr>
<tr>
<td>Non-PCOS</td>
<td>12 (3.8)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Preterm birth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS Low</td>
<td>1 (1.9)</td>
<td>0.50 (0.06–3.90)</td>
<td>0.38 (0.05–3.06)</td>
</tr>
<tr>
<td>Medium</td>
<td>1 (1.9)</td>
<td>0.50 (0.06–3.90)</td>
<td>0.33 (0.04–2.71)</td>
</tr>
<tr>
<td>High</td>
<td>5 (9.6)</td>
<td>2.70 (0.91–8.00)</td>
<td>2.18 (0.71–6.73)</td>
</tr>
<tr>
<td>Non-PCOS</td>
<td>21 (6.6)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Large for gestational age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS Low</td>
<td>2 (3.8)</td>
<td>0.56 (0.13–2.47)</td>
<td>0.53 (0.18–2.37)</td>
</tr>
<tr>
<td>Medium</td>
<td>2 (3.8)</td>
<td>0.56 (0.13–2.47)</td>
<td>0.71 (0.16–3.22)</td>
</tr>
<tr>
<td>High</td>
<td>3 (5.8)</td>
<td>0.86 (0.25–2.99)</td>
<td>0.91 (0.26–3.25)</td>
</tr>
</tbody>
</table>

Testosterone tertiles: low < 1.44 nmol/L, medium 1.44 – 2.36 nmol/L, high >2.36 nmol/L.
PCOS, polycystic ovary syndrome; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; LGA, large for gestational age.
*Adjusted for maternal age, parity, country of birth and smoking.

Paper III

Overall, 6 172 (4.5%) women in the study population had preeclampsia; 154 (2.5%) developed eclampsia and 100 (1.6%) had superimposed preeclampsia.

Table 6 illustrates rates and risks of preeclampsia by PCOS diagnosis and treated or non-treated clinical hyperandrogenism. Women with PCOS had higher rates of preeclampsia than non-PCOS controls, (5.7% and 4.2%, respectively, \( p < 0.001 \)). In the causal approach, women with PCOS had 33% higher risk for preeclampsia after adjustments for confounders. In the prediction model, women with PCOS had 29% higher risk of preeclampsia after adjustment for the predictors maternal age, education, country of birth, year of delivery, type 1 or 2 diabetes mellitus, chronic hypertension, chronic kidney disease, systemic lupus erythematosus and antiphospholipid syndrome (Model 1). When BMI was added to the adjustments, no association was found (Model 2). With imputation of missing values women with PCOS had 10% higher risk for preeclampsia in the causal model, but no association was found
in the prediction model (Model 3). Similar associations were found for women with and without treatment for clinical hyperandrogenism.

Table 7 illustrates rates and risks of early and late preeclampsia and preeclampsia in women with or without an SGA infant by PCOS status. In comparison with non-PCOS controls, women with PCOS had higher rates of all subtypes of preeclampsia (early/late, with/without an SGA infant). However, stronger associations were seen with early preeclampsia and preeclampsia in women with an SGA infant than with their counterparts. With the causal approach women with PCOS had 73% and 30% increased risks of early and late preeclampsia, respectively, in relation to non-PCOS controls. The prediction approach suggested 64% increased risk of early preeclampsia and 26% increased risk of late preeclampsia. No association was found when BMI was added to the adjustments except in the causal approach for early preeclampsia (Model 2). However, with imputed values for missing data (Model 3), women with PCOS had 49% increased risk of early preeclampsia compared to non-PCOS in the causal approach versus 44% in the prediction approach. The associated risks for preeclampsia in PCOS women with an SGA infant increased by 43% in the causal approach and 40% in the prediction approach compared to non-PCOS controls. Corresponding numbers for PCOS women without an SGA infant were 31% and 27%, respectively (Model 1).
Table 6. Rates and odds ratios for preeclampsia in a cohort of nulliparous women giving birth in Sweden 1997–2015, stratified by PCOS and treated clinical hyperandrogenism.

<table>
<thead>
<tr>
<th>PCOS status</th>
<th>N</th>
<th>n (%)</th>
<th>Crude (OR 95%CI)</th>
<th>n (%)</th>
<th>Model 1 (OR 95%CI)</th>
<th>n (%)</th>
<th>Model 2 (OR 95%CI)</th>
<th>n (%)</th>
<th>Model 3 (OR 95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-PCOS</td>
<td>115 272</td>
<td>4860  (4.2)</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS (all)</td>
<td>22 947</td>
<td>1312  (5.7)</td>
<td>1.36 (1.32–1.40)</td>
<td>1306  (5.7)</td>
<td>1.33 (1.23–1.43)</td>
<td></td>
<td>1184  (5.2)</td>
<td>1.08 (0.994–1.17)</td>
<td></td>
</tr>
<tr>
<td>Predictorsb</td>
<td>130 (5.6)</td>
<td>1.39 (1.09–1.78)</td>
<td></td>
<td>119 (5.2)</td>
<td>1.07 (0.82–1.40)</td>
<td></td>
<td>1.12 (0.88–1.44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatedc</td>
<td>2301</td>
<td>130   (5.6)</td>
<td>1.45 (1.31–1.59)</td>
<td>130   (5.6)</td>
<td>1.49 (1.17–1.90)</td>
<td></td>
<td>119   (5.2)</td>
<td>1.07 (0.79–1.36)</td>
<td></td>
</tr>
<tr>
<td>Predictorsb</td>
<td>1176 (5.7)</td>
<td>1.31 (1.21–1.41)</td>
<td></td>
<td>1065 (5.2)</td>
<td>1.08 (0.99–1.17)</td>
<td></td>
<td>1.09 (1.01–1.18)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCOS, polycystic ovary syndrome; OR, odds ratio; CI, confidence interval; BMI, body mass index.
Model 3: Same adjustments as in Model 2 with multiple imputed values for missing data.

aConfounder adjusted models. Confounders: maternal age, educational level, country of birth, year of delivery.
bPredictor adjusted models. Predictors: maternal age, educational level, country of birth, year of delivery, type 1 or 2 diabetes mellitus, chronic hypertension, chronic kidney disease, systemic lupus erythematosus and antiphospholipid syndrome.
cTreatment for clinical hyperandrogenism defined as two dispensations of prescribed anti-androgenic drugs during 2005–2017.
Table 7. Rates and odds ratios for early and late onset preeclampsia and preeclampsia with or without an infant born SGA in a cohort of nulliparous women giving birth in Sweden 1997–2015, by PCOS status.

<table>
<thead>
<tr>
<th>PCOS status</th>
<th>Early onset preeclampsia</th>
<th>Late onset preeclampsia</th>
<th>Preeclampsia in women with an SGA infant</th>
<th>Preeclampsia in women without an SGA infant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n (%)</td>
<td>Crude (OR 95%CI)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Non-PCOS</td>
<td>115 272</td>
<td>441 (0.4)</td>
<td>Reference</td>
<td>113 421</td>
</tr>
<tr>
<td>PCOS</td>
<td>22 947</td>
<td>169 (0.7)</td>
<td>1.84 (1.50–2.25)</td>
<td>1138 (5.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Confounders&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>168 (0.7)</td>
<td>1.73 (1.40–2.13)</td>
<td>Model 1</td>
<td>1046 (4.7)</td>
</tr>
<tr>
<td></td>
<td>138 (0.6)</td>
<td>1.29 (1.01–1.64)</td>
<td>Model 2</td>
<td>1025 (4.5)</td>
</tr>
<tr>
<td></td>
<td>138 (0.6)</td>
<td>1.24 (0.98–1.58)</td>
<td>Model 3</td>
<td>1025 (4.5)</td>
</tr>
</tbody>
</table>

SGA, small for gestational age; PCOS, polycystic ovary syndrome; OR, odds ratio; CI, confidence interval; BMI, body mass index.

Model 1: Adjusted for confounders/predictors. Model 2: BMI added to adjustments in Model 1. Model 3: Same adjustments as in Model 2 with multiple imputed values for missing data.

<sup>a</sup>Confounder adjusted models. Confounders: maternal age, educational level, country of birth, year of delivery.

<sup>b</sup>Predictor adjusted models. Predictors: maternal age, educational level, country of birth, year of delivery, type 1 or 2 diabetes mellitus, chronic hypertension, chronic kidney disease, systemic lupus erythematosus and antiphospholipid syndrome.
Main results are presented in Table 8. Women with PCOS, GDM and a combination of PCOS and GDM had higher odds of gestational hypertension and preeclampsia than unexposed controls. Women with both PCOS and GDM exhibited a tendency towards higher risk estimates for preeclampsia than the other exposed groups. The risk estimates were attenuated following adjustments but were still significant when BMI was added to the models (Model 2). A slightly increased odds of postpartum haemorrhage was found in women with PCOS-only and women with GDM-only compared with controls, although this was no longer statistically significant when BMI was included in the adjustments. No increased odds for sphincter injury were found in the adjusted analyses.

Figure 8. Graphical abstract Paper IV.

<table>
<thead>
<tr>
<th>Maternal outcomes</th>
<th>n</th>
<th>%</th>
<th>Crude</th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>3232</td>
<td>1.1</td>
<td>1.1</td>
<td>1.38 (1.26–1.51)</td>
<td>1.17 (1.06–1.29)</td>
</tr>
<tr>
<td>Controls</td>
<td>2507</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS-only</td>
<td>633</td>
<td>1.6</td>
<td>1.50 (1.37–1.65)</td>
<td>1.50 (1.37–1.65)</td>
<td>1.38 (1.26–1.51)</td>
</tr>
<tr>
<td>GDM-only</td>
<td>61</td>
<td>2.7</td>
<td>2.64 (2.02–3.49)</td>
<td>2.89 (2.20–3.81)</td>
<td>1.98 (1.48–2.64)</td>
</tr>
<tr>
<td>PCOS and GDM</td>
<td>31</td>
<td>3.0</td>
<td>2.90 (2.01–4.19)</td>
<td>2.82 (1.93–4.11)</td>
<td>1.61 (1.08–2.39)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>8428</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>6513</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS-only</td>
<td>1679</td>
<td>4.2</td>
<td>1.55 (1.46–1.64)</td>
<td>1.44 (1.36–1.53)</td>
<td>1.18 (1.11–1.26)</td>
</tr>
<tr>
<td>GDM-only</td>
<td>143</td>
<td>6.4</td>
<td>2.43 (2.03–2.91)</td>
<td>2.63 (2.19–3.16)</td>
<td>1.77 (1.45–2.15)</td>
</tr>
<tr>
<td>PCOS and GDM</td>
<td>93</td>
<td>9.0</td>
<td>3.51 (2.81–4.37)</td>
<td>3.47 (2.76–4.36)</td>
<td>1.86 (1.46–2.36)</td>
</tr>
<tr>
<td>Postpartum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>haemorrhage</td>
<td>14309</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>11943</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS-only</td>
<td>2181</td>
<td>5.4</td>
<td>1.09 (1.03–1.14)</td>
<td>1.05 (1.00–1.10)</td>
<td>1.03 (0.98–1.09)</td>
</tr>
<tr>
<td>GDM-only</td>
<td>137</td>
<td>6.1</td>
<td>1.24 (1.03–1.48)</td>
<td>1.22 (1.01–1.46)</td>
<td>1.15 (0.98–1.09)</td>
</tr>
<tr>
<td>PCOS and GDM</td>
<td>48</td>
<td>4.6</td>
<td>0.92 (0.68–1.24)</td>
<td>0.87 (0.64–1.18)</td>
<td>0.86 (0.64–1.17)</td>
</tr>
</tbody>
</table>

PCOS, Polycystic ovary syndrome; GDM, gestational diabetes mellitus.
<sup>a</sup>Adjusted for maternal age, parity, education and country of birth.
<sup>b</sup>Adjusted for maternal age, parity, education, country of birth and body mass index.

Neonatal outcomes are presented in Table 9. The likelihood of preterm birth was higher in all exposed groups than in the control group, both regarding overall preterm birth and spontaneous preterm birth. Women with both PCOS and GDM seemed to have the highest odds for overall preterm birth. Women with PCOS-only had 52% increased risk of stillbirth compared with the control group. Increased risk for shoulder dystocia was found in adjusted analyses in women with PCOS-only and GDM-only compared with controls. For women with both diagnoses, it was only significant in the crude analysis.

Women with PCOS-only had an increased risk of giving birth to an SGA infant in comparison with the control group. All exposed groups had an increased risk of giving birth to an LGA infant compared with the control group, especially the groups with a GDM diagnosis, with highest odds in the group with both PCOS and GDM. The same trend was seen for macrosomia. The odds of having an infant with an Apgar score <7 at 5 minutes was increased in women with PCOS-only and seemed further increased in women with both PCOS and GDM compared with the controls. Increased odds of neonatal hypoglycaemia were found in all exposed groups compared with the control group, but were dependent on maternal BMI among women with PCOS-only. A sensitivity analysis excluding pregnancies conceived through ART treatment gave similar risk estimates in all analyses.

<table>
<thead>
<tr>
<th>Neonatal outcomes</th>
<th>n</th>
<th>%</th>
<th>Crude</th>
<th>Model 1a</th>
<th>Model 2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm birth overall</td>
<td>14460</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>11386</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS-only</td>
<td>2777</td>
<td>6.9</td>
<td>1.48 (1.41–1.54)</td>
<td>1.42 (1.35–1.48)</td>
<td>1.34 (1.28–1.41)</td>
</tr>
<tr>
<td>GDM-only</td>
<td>180</td>
<td>8.1</td>
<td>1.74 (1.49–2.04)</td>
<td>1.70 (1.45–1.99)</td>
<td>1.64 (1.39–1.93)</td>
</tr>
<tr>
<td>PCOS and GDM</td>
<td>117</td>
<td>11.3</td>
<td>2.52 (2.06–3.09)</td>
<td>2.37 (1.93–2.91)</td>
<td>2.08 (1.67–2.58)</td>
</tr>
<tr>
<td>Spontaneous preterm birth</td>
<td>9011</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>7168</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS-only</td>
<td>1672</td>
<td>4.2</td>
<td>1.41 (1.33–1.49)</td>
<td>1.36 (1.29–1.45)</td>
<td>1.34 (1.26–1.43)</td>
</tr>
<tr>
<td>GDM-only</td>
<td>101</td>
<td>4.5</td>
<td>1.55 (1.27–1.90)</td>
<td>1.58 (1.29–1.94)</td>
<td>1.61 (1.30–1.99)</td>
</tr>
<tr>
<td>PCOS and GDM</td>
<td>70</td>
<td>6.8</td>
<td>2.40 (1.85–3.11)</td>
<td>2.32 (1.79–3.02)</td>
<td>2.26 (1.71–2.98)</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>986</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>725</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS-only</td>
<td>245</td>
<td>0.6</td>
<td>2.01 (1.73–2.32)</td>
<td>1.87 (1.61–2.16)</td>
<td>1.52 (1.29–1.80)</td>
</tr>
<tr>
<td>GDM-only</td>
<td>7</td>
<td>3.0</td>
<td>1.03 (0.49–2.17)</td>
<td>0.89 (0.42–1.88)</td>
<td>0.58 (0.24–1.39)</td>
</tr>
<tr>
<td>PCOS and GDM</td>
<td>9</td>
<td>0.9</td>
<td>2.87 (1.49–5.55)</td>
<td>2.29 (1.19–4.43)</td>
<td>1.59 (0.79–3.23)</td>
</tr>
<tr>
<td>Shoulder dystocia*</td>
<td>692</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Controls</td>
<td>523</td>
<td>0.3</td>
<td></td>
<td></td>
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<tr>
<td>PCOS-only</td>
<td>146</td>
<td>0.5</td>
<td>1.77 (1.47–2.13)</td>
<td>1.78 (1.48–2.15)</td>
<td>1.52 (1.25–1.85)</td>
</tr>
<tr>
<td>GDM-only</td>
<td>18</td>
<td>1.1</td>
<td>4.17 (2.60–6.69)</td>
<td>3.55 (2.20–5.72)</td>
<td>2.60 (1.57–4.32)</td>
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<tr>
<td>PCOS and GDM</td>
<td>5</td>
<td>0.7</td>
<td>2.79 (1.16–6.73)</td>
<td>2.37 (0.98–5.77)</td>
<td>1.34 (0.50–3.61)</td>
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<tr>
<td>SGA (-2SD)</td>
<td>6638</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5483</td>
<td>2.3</td>
<td></td>
<td></td>
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<tr>
<td>PCOS-only</td>
<td>1098</td>
<td>2.7</td>
<td>1.19 (1.11–1.27)</td>
<td>1.08 (1.01–1.15)</td>
<td>1.08 (1.01–1.16)</td>
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<tr>
<td>GDM-only</td>
<td>36</td>
<td>1.6</td>
<td>0.70 (0.50–0.97)</td>
<td>0.55 (0.38–0.78)</td>
<td>0.53 (0.36–0.77)</td>
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<td>21</td>
<td>2.0</td>
<td>0.88 (0.56–1.38)</td>
<td>0.67 (0.42–1.06)</td>
<td>0.61 (0.38–0.98)</td>
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<tr>
<td>LGA (+2SD)</td>
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<td>3.1</td>
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<tr>
<td>PCOS-only</td>
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<td>4.1</td>
<td>1.32 (1.24–1.40)</td>
<td>1.41 (1.33–1.50)</td>
<td>1.15 (1.08–1.23)</td>
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<tr>
<td>GDM-only</td>
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<td>14.6</td>
<td>5.29 (4.64–6.04)</td>
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<td>PCOS and GDM</td>
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<td>18.0</td>
<td>6.78 (5.71–8.06)</td>
<td>7.83 (6.56–9.35)</td>
<td>4.45 (3.71–5.35)</td>
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<td>Apgar &lt;7 at 5 minutes†</td>
<td>2528</td>
<td>1.0</td>
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<tr>
<td>Controls</td>
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<td>PCOS-only</td>
<td>457</td>
<td>1.2</td>
<td>1.37 (1.24–1.52)</td>
<td>1.28 (1.15–1.42)</td>
<td>1.16 (1.04–1.30)</td>
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<tr>
<td>GDM-only</td>
<td>26</td>
<td>1.3</td>
<td>1.42 (0.96–2.10)</td>
<td>1.38 (0.94–2.05)</td>
<td>1.21 (0.81–1.81)</td>
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<tr>
<td>PCOS and GDM</td>
<td>19</td>
<td>2.1</td>
<td>2.33 (1.48–3.68)</td>
<td>2.10 (1.33–3.33)</td>
<td>1.64 (1.03–2.60)</td>
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<tr>
<td>Neonatal hypoglycaemia†</td>
<td>4583</td>
<td>1.7</td>
<td></td>
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<td></td>
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<tr>
<td>Controls</td>
<td>3537</td>
<td>1.6</td>
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<tr>
<td>PCOS-only</td>
<td>745</td>
<td>2.0</td>
<td>1.28 (1.18–1.39)</td>
<td>1.19 (1.09–1.29)</td>
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<td>9.8</td>
<td>6.88 (5.90–8.02)</td>
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<td>10.8</td>
<td>7.62 (6.15–9.44)</td>
<td>7.00 (5.61–8.73)</td>
<td>5.16 (4.08–6.53)</td>
</tr>
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</table>

PCOS, polycystic ovary syndrome; GDM, gestational diabetes mellitus; SGA, small for gestational age; LGA, large for gestational age.

aAdjusted for maternal age, parity, education and country of birth

bAdjusted for maternal age, parity, education, country of birth and body mass index

*Only vaginal deliveries †Gestational length ≥ 37+0
Discussion

Main findings

The main findings of this thesis are that women with PCOS have higher levels of AMH and testosterone and a higher FAI during the second trimester of pregnancy than do non-PCOS controls. High AMH levels were not associated with adverse pregnancy outcomes or birthweight, whereas women with PCOS with the highest testosterone levels also had the highest risk of preeclampsia. Women with PCOS had an increased risk of preeclampsia, especially early preeclampsia and preeclampsia with an SGA infant; however, the risk was not further increased in women who had been treated for hirsutism. The combination of PCOS and GDM is associated with further increased risk of adverse pregnancy outcomes for both the mother and her infant compared with women with either PCOS or GDM.

Methodological consideration

The studies in this thesis are all retrospective cohort studies that vary in size with regard to study population and matching between women with PCOS and controls. The aim of epidemiological studies is to identify risk factors in the pathogenesis of disease and thereby increase knowledge and open up for the development of possible treatments. In this thesis, different exposures and outcomes were used to investigate the association between PCOS and pregnancy complications.

Papers I and II were retrospective matched cohort studies based on material from the Uppsala Biobank of Pregnant Woman, which contains blood samples and information on women taken early in pregnancy. Using already collected biobank material is an advantage as relatively large study populations may be acquired, but the retrospective design has its disadvantages too. Phenotyping of patients relied on medical records, which may or may not have been sufficiently detailed for this purpose. Most likely, the hyperandrogenic phenotype was underreported because absent information on hirsutism may represent ignorance/neglect as well as there being no problem at all. Misclassification of undiagnosed women with PCOS in the control group may have occurred and potentially affected the association between exposure and obstetric complications. In order to decrease that risk, we scrutinised medical records for clinical
signs of PCOS in all controls that either achieved pregnancy by ART or had high levels of AMH.

One of the strengths of Papers I and II is that liquid chromatography with tandem mass spectrometry was used for measuring testosterone, as opposed to using an immunoassay, which is no longer considered to be the gold standard method (69, 70). However, this and the other hormone analyses were based on a single blood sample from each participant taken in early second trimester, and unfortunately, no pre-pregnancy values were available. The ideal would be a hormone profile in each participant over a longer time period, i.e. prior, during and after pregnancy, to be able to classify hyperandrogenic phenotype with greater accuracy and also to be able to analyse the changes in hormone levels during pregnancy. Also, when the PCOS group was subdivided according to testosterone tertiles, there were quite a few women in each group.

On the basis of the studies in Papers I and II, we were unable to draw conclusions that would apply to all women with PCOS, because our results might only be applicable to groups of women with similar demographics, although they still yielded important insight into the subject area.

Papers III and IV were retrospective population-based cohort studies with large study populations from national registers, which opens up for a greater generalisability of results. The quality of register studies depends on the registration of information. Most of the information used in Papers III and IV came from prospectively collected data in the MBR, where registration is performed by trained healthcare professionals. As such, this information can be deemed to be of high-quality and reliable regarding measurements and diagnoses because it is done in accordance with formal procedures in a controlled manner. Despite this, the possibility of incorrect registration cannot be entirely ruled out. Prospective data collection eliminates the risk of both recall bias and information bias because future research targets are unknown when the data are registered.

The main strength in all studies presented in this thesis is that they were rooted in large study populations. In the matched cohort studies, we were able to analyse blood samples from 471 women, which is a relatively large study population compared to other studies in this field during the same time period, which all had fewer participants. In the register studies, Papers III and IV, sample sizes were large, births in over 138 000 nulliparous women in Paper III and more than 280 000 births in Paper IV. These large study populations yielded the opportunity to explore rare outcomes and to adjust for several confounders.

Missing data in register studies must be mentioned as a limitation, especially regarding BMI (9.1% in Paper III and 9.7% in Paper IV). In Paper III, missing values were imputed because the model with BMI adjustments may have been underpowered.
The risk of misclassification bias was limited by the chosen exclusions in the study populations and by including women with anovulatory infertility in the PCOS group because the majority of women with that diagnosis have PCOS (15, 16). We chose to define use of medications, both anti-androgenic drugs in Paper III and metformin in Paper IV, as at least two filled prescriptions in order to ensure medication adherence and thereby decrease the risk of misclassification.

For a systematic overview of possible causal pathways in Paper III, a DAG was constructed to illustrate confounders and associated pathways. In an associative analysis, such as DAG, non-causal pathways must be blocked by adjusting for confounders. DAGs can be used to identify confounders, colliders and mediators in the association between exposure and outcome by drawing unidirectional, non-circular arrows between factors (Figure 9). The DAGitty web application available at www.dagitty.net was used to construct the DAG in study III (Figure 5).

![Figure 9. The relationship between confounders, mediators and colliders and exposure and outcome.](image)

ART is considered to be a mediator in the context of PCOS. PCOS is commonly the underlying reason for using ART, which in turn can increase the risk for adverse outcomes. To exclude the effect of ART, we did a sensitivity analysis in Paper IV in which all pregnancies achieved by ART were excluded, and this analysis yielded similar results.

In the relationship between PCOS and preeclampsia, BMI can be considered both as a confounder and a mediator: a confounder, because high BMI can give rise to PCOS symptoms, and a mediator, as overweight and obesity are common consequences of PCOS and well-known risk factors for preeclampsia. Therefore, we decided to adjust for BMI in a second step and
do our analyses in Papers III and IV with and without BMI adjustments to shed light on how BMI affects the relationship.

In antenatal care, prediction models are used to estimate the individual risk for preeclampsia. Therefore, the prediction approach was used with high and moderate risk factors from the NICE preeclampsia guidelines. This approach yielded results similar to the causal approach after adjustments with confounders.

In Paper IV, the prevalence of GDM in the study population was 1.2%, which is in concordance with a previously reported prevalence of 1–3% in Sweden during that time (101). However, the prevalence of GDM has been rising due to increasing obesity and is estimated today to be 14% globally and 7.8% in Europe. The low prevalence in Sweden during the study period can be explained by higher cut-off levels for a diagnosis of GDM than the World Health Organisation (WHO) recommendations in 2013. In addition, no national consensus regarding GDM screening and diagnosis existed in Sweden during the study period, resulting in varying cut-off values between regions within the country. Screening for GDM with OGTT was based on risk factor assessment rather than being a general screening method in almost all regions in Sweden. Therefore, we can assume that if screening and diagnostic criteria were to be aligned with WHO recommendations, the prevalence of GDM in Sweden would be higher than in this study. That could affect the associations presented in Paper IV in both directions. If more women were diagnosed with GDM due to lower cut-offs levels, the association could be weakened because they would have lower glucose levels and presumably a lower risk for adverse outcome.

The main limitations are associated with use of data from registers. The most important limitation is the fact that PCOS is generally underdiagnosed and underreported. Therefore, women with PCOS in the studies are probably the most severe cases of PCOS with the most disturbed metabolism, hormonal and immune profile. This also means that there are most likely undiagnosed women with PCOS in the control group. This may have affected our results, either underestimating the risk associations or overestimating them so that the associations in our results are only true for the most severe cases of PCOS but not for healthier women with PCOS. GDM is also underdiagnosed in our study due to higher cut-off levels for diagnosing GDM in Sweden than the levels recommended by WHO in 2013, lack of national guidelines, and screening that was based on risk factors in almost all regions in Sweden during the study period.

An important limitation is the definition of hyperandrogenism used in the studies. Unfortunately, there are no ICD-10 codes for PCOS phenotypes, and due to the emphasis on phenotypes in PCOS studies, a stratification was needed. Paper I used clinical data from medical records and not full clinical assessments. Paper III used pharmacological anti-androgenic treatment as a
proxy for hyperandrogenic PCOS phenotype. Most probably hyperandrogenism is underreported in our studies, and therefore its possible association with pregnancy outcomes may not have been adequately investigated.

General discussion

When this thesis was planned, the evidence regarding obstetric complications in women with PCOS was limited. Methodological differences across studies made it difficult to compare results and draw conclusions. A number of studies had found increased risk for adverse pregnancy and neonatal outcomes in women with PCOS, but risk estimates and results for some of the outcomes are inconsistent. The studies in the field and guidelines pointed out the need for more studies that included phenotypic stratification and matching. Therefore, as previously mentioned, we decided to do BMI matching between PCOS women and controls in the material from the Uppsala Biobank of Pregnant Women. We examined the phenotypes specifically in Paper I, where the PCOS group was subdivided according to pre-pregnancy phenotypic presentation. In Paper III, we stratified the PCOS group according to anti-androgenic treatment as a proxy for hyperandrogenism.

Papers I and II

In Paper I, we found higher AMH levels during the second trimester in women with PCOS compared to controls. This finding is in concordance with other studies (42-44).

No association was found between high AMH levels and pregnancy complications or birthweight. In fact, our results showed that high levels of AMH were associated with decreased risk of gestational hypertension. Previous studies on AMH and hypertensive disorders in pregnancy have been inconclusive; some have been in concordance with our results regarding lower levels of AMH being associated with hypertensive disorders in pregnancy (45, 46), while others have found no effect of AMH (47). However, none of these studies focused specifically on women with PCOS. It has been speculated that impaired blood flow to the ovaries due to hypertension could induce ischaemic damage and thereby increase follicle demise and lower AMH levels (109). More recent studies did not find lower AMH levels in women with a history of hypertensive disorders of pregnancy (110, 111). Our findings, however, may also be the result of a rate of gestational hypertension that was lower than expected in our patient group (112).

In our study, no significant difference was found between preterm births in women with PCOS and controls. Also, women with preterm birth had AMH levels that were similar to women with normal pregnancy length. However,
recent studies on IVF pregnancies have found an association between elevated AMH levels and preterm birth in women with PCOS (113, 114).

AMH levels were moderately, positively correlated with total testosterone levels and FAI in the whole group. The main explanatory factors for AMH levels in women with PCOS were low BMI and high testosterone levels. Given the positive correlation between AMH and testosterone, our results in Paper I suggest that the ovaries may contribute to the maternal serum concentrations of testosterone in early second trimester, and others have found the same correlation in late pregnancy (42). In conclusion, based on our results, it seems that it is not the polycystic ovarian morphology, expressed as high AMH levels, that drives the risk for adverse obstetric outcomes in women with PCOS.

The main finding of Paper II was that women with PCOS have higher testosterone levels during the second trimester than women without PCOS. This finding is in line with previous studies on pregnant women with PCOS, where high testosterone levels also in late pregnancy have been reported (42, 43, 115). At the same time, it is important to consider that the elevated testosterone levels have little clinical consequence for the symptoms of PCOS, as free testosterone levels are much lower during a pregnancy than at other times due to a sharp increase in SHBG levels during pregnancy. Women with PCOS more commonly develop preeclampsia, and high testosterone in women with PCOS is potentially associated with increased risk of preeclampsia. The increased risk of preeclampsia in women with PCOS with the highest levels of testosterone should be interpreted with great caution because it is based on only a few cases and the confidence interval for the estimate was wide. The association between manifest preeclampsia and testosterone levels has been described in the literature, although not specifically in women with PCOS, but it remains unclear whether testosterone has a causal role in the pathophysiology of preeclampsia (55, 57, 59, 60).

During pregnancy, the fetoplacental unit is the main source of maternal testosterone, but the ovaries, the adrenals and the adipose tissue may also contribute to the circulating testosterone levels in women with PCOS (49, 106). Androgens are metabolised to oestrogens by placental aromatase (116), and a previous study from our group has demonstrated that adipose tissue aromatisation contributes to maternal testosterone levels in human pregnancy (106). No association was found between high levels of testosterone and maternal metabolic factors during pregnancy or any other obstetric complications than preeclampsia. Regarding birthweight, there was no association between high testosterone and female offspring birthweight; however, a weak association between FAI and male offspring birthweight was noted. Maternal testosterone levels are unaffected by fetal sex, but slight increases in testosterone levels in women carrying male foetuses may be seen under certain circumstances (106). Furthermore, animal studies on AMH exposure during pregnancy suggest that AMH inhibits expression of CYP19A1, causing impaired placenta aromatase
function and subsequently affecting the conversion of testosterone to oestrogens, thereby opening up for a transfer of testosterone levels between mother and foetus, and vice versa (44). We speculate that the association between FAI and male offspring birthweight is a reflection of higher testosterone levels in the male fetuses with greater growth potential, spilling over to the maternal circulation.

Papers III and IV

In Paper III, the results corroborate previous findings on increased risk of preeclampsia among nulliparous women with PCOS, in both causal and prediction approaches. Our results suggest a great impact of BMI on the risk association because the association diminished when BMI was added to the model. Obesity is a strong risk factor for preeclampsia, and approximately half of PCOS women are obese. However, BMI-matched studies found significantly higher odds for preeclampsia in women with PCOS (1).

Based on the results from Paper II and previous studies, there is a positive relationship between androgen levels and preeclampsia (53, 57, 60). We hypothesised that hyperandrogenism would increase the risk estimates for preeclampsia in women with PCOS. We were unable to confirm our hypothesis with the use of anti-androgen treatment as a proxy for hyperandrogenism. However, a previous study has reported that it is more likely biochemical hyperandrogenism which increases the complication risk during pregnancy in women with PCOS and that clinical hyperandrogenism has no effect (65). Using treatment of clinical hyperandrogenism, defined as two filled prescriptions, as a proxy for hyperandrogenism was rooted in the lack of diagnostic codes for PCOS phenotypes in ICD-10. The definition used resulted in only 10% of the study population being classified as hyperandrogenic. This deviates from current PCOS guidelines, which estimate that 60 to 100% of PCOS women have hyperandrogenism, though slightly lower in a Nordic population (1, 15, 117). A broader spectrum of anti-androgenic drugs might yield different results, for instance, if combined hormonal contraceptives with anti-androgenic progestogens like drospirenone, Dienogest or desogestrel were included. Clearly, the definition did not represent all hyperandrogenic PCOS women in the study population.

Intriguingly, we discovered a stronger association between PCOS and the more adverse subtypes of preeclampsia, i.e. early-onset and birth with an SGA infant compared with late-onset and birth without an SGA infant. To our knowledge, we are the first to report this relationship between PCOS and subtypes of pre-eclampsia linked to placental hypoperfusion. That opens up for two rather plausible speculations. Firstly, that women with PCOS would benefit from prophylactic aspirin to prevent development of preeclampsia and, secondly, that PCOS should be included in the prediction models used in clinical practice.
Ultimately, our finding suggests that PCOS impacts placentation in early pregnancy. Placental pathology linked to a hypoxic state has been reported in women with PCOS as has placental thrombosis in women with PCOS who developed either gestational hypertension or pre-eclampsia (118). Even in uncomplicated pregnancies in women with PCOS, placentas have altered morphology and microscopic structures. Alterations in early trophoblast invasion and placentation vary according to PCOS phenotype (64, 119).

Our results in Paper IV indicate that the combination of PCOS and GDM in pregnant women increases the risk of gestational hypertensive disorders and preterm birth compared to pregnant women with either PCOS or GDM. The finding on preterm birth is in concordance with a Finnish register study (89). However, a randomised trial in Norway in women with PCOS did not find a difference in the prevalence of preterm birth with or without GDM. They only found increased risk for late miscarriage in women with PCOS in whom GDM had already been diagnosed in the first trimester (90). Varying results might be explained by differences in study design. Presumably both PCOS and GDM are underdiagnosed and underreported in the register studies so that those included can present the most severe cases with highest probability of adverse outcome.

Paper IV also reported a higher risk of shoulder dystocia in all exposed groups (PCOS or GDM and both) in crude analyses. On the other hand, no association was found for the group with both PCOS and GDM in the adjusted analyses, most likely because the small number of cases ($n = 5$), yielding low statistical power.

Women with PCOS had increased risk of giving birth to SGA infants in Paper IV, which can be explained by prenatal hyperandrogenic exposure and aberrant placentation (76). The previously mentioned placental pathologic findings of thrombosis and infarction in women with PCOS can reflect fetal hypoxia and vascular damage, which are often seen in relation to fetal growth restriction (118).

All exposed groups in Paper IV had increased risk of having an LGA infant, and as expected, the GDM groups had higher risk estimates, with or without PCOS. Our result showed that women with PCOS had a slightly increased risk of LGA infants, a finding in accordance with a recent Swedish study (120). In contrast, the meta-analysis in the PCOS guidelines from 2023 concluded that women with PCOS do not have increased risk for an LGA infant (1). In our study we have minimised the effects of maternal weight, GDM and ART by adjustments and sensitivity analyses. The pathophysiology is not fully understood yet, but we can assume that it lies in insulin resistance, oxidative stress and activated immune response in women with PCOS (62, 121, 122).

In Sweden, all term-born infants of mothers diagnosed with diabetes are screened for neonatal hypoglycaemia 3 hours after birth. That could have caused a surveillance bias because, as mentioned previously, both PCOS and GDM are underdiagnosed and underrepresented in our study population. Our
results point towards undiagnosed cases of diabetes within the PCOS group, which underpins the importance of OGTT screening of women with PCOS.

Clinical implications and future perspectives

It is important to improve professional as well as general awareness about the increased risk for adverse pregnancy outcomes in women with PCOS because PCOS is seldom considered in everyday obstetric care. PCOS has a large spectrum of symptoms and four phenotypes with differing risk profiles. Future research needs to focus on prospective clinical studies in which the diagnosis of PCOS and its phenotypes is accurately determined in every participant prior to pregnancy to be able to explore the mechanism behind these findings and elucidate the risk estimates for each PCOS phenotype in relation to obstetric outcomes. As of today, obstetrical guidelines do not report PCOS as a risk factor for either preeclampsia or GDM. Recommendations from PCOS guidelines regarding pregnancy outcomes should be implemented in everyday clinical care.

It would be ideal to conduct extensive prospective cohort studies with exact diagnosing and phenotyping of PCOS and control for confounding variables. Also, a clinical study on the effect of prophylactic aspirin in women with PCOS would be interesting and hopefully improve pregnancy outcomes. Comparative analysis between practice guidelines and evidence of preeclampsia risk factors deemed the association between PCOS and pre-eclampsia as being probable although the quality of evidence was low (123). More studies on this subject are therefore warranted to conclude whether PCOS should be included in the prediction models for pre-eclampsia.

Nevertheless, our findings in the large register studies support the recommendations of the recently updated PCOS guidelines that blood pressure measurement and early screening with OGTT should be performed when women with PCOS plan for pregnancy or seek fertility treatment. If not performed prior to pregnancy, OGTT could be offered at the first antenatal visit, and all women with PCOS should be offered OGTT at gestational weeks 24–28 (1). This screening method would contribute to the early identification of hypertensive disorders and insulin resistance in women with PCOS who have not yet developed overt diabetes. Early detection and increased monitoring and medical treatment would be beneficial for both mother and infant.
Conclusions

Women with PCOS have higher levels of plasma AMH, testosterone and FAI in the second trimester pregnancy than non-PCOS women. AMH levels are positively correlated with total testosterone levels. Among women with PCOS, a high testosterone level and a low BMI were independently associated with AMH levels. High AMH levels per se are not associated with increased risk for PCOS-associated maternal and neonatal complications. High AMH levels were associated with reduced odds of developing gestational hypertension. Hence, high AMH in women with PCOS should not be considered as the underlying factor in the association between PCOS and pregnancy complications.

Women with PCOS who had the highest maternal testosterone levels were those who had the highest risk of developing pre-eclampsia. FAI was marginally positively associated with standardised birthweight in male offspring.

Women with PCOS face a higher risk of preeclampsia than non-PCOS controls; however, this risk seems partly dependent on comorbid overweight/obesity. On the basis of the available data, the study was unable to determine whether hyperandrogenism in PCOS increased the risk of pre-eclampsia. The association between PCOS and preeclampsia was stronger when we restricted the outcomes to early preeclampsia and preeclampsia in women with an SGA infant, suggesting that pre-existing PCOS impacts placentation in early pregnancy. Similar results were achieved in models using causal and prediction approaches.

The combination of PCOS and GDM indicates further increased risk of adverse pregnancy outcomes for both mother and infant in comparison with the risks associated with having only one of these conditions.

Awareness of PCOS needs to be enhanced among both the general population and professionals. Increased surveillance and routine screening during antenatal care will lead to greater detection of high-risk pregnancies, thereby decreasing the risk of adverse outcomes for both mother and infant.
Polycystiskt ovariesyndrom (PCOS) är den vanligaste endokrina rubbningen bland kvinnor och drabbar runt 10–13% av kvinnor i fertil ålder och många är fortfarande odiagnostiserade. PCOS karakteriseras av mensstörningar och symtom på förhöjda nivåer av androgener såsom ökad kroppsbehåring av manlig karaktär, akne och manligt håra vfall. Vanligaste symtomet på PCOS är oregelbundna eller helt uteblivna menstruationer som tecken på ägglossningsproblem, vilket också leder till svårigheter att bli gravid och ofta behov av fertilitetsbehandling. Diagnosen PCOS ställs med Rotterdam kriterium där minst två av tre följande kriterier ska vara uppfyllda samt att andra orsaker ska vara uteslutna: 1) ägglossningsstörning (oligo- eller anovulation), 2) överskott av androgener (hyperandrogenism), antingen kliniska symtom eller biokemisk som mäts via blodprov, 3) polycystiska äggstockar vid ultraljudsundersökning. Hyperandrogena fenotyper anses vara mer allvarliga och verkar ha högre metabolisk risk.


Anti-Müllerskt hormon (AMH) produceras i små omogna äggblåsor och är starkt korrelerat med antal äggblåsor i äggstockarna. AMH nivåer används för att mäta äggreserv. Kvinnor med PCOS har två till tre gånger högre AMH nivåer än icke-PCOS kvinnor.

Orsaker till utveckling av PCOS är inte helt kända men det är förmodligen ett komplext samspel mellan ärftlighet och livsstilsfaktorer. Även miljön i fosterlivet kan ha betydelse.

Kvinnor med PCOS har ökad risk för graviditetskomplikationer såsom missfall, graviditetsdiabetes (GDM), högt blodtryck och havandeskapsförgiftning. Av dessa är GDM vanligast. Barn till mödrar med GDM har ökad risk för lågt blodsocker efter förlossningen, missbildningar och att födas stora, vil-

Denna avhandling syftar till att studera associationen mellan förhöjda nivåer av AMH och testosteron och graviditetsutfall. Dessutom att öka kunskapen mellan PCOS och havandesksförgiftning och GDM.


Första delarbetet fokuserade på AMH och visade högre nivåer hos kvinnor med PCOS jämfört med kontroller samt att AMH nivåer är korrelerade med testosteron-nivåer. Högt AMH var inte associerat med ökad risk för graviditetskomplikationer eller födelsevikt.

I andra delarbetet undersöktes hur testosteron påverkar graviditet hos kvinnor med och utan PCOS samt barnens födelsevikt. Kvinnor med PCOS hade högre testosteron-nivåer och högre fritt androgen index under mitten av graviditeten jämfört med kontroller. Högst risk för havandesksförgiftning fanns bland kvinnor med PCOS som hade de högsta nivåerna av testosteron.


I fjärde delarbetet undersöktes, i en stor registerstudie med över 281 000 födslar, om samsjuklighet med PCOS och GDM påverkar graviditetsutfall och det nystödda barnet. Resultaten tyder att kvinnor med både PCOS och GDM har högre risk för graviditetskomplikationer än kvinnor med antingen PCOS eller GDM.

Sammanfattningsvis fann vi att kvinnor med PCOS hade högre nivåer av både AMH och testosteron under mitten av graviditeten än kvinnor utan PCOS. Höga nivåer av testosteron hos kvinnor med PCOS är kopplade med
Fjölblöðrueggjastokkaheilkenni (PCOS) er algengasta innkírtlaröskun meðal kvenna og hefur áhrif á um 10–13% kvenna á frjósemisaldri og margar eru enn ógreindar. PCOS einkennist af tíðaóreglu og einkennum hækkaðs magns andrógena eins og auknum hárvexti, bóulum og hárþynningar á höfði. Algengasta einkenni PCOS eru öreglulegar blæðingar eða stöðvun blæðinga vegna egglostruflunar, sem leiðir svo til skertrar frjósemi og þörf á frjósemismeðferð. Greining á PCOS er gerð með Rotterdam greiningarviðmiðum þar sem að minnsta kosti tvö af þremur eftirtalinna einkenna þurfa að vera til staðar og aðrar orsakir útilokaðar: 1) egglostruflun með öreglulegum eða engum egglosum, 2) aukið andrógen, annað hvort sem klinísk einkenni eða hækkin mæld í blóðprufu, 3) fjölblöðrueggjastokkar við ómskoðun. Birtingarmyndir með háum andrógenum eru taldar alvarlegri og eru líklegri til að bera með sér aukna hættu á efnaskiptavillu.

Ofþyngd og offita eru algengur fylgikvilli hjá konum með PCOS, talið er að helmingur kvenna með PCOS séu með offitu. Insúlínviðnám er beintengt PCOS og talið vera til staðar hjá 50–75% kvenna með PCOS, í herra hlutfalli hjá konum sem eru of þungar en einnig til staðar hjá konum í eðlilegri þyngd. Þetta leiðir til auknar hættu á efnaskiptavillu eins og sykursýki af tegund 2, hjarta- og æðasjúkdönum, þeim herre þeim hærri AMH gildi eru notuð til að meta eggjafornið. Konur með PCOS hafta tvisvar til þrisvar sinnnum hærri AMH gildi en konur sem ekki eru með PCOS.

Orsakir PCOS eru ekki þekktar að fullu en það er flókið samþykkt. Umhverfi á fösturskeiði getur einnig haft áhrif.

fæðast andvana eða sem léttburar og leggjast oftar inn á vökudeild. Það eru margir þættir sem hafa áhrif á þessa fylgikvilla eins og efnaskiptatriðinir, ínsúlínviðnám, langvarandi bólgur, há gildi andrógena og þættir sem tengjast ófrjósemi.

Markmið þessarar ritgerðar var að rannsaka sambandið á milli háekkaðra gilda AMH og testósteróns og meðgöngufylgikvillas. Einnig auka þekkingu á tengslum PCOS við meðgöngueitur og meðgöngusykursýki.


Fyrsta rannsóknin sýndi að konur með PCOS höfðu hærra AMH samanborið við viðmiðunarhóp og að fylgni sé milli AMH gilda og testósterónmagns. Há AMH gildi tengdust ekki aukinni hættu á meðgöngufylgikvillum eða fæðingarþyngd.


Fjórða rannsóknin var afturskyggn ferilrannsókn með meira en 281 000 fæðingum. Hér voru könnuð áhrif PCOS og meðgöngusykursýki, annars vegar sitt í hvoru lagi og hins vegar samhliða, á meðgöngufylgikvilla og útkomu nýbura. Niðurstöðurnar benda til þess að konur með bæði PCOS og meðgöngusykursýki súe með hærri likur á meðgöngufylgikvillum en konur með annað hvort PCOS eða meðgöngusykursýki.

Niðurstöður rannsóknanna í þessari ritgerð sýna að konur með PCOS höfðu hærra magn af bæði AMH og testósteróni á miðri meðgöngu samanborið við konur án PCOS greiningar. Hátt testósterón gildi hjá konum með PCOS er líklega tengt aukinni hættu á meðgöngueitr. Samanborið við konur án PCOS eru konur með PCOS í aukinni hættu á meðgöngueitr og þá sérstaklega
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