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STRESS, DEPRESSION, AND OTHER PSYCHIATRIC DISORDERS IN WOMEN:
An Epidemiological Approach To Studying Causes and Consequences
for In Vitro Fertilization and Polycystic Ovary Syndrome

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Stockholm 2017

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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ABSTRACT

Reproductive epidemiology, psychiatric epidemiology, and pharmacoepidemiology come together in this thesis which is comprised of four studies and divided into two parts.

Part 1 examines the impact of stress and depression on the outcomes of infertility treatment in women undergoing in vitro fertilization (IVF). Up to 30% of couples will experience difficulties conceiving, and women undergoing infertility treatment report high levels of stress and symptoms of depression and anxiety. There is concern that stress affects the success of the infertility treatment, and little is known about the influence of depression and treatment with antidepressants on fertility.

Using the Swedish national registers, **Study I** investigated the association between depression, anxiety, and antidepressant use and IVF cycle outcome in 23,577 nulliparous women undergoing their first recorded IVF cycle. Overall, 4.4% of the women had a diagnosis of depression or anxiety, or a dispensation for antidepressants prior to IVF cycle start. Findings suggest that women with the most complex and severe cases of depression had reduced chances of becoming pregnancy, independent of treatment with antidepressants. **Study II** examined the influence of multiple measures of stress on IVF cycle outcomes in the Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study. Perceived life stress, infertility-related stress, and biological stress measured by cortisol were not associated with indicators of oocyte and embryo quality, or pregnancy rate in the UppStART participants, a finding which is potentially reassuring to both patients and clinicians.

Part 2 investigates psychiatric disorders in women with polycystic ovary syndrome (PCOS). PCOS is a common endocrine disorder, affecting up to 15% of reproductive-aged women and is often accompanied by metabolic disorders and depression.

Study III utilizes diagnoses recorded in the Swedish National Patient Register to investigate both common and rare psychiatric disorders in women with PCOS, as well as in their siblings. Women with PCOS were found to have a 50% increased odds of having a psychiatric disorder compared with women from the general population without PCOS. Higher odds for some psychiatric disorders in the sisters and brothers reveal that common familial factors could be responsible for these findings, and indirect evidence for the role of elevated androgens in the risk for psychiatric disorders was found. **Study IV** provides evidence of common genetic and environmental factors between PCOS and major depression by utilizing data in a cohort of Swedish female twins. Findings suggest that part of the comorbidity between depression and PCOS is attributable to common factors between these two traits and neuroticism – a personality trait associated with depression and also found to be higher in women with PCOS.

Svensk sammanfattning

Den här avhandlingen består av fyra studier och omfattar reproduktiv epidemiologi, psykiatrisk epidemiologi och farmakoepidemiologi. Avhandlingen är uppdelad i två delar.

I **del 1** undersökte vi effekterna av stress och depression på befruktningen hos kvinnor som genomgår in vitro fertilisering (IVF). Upp till 30 % av alla par upplever svårigheter att bli gravida, och kvinnor som genomgår fertilitetsbehandling rapporterar höga stressnivåer och symtom på depression och ångest. Det är möjligt att stress påverkar hur framgångsrik fertilitetsbehandlingen blir och väldigt lite är känt om hur depression och behandling med antidepressiva läkemedel påverkar fertiliteten. Med hjälp av svenska nationella register undersökte vi i **Studie I** sambanden mellan depression, ångest och användning av antidepressiva läkemedel med utfallet av IVF-behandling hos 23,577 kvinnor utan barn som genomgick sin första IVF-behandling. Totalt hade 4.4% av kvinnorna diagnosticerats med depression eller ångest, eller hade fått antidepressiva läkemedel utskrivna före behandlingsstarten med IVF. Våra resultat visade på att kvinnorna med mest komplex och allvarlig depression hade minskad chans att bli gravida, oavsett behandling med antidepressiva läkemedel. I **Studie II** undersökte vi hur stress – mätt på flera olika sätt – påverkade IVF-behandlingens utfall i studien "Uppsala-Stockholm Assisted Reproductive Techniques" (UppStART). Vi fann inget samband mellan upplevd livsstress, infertilitetsrelaterad stress och biologisk stress, uppmätt i kortisolnivåer från saliv, med antalet eller kvaliteten på ägg eller embryon, eller med sannolikheten att bli gravid hos deltagarna i UppStART, ett fynd som kan vara lugnande för både patienter och läkare.

I **del 2** undersökte vi psykisk sjukdom bland kvinnor med polycystiskt ovarialsyndrom (PCOS). PCOS är en vanlig endokrin sjukdom som påverkar upp till 15% av kvinnor i reproduktiv ålder och uppträder ofta tillsammans med andra metabola sjukdomar och depression. I **Studie III** använde vi diagnoser från det svenska Patientregistret för att undersöka både vanliga och ovanliga psykiska sjukdomar bland kvinnor med PCOS och deras syskon. Kvinnor med PCOS visade sig ha 50 % ökade odds för psykisk sjukdom jämfört med kvinnor från den övriga befolkningen utan PCOS. Högre odds för vissa psykiska sjukdomar hos kvinnornas systrar och bröder visade att gemensamma familjära faktorer kan ligga bakom dessa fynd, och vi fann indirekta bevis för att förhöjda nivåer av androgener kan spela en potentiell roll. I **Studie IV** visade vi på gemensamma genetiska och miljömässiga faktorer bakom PCOS och djup depression med hjälp av data från en kohort av svenska kvinnliga tvillingar. Våra fynd tyder på att en del av samsjukligheten mellan depression och PCOS beror på faktorer som delas av dessa två egenskaper och neuroticism (emotionell instabilitet) – ett personlighetsdrag associerat med depression – som även visat sig vara högre hos kvinnor med PCOS.

LIST OF SCIENTIFIC PAPERS

I. **Cesta CE**, Viktorin A, Olsson H, Johansson V, Sjölander A, Bergh C, Skalkidou A, Nygren KG, Cnattingius S, Iliadou AN. Depression, Anxiety, and Antidepressant Treatment in Women: Association with In-vitro Fertilization Outcome. *Fertility and Sterility.* 2016; 106: 1594-1602.

II. **Cesta CE**, Johansson AL, Hreinsson J, Rodriguez-Wallberg KA, Olofsson JI, Holte J, Wramsby M, Cnattingius S, Skalkidou A, Iliadou AN. Perceived stress, infertility-related stress, and cortisol levels in women undergoing in vitro fertilization and its influence on embryo quality and cycle outcome. 2017 (*submitted*)

III. **Cesta CE**, Månsson M, Palm C, Lichtenstein C, Iliadou AN, Landén M. Polycystic Ovary Syndrome and Psychiatric Disorders: Co-morbidity and Heritability in a Nationwide Swedish Cohort. *Psychoneuroendocrinology*. 2016; 73: 196-203.

IV. **Cesta CE**, Kuja-Halkola R., Lehto K., Iliadou AN, Landén M. Polycystic Ovary Syndrome, Personality, and Depression: A Twin Study. 2017 (*submitted*)

CONTENTS

LIST OF ABBREVIATIONS

ADHD   attention deficit hyperactivity disorder
AE-PCOS   Androgen-Excess PCOS Society
AFC   antral follicle count
ART   assisted reproductive techniques
ASD   autism spectrum disorder
ATC   Anatomical Therapeutic Chemical Index
BMI   body mass index
CBT   cognitive behavioral therapy
CI   confidence interval
CIDI-SF   Composite International Diagnostic Interview-Short Form
COMPI   Copenhagen Multi-Centre Psychosocial Infertility Research Program
COMPI-FPSS   COMPI Fertility Problem Stress Scale
DSM-IV   Diagnostic and Statistical Manual of Mental Disorders, 4th edition
DZ   dizygotic
EPI-Q   Eysenck Personality Inventory
FAI   free androgen index
FertiQoL   Fertility Quality of Life Tool
FPI   Fertility Problem Inventory
FSH   follicle stimulating hormone
GID   gender identity disorder
HPA   hypothalamic-pituitary-adrenal axis
ICD   International Classification of Diseases
ICSI   intra-cytoplasmic sperm injection
IVF   in vitro fertilization
LH   luteinizing hormone
MDD   major depressive disorder
MZ   monozygotic
NIH   National Institute of Health, USA
NPR   National Patient Register
OR   odds ratio
PCOS   polycystic ovary syndrome
PSS   Perceived Stress Scale
Q-IVF   The National Quality Register of Assisted Reproduction
SSRI   selective serotonin reuptake inhibitors
STAGE   Study of Twin Adults: Genes and Environment
STR   Swedish Twin Registry
UppStART   Uppsala-Stockholm Assisted Reproductive Techniques study

1   INTRODUCTION

*It is only recently – in this last year of my doctoral studies - that I have come across a definition of epidemiology that articulates why I have chosen this path and resonates with my goals as a researcher: **Epidemiology** is the science of understanding the **distribution and causes** of population health,* with the ultimate goal of using this knowledge to intervene and prevent disease.[1] *This section is a brief summary of the general steps required to take an epidemiological approach to a research question. The methods of epidemiology can be applied to all areas of health and illness. Within this thesis, three fields are merged: reproductive epidemiology, psychiatric epidemiology, and pharmacoepidemiology. Although each field has specific methodological challenges, the main preoccupation of epidemiologists is to address and minimize bias with the goal of maximizing the evidence towards causation in their respective research fields.*

1.1   AN EPIDEMIOLOGICAL APPROACH

The origins of epidemiology can be found in the 18th and 19th centuries when finding the sources of the infectious diseases that were plaguing society was vital and urgent. However, the advent of **modern epidemiology** – the science and methodology we practice now – was a mere three decades ago in the 1980's. With the establishment of large-scale prospective longitudinal studies new methods were needed to account for such issues as varying follow-up periods, differential loss of follow-up, person time, competing risks, et cetera.

Also, in these last decades, knowledge has increased rapidly on the biological (e.g., the human genome) and social influences on the variation in human health and disease, and it has become evident that determinates of health are dispersed throughout the life course, beginning even before conception. Epidemiological methods continue to evolve to be able to take into account that exposures are part of a complex web of biological and social influences. However, the fundamentals concepts remain for a solid epidemiologic approach.

Seven Steps in Conducting an Epidemiological Study
Adapted from Keyes & Galea 2014 [2]

Step 1: Define the population of interest

Step 2: Conceptualize and create measures of exposure and health outcomes

Step 3: Take a sample of the population

Step 4: Estimate measures of association between exposures and health outcomes

Step 5: Rigorously evaluate whether the association observed suggests a causal association

Step 6: Assess the evidence for causes working together

Step 7: Assess the extent to which the results matter to other populations

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The concept of **bias** – the lack of **internal validity** or an incorrect assessment of the association between exposure and outcome – is a central issue in **observational studies** in epidemiology, and can occur at any step in the study process from the initial literature review of the topic, to the selection of the study sample, measurement of exposure/outcome, data analysis, result interpretation, and even publication.[3]   Bias in each step of the epidemiological approach is discussed briefly below, and then in regards to each study in this thesis in later sections.

Step 1: Define the population of interest

Populations are defined by eligibility criteria – the characteristics of individuals that make them of interest for study. These criteria generally involve:
- A geographic area and time period
- Characteristics, events, or specific exposures or health related factors
- Specific factors that promote successful study completion, if recruiting participants

Inclusion criteria for the study population can be minimal (e.g., women) or extensive (e.g., nulliparous women, aged 20-45, born in Sweden, with history of infertility). The defining of the population is directly related to the **external validity** and **generalizability** of the study findings addressed in step 7.

Step 2: Conceptualize and create measures of exposure and health outcomes

Both exposures and outcomes can be binary, ordinal, or continuous measures. Exposures can be innate, acute, chronic, time varying, of certain duration, or may occur during a critical window. It is therefore important to be clear about what is being measured. Methods of measurement should be reliable and valid, represented by the concepts of **sensitivity** and **specificity**, and directly related to the internal validity of a study.

Step 3: Take a sample of the population

The sample required depends on the goal of the study. For example, to estimate accurate population parameters a **representative** sample is needed. However, to estimate a causal effect of an exposure on an outcome, a **purposive** sample may be desired, where the principle concern when taking the sample is whether exposed individuals are comparable with non-exposed individuals.[4]   Logistical and practical consideration are also needed when taking a study sample, including availability of previously collected data, required sample size, resources available and feasibility and ethics of prospective collection. Including the entire population in a study is ideal. This is sometimes possible with the Nordic population registers. However, while the numbers are large in these registers, the data is not necessarily rich, and conducting a prospectively collected cohort study may therefore be worthwhile, as in the case of the UppStART study utilized in Study II of this thesis.

Step 4: Estimate measures of association between exposures and health outcomes

Analysis is usually performed in two steps. First is a descriptive assessment of the exposure and outcome, reported as incidence, prevalence, incidence rate, risk, et cetera. The next step is an estimation of the association producing a ratio measure (e.g., odds ratio, risk ratio, hazard ratio) with statistical parameters to determine the significance of the estimate.

Step 5: Evaluate whether the association observed suggests a causal association

Ideally we would like to observe the same individual under the exposure, and then in identical conditions without the exposure. The difference between the outcomes would be a perfect measure of the **effect** of the exposure. However, this is clearly impossible as everything cannot be held constant, especially time. One state occurs in reality, and the alternative state to what is actually observed is called the **counterfactual**, and is a concept that has become central to **causal inference theory** in epidemiology.[5] The main task for cause-examining research is to create an approximation of the counterfactual. To do this, various approaches are used in an attempt to ensure that all factors are equally distributed between exposed and unexposed individuals, thereby maximizing the **internal validity** of the study. Violation of internal validity arises by **non-comparability** between the study groups and is caused by two categories of bias: **systematic bias** (i.e., selection bias, information bias, and confounding) and **random error** (i.e., caused by chance). Systematic bias can be handled by the researcher through study design and adjustments in the analysis as discussed below. Random error cannot be corrected for, but decreases with increasing sample size. Statistical analyses assess the variability in the data in an attempt to distinguish the chance findings from true results.

Selection bias occurs when the distribution of exposure in the study participants is different from the distribution of exposure in the population. Examples include inappropriate selection of controls in a case-control study, volunteer bias, non-response bias, and differential loss of follow up in a longitudinal study. In the latter cases, it is prudent to conduct a loss of follow up and non-responder analysis, where differences in their exposure status compared with those participants remaining in the study. The consequence of selection bias is that the study sample obtained is not representative of the underlying population, and therefore affects both the internal and external validity of a sample.

Information bias occurs when the methods for obtaining information about the study subjects are inadequate and as a result the classification of subjects as exposed/unexposed or diseased/disease-free is compromised. Misclassification can occur in two forms: **differential misclassification** where the rate of misclassification differs in the different study groups, and **non-differential misclassification** where misclassification is not related to exposure or outcome status. Differential misclassification can result in either an over-estimation or under-estimation of the effect, whereas non-differential misclassification

leads to a larger variability in the data and most often results in an attenuation of the true effect. Sources of information bias are: **recall bias, ascertainment bias, surveillance bias,** and **reporting bias,** some of which are relevant for the studies in this thesis.

Information bias is relevant in the case of **reverse causation,** where the association between the exposure and outcome is actually due to the outcome exerting an effect on the exposure. Logically, an exposure needs to precede the outcome to affect the outcome. However it can be difficult to ascertain the timing of the exposure relative to the *onset* of the outcome, thereby leading to the potential for reverse causation.

While an association between the exposure and outcome may be found, it may not be the exposure *per se* that is causing the outcome. **Confounding** of an association occurs when there is a common factor causing both the exposure and outcome, as illustrated in Figure 1a. Confounding can cause bias in either direction (i.e., an over- or underestimation of the effect), or even a reversed estimation. Confounding can be dealt with entirely by **randomization** of study individuals to exposed and unexposed groups. However, this is not feasible or ethical in many cases. In observational studies, **matching** or **stratification** on the confounding factor can be performed. Alternatively, **adjustment in regression analysis** allows for estimates of the effect to be averaged over different levels of the confounder. By adjusting for the confounding factor, the pathway from the confounder to the outcome via the exposure is closed. However, one must take care in correctly identifying confounders. If the factor is instead a **mediator** i.e., a factor caused by the exposure on the pathway to the outcome (Figure 1b), the total causal effect of the exposure cannot be estimated, and will typically be biased to the null.[6]

Figure 1: Direct Acyclic Graphs demonstrating (a) a confounder and (b) mediator

a) Confounder → Exposure → Outcome

b) Mediator → Exposure → Outcome
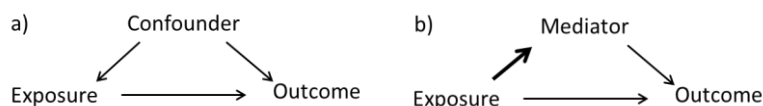
Step 6: Assess the evidence for causes working together

Rarely, there is a single cause of a disease and multiple factors may individually contribute to a health outcome. **Interaction**, or **effect modification**, can be seen in study data as an effect of an exposure on the outcome that varies across the levels of another factor.

Step 7: Assess the extent to which the results matter to other populations

This last step is concerned with the **external validity**, or **generalizability**, of the findings in populations other than the study sample. A requisite for good external validity is high internal validity. However, a trade-off is required because a more narrow and uniform study population achieves higher internal validity, but decreases the generalizability of the study results.

1.2   REPRODUCTIVE EPIDEMIOLOGY

Reproduction is a fundamental element in the life of every individual. If you are reading this then you have experienced and survived the precarious time *in utero* and the vulnerable first years of life, to be forever influenced by events that occurred during this time.   Further, you will have experienced puberty and spent some time considering how not to become pregnant (or how not to impregnate someone), and may have decided on whether or not to have children of your own.

Reproductive epidemiology is a broad field encompassing an epidemiological approach to study the distribution and determinants of health-related states or events related to reproductive health and fertility in women and men, pregnancy, immediate and even long term outcomes of the mother and offspring.

By this definition, one of the commonly noted pioneers of epidemiology, Ignasz Semmelweis, was in fact a reproductive epidemiologist. Through keen observation in his clinic over time, he discovered the source of puerperal fever that was raging through the maternity wards of Europe in the mid-19th century, leading to high rates of maternal death. Puerperal sepsis risk was high when deliveries were handled by medical students or doctors who went directly from the autopsy room to the delivery ward, but low when only midwives attended the delivery. He then went on to intervene by implementing a handwashing policy amongst the clinical staff thereby reducing the risk of puerperal fever and saving countless mothers lives.[7]

Reproductive epidemiologists have often led the progress in the field of epidemiology. For example, it is from the work of Janet Lane-Claypon in the early 1900s that we have some of the most important epidemiological study designs. By designing and preforming a study to examine breast feeding versus bottle feeding in relation to infant weight gain, she has been attributed as being the first researcher to use a retrospective cohort study design, which included elements to address both systematic and random error, such as the exclusion of sick infants to prevent confounding and noting that the potential for confounding by social class could not be controlled for with the data available. Later, she published results from the first modern case-control study, aimed to identify risk factors for breast cancer.[8]

Epidemiology has made substantial contributions to our knowledge of human reproduction. It's little known that so much of our knowledge about the timing of the human menstrual cycle come from observational studies performed by dedicated epidemiologist and even more dedicated study participants. Modern textbooks still describe the 'normal' menstrual cycle to be 28 days long, however from at least 1962 onward we have known this not to be true. A study of 700 Japanese women who recorded their menstrual cycles for over 2 years demonstrated that only 12% of cycles are actually 28 days long with wide variation in cycle length.[9, 10] In 1934, the Menstruation and Reproductive History Study was initiated by

Alan Trelaor at the University of Minnesota. He recruited thousands of college students to keep diaries of their menstrual cycles with the goal to *'define quantitatively the rhythm pattern of the human menstrual cycle through current recording of dates of onset and cessation of flow'.* This prospective cohort study allowed for the first time the detailed observation of the normal variability in the menstrual cycle length throughout reproductive life, from menarche to menopause.[11] Now known as the TREMIN Research Program on Women's Health, it is one of the oldest ongoing research programs. After conducting multiple waves of enrollment, data collection ended in 2009, and now includes data on more than a half-million menstrual cycles.[12]

It was almost three decades after the discovery of menstrual cycle length that the fertile days of the menstrual cycle were identified (i.e., the 5 days before ovulation and the day of ovulation), in a 1995 study published by a devoted and prominent reproductive epidemiologist, Alan Wilcox.[13] Again, this important discovery was made possible by the participation of dedicated women who rigorously recorded their daily information on ovulation, intercourse, and resulting conceptions.

Observational epidemiological studies prevail as a leading method for discovery in human reproduction, and there are specific challenges and advantages within reproductive epidemiology not necessarily experienced by other epidemiological fields.


Common challenges in Reproductive Epidemiology
Adapted from Wilcox, 2010 [9]

- Reproduction involves 2-3 individuals: the mother, the father, and the offspring

- Individuals can experience infertility but be healthy in every other way, and only half of couples experiencing infertility will seek medical care

- Many aspects of reproduction do not occur within the medical sphere (e.g., not all miscarriages are reported to the doctor)

- Data quality ranges from nearly perfect to nothing and even unmeasureable

- Denominators are not often available (e.g., incidence of birth defects in fetuses) or contraversial (e.g., clinical pregnancy versus live birth in infertility treatment studies)[14]

- Exposures and outcomes can happen closely together, making reverse causation a persistent threat

- Alternatively, the time between exposure and outcome can be very long; even decades

- Events range from extremely common (e,g., preterm birth) to extremely rare (e.g., birth defects)

1.3   THIS THESIS

Globally, more than 350 million individuals currently live with depression, a number that has increased by 18% between 2005 and 2015.[15] Major depression is more common in women than men (Figure 2), and women of childbearing age are at their greatest lifetime risk for developing depressive and anxiety disorders.[16-18]
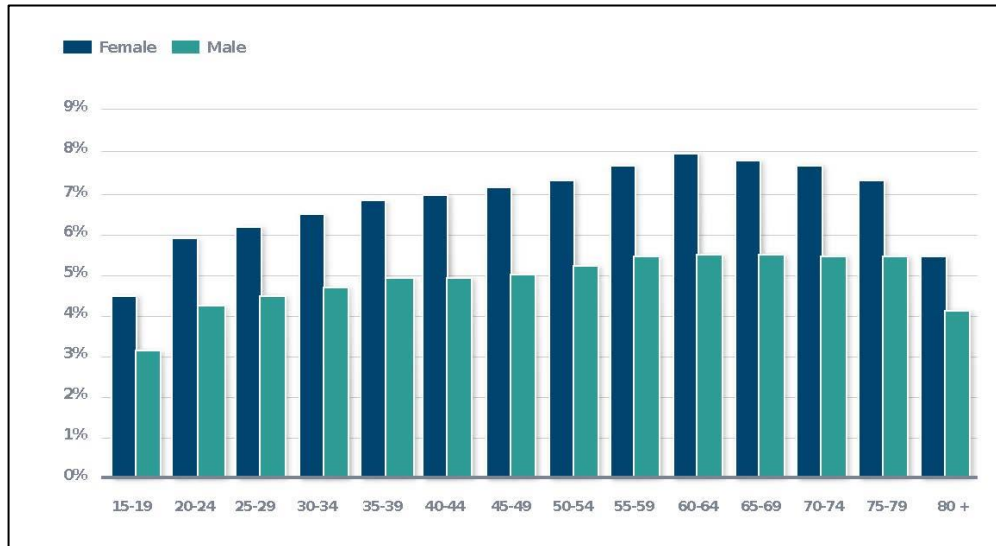
Figure 2: Global Prevalence of depressive disorders, by age and sex (%)
Source: Global Burden of Disease Study 2015 (http://ghdx.healthdata.org/gbd-results-tool)[15]

Four studies comprise this thesis, which is divided into two parts. **Part 1** examines the potential impact of stress and depression on the ability to conceive in women undergoing in vitro fertilization (IVF), a setting which provides a unique opportunity to view human fertility and reproduction through assisted reproduction techniques. **Part 2** investigates depression in women with polycystic ovary syndrome and the possible causes behind the common comorbidity between the two disorders including elevated levels of androgens and personality, thereby potentially shedding some light on factors related to the development of psychiatric illness.

1.4   ETHICAL CONSIDERATIONS

Research ethics encompasses the discussion surrounding and the regulations ensuring the protection of participants in research. Participants should be protected against the risk of injury, violation of integrity, and should be afforded autonomy – the right to choose to participate in research with clear knowledge of the risks involved. Risks should be balanced with the potential benefit of the knowledge to be gained.

Sweden has signed the European Council's Convention on Human Rights and Biomedicine which requires legally enforced regulation of the ethical vetting process and dictates the activities of the regional ethical review boards. Regulations in the Ethical Review Act (2003:460) that are important for studies included in this research plan are those addressing the handling of sensitive personal data, the collection of biological samples, and informed consent.

All studies in this thesis have been approved by the regional ethics review board and granted the following approval numbers:

- **Study I & III:** National Register linkage = Dnr 2013/1849-31/2, 2015/876-32
- **Study II:** UppStART study = Dnr 2011/230-31/1, 2011/1427-32, 2012/131-32, 2012/792-32, 2013/1700-32
- **Study IV:** STAGE twins = Dnr 03-224, 2016/1358-32

Use of register-based data is one of the few areas in medical research where exceptions are made to the requirement of informed consent; however, approval is needed from the regional ethics review board.[19]

In Study II, data were collected from UppStART study participants. Fertility clinic patients fulfilling the inclusion criteria for the study were approached by a midwife and informed about the study. Participants were informed that their participation was voluntary and that they could withdraw from the study at any time without consequence to their medical care. Those patients who were willing to participant provided their written consent. Upon enrollment, study participants were assigned a participant ID number and all personal identifiers (e.g. Swedish personal identification number, name) were removed from the data and biological samples.

In study IV, data was used from the Swedish Twin Registry. All Swedish twins born between 1959 and 1985 were sent an invitation letter by the Swedish Twin Registry asking for their participation in the Study of Twin Adults: Genes and Environment (STAGE) study. Informed consent was given online before completing the online survey. Participants were informed that they could withdraw from the study at any time.

For both STAGE and UppStART only a small number of study personnel have access to the key linking the study participant ID number to personal identifiers.

2 PART I: STRESS, DEPRESSION, AND IVF OUTCOME

2.1 BACKGROUND

2.1.1 Stress

The ability to respond to stress and adapt to adversity is a central aspect of human functioning. The first description of the 'stress response' and the advent of stress research is attributed to Hans Selye in the 1930s.[20] While certain amounts of stress are normal and necessary, too much stress can have harmful physiological and psychological consequences. There are two major components to stress: **the cause and the effect**, the latter of which includes an appraisal of the stressor and an emotional response (Figure 3). Lazarus & Folkman, two pioneers in defining the most well-known construct of stress, proposed that *negative* stress occurs when an individual perceives that the demands of a situation exceed their resources and capacity to cope with those demands.[21] The appraisal process determines the type, direction, and intensity of the stress-related emotions (e.g. anxiety, anger, guilt, sadness) which vary based on individual characteristics such as personality traits, learned coping strategies, past experience, and perceived abilities.[22] *Note: As the word 'stress' is so commonly used to describe the negative impact of stress, also referred to as 'distress', these words will be used interchangeably throughout this thesis.*
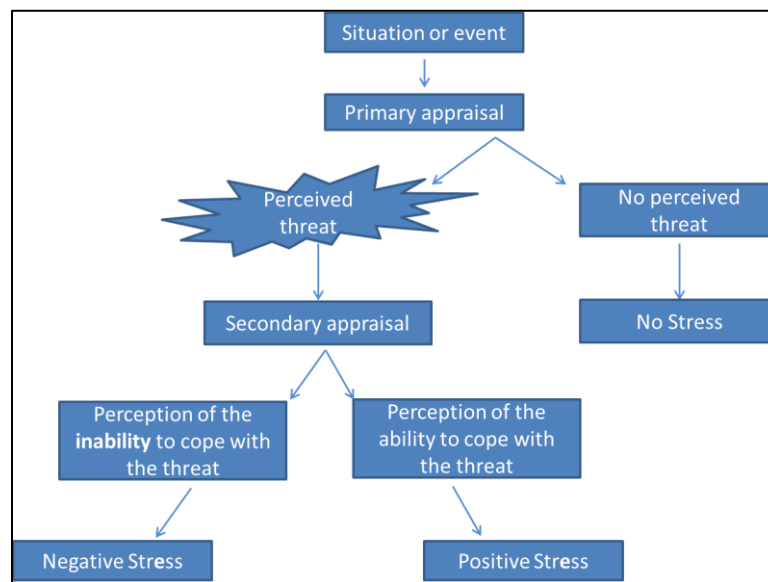
Figure 3: *Interactional Model of Stress, adapted from Lazarus & Folkman 1984*

Lazarus & Folkman proposed the concept of a Transactional Coping Model, defined as "the constantly changing cognitive and behavioral efforts to manage specific external demands that are appraised as taxing or exceeding the resources of the person" and suggested that there are four main coping strategies: Active-avoidance (e.g., avoiding specific people/places), Active-confronting (e.g., asking for advice), Passive-avoidance (e.g., hoping for a miracle), and Meaning-based coping (e.g., find other goals in life).[22] Some consider

the coping response to be anchored in one's personality as a personality trait, others believe it to be a result of psychosocial resources, and others believe that elements of coping may be learned from one's reference groups.[23] Importantly, certain coping strategies are known to reduce stress (e.g., meaning-based), and others to increase stress levels (e.g., active-avoidance).

Stress is pervasive in today's society. The sources of stress differ for each individual ranging from daily hassles, to major life events, to more pervasive factors such as discrimination and structural inequalities resulting in societal disadvantage. Increasingly there is an interest in how chronic exposure to stress can increase the risk for both physical and mental health illness.[24] Yet after many decades of attempting to determine the extent to which physiological and psychological processes are involved, defining and measuring stress remains a limiting challenge.

Methods of Measuring Stress in Epidemiological Research

There are three general approaches for assessing stress: an **environmental** approach which assesses the stressor (e.g., work-related stress), a **psychological** approach which assesses an individual's ability to cope or affectively respond to stress, and a **biological** approach which measures activation of the physiological systems responsible for the stress response.[25] The duration dimension of stress - acute versus chronic stress - is also important to take into account when designing a research question and method of assessment.

A multitude of questionnaires have been created to evaluate stress using the environmental and the psychological approach. An example of the former are checklists of common stressful life events designed for use in researcher-participant interviews or self-reported questionnaire and is a widely accepted method of assessing overall stress levels.[25] In-line with the psychological approach, the Perceived Stress Scale (PSS) was developed in the early 1980's by Sheldon Cohen, and is still commonly used in medical research. The PSS measures the degree to which individuals appraise their lives as stressful via questions asking the respondent to evaluate non-specific situations in their life in the last few months as unpredictable, uncontrollable, and overloaded.[26] The PSS has been used to evaluate the influence of stress on smoking behavior, cardiovascular disease, asthma, cancer, and many more health conditions. However, Lazarus & Folkman recommend that stress and coping methods should be measured in relation to a specific stressor,[21] and perhaps more than in any other field, researchers of infertility have taken this advice to heart. A number of infertility-stress specific questionnaires have been developed over the last few decades, including the Fertility Quality of Life (FertiQoL) tool [27], the Fertility Problem Inventory (FPI) [28], and the Fertility Problem Stress Scale

developed by the Copenhagen Multi-Centre Psychosocial Infertility Research Program (COMPI-FPSS) [29], the latter of which is used in Study II of this thesis.

However, collecting self-reported data in general is vulnerable to recall bias and the source of stress cannot be measured in an objective manner. Therefore, the biological approach to measuring stress in epidemiological research removes the potential of this form of bias but requires knowledge of the stress-related pathways and access to biological samples. The hypothalamic-pituitary-adrenal (HPA) axis is the main physiological pathway through which the stress response is mediated, and when activated by a physical or psychological stressor, the end result is the secretion of **cortisol**, a glucocorticoid, by the cortex of the adrenal gland. Chronic activation of this stress response pathway can lead to dysregulation of the HPA axis via damage to various brain structures and hormonal feedback systems,(reviewed in [30]). Chronically elevated levels of cortisol have been associated with impaired cognition, decreased thyroid function, lowered immune function, accumulation of abdominal fat, and lower levels of general health. [31, 32]

While there are many genes and hormones involved in the physiological response to stress , the most common hormone measured in stress research is cortisol.[33] Cortisol is a relatively easy hormone to collect and measure in saliva, blood, and more recently in hair, but it is not always as easy to interpret the results, as there is much intra-cortisol variation within an individual and much inter-cortisol variation within a population.[34, 35] However, more appropriate biomarkers for stress have yet to be established.

In addition to biological samples and questionnaire responses, **proxies for stress** can be used, particularly when population register data are available. For example, identifying stressful life events (e.g., death of a family member, divorce) can be assumed to produce an elevated level of stress for an individual over a period of time and then related to a specific later-emerging health issue.[36] Additionally, as the symptoms of psychological distress are also the symptoms of depression and anxiety, it is therefore plausible to use diagnoses of depression and anxiety to flag an individual with high levels of stress.

2.1.2   Depression and Anxiety Disorders

The core symptom of **major depressive disorder** (MDD) is persistent sadness or loss of pleasure which can be accompanied by specific somatic symptoms including disturbances in appetite, sleep, which results in problems with weight, fatigue, and lack of concentration. To fulfill the criteria for a diagnosis, the symptoms must cause significant distress or impairment of functioning and be unremitting for at least 2 weeks, yet episodes often last for several months.[37] MDD is an episodic illness and over a lifetime an individual may experience only a single episode, or suffer from recurrent episodes separated by periods of

normal mood. MDD can be extremely debilitating due to the psychological, emotional, social, and physical problems that accompany this disorder, and individuals will often feel worthless, guilty, a sense of dread, and may at its extreme, be at risk of harming themselves.

Across cultures, women are more than twice as likely to suffer from MDD than men.[16] Within women, rates of depression vary by age and increase with reproductive events such as puberty, the premenstrual period, pregnancy, the postpartum period, and menopause indicating an importance of hormones and biology in its etiology. However, biological differences cannot be the sole reason for the greater vulnerability of women to depression, as social roles and expectations, gender inequality, and vulnerability to domestic violence are potentially important risk factors.[30]

Anxiety is a common emotion which manifests itself through worries that something negative will occur and physical symptoms such as rapid heartbeat and feelings of tension. Anxiety disorders arise when anxiety becomes out of proportion to the situation. **Generalized anxiety disorder** is diagnosed when excessive, uncontrollable anxiety and worry occurs on more days than not for at least 6 months, and results in significant impairment in daily functioning with additional symptoms such as restlessness, irritability, sleep disturbances, muscle aches and tension, fatigue, and difficulty concentrating.[37]

The lifetime prevalence in the general population for major depression and anxiety disorders is between 16-30%.[38] Interestingly, genetic correlation between MDD and GAD is 100%, indicating that the same genetic factors influence the risk for both disorders, and that it is environmental factors that influence whether an individual develops one or the other, or both.[39] Symptoms of depression and anxiety have, even at low levels, been associated with adverse health outcomes, including increased risks of mortality from cardiovascular disease, cancer, and all-cause mortality.[31] As stress levels rise and rates of depression and anxiety diagnoses increase globally, it is important to consider the impact on general health, fertility included.

Antidepressants

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed class of antidepressants for treatment of depression and anxiety.[40] Treatment with SSRIs has increased both in general,[41] among women of reproductive age and during pregnancy,[42] with reports that 3% of women fill a prescription for an SSRI in the 3-month period before conception.[43, 44] Still, little is known about the effect of SSRIs on fertility.[45]

2.1.3   Effects on Fertility: Physiology to Epidemiology

There are many crucial steps between conception and delivery, and interference in any of these steps can result in the failure to achieve the delivery of a healthy live infant. Ovulation, fertilization, and implantation lead to pregnancy, where elective and spontaneous abortion and stillbirth can end it prematurely. Mechanisms by which stress and depression may impair fertility are likely to be multi-factorial.   While not fully characterized, evidence exists for the role of altered hormonal signaling pathways, impaired immune defense, antidepressant use and behavioral patterns associated with stress and depression (e.g., smoking, nutrition, alcohol use, sexual behavior) that may result in disrupted ovulation, early menopause, and possibly even spontaneous abortion.[46, 47]

Anovulation

The first physiological evidence that stress influences fertility was reported in 1939, when Hans Selye noted that the rat ovary atrophied when rats were exposed to various stressors including "excessive muscular exercise, inadequate diet, and toxic doses of different drugs".[48]   Since then, physiological research has had an emphasis on exploring the interactions between the HPA axis (which regulates the stress response) and the hypothalamic-pituitary-gonadal (HPG) axis which regulates reproductive function.   The intersection of the HPG – HPA axes may result in a reduced production of ovarian steroids required for follicular development and subsequent disruption to ovulation, thereby providing one mechanism through which stress could affect reproductive function.

Clinical studies of reproductive-aged women with depression have shown results consistent with a compromised HPG axis leading to anovulation.   Meller et al. (1997) found a significantly lower, dysrhythmic luteinizing hormone (LH) pulse frequency in women with depression compared to non-depressed women,[49] which was subsequently shown to result in lower ovulation rates and menstrual dysfunction.[50]   Additionally, a lifetime history of major depression was reported to result in higher follicle stimulating hormone (FSH) and LH levels and lower estradiol levels,[51, 52] which could potentially affect ovulation. Lastly, depression and depressive symptoms have been associated with changes in menstruation including secondary amenorrhea and irregular menstrual periods,[53, 54] which result from anovulation or oligoovulation.

A large prospective study in healthy women showed that high versus low daily stress influenced hormone levels: for each unit of increase in daily stress level the women had 70% higher odds of experiencing anovulation.[55]   In an early study, long and irregular menstrual cycles were more common amongst women with depression, and subsequently, long and irregular menstrual cycles were associated with a history of infertility.[56]

Early Menopause

Another potential mechanism by which stress and depression may decrease fertility is by causing earlier menopause.[57] The Harvard Study of Moods and Cycles found an association between a lifetime history of depression and an earlier transition to perimenopause.[51] Chronic lifetime psycho-social stressors including a personal history of abuse, recreational drug use, and/or family history of drug use, were found to be determinants of decreased ovarian reserve, while current stress was not.[58] A more recent study has shown that women with greater psychological stress experienced earlier reproductive aging measured by higher antral follicle counts (AFC) and a greater AFC decline, and were therefore at greater risk for difficulties conceiving.[59]

Of note, there is an increasing interest in the role of the immune system with respect to pregnancy and infertility. Elevated stress and depression decrease immune function [60] and has therefore the potential to act through pathways of the immune system to interfere with fertilization, implantation, and the maintenance of a healthy pregnancy.[61] However, the role of the immune system will not be discussed in this thesis.

Spontaneous abortion

Spontaneous abortion occurs in 10-15% of clinically recognized pregnancies.[62] Elevated stress during the pre-conception period and early pregnancy due to rocket attacks,[63] intimate partner violence,[64] economic crisis,[65] and an accumulation of stressful life events[66] have been associated with increased risk for spontaneous abortion. Yet, attempts to identify biological pathways by which this may occur have not been rewarding.[67] Additionally, antidepressant use for treatment of depression has been associated with spontaneous abortion in a number of studies,[47] however recent large population register-based studies have stressed that the severity of depression and comorbid mental health issues and the associated lifestyle factors are likely confounding this association.[68-70]

The Role of Lifestyle Factors and Behavior

Lifestyle factors, including cigarette smoking, alcohol use, very low and very high body mass index (BMI), have the potential to reduce the ability to become pregnant.[71] Women suffering from depression are more likely to be smokers,[51] and smoking is associated with an increased risk of spontaneous abortion,[72] as high daily caffeine intake [73, 74] and alcohol consumption.[75] Additionally, smoking is also one of the strongest risk factors for early onset of menopause. However, a substantial amount of the effect of depression on early menopause has been found to be independent of smoking.[51]

Pregnancies of women taking or having recently discontinued antidepressants have been reported to be different on almost all measured lifestyle factors including smoking, alcohol

intake, BMI, and the pregnancies were less often planned.[70] Women not attempting to become pregnant are less likely to be taking recommended folate supplementation. Since lower levels of maternal serum folate have also been associated with increased risk of spontaneous abortion,[76] this may be a mechanism through which increased pregnancy loss occurs.

Adolescents with a diagnosis of depression and other psychiatric disorders are more likely to report risky sexual behavior and the contraction of sexually transmitted infections which may have consequences on their future ability to conceive.[77]   Additionally, common symptoms of depression include decreased energy, self-esteem, and libido, which may reduce the ability to find companionship and desire to engage in sexual activity. Women with lifetime history of major depression have an increased risk of being divorced or separated,[51] which potentially provides less opportunity for attempting pregnancy.

Epidemiological studies where fertility is the end point

The terms *fertility* and *fecundity* refer to the capacity to conceive and to produce offspring, respectively. *Infertility* describes a couple who has difficulty conceiving or maintaining a pregnancy. *Fecundability* is a quantitative measure of a couple's capacity to conceive, defined as the probably of conceiving in one menstrual cycle. Fecundability is calculated for a population, based on a measure of the *time to pregnancy.* [78]

While evidence from physiological studies and clinical research suggest various pathways by which stress and depression may be able to affect female fertility, results from epidemiological studies with *fertility as the endpoint* are few and have been less conclusive in identifying the effect of depression. Each study in Table 1 has a different method of measuring fertility, and while age is generally accounted for, factors such as infertility in the male partner, antidepressant treatment, length of depression, are not.

Table 1: Epidemiological studies of fertility in women with depression

Author	N	Depression	Fertility Results
Ødegård, 1980 [79]	30,428 individuals with psychiatric diagnoses, Norway	Norwegian inpatient register diagnoses for unipolar depression and bipolar disorder 1936-1975	Number of children born not different from the general population.
Lapane et al., 1995 [46]	339 women, USA	Self-reported history of depressive symptoms	2-fold increase in risk for self-reported infertility, however adjustment for confounders results in a non-significant odds ratio
Laursen et al., 2010 [80]	2,819,941 individuals followed from 1970 – 2006, Denmark	Danish inpatient register psychiatric diagnosis of depression	Decreased incidence rate ratio of fertility for women with unipolar depression compared to general population IRR=0.57 95%CI (0.55, 0.60) Higher rates of induced abortion in women with psychiatric disorders found, however this did not account for the decreased fertility
Lynch et al., 2012 [81]	339 women attempting to conceive, United Kingdom	Self-reported current psychosocial stress, anxiety, depression	Not associated with becoming pregnant
Power et al., 2013 [82]	2.3 million individuals born 1950 to 1970, Sweden	Swedish inpatient register diagnoses of depression	Women with depression had similar fecundity, defined as the number of children, as the general population

### 2.1.4 Infertility and Assisted Reproduction

Assisted reproduction techniques (ART) provide a unique opportunity to study human reproduction in ways that are impossible to do in a more 'spontaneous' setting. As discussed, anovulation is an important mechanism by which stress and depression may lead to decreased fertility in women. Infertility treatments attempt to overcome the issue of anovulation via ovarian stimulation and ovulation induction. Overcoming a diminished ovarian reserve and early menopause is a harder feat. If it can be shown that stress and depression do not lead to further difficulties in becoming pregnant and delivery of an infant after ovarian stimulation procedures, this provides additional evidence for the main influence of stress on fertility to be via anovulation.

Globally, it is estimated that up to 30% of couples attempting to become pregnant will experience infertility, defined as the inability to conceive following one year of unprotected intercourse.[83] In both more-developed and less-developed countries, only half of these couples will seek medical care, and only around 20% will receive infertility treatment.[84, 85]

In Europe, treatment seekers are more likely to be better educated, employed in a higher status occupation, and to have decided to become parents later in life, [85] even in countries like Sweden where infertility treatment is publically funded.[86] The latest European report on ART in 17 EU countries reports that in 2011, 361 972 ART cycles were performed, corresponding to 1269 cycles per million inhabitants. For IVF cycles (including Intra-cytoplasmic sperm injection; ICSI), the clinical pregnancy rate per aspiration was 28-29% and per embryo transfer was 32-33%. Results from Sweden were comparable to those in the rest of Europe, with a final live infant delivery rate of approximately 24% per cycle.[87]

Lifestyle factors including cigarette smoking, alcohol use, and BMI, have the potential to significantly reduce the ability to become pregnant via ART but the most important factor is the woman's age.[71] Still, it is important to recognize that infertility occurs within a couple, and that the cause of infertility at least half the time is attributable to male issues. The psychological aspects of male fertility are understudied, as reviewed by Hall et al., (2012).[88] However, this will not be addressed in the studies in this thesis.

Figure 5: Summary of the steps in an ART treatment cycle
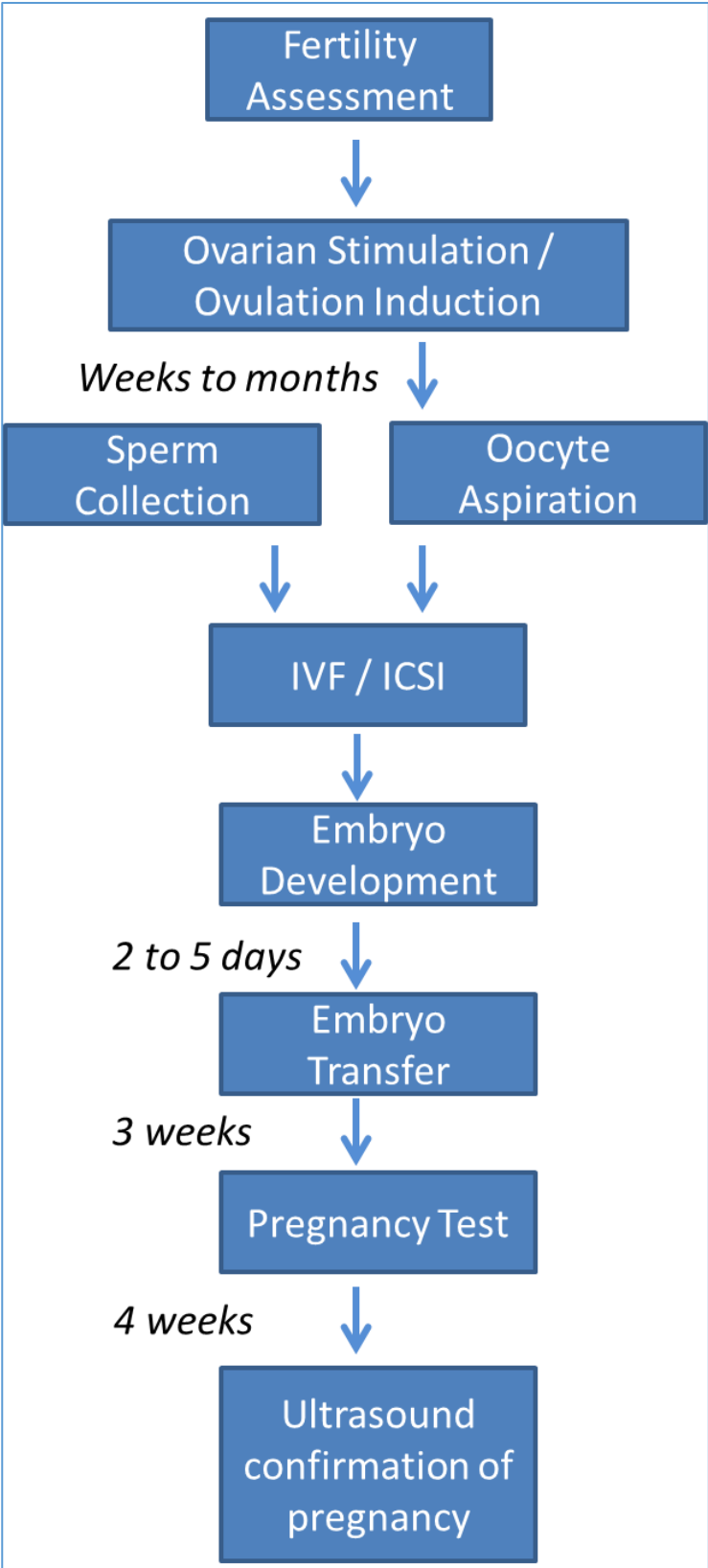
Psychological aspects of infertility

The World Health Organization recognizes that both women and men suffer considerable psychological distress when experiencing reproductive health issues, including low self-esteem, isolation, loss of control, sexual inadequacy, and depression.[89] Furthermore, the psychological impact of infertility has been shown to be similar to other chronic medical conditions such as cancer, cardiac rehabilitation and hypertension.[90]

When young adults attending universities in Sweden and the US were polled by a Swedish researcher regarding their future family planning strategy, they stated that they want to begin having children between the ages of 30 and 35, to have 2-3 children within a stable relationship and with stable financial resources, and that the number one priority was to have a genetically related child conceived within the relationship. Most also believed that they would be able to have children when they want to, and overestimated both the age at which female fertility starts to decline and the efficacy of infertility treatment.[91, 92] When unexpected infertility is revealed to individuals who have delayed childbearing until their mid-30s, individuals transition quickly from having a sense of being in control to feeling a loss of control with limited time to solve the problem.

Infertility is a complex existential crisis within a couple, beginning with shock and disbelief and leading to frustration and depression. Each month becomes an emotionally exhausting rollercoaster where the first half is met with hope of pregnancy, and the second – after menstruation begins - with grief and distress.

Further, ART procedures encompass many different stressful procedures within a single cycle (Figure 4), potentially including daily self-injection with hormonal drugs, retrieval of oocytes via transvaginal ovarian puncture, and transfer of the cultured embryo to the uterus. Patients then have to wait up to 3 weeks to determine if the treatment has successfully resulted in pregnancy, and another 4 weeks to confirm it by ultrasound. Additionally, couples must cope with their infertility diagnosis, balance a career and marriage while undergoing these highly invasive and time consuming infertility treatments, face potential financial burdens of treatment, and experience the recurring cycle of hope, anxiety, and disappointment that accompanies each ART cycle.[93] Retrospective studies have identified that the psychological burden of ART to be the most common reason for treatment cancellation.[94-96]

Psychosocial factors associated with poor emotional adjustment to infertility are neuroticism, high self-criticism, escapist coping strategies, and marital dissatisfaction, while factors associated with good emotional adjustment are optimism, problem focused coping strategies, marital satisfaction, and social support.[97] It has been found that the relationship of the couple can be either strained or strengthen by dealing with infertility.[98, 99]

Anecdotal evidence of the negative effects of stress on fertility is widespread in stories of spontaneous conception in infertile couples when the strain to continue trying to conceive lessens, such after adopting a child or after discontinuing infertility treatment. Generally, this phenomenon is not well explored, although one study has reported that spontaneous pregnancy and subsequent live birth occurred in 24% of couples who remained childless after their ART attempts. These findings were higher amongst younger women with unexplained infertility who discontinued ART after fewer cycles, indicating that time, not stress, is likely the key factor behind these findings.[100, 101]

Prevalence of Depression and Anxiety in Women undergoing ART

It is not surprising that a multitude of studies have been published reporting higher stress levels and higher prevalence of symptoms of depression and anxiety among infertile women receiving fertility treatment compared to the general population.[102-104] Nor is it surprising that the prevalence of these symptoms increase in women undergoing repeated IVF cycles after failed attempts.[105, 106] However, the prevalence of diagnosable depression and anxiety disorders in women undergoing ART is lower than expected. A Swedish study assessing psychiatric disorders in women undergoing ART reported a prevalence of 11% for major depression and 15% for generalized anxiety disorder, and that the majority of cases were undiagnosed at time of the study.[104] Further, two Nordic registry-based studies suggest a possible 'healthy patient effect' in women undergoing infertility treatment after finding that women undergoing ART were less likely to have been hospitalized for psychiatric conditions, including depression, prior to infertility treatment compared to the general population[107] and the incidence rate of first and recurrent depression diagnoses was significantly lower.[108] One potential reason for this is that depression has been reported as an obstacle to seeking medical care for infertility,[109] and could therefore result in overall lower rates of depression and anxiety in women that do undergoing ART.

The Influence of Stress, Depression, and its treatment on ART outcome

Women with infertility to a large extent believed that stress had a role in their inability to conceive,[110] and patients undergoing infertility treatment are very often concerned that stress may decrease the likelihood of becoming pregnant.

In 2011, two meta-analyses combining over 30 prospective studies assessing the associations between psychological distress in women and ART outcomes were published. Boivin et al., [103] reported that emotional distress (measured by depression or anxiety) prior to beginning ART was not associated with pregnancy outcome, whereas the larger meta-analysis by Matthiesen et al., [102] found a small but significant negative association

between depression symptoms and clinical pregnancy. Authors of the latter study noted that their results were limited by large between-study heterogeneity, and that mean age, duration of infertility and percentage of first time ART users were found to moderate the associations.

While there are recommendations from governing bodies and requests from patients for psychological care during the ART process,[111] many clinics do not routinely provide psychosocial care to their patients. This is despite a recent meta-analysis showing that psychosocial interventions for couples in infertility treatment, cognitive behavioral therapy (CBT) in particular, is effective in both reducing psychological distress and potentially improving clinical pregnancy rates.[112]

Further, there is a large gap in our knowledge regarding antidepressant use in women undergoing infertility treatment including the prevalence of use, the efficacy in treating depression, and the risks, if any, associated with their use on ART outcome. A recent in vivo study has demonstrated that a specific SSRI, fluoxetine, has an influence on the timing of the developmental stages in human embryos.[113] Timing of different developmental stages is associated with embryo quality and with implantation rate,[114] and thereby providing a mechanism by which SSRI use could potentially influence the outcome of infertility treatment. However, clinically it remains unknown if antidepressant use influences embryo quality, implantation and pregnancy rate.[45]

Four small studies have previously been published on this topic, with no definitive conclusions. Two retrospective chart reviews found no statistically significant differences in pregnancy rates in women taking SSRIs and those not taking SSRIs.[115, 116] A double-blinded study of 152 women randomized to fluoxetine or placebo at the start of their IVF cycle found no difference in pregnancy and live birth rates between the two groups, however treatment was limited to only 26 days.[117] In a second such study, 140 couples in whom at least one partner was diagnosed with depression were randomized into three treatment groups: CBT, CBT plus and SSRI, or no treatment. While depression scores decreased and pregnancy rates were overall higher in the treatment groups, the pregnancy rates per treatment group were not reported.[118] Thus, there is a clear need for more research into the prevalence and consequences of antidepressant use in women undergoing ART.

Additionally, clinical pregnancy and live birth rates are the most common outcomes reported in studies investigating the association between stress and ART. Fewer studies assess the quality of oocytes and embryos, despite the valuable contribution these results can make to elucidating potential physiological mechanisms by which stress may influence reproduction.

2.2 AIMS

The aims of the first two studies in the thesis were:

- **Study I:** To investigate the association between depression, anxiety, and treatment with antidepressants and IVF cycle outcome including pregnancy, live birth, and miscarriage.

- **Study II:** To examine the influence of multiple measures of stress on indicators of oocyte and embryo quality and IVF cycle outcome.

2.3   MATERIALS AND METHODS

Table 2: Summary of Data Sources and Analysis Methods for Studies I and II

Population	Data Sources	Exposure / Outcome	Study Design and Analysis Method
Study I: **Depression, anxiety, and antidepressant treatment in women: association with in vitro fertilization outcome**			
Nulliparous women undergoing their first recorded IVF cycle, January 2007 to December 2012 (n = 23,557)	The National Quality Register of Assisted Reproduction (Q-IVF) Swedish Population Registers: • National Patient Register • Prescribed Drug Register • Medical Birth Register • Total Population Register • Longitudinal Integration Database for Health Insurance and Labor Market Studies See Figure 5	*Exposure:* • Diagnosis of depression and anxiety in the 2 years prior to IVF cycle start • antidepressant dispensation in the 6 months prior to cycle start *Outcome:* • pregnancy • livebirth per cycle • live birth per pregnancy • miscarriage	Cohort Study, cross-sectional data Associations evaluated using logistic regression, adjusted for: • age • country of birth • psychosis
Study II: **Perceived stress, infertility-related stress, and cortisol levels in women: influence on embryo quality and in vitro fertilization cycle outcome**			
Women receiving infertility treatment in the greater Stockholm region recruited September 2011 to December 2013 and followed to December 2014 (n = 485)	The Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study • baseline questionnaire (demographics, health history and lifestyle) • Clinical medical records for IVF cycles from fertility clinics • Saliva samples for cortisol measurement	*Exposure:* • Perceived Stress Scale score • COMPI Total Fertility Stress Scale • Morning and evening salivary cortisol *Outcome:* • Oocyte Aspiration • Embryo Transfer • Clinical Pregnancy • Oocyte Maturation ratio • Embryo Utilization ratio • Embryo Quality ratio	Cohort Study, cross-sectional data Associations evaluated by logistic and linear regression, and adjusted for: • age • BMI • education, smoking • alcohol use • daily caffeine intake • shiftwork • night work

2.3.1 Data sources: Swedish National Registers and UppStART

Swedish National and Quality Registers

Study I utilizes data from multiple population based registers and one quality health register in Sweden (Figure 5). Sweden has a long history of collecting population statistics, with the first parish registries dating back to 1749. The personal identity number (*personnummer*) was introduced in 1947 and from that point onward every individual residing in Sweden has been assigned a unique registration number and recorded in the Total Population Register either at birth or upon immigration. This personal identity number enables the link of population registers at the individual level and is the keystone of epidemiological research in Sweden.[119] Statistics Sweden (*Statistiska centralbyrån*) is the governmental agency responsible for maintaining these registers, and collects data from register holders, such as the National Board of Health and Welfare (*Socialstyrelsen*), the quality registries, and the Swedish Tax Agency (*Skatteverket*). Researchers at Swedish universities are granted access to de-identified data for research following approval from an ethical review board.[19]

The National Quality Register of Assisted Reproduction (Q-IVF; Nationellt kvalitetsregister för assisterad befruktning; http://www.medscinet.com/qivf/) was initiated in 2007 and collects data on all IVF and ICSI treatments for the purpose of monitoring treatment outcome and any medical risk for both the couple undergoing treatment and any subsequent children. Prior to 2007, data on assisted reproduction was only collected for women who delivered live infants.

The *Total Population Register (Statistics Sweden)* was established in 1967 and contains demographic data including sex, birth date, country of birth, civil status, dates of migration, and place of residence for all residents of Sweden born after 1932.

The *National Patient Register (National Board of Health and Welfare)[120]* contains diagnosis codes classified according to the World Health Organization's International Classification of Diseases: ICD-8 (1969-1986), ICD-9 (1987-1996), ICD-10 (1997-present). Diagnoses codes are assigned by the treating physician upon discharge from the hospital. Data on outpatient hospital-based care are available from 2001 onward.

The *Prescribed Drug Register (National Board of Health and Welfare)*[121] was established in 2005 and contains complete national data on all pharmaceutical dispensed on prescription. Dispensations are recorded according to the Anatomical Therapeutic Chemical (ATC) index, with the date of dispensation, the dose, and quantity.

The *Database for health insurance and labor market (Statistics Sweden)* is a longitudinal integrated database and since 1990 contains information from the labor market, educational and social sectors for all individuals registered in Sweden 16 years of age and older.

The *Medical Birth Register (National Board of Health and Welfare)*[122] covers 99% of all births in Sweden since 1973, with data collected from antenatal care clinics, delivery units and pediatric examination of the infant.

Figure 5: Flowchart of the derivation of the study population from a linkage of the national registers
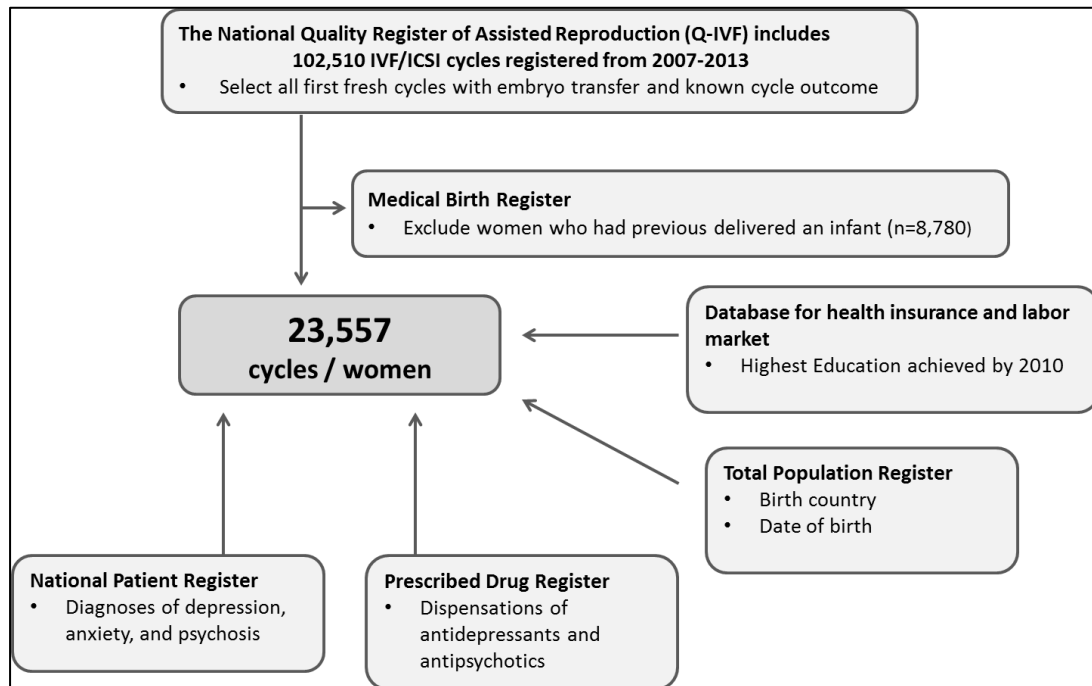
The UppStART Study

The Uppsala-Stockholm Assisted Reproductive Techniques (UppStART) study is a prospective cohort study of couples undergoing infertility treatment in the greater Stockholm region. The UppStART study was initiated with multiple aims; one being to investigate possible risk factors for pregnancy rates and other ART outcomes.

Participants were recruited from three of the four fertility and reproductive health clinics (one public, two private) in Stockholm and one private clinic in Uppsala County, which serves a large volume of patients from Stockholm. Women and their partners were approached by the clinic nurse and asked to participate. To facilitate the process of informed consent, the participant was provided with information approved by the regional ethics board, both verbally and in written format about the purpose of the study, design, methods, and possible risks of participation. Requirements for inclusion in the study were an understanding of the Swedish language and being part of a heterosexual couple. At the time when informed consent was given, the clinic nurse withdrew blood samples and provided with participant with a kit for at home saliva collection. The participants were asked to answer an extensive web-based questionnaire within a few days of their clinic visit and prior to their IVF treatment start, which included questions on sociodemographic, anthropometric and life-style factors. Recruitment took place from September 2011 to December 2013 and participants were followed until December 2014.
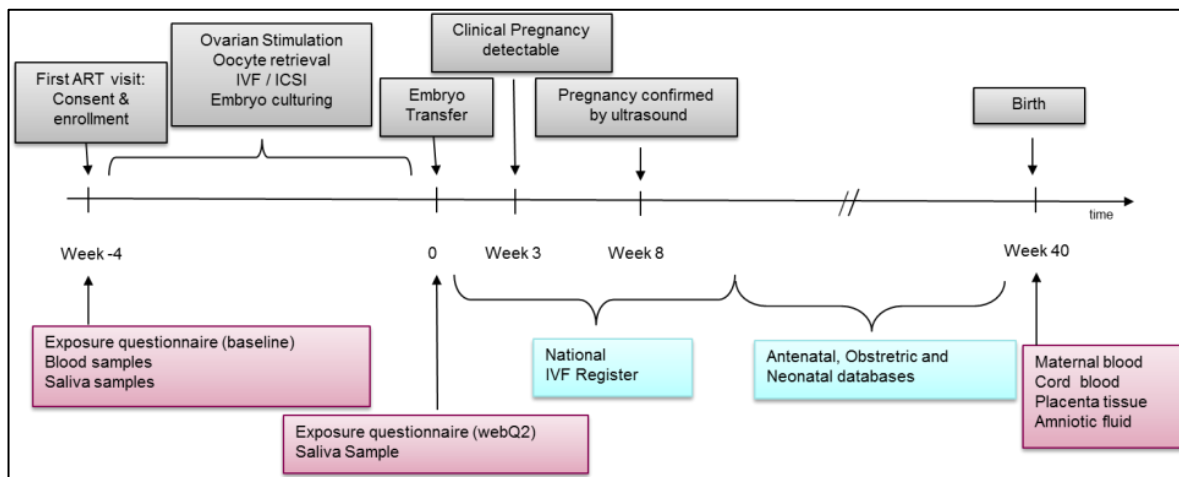
Figure 6: UppStART study timeline of participant data collection

Signed consent forms were sent to the UppStART research nurse at Karolinska Institute (KI) who monitored recruitment and questionnaire responses, reminding participants via email 2 weeks after signing the consent form to answer the baseline questionnaire if they had not done so. A second reminder by email was sent two weeks after the first, and if participant still had not completed the online questionnaire, the research nurse attempted to contact

them by phone. No other attempts were made after the telephone call and participants were marked as non-responders.

Saliva samples

Participants were provided with two pre-labelled Salivette® (Sarstedt, UK) kits, a pre-paid envelope for the return of samples to the Karolinska Biobank, and saliva collection instructions: samples were to be collected at home at 7:00 (upon awakening and before breakfast and teeth brushing) and at 21:00 the same day, with no eating, drinking, gum chewing, snus use, or smoking in the 30 minutes prior to collection.   Saliva samples were returned to the KI Biobank by the participant via post, with a Sample Identification Form on which the participant wrote the date and time of sample collection and their personal identification number.   The KI biobank recorded this information, provided it to the UppStART database administrator, and was linked to the study participant id in the database. Samples were stored at the KI Biobank at -80°C until analysis.

Creating the UppStART database

The UppStART **questionnaire** was created by a database developer with the Department of Medical Epidemiology and Biostatistics using LIME, an Oracle Management Software. When participants logged into to the questionnaire system for the first time, they were requested to provide their Swedish personal identification number, from which their signed consent form could be identified, and the system assigned them a study participant identification number. All data within the study participant's **medical record** were collected from the four participating clinics by data extraction from clinical software and secure transfer of the files to the UppStART database administrator.

Dataset preparation

Study II is the first study to use the data collected by the UppStART study. The first and largest challenge was to create a dataset for analysis. Hundreds of data variables were provided by the four clinics, which used two different types of clinical software, and consequently the data were in different formats. Variables were often difficult to identify from the label alone. In consultation with clinical staff, codebooks were created and the data required for analysis were identified. In the case where both the questionnaire and clinical data collected the same type of data on the participants (e.g., smoking status, reason for infertility, BMI) it had to be decided which data was best to use, especially in cases where the data from the two sources differed. For example, self-reported smoking may be more reliable than smoking status reported to clinical staff because of the stigma surrounding smoking, especially in the setting of infertility treatment. Conversely, clinic measured height and weight may be more reliable than self-reported measurements.

Exposures

Salivary Cortisol

Saliva cortisol levels were measured by radioimmunoassay (Orion Diagnostic, Finland) by the Study Center of Laboratory Medicine at the Karolinska University Laboratory, and data returned for inclusion in the UppStART database. In consultation with laboratory staff with expertise in cortisol measurement, morning cortisol values above 100 nmol/L (n=2) were removed.

Perceived Stress Scale (PSS)

The 10-item version of the PSS,[26, 123] translated into Swedish was used to assess the degree to which the participants perceived their lives as unpredictable, uncontrollable and overloaded in the previous 3 months.

Perceived Stress Scale

10 item version

How often in the last 3 months have you:

1) Become upset about something that happened unexpectedly?
2) Felt that you had no control over the important factors in your life?
3) Felt nervous and stressed?
4) Felt that you could not handle everything that needs to be done?
5) Become angry about things that have happened and that were beyond your control?
6) *Felt confident in your ability to handle your personal problems?
7) *Thought that things have developed that you wanted?
8) *Felt that you had control of irritating moments in your life?
9) *Felt that you had control over things?
10) Felt that the problems have become so numerous that you could not overcome them?

Response key: (1) Never; (2) Rarely; (3) Often; (4) Very Often
*Reverse score for these four 'positive' items

Fertility Problem Stress Scale

UppStART participants responded to the questions in an infertility-specific stress questionnaire developed within a sample of 1,169 infertile Danish couples as part of the Copenhagen Multi-Centre Psychosocial Infertility (COMPI) research program.[47] The COMPI Fertility Problem Stress Scale (COMPI-FPSS) measures the strain related to infertility produced in the personal, social and marital domains. Items from all subscales are summed to produce a total score.

Outcomes

All outcome variables were derived from data in the participant's medical record.
The binary (yes/no) outcomes were:

- Whether or not a participant reached the **oocyte aspiration** step of the treatment;
- If yes, then whether the participant underwent an **embryo transfer**;
- If yes, then whether or not **clinical pregnancy** was achieved, defined by a clinically confirmed pregnancy by ultrasound;
- Clinical pregnancy per cycle started.

All oocyte and embryo quality outcomes were continuous:

- **Oocyte Maturation ratio**
  = # of oocytes used for the IVF/ICSI procedure / the total number of aspriated oocytes

- **Embryo Utilization ratio**
  = # of embryos transferred or frozen / the total number of embryos created

- **Embryo Quality ratio**
  = # of top and average quality embryos / the total number of scored embryos
  Embryo score of 'top', 'average', or 'poor' was determined using criteria set at the The Istanbul Consensus Workshop on Embryo Assessment [124] for a subset of the cycles where embryo parameter data were available.

2.3.2 Cohort Studies and Regression analysis

Cohort Studies

Conducting a cohort study begins by selecting a study population and identifying the exposed and the unexposed. All individuals in the cohort are then followed until a point in time i.e., the occurrence of the outcome or censoring (e.g. the end of a study period). Association between the outcome and exposure is estimated via regression modeling where it is possible to adjust for potential confounding variables. It is possible to use the data in a cohort study in a cross-sectional manner, ignoring the aspect of time, as in Study I and II.

Linear regression

A linear regression model is commonly used for cross-sectional data when the outcome is measured as a continuous variable. Linear regression models attempts to fit the observed data to a linear equation (i.e., $y = a + bx + e$, where a and b are regression coefficients, and e is the error term), and estimates the mean value of the outcome (y) for each value of the exposure (x). Assumptions of linear regression analysis are:

- Linearity – there is a linear relationship between x and y
- Independence – the observations in the sample are independent
- No multicollinearity – variables in the model are independent from each other
- Normality – the errors follow a normal distribution
- Homoscedasticity – the variance is constant; i.e., the variability of the distribution of the y values in the population is the same for all values of x.

Linear regression analysis produces the b coefficient which is the mean difference of the outcome for a given level of the exposure. The standard error of b can be used to calculate 95% confidence intervals for the mean difference.

Logistic Regression

When the outcome is measured as a binary variable, a logistic regression model can be used to measure the association. The model assumes that the log of the odds of the outcome has a linear relationship with the exposure variables, and the estimated regression coefficients are log odds ratios. The exponentiated coefficient – the odds ratio (OR) is most often presented. The OR is the odds of the outcome in the presence of the exposure, compared to the odds of the outcome without the exposure. An OR value above 1 indicates an increased odds of having the outcome, and a value below 1 indicates a decreased odds of having the outcome. Both linear and logistic regression models can be adjusted for potential confounders, which allow for estimation of the effect within levels of the confounder and produces an average effect over those levels.

2.4   STUDY RESULTS AND EPIDEMIOLOGICAL CHALLENGES

2.4.1   Study I: Depression, anxiety, and antidepressant treatment in women: association with in vitro fertilization outcome

Results

- Of the 23,557 women undergoing an IVF cycle, **4.4%** (n=1,044) had been diagnosed with depression/anxiety and/or had a dispensed antidepressants before IVF cycle start. Overall their odds for pregnancy (adjusted OR=0.86; 95%CI 0.75, 0.98) and live birth (adjusted OR=0.83; 95%CI 0.72, 0.96) were decreased compared to women with no diagnosis or dispensation.

- Women with a prescription for an **SSRI only** (n=829) had no statistically significant associations with any of the outcomes.

- Women with **non-SSRI antidepressants** (n=52) had lower education, were more often from a non-Nordic birth country, and were more likely to have a diagnosis of a psychotic psychiatric disorder and/or dispensations of anti-psychotic medication. They were at reduced odds of pregnancy (adjusted OR=0.41; 95%CI 0.21, 0.80) and live birth (adjusted OR=0.27; 95%CI 0.11, 0.68). Those that became pregnant had a higher risk for miscarriage (adjusted OR=3.56; 95%CI 1.06, 11.9).

- Women with a depression/anxiety diagnosis with **no antidepressant** (n = 164) were more likely to have an ICD code reflecting a more severe depression diagnosis, and had reduced odds of pregnancy (adjusted OR=0.58; 95%CI 0.41, 0.82) and live birth (adjusted OR=0.60, 95%CI 0.41, 0.89).

Internal Validity

Use of the national register reduces many types of bias including bias produced by loss to follow up or non-responders, and recall bias by participants.   However, their use can produce some forms of information bias.

Ascertainment bias

- Acertainment bias is produced when the cases gathered do not represent the cases that originate in the intended study population and is a result of many possible sources, including health care access/utilization bias. When using national registers, only those that have sought out hospital-based specialist care will have a diagnoses registered in the National Patient Register. Therefore those not seeking help and cases of depression or anxiety treated by general practitioners or other health care providers will not be represented in the study. Indeed, 4.4% of the study participants were either treated with antidepressants or had a diagnosis of depression or anxiety. This is lower than

estimates reported of 10% and 14% for major depression and generalized anxiety disorder in women undergoing ART in Sweden.[104] This implies that there are women in the healthy reference group with depression and anxiety (i.e., misclassification) which could lead to an underestimation of the associations.

- A prescription for an antidepressant does not guarantee that the medication was actually consumed, which could lead to women with a dispensation being misclassified as exposed. Women planning to become pregnant are often conscientious about lifestyle factors that could affect conception [125], and they may decide to discontinue their medication directly before or during their IVF cycle.

- To account for the possibility that women discontinue taking their antidepressant medication when starting an IVF cycle, we restricted the SSRI-only group to women who had both an SSRI dispensation in the 6 months before cycle start AND an SSRI dispensation in the 3 months after cycle start (n=438; 52% of the SSRI-only group), and assumed that this group was taking their antidepressant throughout their cycle. When compared with the reference group, the odds were significantly lower for cycles to result in pregnancy (n=148; adjusted OR=0.80, 95%CI 0.65, 0.98) and live birth (n=94; adjusted OR=0.73; 95%CI 0.60, 0.93), which was not seen in the main analysis.

Reverse causation

- While the goal of this study was generally unconcerned about the cause of the depression or anxiety, we did conduct a sensitivity analysis where we excluded the women who had a depression or anxiety diagnosis *after* an infertility-related diagnosis in the National Patient Register (n=182; endometriosis, PCOS, or infertility) to account for the possibility that the infertility was the cause of the depression or anxiety diagnosis. The odds ratios for pregnancy did not change significantly from the main analysis.

Confounding

- The regression models were adjusted for age at cycle start, birth country, and evidence of psychosis, but we were unable to account for potential confounding of specific infertility diagnoses. Some causes of infertility, such as endometriosis and PCOS, are associated with increased rates of depression. Further there was no available data to adjust for lifestyle factors such as smoking and BMI that are important for fertility, and also may be increased in individuals with depression.

- Initially, the aim of this study was to investigate the association between antidepressant use and IVF outcome. However it quickly became evident that depression and anxiety needed to be accounted for in the study due to *confounding by indication* – a type of confounding where the disorder underlying the need for the medication may be the source behind the association between drug exposure and outcome. In this study, women with dispensations of non-SSRI antidepressants had reduced odds for pregnancy

and livebirth. As non-SSRI antidepressants are prescribed for more complex cases of depression and because this group of women had more evidence for psychosis, it is suspected that the severity of their diagnosis is likely to be behind these findings. In support of this, women with diagnoses without any antidepressant dispensations were considered separately, and were found to have decreased odds of pregnancy and livebirth, indicating that the underlying disorder does have an influence on the outcome, independent of the medication.

2.4.2 Study II: Perceived stress, infertility-related stress, and cortisol levels in women: influence on embryo quality and in vitro fertilization cycle outcome

Result Summary

- The mean age of the participants was 33.8 years. The majority of women had a BMI within the normal category, were non-smokers, had achieved a university level education, had moderate physical activity levels, and low to moderate daily caffeine intake. Infertility was most frequently due to combined or unknown cause, and 20% of the women had at least one previous child.

- After IVF cycle start, 10.3% of women did not progress to an oocyte aspiration procedure. These women presented with higher BMI were more likely to have female factor infertility and had fewer follicles after stimulation compared to those women that underwent aspiration.

- Of the women who did have an oocyte aspiration, 90% (n=391) underwent subsequent embryo transfer. Pregnancy rate per embryo transfer was 32.9% (n=129) and 26.6% per IVF treatment started for all women in the study.

- Of the four measures of stress, scores on the two questionnaire-based methods (PSS and COMPI-FPSS) were positively and significantly correlated to each other, but not with cortisol levels.

- No measure of stress was associated with whether a woman had an oocyte aspiration, embryo transfer, or pregnancy.

- Stress measures were not associated with oocyte maturity rate or the embryo utilization rate. There seemed to be a negative trend between mean embryo utilization rate and self-reported stress, with the lowest mean values in the highest PSS and COMPI-FPSS categories. For the subset of 145 women with quality scored embryos, there seemed to be a similar trend where increasing levels of self-reported stress were associated with decreasing mean embryo quality ratio.

Internal validity

Volunteer bias

- a study about stress may attract study participants who feel more stress or are inclined to believe that stress will affect their fertility. However, the UppStART study was

presented as a study of general lifestyle factors and may not have influenced the decision to participate in this manner. More likely is the converse situation, where those women that were most stressed were less likely to take on the burden of participating in a research study. If this is true for this study, an underestimate the effect of stress on the ART outcomes would be the result. While some clinics kept record of the number of women who declined to participate in UppStART, there is no information available to determine characteristics of these women.

Non-responder bias

- Of the total number of women who gave consent to participate in UppStART, 14% did not respond to the baseline questionnaire. Linkage of the UppStART database to the national registers currently being undertaken to determine if any demographic differences exist between these non-responders and the participants that did respond to the questionnaires.
- For 34% of the women, no saliva samples were provided. Compared to women who provided samples, women with missing samples had lower rates of oocyte aspiration, but did not differ in stress levels measured by the baseline questionnaire (PSS and COMPI-FPSS).

Lost to follow-up

- For 29 women (6%), clinical medical records were not retrieved and outcome data was therefore not available, leading to their exclusion from the study population. However, this loss of follow up was not related to participant exposure or outcome status, and it is unlikely to bias the study findings.

Information bias

- Multiple measures of stress were used to capture different aspects of stress. Both questionnaire methods (PSS and COMPI-FPSS) had good Cronbach's alpha scores (0.82 and 0.86, respectively) in the UppStART study data. Cronbach's alpha measures the internal consistency of the items in the scale and these scores indicate that the scales were appropriate to use in this study population.
- The COMPI-FPSS has been evaluated in relation to other fertility-stress questionnaires and found to offer practical advantages compared to the FPI and FertiQoL because it has considerably less items, is quick to complete, and fulfils our research goal of accurately measuring infertility-related stress.[126] Additionally, while developed in Denmark, a cross-cultural validation study including data from the UppStART study, concluded that the COMPI-FPSS is reliable and valid for use in Swedish populations.[29]
- Circadian rhythm dictates that cortisol levels peak in the morning, decreasing throughout the day, and plateau by around 18.00 until they beging to rise again in the

early hours of the morning. UppStART participants were instructed to collect morning samples between hours of 07:00 and 09:00, and 82% of the participants did so. The main analysis included all cortisol measures, and a sensitivity analysis was conducted including only the 82% (n=266). Results from the crude and adjusted analyses did not significantly differ from the main analysis.

- Salivary cortisol is considered a reliable correlate of the unbound biologically active cortisol concentrations circulating in blood.[33] However, salivary cortisol is ideally used as an acute measure of stress and is a hormone with large intra- and inter-individual variation in response to stressful situations and is influenced by lifestyle factors, exposure to chronic stress, and genetics.[34, 35] As we are interested in the effect of longer-term stress on IVF outcome, salivary cortisol is not an ideal measure. New techniques of extracting cortisol from hair have emerged since this study was designed and implemented, which may provide a better way of measuring the influence of longer term cortisol levels on IVF treatment outcome.

Recall bias

- The majority of the questions in the UppStART baseline questionnaire asked about lifestyle during the previous three months, and are therefore at the risk of recall bias. Women experiencing infertility and planning to undergo infertility treatment often make changes in their lifestyle based on what they believe will maximize the chances of conception [125], which may make them be able to respond to the questions more accurately.

- No recall bias is present for the outcomes since they were extracted from the participant's medical records.

Confounding

- The regression models were adjusted first for just age (Model 1), and then for age, BMI, education, smoking, alcohol use, daily caffeine intake, shiftwork, and night work (Model 2). Estimates from the two adjusted models differ very little from each other and from the crude analysis. However, none of these covariates were associated with both the exposures and outcomes, and may therefore not be true confounders.

- There is a large degree of homogeneity in the UppStART sample of women, with generally low rates of factors known to affect fertility and IVF outcome (e.g., high BMI and smoking), and confounding of lifestyle should therefore not be a major concern. As with the known covariates, we speculate that the study sample is also likely to be relatively homogeneous with respect to prevalence of any unmeasured confounders.

2.5   CONCLUSIONS

In Study II, women with the highest levels of stress did not have any difference in IVF outcomes compared to women with low to moderate stress. In Study I, only women with the most complex and severe cases of depression had reduced odds of pregnancy and livebirth following an IVF cycle.

Previous research has demonstrated that anovulation is an important mechanism by which stress and depression may lead to decreased fertility in women. While the biological pathways behind this are not clear, assisted reproduction techniques overcome anovulatory infertility through ovarian stimulation procedures. Neither Study I nor II include data on ovulatory status of the women in the study. However results from these studies indicate that there is no additional effect of stress and depression once the IVF cycle begins, except when the depression and psychiatric burden is severe.

In the cases of more severe depression, mechanisms behind the failure to achieve pregnancy and live birth via IVF remain unknown, with only weak evidence from Study I that increased rates of miscarriage may be a contributing factor. However, unhealthy lifestyle factors associated with depression may be behind the lower pregnancy rates from IVF cycles. Such data were not available in the national registers for Study I, and in Study II where rich lifestyle data were available, very few cases of depression were reported, and thus this hypothesis could not be further explored.

In combination with the majority of other studies that also finding very little influence of stress and depression on IVF pregnancy rates, our results are potentially reassuring to women undergoing infertility treatment and their clinicians. Study I is the first large-scale study to investigate the influence of antidepressants on IVF outcome. While replication of these findings is needed in other populations, results are reassuring for those who are concerned about taking medication during their treatment cycles, and highlight the importance of identifying and treating depression in women.

ART provides a unique opportunity to study fertility and human reproduction. However, those that are most stressed or most depressed are less likely to seek medical care for their infertility [109] and if they do, they have higher rates of drop out from treatment.[94-96] Therefore, population samples of ART women are not representative of all women with respect to stress and depression levels, and the true influence of stress and depression on fertility may not be detectable in this setting.

3 PART II: POLYCYSTIC OVARY SYNDROME AND PSYCHIATRIC DISORDERS

3.1 BACKGROUND

3.1.1 Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women, and is characterized by symptoms of hyperandrogenism (e.g., hirsutism, adult acne, abdominal obesity), oligoovulation or anovulation, and polycystic ovaries.[127] Depending on the diagnostic criteria used, the prevalence of PCOS varies from 5 to 15% in women of reproductive age.[128] PCOS is often accompanied by insulin resistance and related co-morbidities including obesity, Type II diabetes mellitus, metabolic syndrome, and hypertension.[129, 130]

PCOS was first described in medical literature in 1935.[131] However, there was little interest in this syndrome for most of the 20th century. A basic PubMed search shows that approximately 2600 studies were published on PCOS prior to 1990 when the first clinical guidelines for diagnosis were drafted. As the health consequences of PCOS throughout the lifespan of women have become apparent, the publication rate has increased exponentially, with 11,600 more publications from 1991 to present attempting to characterize the pathophysiology, etiology, consequences and potential treatment of PCOS. It has been argued that the multiple diagnostic criteria proposed and implemented over the last few decades have hindered progress in understanding and management of this prevalent syndrome. The name 'PCOS' itself is misleading as it is based on a specific criterion is that not necessary for the syndromal diagnosis. [132]

Diagnostic Criteria and Prevalence

PCOS was first described by Stein and Leventhal in 1935 as a syndrome of oligo-amenorrhea and polycystic ovaries that was often accompanied by hirsutism, acne, and obesity.[131] In 1990, the first diagnostic guidelines for PCOS were developed by the National Institute of Health (NIH) (Table 3).

In 2003, the heterogeneity of the PCOS phenotype was widened by the Rotterdam criteria, which were developed by consensus (hence, not necessarily on evidence) at a joint meeting for the European Society for Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM). These criteria are currently in use clinically. They build on the NIH criteria by adding polycystic ovaries detectable by ultrasound as a possible criteria for diagnosis, thereby making four PCOS phenotypes possible.[133]

Table 3: Diagnostic Criteria for PCOS

NIH 1990	Rotterdam 2003	AE-PCOS Society 2006
• Clinical and/or biochemical signs of hyperandrogenism • Chronic anovulation • exclusion of other etiologies, e.g., congenital adrenal hyperplasia	• Clinical and/or biochemical signs of hyperandrogenism • Oligo- and/or anovulation • Polycystic ovarian morphology *(Two of three criteria needed)*	• Clinical and/or biochemical signs of hyperandrogenism • Ovarian dysfunction (Oligo-anovulation and/or polycystic ovarian morphology)

In 2006, the Androgen-Excess PCOS (AE-PCOS) Society proposed a set of criteria where hyperandrogenism is a key criterion.[134] While succeeding in highlighting the importance of elevated androgens in PCOS, the criteria have not been adopted clinically. Most recently, an independent review panel recommended that the Rotterdam criteria should be supplemented with specific identification of phenotype. Table 4 lists the phenotypes in order of decreasing clinical severity that correspond to decreasing hyperandrogenism as well as the decreasing severity of insulin resistance and obesity.[132] While these phenotypic categories are being increasingly considered in new clinical and epidemiological research publications, it remains to be seen what is evolving clinically.

Table 4: Four Phenotypes of PCOS under the Rotterdam 2003 criteria

	Clinical and/or biochemical signs of hyperandrogenism	Oligo- and/or anovulation	Polycystic ovaries
Phenotype 1 ('classic' PCOS)	✔	✔	✔
Phenotype 2 (NIH Criteria)	✔	✔	-
Phenotype 3 (ovulatory PCOS)	✔	-	✔
Phenotype 4 (non-hyperandrogenic PCOS)	-	✔	✔

Overall, the prevalence of PCOS ranges from 5 – 15%, and even up to 21% in one study of Australian indigenous women, and clearly depends on the diagnostic criteria used: Prevalence of PCOS ranges from 5-10% according to NIH criteria, from 10-15% according to the AE-PCOS 2006 criteria, and from 6%-21% under the Rotterdam criteria.[135] The influence of ethnicity and sociodemographic factors on the prevalence of PCOS has not been explicitly studied, but seems to be relatively similar among different ethnic populations and geographic locations.[136]

PCOS and hyperandrogenism

Hyperandrogenemia can be clinically assessed by the presence of hirsutism, acne, or androgenic alopecia, or measured biochemically in serum via the free androgen index (FAI). The FAI is the ratio of total testosterone to the sex hormone binding globulin (SHBG) level and provides a measure of free testosterone - the form of the hormone that is available to bind to receptors in tissue to exert its effect.

Hyperandrogenemia is the most deleterious feature of PCOS; however the source of the elevated androgens has not been entirely elucidated. In female with normal physiology, the ovaries and the adrenal glands produce approximately equal amounts of testosterone and its precursor androstenedione, which is converted peripherally in liver, skin and fat. Evidence suggests that in 87% of cases, elevated androgens are due to ovarian overproduction (functional ovarian hyperandrogenism), 5% due to adrenal overproduction (functional adrenal hyperandrogenism), and the remaining 8% is idiopathic and associated to obesity.[137] In the most simplest terms, ovarian theca cells produce testosterone, which diffuse to the ovarian granulosa cells where it is converted into estrogens. The granulosa cell is also responsible for producing a negative signal for the theca cell to inhibit testosterone function as needed in follicular development. In PCOS, there is some *in vivo* experimental evidence for an intrinsic defect in the theca cells of the ovary leading to excess androgen production, and there is also evidence for deficiencies in the granulosa cell-initiated negative feedback signaling.

This unclear picture is further muddled by the influence of insulin resistance and subsequent hyperinsulinemia that develop in women with PCOS independent of obesity. Hyperinsulinemia leads to higher levels of free circulating testosterone through various mechanisms including stimulation of androgens secretion from the ovaries, inhibition of SHBG in the liver, and stimulation of testosterone formation in fat.[137]

In summary, while the exact pathogenesis of PCOS is unknown, evidence points to ovarian and adrenal hyperandrogenemia, hyperinsulinemia and altered intra-ovarian signaling that interrupts follicle growth. This combination leads to anovulation and subsequent menstrual irregularities and the accumulation of small antral follicles on the periphery of the ovary, giving rise to the polycystic morphology. Further details can be found in an extensive review of PCOS pathogenesis by *Rosenfield & Ehrmann [137]*.

Etiology of PCOS

With undecided pathophysiology, it is not surprising that the etiology of PCOS remains elusive. Twin and family studies have demonstrated a genetic component to PCOS: 22-40% of first-degree female relatives of women with PCOS have PCOS themselves.[138, 139] Vink and colleagues (2006) determined the heritability to be approximately 70% in a cohort of Dutch twins.[140] More recent genome-wide association studies in cohorts of European

and Chinese ancestry have identified 16 loci for PCOS, with some having a clear role in reproductive and metabolic functioning.[141]

Efforts to find environmental risk factors for PCOS have been less fruitful (reviewed by Merkin et al., 2016 [142]). As PCOS typically first presents at puberty, investigating environmental risk factors during the prenatal and postnatal periods have taken precedent. Findings suggest that in utero exposure to elevated testosterone exerts a risk on female offspring above and beyond the genetic risk that may be passed on through the mother, who is likely to have PCOS herself.[143]

PCOS in an evolutionary context

The relatively similar prevalence rates among geographic locations, as well as the emerging evidence for shared genetic susceptibility among different ethnic populations suggest that these genetic factors associated with PCOS were present in early humans before their migration out of Africa. However, PCOS – the most common cause of anovulatory infertility – is considered an evolutionary paradox. Theories have been proposed that PCOS developed to preserve metabolic and reproductive capacity via increased insulin and androgen production at times of famine and high energy expenditure, but has become pathogenic with the transition to a sedentary lifestyle.[144, 145] Alternatively, genes related to PCOS may have remained in the population because they improve the fitness in related males by promoting male hyperandrogenism, which could potentially compensate for reduced female fertility, a phenomenon known as *intralocus sexual conflict.*[146]

PCOS and Fertility

Anovulation or oligoovulation resulting in a reduced number of ovulatory cycles per year is likely the cause of the subfertility in women with PCOS. However, their desired family size is not necessarily compromised.[147] Increasing age has been shown to be accompanied by a decrease in the prevalence of both clinical and biochemical hyperandrogenism,[148] and women with PCOS show prolonged fertility with advancing age when compared to women with regular menstrual cycling.[149] Only women with both obesity and PCOS experience overall lower fertility,[147] although the exact mechanism here is unclear.

It is estimated that 20% of all ART patients are women with PCOS, however, there is a lack of studies concerning specific outcomes of IVF in women with PCOS.[150] Generally, controlled ovarian stimulation is a challenge in women with PCOS and the risk of ovarian hyperstimulation syndrome is high.[150] A meta-analysis of nine studies reported increased cancellation rates, more oocytes retrieved per retrieval, and a lower fertilization rate in women with PCOS undergoing IVF, and while BMI was not taken into consideration, PCOS and control patients achieved similar pregnancy and live birth rates per cycle overall.[151]

3.1.2 Depression, Psychiatric Disorders and Androgens

It's been well documented that women with PCOS report a reduced quality of life, score higher on depression and anxiety symptom scales, and have higher rates of depression and anxiety diagnoses.[152-160] However, causes behind these common comorbidities are only speculative, and may be a mixture of psychosocial factors, hormonal, and genetic factors.

One hypothesis is that the physical manifestations of PCOS including hirsutism, acne, obesity, and infertility result in symptoms of depression and anxiety.   A second hypothesis is that the elevated risk of depression and anxiety is attributable to the elevated androgens in women with PCOS, as higher levels of circulating androgens have been related to mood disorders in women.[159, 161, 162]   These two hypotheses are intricately linked, as increasing levels of hyperandrogenism are also associated with increasing clinical severity and rate of comorbidities in PCOS.

Studies comparing androgens levels in relation to symptoms of depression in PCOS patients are limited and show no association.[154, 158, 160] In terms of anxiety related disorders, Månsson *et al.*,[154] found that social phobia was highly correlated the FAI and BMI amongst women with PCOS. Jedel *et al.*,[163] found that the burden of anxiety symptoms and specifically phobic symptoms - including unreasonable fear in specific situations such as busses, grocery stores, crowds, or when feeling enclosed and being alone - were significantly higher in women with PCOS independent of BMI.

Less is known about the role of androgens in the development of other psychiatric disorders. There is some evidence for the influence of androgens in the development of some disorders such as attention deficit hyperactive disorder (ADHD), autism spectrum disorder (ASD), tics, bulimia nervosa, and gender identity disorder (GID).[164-167] Additionally, only two smaller population based studies and a handful of small clinical case-control studies have explored the association between PCOS and psychiatric disorders other than depression and anxiety.[154, 156-158, 160, 168-171] Table 5 summarizes these previous findings.

An as yet unexplored hypothesis is that the high rate of co-occurrence of PCOS and depression may indicate common genetic or environmental risk factors between the two conditions.

Table 5: PCOS, androgens, and psychiatric disorders: Summary of previous findings

	Previous findings on the associations between PCOS, androgens, and psychiatric disorders
Alcoholism	• Not investigated
Attention deficit hyperactivity disorder	• Women with PCOS scored higher on self-rated ADHD symptoms and more often had ADHD in childhood.[172] • Offspring of women with PCOS have increased rates of ADHD.[173]
Autism spectrum disorder	• Females with ASD have increased rates of PCOS and PCOS symptoms, including irregular menstruation and hirsutism.[174] • Offspring of women with PCOS have increased rates of ASD.[175]
Tics	• Prenatal exposure to androgens hypothesized to be a risk factor for tic-related disorders.[167]
Bipolar disorder	• No association detected between bipolar disorder and PCOS.[157] • PCOS-like symptoms in clinical samples of women treated for bipolar disorder with valproate, but evidence also suggests that these symptoms are present before treatment. [176, 177]
Personality disorders	• Personality disorders are significantly more common in women with PCOS compared with controls. [126, 148]
Eating disorders	• Substantial evidence linking the menstrual and hyperandrogenic symptoms of PCOS with binge eating and other bulimic behavior, [178, 179] although it is unclear which is a cause or a consequence of the other. • Circulating levels of testosterone have been found to be elevated in women with bulimia.[180] • Androgens have appetite-stimulating effects and could stimulate bulimic behaviors via its role in appetite and impairment of impulse control.[181] • A placebo-controlled trial found that the androgen antagonist flutamide reduced symptoms in bulimia nervosa. [182]
Gender identity disorder	• Women with PCOS more often viewed themselves as sexually undifferentiated, less likely to identify with a female gender scheme, and more likely to view themselves as androgynous.[170] • A high proportion of female-to-male transsexuals have some features of PCOS before sex reassignment. [164, 183, 184] • However, not all studies have found a higher prevalence of PCOS in female-to-male transsexuals. [183]
Schizophrenia	• No association found in a Taiwanese study of over 5000 PCOS women and matched population controls.[157]
Suicide	• Risk of self-harm and attempted suicide in women with PCOS is elevated but was not statistically signficant. [154-156]

3.1.3 Depression and Personality Traits

Modern psychology uses five personality traits to describe personality: openness, conscientiousness, extraversion, agreeableness, and neuroticism.[185] Personality traits influence an individual's ability to react and interact with their environment. Neuroticism is defined as the tendency to respond with negative emotions to threat, frustration, or loss. Individuals with high levels of neuroticism experience tension and emotional insecurity, are more likely to perceive stress,[186] and are more sensitive to adverse effects of stress.[187] Neuroticism is strongly associated with lifetime risk for major depression and anxiety in adults, and is thought to include a genetic predisposition for various psychiatric disorders.[188]

In a study of 448 women with PCOS, passive coping mechanisms were found to be used more often and were associated with greater anxiety and depression, as well as reduced psychological quality of life.[189] Scaruffi *et al.,* [156] reported that in their sample of women with PCOS, 53% had reduced coping abilities and social skills, 24.5% reported being constantly alert and worried, and approximately 50% had chronic stress. Stress response has also been reported to be altered in women with PCOS.[190]

Further, it has been reported in a small clinical sample of women with PCOS that they experience more neuroticism compared with sub-fertile controls.[191] Therefore, it is conceivable that personality traits, such as neuroticism, may be the source of the increased risk for depression in women with PCOS; however this has yet to be investigated.

3.2   AIMS

The aims of the last two studies in the thesis were:

- **Study III:** To investigate the association between PCOS and psychiatric disorders, to elucidate if any potential associations are due to common familial factors, and to indirectly test if elevated androgens can be implicated in the associations found.

- **Study IV:** To test if the cormorbidity between PCOS and depression is attributable to higher levels of neuroticism in women with PCOS; to investigate if shared genetic factors underlie the comorbidity between neuroticism, PCOS and MDD.

3.3   MATERIALS AND METHODS

Table 6: Summary of Data Sources and Analysis Methods for Studies III and IV

Population	Data Sources	Exposure / Outcome	Study Design and Analysis Method
Study III: Polycystic ovary syndrome and psychiatric disorders: Co-morbidity and heritability in a nationwide Swedish cohort			
All women in Sweden diagnosed with PCOS between 1990 and 2013 (n = 24,385), their full-siblings (n = 25,921), plus matched individuals (1:10/100) from the general population and their full-siblings.	Swedish Population Registers • National Patient Register • Prescribed Drug Register • Multi-Generation Register • Migration Register • Cause of Death Register	*Exposure:* Main analysis: • Diagnosis of PCOS Sibling Analysis: • A sister with a diagnosis of PCOS *Outcome:* Psychiatric disorders : • Schizophrenia • bipolar disorder • depressive disorder • anxiety disorders • eating disorders • personality disorders • gender identity disorder • transsexualism • autism spectrum disorder • attention-deficit hyperactivity disorder • tics • attempted suicide • suicide	Matched Cohort Study Conditional Logistic Regression adjustment for comorbid psychiatric disorders.
Study IV: Polycystic Ovary Syndrome, Personality, and Depression: A Twin Study			
A cohort of 12,628 Swedish female twins	Swedish Twin Registry • The Study of Twin Adults: Genes and Environment (STAGE), a web-based survey performed in 2005 among all Swedish twins born between 1959 and 1985	PCOS status determined by questionnaire responses regarding symptoms Neuroticism assessed by the 9 question short form of the Eysenck Personality Inventory Lifetime prevalence of Major Depressive Disorder (MDD) assessed by the Structured Clinical Interview for DSM-IV Disorders	Twin Study Conditional logistic regression was used to measure the association between the three traits in the cohort of female twins. Structural equation modeling was used to study the genetic and environmental sources of the variation within, and covariation between neuroticism, PCOS, and MDD.

3.3.1 Data Sources: Swedish National Registers and Twin Registry

Study III: Swedish National Registers

The *National Patient Register* was described in detail in section 2.3.1, and for Study III was used to identify both the diagnoses of PCOS and psychiatric disorders. Women with PCOS were identified by having at least one of the PCOS ICD codes recorded in the NPR: ICD-9: 256E; ICD-10: E28.2, between 1990 and 2013. Cases were excluded if:

- they had a condition that may cause symptoms similar to PCOS
- the PCOS diagnosis was made before the age of 13 without an additional diagnosis in adolescence or adulthood

Study subjects were considered to have a psychiatric disorder if they had at least one recorded diagnosis from 1973 to 2013, identified using ICD-8, -9, and -10 codes.

The Cause of Death Register (National Board of Health and Welfare) registers all deceased individuals registered in Sweden at the time of death regardless of whether the death occurred in Sweden or abroad, and is considered complete from 1961. Information on the date of death and the cause(s) of death coded according to ICD codes. For Study III, data on suicide for the study subjects was extracted.

The *Prescribed Drug Register*, described in Section 2.3.1, was used to extract information about valproate dispensations for individuals with bipolar disorder.

The Multi-Generation Register (Statistics Sweden)[192] links all individuals (index persons) born since 1932 and alive in 1961 to their biological and adoptive parents. From 1961 onward, the register has excellent coverage with information on 100% of the biological mothers, and 98% of fathers born in Sweden. The coverage is less complete for index persons born outside Sweden as information on their parents is only included if they immigrated before the age of 18 years. The link between children and parents makes it possible to construct large pedigrees of different family relationships: full and half-siblings, aunts/uncles, cousins, and even grandparents. More than 47% of index persons have information on two generations or more. For Study III, full siblings of women with PCOS and their matched unaffected individuals were identified using this register.

Small Area Marketing Statistics Register (Statistics Sweden) is a geographical classification system including 9,200 residential areas in Sweden, and was used to identify the county of residence of the individuals in the study population.

Study IV: Swedish Twin Registry

The Swedish Twin Registry (STR) was established in the 1950s and now includes data on over 97 000 pairs of twins born since 1886 with zygocity determined by an intra-pair similarity algorithm and/or DNA. The STR is the largest twin registry in the world and has become an integral epidemiological resource for studying genetic and environmental influences on a large number of traits and diseases.[193] The STR is composed of a number of specific cohorts, including **The Study of Twin Adults: Genes and Environment (STAGE)** used in Study IV of this thesis.

STAGE is a web-based survey performed in 2005 among all Swedish twins born between 1959 and 1985 (n=42,852) that comprised approximately 1,300 questions about common complex disease and common exposures including an extensive list of mental health and women's health and reproduction question.[105] STAGE female twins (n=14,180) were of reproductive age (20-46 years) when responding to the survey.

PCOS

Validation studies in a large Finnish birth cohort have concluded that the presence of PCOS including hyperandrogenemia can reliably be established through self-completed questions concerning hirsutism and oligomenorrhea [194, 195]. Therefore, female twins with PCOS in the STAGE cohort were identified by their responses to questions addressing menstrual irregularities and hirsutism, which included answering 'yes' to the following questions:

"Have you, during any period of your life, had irregular menstrual periods – more than 5 weeks between periods?"
OR
"Have you ever missed more than three periods without any natural causes such as pregnancy or menopause, etc?"
AND
"Do you feel that you have abnormal hair growth on parts of the body?"

In total, 12,628 (89%) had data available to determine PCOS status, of which 752 (6.0%) were identified to have PCOS.

Major Depressive Disorder (MDD)

Lifetime prevalence of MDD was measured using a computerized version of the Composite International Diagnostic Interview-Short Form (CIDI-SF)[196] which was adapted from its original version to assess lifetime prevalence of major depression according to criteria A, C and E in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV).[37] For this study, only criteria A and C had to be fulfilled in order to be classified as having MDD.

To meet A criteria, at least 5 of the following symptoms, including depressed mood or diminished interest, had to have been present during the same 2 week period at some point in life:

> *depressed mood, sleep disturbance, markedly diminished interest or pleasure from almost every activity, significant weight change or change of appetite, feeling inhibited or agitated, feeling of lack of energy, feeling of guilt or worthlessness, diminished ability to concentrate, recurrent thoughts about death or suicide.*

To fulfill criteria C, these symptoms had to cause clinically significant distress or impairment in daily functioning.

For 91.3% of all female twins in STAGE, there was sufficient data to determine their lifetime prevalence of MDD.

Neuroticism

The STAGE questionnaire contained questions from the short form of the Eysenck Personality Inventory (EPI-Q) [197]. This short version of the questionnaire includes 9 yes/no questions, from which a score from 0 to 9 was calculated. If the participant answered fewer than 7 questions, the score was set to missing. For 78% of all female twins in STAGE, there was sufficient data to determine a neuroticism score.

EPI-Q questions used in STAGE to assess neuroticism score

1) Are you often uneasy and feel that there is something you want without knowing what it is?
2) Are you sometimes happy or sometimes sad without any special reason?
3) Do you often reach decisions too late?
4) Do you often feel tired and listless without any special reason?
5) Are you often lost in your own thoughts?
6) Are you extremely sensitive in any respect?
7) Are you ever too restless to sit still?
8) Do you have any nervous problems?
9) Do you worry too much after an embarrassing experience?

Response Key: Yes/No

3.3.2 Matched Cohort Studies and Conditional Logistic Regression

Matched Cohort Study

In a matched cohort study design, exposed subjects, known as *probands*, are identified and matched with randomly selected unexposed individuals from the population, on the same level of the matching variables. As with a regular cohort study, the probands and unexposed individuals are followed, and the rates of the outcome(s) are compared between them. By matching, the clusters are constructed so that each member has the same level of the confounder. The confounder can then vary between clusters, but not within the matched cluster, and therefore the confounder is equally distributed among the exposed and unexposed in a matched cohort. This breaks the association between the exposure and the confounder, hence the matching scheme is exposure driven. The matched cohort study design is gaining in popularity and is suitable for when large population data sources with exposure data are available.[198]

Study III is a matched cohort study with cross-sectional data, as in Study I and II. For each woman with PCOS and for each sibling of these individuals, 10 individuals matched on birth year, sex and county of residence within the decade of diagnosis were randomly selected from the general population listed in the Total Population Register. Individuals had to be alive, living in Sweden and without a diagnosis of PCOS ever during life. Where the psychiatric disorder of interest was rare, the matching was 1:100 to have sufficient numbers to run the analysis.

Family Study

Study III also contains a family study, where sisters and brothers of probands are matched to unexposed individuals. Family studies rely on the assumption of similarity within a family and are used to examine the familial causes (i.e., genetic and shared environment factors) of comorbidity between two disorders. In this study, if the occurrence of the comorbid psychiatric disorder is increased in women with PCOS and not in their unaffected siblings, then it suggests that PCOS is linked to the comorbid psychiatric disorder. But if the psychiatric disorder is also increased in the siblings, this suggests that familial factors are likely behind the association, and not PCOS per se.

Conditional Logistic Regression

In Study III, random sampling was performed within strata of the matching variables, and therefore the analysis must be performed within strata of matching variables to maintain the assumption of independence between observations. Conditional logistic regression is an efficient way to perform a "conditional/stratified" analysis. In conditional logistic regression, the data are grouped and the likelihood is calculated relative to each matched group.

3.3.3 Twin Modeling and Quantitative Genetic Analysis

The aim of quantitative genetic studies is to explore the relative contribution of genetic and environmental influences to individual differences in one or more traits using family or twin data. Structural equation modeling is one method by which to separate the genetic and environmental sources of variation within a trait and the covariation between traits in a population. Using this method, (co)variation in the population can be attributed to:

- A = additive genetic effects, i.e., additive effects across alleles. A is assumed to be 100% shared between MZ twins, and 50% shared in DZ twins, because monozygotic twins (MZ) are genetically identical and dizygotic (DZ) twins on average share half of their co-segregating alleles.
- D = dominant genetic effects, i.e., interactions between alleles at the same gene locus. Assumed to be 100% shared between MZ twins, and 25% shared for DZ twins
- C = shared environmental effects, i.e., environmental factors which make members of the twin pair more similar. Assumed to be 100% shared within both MZ and DZ pairs.
- E = unique environmental effects, ie., the environmental factors that contribute to dissimilarity among members of the twin pairs. Assumed to have a correlation of 0 for both MZ and DZ twins, and accounts for measurement error.

Classical twin analyses, based on contrasting twin pair correlations between MZ and DZ twins are based on the assumptions of the classical twin study design: 1) MZ and DZ pairs share 100% of their common environmental factors; 2) there is no gene-environment (GxE) interaction for the traits; 3) the trait does not differ between twins and singletons; 4) random mating occurs in the population so that DZ pairs share only 50% of their segregating genes.[199]
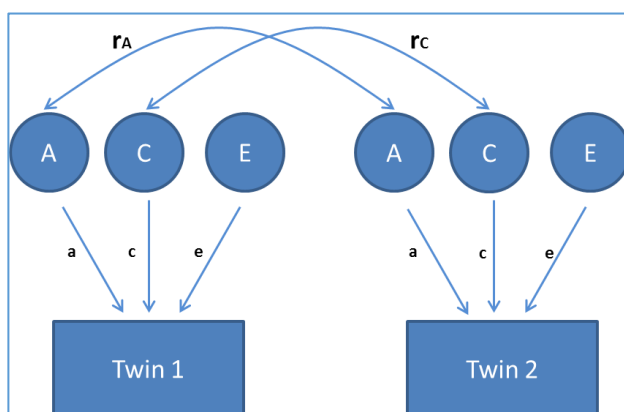
Figure 7: The classic ACE twin model for one phenotype. Squares represent the observed trait; circles represent latent variables; single headed arrows represent causal pathways; double headed arrows describe covariance between twins. rA = genetic correlation [MZ=1.0; DZ=0.5]; rC = shared environmental correlation [MZ=1.0; DZ=1.0].

Figure 7 shows a path diagram of the basic twin model of one trait, which illustrates how the phenotypic variance is partitioned into effects of A, C, and E. The lower case letters (a, c, e) are regression coefficients that estimate the effect of the latent variables on the observed phenotypic variance.

The classical twin design cannot estimate the C and D components simultaneously because C and D are confounded. They are estimated from the same information – the differences between the within pair correlation in MZ pairs relative to DZ pairs. Therefore, C components *increase* the DZ correlation relative to the MZ, whereas D components *decrease* the DZ correlation relative to the MZ correlation. Hence, a choice of model (i.e., ACE or ADE) must be made, which depends on the patterns of MZ and DZ correlation: If the DZ twin correlation is *less* than half of the MZ twin correlation then evidence for dominance genetics exists and D should be estimated; if the DZ correlation is *more* than half, then C should be estimated in the model.

Model Fitting

In addition to ACE and ADE models, there is a possibility that models including just AE or CE, or even only E, best represent the variance in the traits. Structural equation modeling of twin data is now most commonly performed in the structural equation modeling software OpenMx.[200, 201]. This program uses maximum likelihood modeling procedures to determine which combination of A, C/D, and E best explain the observed data.

Models can be compared by conducting a likelihood ratio test of the goodness-of-fit statistics (χ^2). A statistically significant likelihood ratio test result suggests that there is a significant difference in model fit. Alternatively, the fit of the models is also summarized by combining information from the test statistics χ^2 and the degrees of freedom (df) into the Akaike's information criterion (AIC, χ^2-2df). The model with the lowest AIC is considered to have a bitter fit to the data.

Multivariate Genetic Modeling

Twin modeling with one trait produces a *heritability* estimate, which is defined as the proportion of phenotypic variance in a population that is attributable to genetic factors. The remainder of the variance is attributable to environmental components. By including more than one variable in the model, the covariance between the two or more traits can be partitioned into those due to A, C/D, and E, in the same way we do for the variance of a single trait.

For Study IV, structural equation modeling was used to study the genetic and environmental sources of the variation within, and covariation between, neuroticism, PCOS, and MDD, where PCOS and MDD were defined as binary traits. Therefore, the *liability-threshold method* was used in analyzing the data. Here, each individual is assumed to have an underlying normally distributed liability of having the binary trait, with '0' observed if the liability is lower than an estimated threshold and '1' observed if it is above the threshold. The thresholds are determined by the prevalence of the traits. The analyzed measure of association is the correlation between the assumed underlying normal distribution of

liability, also referred to as the tetrachoric correlation. Preliminary analysis of the data tested whether it is possible to equate the threshold for the binary variables, the variances, and covariance between twin order (Twin 1 and Twin 2), and between zygosities (MZ and DZ).

Model Fitting for Study IV

The intra-pair correlations for neuroticism, PCOS, and MDD are presented in Table 7. For all three traits, the correlation in DZ twins was less than half of that in the MZ twins, and therefore only the ADE and the reduced AE trivariate models were fit.

Table 7: Twin correlations for PCOS, Neuroticism and Major Depression

	PCOS	Neuroticism	MDD
Monozygotic	0.61 (0.50, 0.70)	0.54 (0.50, 0.57)	0.49 (0.42, 0.56)
Dizygotic	0.25 (0.05, 0.43)	0.18 (0.12, 0.24)	0.16 (0.06, 0.26)

Data are given as correlation (95% confidence intervals).
Abbreviations: PCOS = polycystic ovary syndrome; MDD = major depressive disorder

The goodness-of-fit for the AE model was evaluated and compared with the ADE model using the likelihood ratio test. The lowest AIC value was used to identify the most parsimonious model, which was the AE model.

Therefore, the A and E sources of variance were modelled in a trivariate *Cholesky decomposition model.* The factors of the first phenotype (neuroticism) loaded on all three traits, while the factors of the second phenotype (PCOS) only loaded on the second and third (MD) traits. The factors of the third phenotype load on only the third trait (Figure 8). The univariate heritability and bivariate heritability were first calculated. Next, the genetic and environmental correlations between 1) neuroticism and PCOS, and 2) PCOS and MDD, were calculated. Lastly, the model assessed the fraction of the phenotypic correlation between PCOS and MDD that is explained by genetic and environmental factors shared across neuroticism, PCOS, and MDD, and factors that are shared by PCOS and MDD, but not neuroticism.
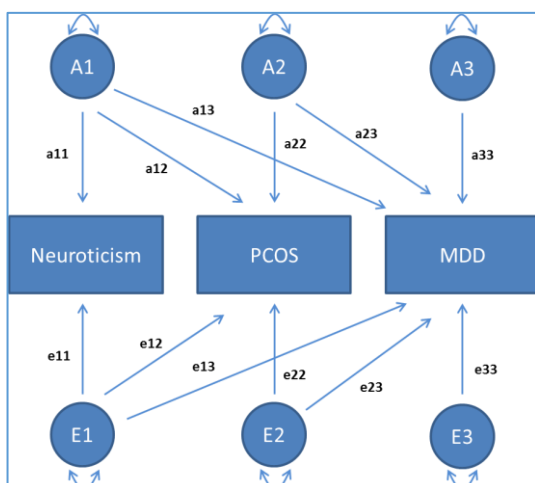
Figure 8: Trivariate Cholesky decomposition, including additive genetic (A) and non-shared environmental (E) variance for three traits: neuroticism, polycystic ovary syndrome (PCOS), and major depressive disorder (MDD).

3.4 STUDY RESULTS AND EPIDEMIOLOGICAL CHALLENGES

3.4.1 Study III: Polycystic ovary syndrome and psychiatric disorders: Co-morbidity and heritability in a nationwide Swedish cohort

Results

- Women with PCOS had an increased odds of having at least one psychiatric disorder (OR= 1.56; 95CI% 1.51, 1.61) compared with the matched unaffected women. Crude ORs showed associations with all psychiatric disorders included in this study, except for alcoholism, anorexia nervosa, transsexualism, and suicide.

- Following adjustment for comorbid psychiatric disorders, women with PCOS were still at a significantly increased risk for bulimia nervosa (OR=1.21; 95CI% 1.03, 1.41), schizophrenia spectrum disorders (OR=1.26; 95CI% 1.10, 1.43), bipolar disorder (OR=1.41; 95CI% 1.28, 1.56), depressive (OR=1.25; 95CI% 1.19, 1.31) and anxiety disorders (OR=1.37; 95CI% 1.32, 1.43), and personality disorders (OR=1.49; 95CI% 1.14,1.94). The highest adjusted ORs were found for ASD (OR=1.55; 95%CI 1.32, 1.81) and tics (OR=1.65; 95%CI 1.10, 2.47).

- A sub-analysis of women diagnosed with PCOS between 1990-2003 (under the diagnostic NIH criteria, where hyperandrogenemia was a manditory crtieria for diagnosis; n=4,286) was conducted. Women with PCOS had a higher increased odds of having at least one psychiatric disorder (OR=1.85; 95CI% 1.72, 1.99), and estimates for each psychiatric disorder were higher compared with the main analysis, with the exception of bulimia nervosa.

- In a second sub-analysis, where PCOS criteria were made from 2004 onward under the Rotterdam diagnostic criteria (n= 20,099), the odds of having having at least one psychiatric disorder were similar to the main analaysis (OR= 1.50; 95CI% 1.45, 1.56), but estimates for each individual psychiatric disorder were attenuated.

- In the sibling analysis, 16.2% of sisters and 11.6% of brothers had a psychiatric diagnosis compared with 14.2% and 9.9% of the matched comparison individuals, respectively. These increases were statistically significant (sisters: OR= 1.18; 95% CI 1.12, 1.25; brothers: OR= 1.16; 95%CI 1.09, 1.24). Specifically, significantly higher adjusted ORs were found for ASD in both brothers (OR=1.32; 95%CI 1.09, 1.60) and sisters (OR=1.32; 95%CI 1.03, 1.70) of women with PCOS, and for depressive, anxiety, and schizophrenia spectrum disorders in the sisters only.

Internal validity

Ascertainment bias

- Similar to Study I, the use of the national registers introduces some forms of bias to this study. With respect to PCOS, the prevalence in the register (approx. 2%) is lower compared with the expected prevalence of PCOS in the general population. In 2010, March et al., [202] reported the prevalence of PCOS to be from 8.7% to 17.8% in a community-based sample of women, depending on whether the NIH or Rotterdam diagnostic criteria were used. However, they also found that approximately 70% did not have a pre-existing diagnosis of PCOS, which would bring the prevalence of actual diagnosed PCOS cases closer to the prevalence rates found in our study.

- Lizena et al.,(2016) [203] have reported significant referral bias for PCOS, where women with more complete phenotypes of PCOS (i.e., with hyperandrogenemia) and higher BMI are more likely to receive medical care. Similarily, the women with PCOS identified in our study are likely the more severe cases of PCOS in the Swedish population, with certain clinical manifestations of the disorder. If these specific phenotypes also have greater risk of being diagnosed for psychiatric disorders, then a bias is introduced into this study and is likely to overestimate the associations.

- The basis of the sibling analysis is that it includes sisters (and brothers) without PCOS. Given the lower prevalence of PCOS in the National Patient Register, and the high heritability of PCOS, it is highly likely that there are sisters with PCOS misclassified as 'unaffected' because they have not received a diagnosis of PCOS, which would result in an overestimation of the associations with psychiatric disorders.

Reverse causation

- Addressing the temporality of PCOS and psychiatric disorders with the data available in the national registers is not possible. The age at first diagnosis of PCOS in our sample was 28 years, which only reflects the age of health seeking behavior, as the onset of PCOS symptoms is at puberty. Therefore, receiving a depression diagnosis prior to the date of a PCOS diagnosis does provide any information on the direction of the association between PCOS and psychiatric disorders, and was therefore not addressed in this study.

- In the case of bipolar disorder, reports have linked valproate treatment to symptoms of PCOS. A sensitivity analysis was conducted where PCOS cases were excluded if they had a valproate dispensation prior to the first diagnosis date of PCOS (n = 110 of 18,641 PCOS cases diagnosed since 2005 when the Prescribed Drug Register began). The association between PCOS and bipolar disorder – while slightly attenuated – still held: adjusted OR=1.26 (95% CI, 1.11–1.42).

Confounding

- The probands and comparison individuals were matched on age, sex, and county of residence to account for differences in healthcare provision in the different counties that could affect both diagnosis of PCOS and a psychiatric disorder. As there are few known risk factors for PCOS, there were no other identifiable covariates to account for in the analysis.

Random error

- For the more rare psychiatric disorders, the numbers in the final analysis were small and estimates could be vulnerable to random error in the study.

3.4.2 Study IV: Polycystic Ovary Syndrome, Personality, and Depression: A Twin Study

Results

- Of the 12,628 female twins with data to determine PCOS status, 752 met the criteria for having PCOS (6.0%). Overall, 37.3% of women with PCOS had MDD compared with 22.9% who did not have PCOS. Women with PCOS had a mean (sd) neuroticism score of 7.90 (4.9) compared with a mean of 5.78 (4.3) in women without PCOS. The odds ratio for the association between PCOS and neuroticism as a continuous variable was 1.11 (95%CI 1.09, 1.13). Between PCOS and MDD, the OR was 2.01 (95%CI 1.70, 2.36), and when the model was adjusted for neuroticism, the estimate attenuated to OR=1.37 (95%CI 1.12, 1.68).

- Both the within pair correlations and the cross-twin-cross-trait correlations were higher in the MZ twin pairs than the DZ twin pairs (Table 7), indicating the presence of genetic factors linking PCOS and neuroticism, as well as PCOS and MDD.

- Heritability estimates for PCOS, neuroticism, and MDD were 60%, 52% and 46%, respectively.

- The phenotypic correlation between PCOS and neuroticism was 0.19, with 75% of the correlation explained by genetic factors, the remainder by unique environmental factors. The phenotypic correlation between PCOS and MDD was also 0.19, with 63% of the correlation attributable to common genetics between the two traits.

- The trivariate estimates revealed that 41.1% and 9.2% of the phenotypic correlation between PCOS and MDD was attributable to genetics and non-shared environment components that are common between neuroticism, PCOS, and MDD, respectively. The contribution unique to PCOS and MDD was 21.8% for genetic and 27.9% for non-shared environmental factors.
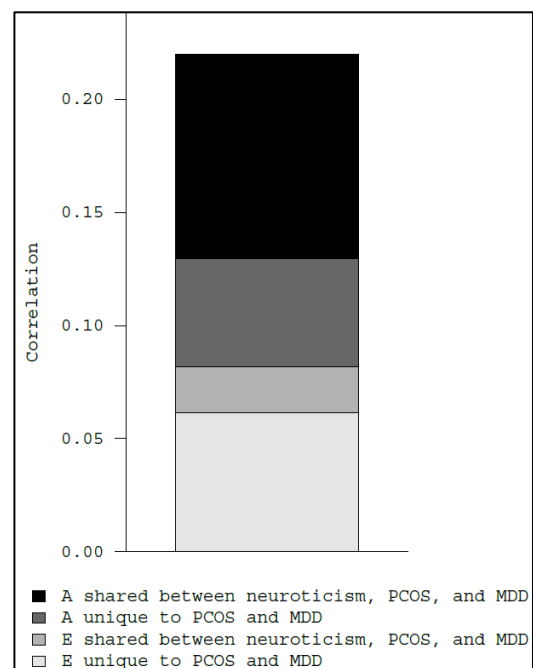
Figure 9: Trivariate model results

Internal validity

Selection bias

- The STAGE invitation letter was sent to all swedish twins born between 1959 and 1985. The total response rate was 59.6%, and for women 66%.[204] The general aim of the STAGE survey was to obtain information regarding several complex diseases, and did not contain a specific a priori hypothesis, reducing the potential influence on individuals with certain disorders to participate. STAGE participants and non-participants do not differ by age, however a higher proporation of the participants were female, Swedish born, and non-participants were had more often been convicted of a crime, had less education, and had more often been diagnosed with a psychiatric disorder.[205]

Information bias

- PCOS in the STAGE twins was identified using an algorithm based on responses to questions regarding the presence of PCOS symptoms, including hirsuitism. This method has been validated in a Finnish birth cohort.[194, 195] However, while hirsutism is often considered the clinical proxy of hyperandrogenemia, half of women with mild hirsuitism and a small portion of women with moderate to severe hirsuitism do not have hyperandrogenemia.[206] This could then result in a differential misclassification of PCOS in women who do not have PCOS, resulting in biased estimates.

Potential consequences of violating the assumptions of the twin model

- Violation of assumption 1 (the equal environments assumption) where MZ and DZ twins do not share 100% of their common environment, will result in an incorrect inflation of the estimate of A. However, there is little evidence for most traits that controlling for environmental similarity reduces heritability, with the exception of neuroticism. However, it remains to be clarified if this is problematic for the equal environments assumption.[207]
- Voilation of assumption 2 (no gene-environment correlation) occurs if individuals actively or passively expose themselves to different environments depending on their genotype, thereby causing the relative contribution of genes and environment to the trait variance to differ by individual. GxE will inflate the estimate of E when the environmental influence is unshared, or inflate the estimate of C when the environmental influence is shared between twins.
- Violation of assumption 3 will bias the estimates of any of the parameters (A, C/D, E) depending on how singletons differ from twins in the traits under study. There are only

a few studies of PCOS in twins, and little to no evidence that PCOS may be different in twins versus singletons. Speculatively, the prevalence of PCOS in twins may be lower than in singletons since PCOS has been associated with higher birth weight,[208] but symptoms of PCOS have also been associated with lower birth weight.[209] For depression, at least one study has shown that twins in Sweden are representative of the general population with respect to their rates of hospitalization for MDD.[210]

- If assumption 4 is violated, non-random mating will result in the genetic relatedness of DZ twins to be higher than 50%, consequently resulting in an inflation of the C estimate, and a subsequent deflation of the heritability. There has been no investigation into the mating patterns of women with PCOS, however a recent study has shown non-random mating amongst individuals with psychiatric disorders, including depression.[211] However, any influence of assortative mating is likely be modest.[212]

3.5   CONCLUSIONS

In Study III, women with PCOS had higher risk for a range of psychiatric disorders, including depression.   There was elevated risk in the unaffected sisters of women with PCOS for depression, which could indicate familial factors (i.e., genetics or shared environment) behind the comorbidity of PCOS and depression.   However, this elevated risk for depression was not elevated in the brothers, and as discussed previously, there is likely to be significant amounts of misclassification in the sisters that might inflate the association.   Therefore, evidence for familial confounding in the case of depression is not robust.   Stronger evidence lies behind the role of androgens from the higher associations between PCOS and depression in the sub-analysis of women diagnosed during the years of the NIH diagnostic criteria.

Study IV provides evidence of common genetic factors between PCOS and major depression.   It also suggests that part of the comorbidity between depression and PCOS is attributable to common factors between these two traits and neuroticism.

Taken together, there are no clear indications of the nature of the etiology behind PCOS and depression, but these studies provide insight into the possible influence of genetics, elevated androgens, and personality traits.   These are the first studies delving deeper into the etiology of this common comorbidity, and future studies can build upon these findings.

As reviewed, androgens have been implicated in the development of some psychiatric disorders and women with PCOS provide an enticing means to test this hypothesis.   In addition to elevated risk for depression, Study III reports increased associations with many psychiatric disorders that are typically found at higher rates in males.   Interestingly, the prevalence of ASD, ADHD, and tic-disorders in women with PCOS was similar to the prevalence in the unexposed male control group.   Fetal exposure to high levels of androgens has been implicated in the development of all these disorders - including PCOS.   Further investigation into this potentially common etiology may be fruitful for further elucidation of the role of sex hormones in the development of psychiatric disorders.

4 FUTURE DIRECTIONS

I began this thesis with a definition of epidemiology that emphasizes generating knowledge *with the ultimate goal of using this knowledge to intervene and prevent disease.* The intersection between reproduction and mental health is fundamental, yet it is greatly understudied and much of the field is still in the descriptive phase - the studies in this thesis included.

The influence of stress may not be discovered until we learn more about biological process underlying human reproduction. I propose that until that time no additional studies on stress and IVF outcome are needed. Study II does not contain a definitive conclusion on the matter, nonetheless many other clinical and epidemiological studies have investigated this topic and there is little evidence of a marked effect on IVF outcome. Already, psychosocial care for women and men undergoing infertility treatment is being highlighted by regulating bodies in Europe.[213] Undeniably, women and men undergoing infertility treatment experience high levels of stress and for that reason alone this psychosocial care is needed and additional resources should be used to bring this aspect of care fully into the clinics. I also propose that additional resources be directed into fertility education in order to distribute more accurate knowledge about the limits of human fertility and expectations of infertility treatments. While this may not decrease the number of couples experiencing infertility, it may decrease some of the emotional distress found in this population of women and men. Additionally, efforts should be directed at discovering the mechanisms behind conditions that infertility treatment is less successful in overcoming such as recurrent miscarriage.

Alarmingly, the number of people suffering from depression has increased by more than 18% between 2005 and 2015.[15] It is of great benefit to society to be able to identify high risk groups and specific causes behind the development of depression, to be able to create more direct prevention and treatment strategies. It has clearly been shown that women with PCOS experience higher rates of depression. Study IV implicates neuroticism in this comorbidity, and while personality traits are not easily reshaped, screening for neuroticism may provide a way to identify the women with PCOS most at risk for depression. Another avenue for future investigation is the efficacy of treatment of depression in women with PCOS. It's been shown that the rate of prescription for antidepressants are higher in women with PCOS,[214] however little is known about the effect of these pharmacotherapies on androgens and other reproductive hormones. Efforts to elucidate the role of androgens in psychiatric disorders have revealed some promising findings in recent years and should be continued, and may results in advancing the prevention, detection, and treatment of psychiatric comorbidity in women with PCOS.

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