Early Life Influences and Female Fertility

Developmental Origins of Adult Health and Disease: Early Life Influences, Polycystic Ovary Syndrome and Ovarian Reserve

Sheda Sadrzadeh
The mythical Garuda hatches fully mature, leaps immediately into the air, and remains forever in flight. According to the Shambhala teachings, Garuda is “Outrageous”: fearless and cheerful in the face of life's vagaries. This commanding representation bursts out of a brilliant red sky, inviting us to join in celebrating the unpredictable adventure of human existence.
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For my Mother,

who loved me enough to let me go
For things to reveal themselves to us,
we need to be ready to abandon our views about them

Thich Nhat Hanh
Table of contents

Section one: Introduction

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>General Introduction</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 2</td>
<td>Bias regarding birth weight in historical and contemporary twin data bases</td>
<td>33</td>
</tr>
</tbody>
</table>

Section two: Ovarian Reserve

| Chapter 3 | Ovarian reserve in young women with low birth weight and normal puberty: a pilot case control study | 47 |
| Chapter 4 | Premature ovarian insufficiency and perinatal parameters: A retrospective case control study | 59 |
| Chapter 5 | Birth weight and age at menopause in Australian female twin pairs: exploration of the fetal hypothesis | 73 |
| Chapter 6 | Early environment and age at menopause: a systematic review | 87 |

Section three: Polycystic Ovary syndrome

| Chapter 7 | Heritability of polycystic ovary syndrome in a Dutch twin-family study | 109 |
| Chapter 8 | Birth weight and age at menarche in patients with polycystic ovary syndrome or diminished ovarian reserve, in a retrospective cohort | 125 |
| Chapter 9 | Developmental origins of PCOS, a case control study comparing birth weight in women with PCOS and controls | 141 |
Chapter 10  Birth weight and the development of PCOS: a systematic review and meta-analysis  153

Conclusion

Chapter 11  Summary, discussion and future perspectives  177
Chapter 12  Samenvatting, Dutch summary  193

Appendix

Dankwoord  203
Curriculum Vitae  207
Abbreviation list  209
Section One

Introduction
Chapter one

General Introduction
Developmental Origins of Health and Disease (DOHaD): Female fertility

Developmental Origins of Health and Disease

The notion that early development affects adult life is not a novel idea. As early as in the 1930s, childhood conditions were thought to influence adult mortality (1). In the 1970s, the east German (DDR) endocrinologist, Günter Dörner, published a series of articles connecting intrauterine and early postnatal conditions with the incidence of obesity and diabetes in adult life (2-4). His publications formed the basis of east Germany’s public health policy to advance maternal health and to stimulate breast feeding. In the 1980s the epidemiological studies of Barker et al. linking low birth weight to increased cardiovascular disease later in life, despite initial skepticism, altered the paradigm that genetics and lifestyle were the only factors involved in cardio-metabolic disease. Initially this phenomena was referred to as ‘The fetal origins of adult disease’, but as it broadened to include the effects of early postnatal environment on later health, the concept is currently known as the ‘developmental origins of adult health and disease’ DOHaD (5-7). Various hypotheses were postulated to explain the epidemiological relation between adverse circumstances early in life and disease later in life. In 2001 ‘The Thrifty Phenotype’ hypothesis was postulated by Hales and Barker as a counterpart to ‘The Thrifty Genotype’ hypothesis (8). The central paradigm is that the fetus receives environmental cues during gestation, which permanently alter its phenotype. This phenomenon is called developmental plasticity and results in a variation of postnatal phenotypes without changing the genetic make-up, genotype, of the individual. Some adaptations may be in direct response to restricted nutrient supply: e.g. decreased growth of organs non-essential for (fetal) survival including kidney, lungs and gastrointestinal tract to ensure immediate survival also called the brain-sparing effect (9, 10). Other adaptations may be aimed at optimizing nutrient uptake of scarce nutrients; these include endocrine or metabolic alterations, e.g. hypothalamic-pituitary-adrenal axis. Another possible explanation for adjustments made during early life in response to environmental cues is the notion that the organism adjusts its phenotype to suit the future while it has no immediate benefit from these adaptations. Anticipatory mechanisms are quite common in
developmental biology (11). Some insects, for example, change the size of their future wings during their larval stage depending on the external circumstances such as temperature or population density. Larva growing in crowded populations for instance tend to develop larger wings, enabling them to fly greater distances in search of resources (12). Even mammals can change their phenotype in anticipation of future events. Some squirrels can adjust their fertility anticipating a future crop and human infants determine the number of sweat glands from environmental cues within a crucial window after birth, anticipating future thermal conditions without any immediate necessity (13-15).

Plasticity is possible by the adaptation of developmental pathways during growth and development. There is evidence that certain organ systems are particularly amenable to environmentally driven alterations during certain periods of rapid growth or differentiation. This concept of ‘critical periods’ may explain why plasticity is primarily a phenomenon of early life and development (16).

To summarize the above, a fetus confronted with adverse intrauterine conditions due to nutritional deficiencies, famine or placental insufficiency may adapt to a nutritionally poor environment, leaving the fetus with endocrine or metabolic boundaries to its fitness to face the modern day’s abundant postnatal environment.

Looking at this from the perspective of evolutionary development, ‘evo-devo’, the goal of many species is to optimize the survival of the species through maximal fertility, which may be at odds with investments in the survival of the individual (17). Fertility is the determining factor of the species’ fitness. In fact, all other fitness components, such as mortality, only affect fitness through the species’ ability to procreate (18). Many species, including humans, make compromises in this principle in two regards (19). The first is investing time and energy in maturation to benefit offspring survival. Variable conditions in this maturation period can be cues for the individual to adjust fertility. In prosperous times, girls are known to enter their fertile life span at a younger age while unfavorable conditions during maturation can delay maturity and negatively affect fertility, at least in the short term (20, 21). The second trade-off is the balance between investment in size and number of offspring. Compared to other species, women can have a very limited number of children. We, as a species, have invested a large amount of energy in delivering
a few healthy offspring which consequently makes female fertility the weakest link in times of adversity and therefore biologically vital to protect. Considering this we can say that the aim of developmental plasticity may be to keep the individual alive and healthy long enough to reproduce.

**Female fertility and DOHaD**

The effect of intrauterine and early childhood conditions on female fertility in humans does not seem to be conclusive and the papers published on the subject are not consistent. Intrauterine growth retardation (IUGR) has been linked to various fertility related outcomes such as precocious puberty, small uterine size, high stimulating hormone (FSH) levels and polycystic ovaries (PCOS) (22, 23). Most of these studies however, were conducted in children and young women who were severely growth restricted at birth and did not fully catch-up. These children’s development could be considered permanently stunted as a result of a poor intrauterine environment, a phenomenon that is known to have impact on many features of physiology. A comparable number of studies dismiss the effect of early life environment on fertility or even detect increased fertility after adverse early life circumstance (24-26).

Rodent studies where food restriction was applied to match specific fetal ovarian development indicate that different developmental mechanism are sensitive to intrauterine food restriction (27). In the early stages of germ cell migration and proliferation maternal food restriction reduces the number of germ cells produced (28). Maternal food restriction in later stages affects the expression of genes that regulate apoptosis (27, 29). These rodent models could be argued to apply undernutrition that differs in two ways from human poor nutritional models: it is more severe and primarily protein restricted. And of course mice are not men, both in terms of physiology and reproductive strategy.

Considering the huge effort of all species to maintain, protect, and adjust fertility according to environmental conditions, in our opinion it is very likely that reproductive fitness in women is protected from intrauterine and childhood adverse conditions. To
preserve fertility, reproductive organs would hypothetically be spared actively or passively.

But one could also argue that the oocyte is at its most vulnerable stage during gestation. In no other developmental period does the oocyte undergo so many changes in number or cellular structure. Depending on the timing of the stress induced by the early life events such as famine, oocyte numbers could rapidly decline due to lower rates of mitosis or increased rates of apoptosis, or increase due to the deregulation of programmed cell death. It also has been hypothesized that, because during embryonic development the ovary and kidney derive their nutrition from the same or closely related blood supplies, fetal growth restriction may lead to smaller number of ovarian follicles due diversion of blood flow to the brain away from the ovary (30, 31).

To fully understand the possible impact of intrauterine conditions on female fertility, one needs to have an overview of intrauterine oocyte and follicle formation as well as active cell death mechanisms.

**Oocyte development**

The first primordial germ cells (PGC) are observed at a gestational age of 4 weeks when the embryo has a developmental age of 17 days (32). By the 5th week of gestation PGC start to migrate from their extra embryonic location to the gonadal ridges arriving at the gonads around the 6th week of gestation (33). At the 6th week of gestation the gonads are bi-potent: they have the ability to develop into either ovary or testes. Initially germ cells form interconnect nests in the ovarian cortex (34). These nests of oogonia are surrounded by a thin layer of pregranulosa cells. At about the 15th week of gestation these nests breakdown and the first follicular formation is detected (35, 36). The migration of oogonia from their extra embryonic location to the genital ridges, is completed during the 8th week of gestation. During this journey the number of the PGC increases from a few hundred to 5000 by the 8th week. This proliferation is mainly due to mitosis. At about 10th week of gestation the first oogonia initiate meiotic division forming primary oocytes (37, 38). Animal studies indicate that retinoic acid (RA), originating from the rete ovary or mesonefros induces meiosis of the oogonia in female ovaries (39). Therefore oogonia
nearest to the medulla initiate meiosis first. They are also the first oocytes to be arrested in meiosis prophase 1 (40, 41).

The time elapsed between meiosis 1 and ovulation seems to be related to the quality of the oocyte later in life. Oocytes entering meiosis first are also the first to ovulate and oocytes furthest from the ovarian medulla are the last oocytes left in the ovarian pool at the end of the fertile period (42).

In contrast to some other species for example mice, human oogonia do not enter meiosis synchronically. Therefore a cross section of fetal ovaries shows oogonia and oocytes in different meiotic and mitotic stages. By the 6th month of gestation all oocytes have initiated the meiotic prophase 1 and are arrested in the diplotene stage entering an extended dormancy which may last up to 50 years.

Germ cell loss is mainly seen in the following stages of ovarian development: (I) Massive loss of oogonia migrating from the yolk sac to the genital ridges while undergoing mitosis in 5th - 6th week of gestation. This is thought to be due to passive as well as active cell death (43). (II) Germ cell loss at the pachytene and diplotene stage initiated after the 10th week of gestation. This atresia is thought to be mainly through active programmed cell death, mediated by various apoptotic agents (38, 42).

**Follicle formation**

When entering the gonadal streak, PGC proliferate extensively forming nests of oogonia separated by cords formed in the primitive gonads. Within these nests the germ cells communicate through cytoplasmic bridges (44). Follicle formation is seen as early as 11-13 weeks of gestation but are more abundant at 16-20 weeks of gestation in the ovarian cortex (38, 45, 46). In the 15-20 week of gestation oocyte nests start to break down losing direct communication (35, 47). At this stage individual oocytes are selected for follicle formation. It is estimated that between one third and two third of the oocytes degenerate while the nests break down (36, 48). This process is an active apoptotic process (49-52).

When the active breakdown is defective multi oocyte follicles (MOF) are seen more frequently. MOFs are defective follicles which contain more than one oocyte. MOFs can
also be induced by high estrogen levels and are associated with lower ratios of fertilization (47, 53). If MOFs can result in a dizygotic pregnancy is disputed (54-56).

There are two types of ovarian somatic cells: theca cells and granulosa cells. Both cell types participate in the regulation of follicular development by actively synthesizing or binding local or systemic hormones (57). Somatic theca cells originate from ovarian stroma cells while somatic granulosa cells originate from the ovarian hilus in the medulla (38). The synthesis of the two ovarian steroids, estrogen and progesterone, is the major function of the granulosa cell as well as the physical support of the oocyte. The steroid synthesis by the granulosa cell is mainly under the influence of hypothalamic gonadotropins, FSH and luteinizing hormone (LH). The primordial (intrauterine) follicle in contrast to the postnatal secondary, pre-antral and antral follicle, however, is gonadotropin and steroid independent (58).

The major function of the theca cells is the production of ovarian androgens under the influence of LH (59). Theca cells are recruited from the ovarian stroma when the primordial follicle is developing into the primary follicle. At this stage prenatal pre-theecal cells are also gonadotrophin and steroid independent.

A women’s ovarian reserve consists of the number of dormant follicles all in mitotic arrest present at birth. While it is assumed that this number is finite, new evidence suggests that post-natal production of oocytes from germline stem cells is possible (60, 61). This concept however, is still controversial and not in the scope of this thesis. The journey from germ cell to primordial follicle is visualized in figure 1.

**Fetal Hypothalamus-pituitary**

The Hypothalamus and the pituitary differentiate during the first weeks of gestation and the hypothalamic-pituitary portal system is functional at about 18 -20 weeks of gestation (62). The hypothalamus secretes gonadotrophin-releasing hormone (GnRH) which travels though the portal system stimulating the pituitary to produce LH and FSH. Fetal blood concentration of these hormones increase until mid-gestation (22-25 weeks) and declines thereafter. The maturation of the hypothalamus and it’s increasing sensitivity to the
negative feedback of placental sex steroids attributes to this drop in hormonal concentration levels (63).

Figure 1. Oocyte development: from germ cell to follicle

LH and FSH seem to play a role in the differentiation of the ovary but do not influence fetal germ cell and follicle development. The fetal ovary also appears to be relatively inactive with respect to steroidogenesis. Fetal Ovarian steroids seem unnecessary for
genital differentiation but are said to have a role in the sex specific differentiation of the hypothalamus-pituitary axis (63).

**Aging and cell death**

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a role in cellular aging. ROS and RNS, also known as free radicals, are highly reactive molecules with an extra free electron in their outer envelope. Mitochondria, the energy factories of cells metabolizing oxygen and producing ATP, are the main cellular energy source and the main site of ROS output. The most reactive form of RNS is produced when the nontoxic intercellular messenger, nitric oxide (NO), reacts with superoxide (O$_2^-$) to form oxidant peroxynitrite (ONOO$^-$)(64). Small amounts of free radicals are necessary for physiological function but free radicals also interacts with macromolecules thereby damaging DNA, lipidmembranes and proteins. The accumulative cellular damage finally results in the cells senescence and cell death (65). Increased oxidative stress is associated with ovarian aging and the decline of ovarian reserve (66).

One of the mechanisms through which free radicals accelerate the aging process, is DNA damage to telomeres. Telomeres are a unique nucleotide repeat sequence at the distal ends of chromosomes essential for the genomic stability and are considered the mitotic clock. Telomeres shorten after each mitotic division and when they reach a critical length the cell seizes to divide (67). There is an inter-individual difference in telomere length at birth and telomere attrition rate there after (68). The average rate of telomeric length attrition is highest during infancy, presenting a ‘critical window’ for programming of senescence by environmental influences (69). Telomerase, an enzyme present in germline cells, some somatic cells and cancer cells, has the ability to increase the length of the telomeric DNA and is highly active in oocytes (70). Ovarian telomerase activity declines with age and there is evidence of lower activity in women with ovarian insufficiency (71, 72). Free radical overload can also directly activate apoptotic pathways.

Cells, however, have the ability to bind free radicals with antioxidants, producing water in the process and buffer the negative effects to the cellular structure (73). Nutritional stress
induced by periods of undernutrition or specific nutritional deficiencies increase oxidative stress rates and thereby cellular aging (74), and could affect ovarian reserve.

To summarize the above, we can state that the most eventful period in the lifecycle of a germ cell is during gestation. In the first weeks of gestation germ cells multiply rapidly, to decline steeply only a few weeks into pregnancy while migrating to the genital ridges. In mid-gestation the proliferation is completely ceased and due to the breakdown of germ cell nests and formation of follicles many germ cells are actively terminated. It is estimated that 85% of the initial pool of germ cells is lost before birth (37). After birth there is a steady decline in germ cells with a post-gestational surge (75). Theoretically environmental factors influencing cell proliferation and active cell death in these sensitive periods could have a distinctive effect on the female germ cell reserve.

**Ovarian reserve**

As laid out in previous sections, the entire ovarian reserve is established at mid-gestation and declines from there on. The most accurate and most likely the only true measure for ovarian reserve is age at natural menopause. A year after spontaneous cessation of menstruation ovarian reserve is depleted down to approximately 1000 follicles. The cessation of menstrual periods is preceded by a decade of declining fertility (76). Following the introduction of hormonal contraception in the 1960s, increasingly couples could determine the number and spacing of their children. With the increasing age of mothers at first pregnancy, women can find themselves confronted with subfertility and in need of assisted reproductive treatment (77). These demographic developments have further stressed the need for a better understanding of the drivers determining age at menopause and premature ovarian insufficiency. Physicians counseling women with fertility issues have an increasing need to determine ovarian reserve as an aid to predict the chances to a successful pregnancy and advise on family planning. Unfortunately accurately measuring ovarian reserve, being the number of oocytes left in the ovary, has proven difficult (78). Different biological markers are currently in clinical use to estimate functional ovarian reserve. Serum levels of LH, FSH, estrogen (E2), anti mullerian hormone (AMH) and the pituitary response to exogenous GnRH, all are established markers for ovarian reserve (79,
While FSH is a good marker of the current state of fertility, AMH seems to have the most predictive value. While cystic ovaries have been described as early as the 17th century, in 1935 Stein en Leventhal published the first article on a series of women demonstrating the combination of polycystic ovaries, hirsutism, oligo/amenorrhea and subfertility. From then onward Stein-Leventhal syndrome was synonymous to PCOS, the latter being the preferred term since the 1990s. Stein en Leventhal considered this syndrome to be rare and insignificant for female fertility, but with a currently documented prevalence of up to 20% depending on diagnostic criteria and geographic location, PCOS is one of the most common causes for reduced fertility in women. The diagnostic criteria for PCOS have been subject to change over the last decades. The National Institutes of Health (NIH) criteria published in 1990, require the presence of hyperandrogenism and menstrual dysfunction to diagnose PCOS. The Rotterdam criteria first published in 2003 require 2 out of 3 of the following features; oligo-anovulation, hyperandrogenism and polycystic ovaries (PCO) by ultrasound. In 2006 the Androgen Excess Society published new diagnostic criteria which required the presence of clinical or biochemical hyperandrogenism in the presence of either PCO or menstrual dysfunction to diagnose PCOS (Table 1).

PCOS is marked by normo-gonadotrophic normo-estrogenic oligomenorrhea or amenorrhea. Women with PCOS have an elevated LH:FSH ratio as well as elevated LH blood levels. An exaggerated LH response to exogenous GnRH with relatively low FSH levels is common in women with PCOS. While PCOS is named for its impact on the ovaries and fertility issues, it is known to have several sequelae for cardio-metabolic health: obesity, insulin resistance, cardiovascular disease and overall mortality are also highly associated with PCOS.
Table 1. PCOS definitions (87-89)

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<th>NIH Criteria 1990:</th>
<th>Rotterdam criteria: 2003</th>
<th>Androgen excess society:</th>
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<td>All of the following</td>
<td>Two of the following</td>
<td>All of the following</td>
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<tr>
<td>1. Clinical and/or biochemical hyperandrogenism</td>
<td>1. Clinical and/or biochemical hyperandrogenism</td>
<td>1. Clinical and/or biochemical hyperandrogenism</td>
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<tr>
<td>2. Menstrual dysfunction</td>
<td>Oligo-anovulation</td>
<td>2. Menstrual dysfunction and/or Polycystic ovaries</td>
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<td>3. Polycystic ovaries</td>
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PCOS seems to have a strong genetic component and a clustering of PCOS within families is common (94). Different modes of inheritance and candidate genes have been brought forward (95, 96). While genetics do not fully explain the rise in PCOS prevalence in recent decades, and the etiology of PCOS remains largely unclear, several hypotheses have been coined on the effects of early life on PCOS. Animal studies in non-human primates indicate that elevated prenatal testosterone levels during critical periods of gestation can cause a PCOS like phenotype, as well as intrauterine growth restriction (97, 98). As the human fetus is protected from excess maternal androgens by Sex Hormone Binding Globuline (SHBG) and placental aromatase, which convert androgens into estrogens, a fetal origin of androgen, ovarian or adrenal, should be taken into account (99). A placental origin of fetal excessive androgen exposure in PCOS mothers has also been postulated (100). A primary effect of maternal hyperinsulinemia should also be considered since insulin, IGF-1 and IGF-2 inhibit placental aromatase secretion at concentrations just above physiological, exposing the fetus to elevated androgen levels (101, 102).

A sedentary lifestyle and dietary habits as well as environmental toxins have been named as exacerbating factors of PCOS (103, 104). Bringing together adult risk factors for PCOS with intrauterine factors, a two-hit-theory has been suggested, where two insults during specific developmental windows are required for the development of PCOS. The first hit being prenatal or early childhood influences such as hyperandrogenism due to genetic or environmental factors (105, 106).
Aim of this thesis

The aim of this thesis is to answer the question whether, and to which extent adverse perinatal and/or early childhood conditions affect ovarian reserved and fertility in women. The thesis is divided in three sections; the first section is an introductory section. The second section is devoted to the effect of adverse perinatal and early childhood conditions on ovarian reserve. We hypothesized that adverse perinatal and early childhood conditions result in a diminished ovarian follicle reserve, either by leading to a reduced primary ovarian follicle pool due to impaired intrauterine follicle formation, or due to accelerated follicular cell death. The third section investigates the association between measures of an adverse environment in early life, including birth weight, and PCOS. PCOS and adverse perinatal conditions both are also established risk factors for insulin resistance and cardiovascular disease the question remains if these two risk factors are related.

Outline of this thesis

Many studies, including several of the studies in this thesis, consult historical databases to gather information on birth parameters such as birth weight and link them to phenotypic outcomes in adulthood. The first question we tackle in chapter 2 (section one) is whether, by using data on birth parameters from historical databases introduces a systematic bias. We hypothesized that more recent databases collecting data from the 1980s onward contain more individuals born (extremely)small for gestational age or preterm due to their increased survival chances compared to historical databases. We also investigated whether selection bias is a feature that differs between volunteer based and population based databases. Population based twin registers include all twins born in a defined region while volunteer based twin registers only enroll twins who actively volunteer to register. The fact that a person was born with a (extremely) low birth weight or preterm may influence their motivation to enroll in a volunteer database, thus leading to selection bias.

In section two, consisting of chapters 3, 4, 5 and 6, we concentrate on the effects of birth parameters on ovarian reserve. Chapter 3 is a pilot case-control study were we quantify ovarian reserve, by measuring LH, FSH, E2, AMH levels and the pituitary response to
exogenous GnRH in adolescent healthy women aged between 18 and 21 years born small for gestational age (SGA) or appropriate for gestational age (AGA). All women had a regular menstrual cycle, no history of gynecological or endocrinological disease and did not use any kind of hormonal anticonception. We hypothesized that women born SGA have a diminished ovarian reserve. In chapter 4 we describe a case-control study comparing birth parameters between women diagnosed with premature ovarian insufficiency (POI), defined as age at natural menopause < 40 years and controls without early cessation of menstruation, in order to assess the role of early environment on the etiology POI. Chapter 5 is a twin study, focusing on the inter-twin difference in menopausal age of monozygotic and dizygotic twins discordant for birth weight in order to assess the contribution of genetic and early environmental influences on age at menopause. In the final chapter of this section, chapter 6 we systematically review publishes literature according to PRISMA and MOOSE criteria on the relation between early life growth and nutritional restriction on age at natural menopause.

Section three including chapters 7, 8, 9 and 10 focusses on the genetic and perinatal environmental influences on the etiology of PCOS. Chapter 7 is a twin study estimating the heritability of PCOS. In chapter 8, a cohort study of women enrolled in one of the 13 IVF centers for treatment between 1980-1995 (OMEGA cohort), we investigated the effect of birth weight and age at menarche on PCOS and diminished ovarian reserve. The case-control study described in chapter 9 compares birth parameters of women diagnosed with PCOS and a control group. We conclude this section with chapter 10: a systematic review and meta-analysis according to PRISMA and MOOSE of all published literature on the relation between early life parameters and PCOS.

Chapter 11 is the general discussion of this thesis. Here we review our findings in light of evolutionary development and discuss the implications for health care professionals, preventive medicine and future research.
Reference


77. OECD Family Database. Available from:: http://www.oecd.org/els/family/database.htm

Chapter two

Potential Bias Regarding Birth Weight in Historical and Contemporary Twin Data Bases.

Sadrzadeh S, Treloar SA, van Baal GC, Lambalk CB.

Abstract

In this study we examine the hypothesis that monozygotic (MZ) twins in historical databases are less discordant for birth weight due to negative selection of severely discordant MZ twins. Furthermore, we test the hypothesis that MZ twins are less discordant for birth weight when comparing a volunteer based twin registry with a population based twin registry, due to selective registration. Data were available on 3927 twin pairs from the volunteer Australian Twin Registry born before 1964, 3059 volunteer twin pairs from the Netherlands Twin Register born 1987-1989 and 454 Belgian twin pairs from The East Flanders Prospective Twin Survey born 1987-1989. Intra-pair relative birth weight differences (RBWD) were computed for MZ and dizygotic (DZ) twins from each twin registry. Comparing birth weight differences between MZ and DZ twins provides support for the hypothesis that MZ twins are subject to a negative selection in historical databases. Furthermore, Australian MZ twins have a lower RBWD compared to Dutch MZ twins when corrected for the RBWD of Australian and Dutch DZ twins, indicating circumstances which only affect MZ twins. Our hypothesis that MZ twins are less discordant for birth weight in a volunteer based twin registry compared to a population based twin registry had to be rejected. We suggest that investigators using historical databases to test the fetal origins hypothesis should be aware of this increased likelihood of selective exclusion of individuals with extreme morphometric parameters at time of birth.
Introduction

Over the last decade there has been increasing evidence that adverse intrauterine conditions are associated with adult onset disease such as high blood pressure and mortality rates (1). Low birth weight is often seen as an indicator for these adverse intrauterine conditions. Twin data have frequently been used to investigate these issues with inconsistent results. When using twin data, some authors fail to find an association between intrauterine growth retardation experienced by twins and increased blood pressure or increased mortality rates in adults (2, 3). Other investigators however find an association between low birth weight and increased blood pressure in adult life when testing the fetal origin hypothesis in twins (4, 5). These inconsistent findings might be due to selection bias, whereby severely discordant monozygotic (MZ) twins are unknowingly excluded from some twin databases and not from others, thereby introducing a bias when comparing research results or data from different databases. MZ twins are known to be at a higher risk of perinatal mortality than dizygotic (DZ) twins (6, 7). This may be due to the effects of circumstances specific to MZ twin pregnancies, in particular monochorionic pregnancies, such as vascular anastomosis, unequal sharing of placenta perfusion zones or Twin-To-Twin Transfusion Syndrome (TTTS) (8, 9). TTTS is a condition with a high mortality rate up to 100% and accounts for large intra-pair birth weight differences between MZ twins (10, 11). The reported incidence of TTTS varies from 5% up to 20% of monochorionic twin pregnancies (12-14).

Birth weight is influenced by genetic factors; however, as demonstrated among extremely discordant twins, environmental factors such as TTTS or unequal sharing of placenta perfusion zones may have a more profound influence (8). Since 1980 improved diagnostic and therapeutic tools, combined with advanced neonatal care, have contributed to increased survival of twins, of MZ twins in particular (15, 16). Accordingly, historical databases in which adult twins are recruited on a volunteer basis could be expected to have a lower rate of discordance among MZ twins, due to the lower survival of low birth weight twins before 1980.

With decreasing mortality rates the number of severely discordant twins surviving extreme intrauterine and neonatal conditions increases. Twins surviving extreme
intrauterine conditions can encounter severe health problems (17). These health problems among the surviving twins might negatively influence the willingness of parents to register their children. Because of this, MZ twins in volunteer twin registers may be less discordant compared to population based twin registers. Data on biometrical birth parameters might therefore be influenced by birth year of the twins and recruitment methods.

In this study we examine the hypothesis that, in historical databases, MZ twins are less discordant in birth weight than MZ twins in contemporary databases, due to negative selection. Furthermore, we test the hypothesis that MZ twins are more discordant in population based twin registries than those enrolled in volunteer twin registries, due to selective registration. We chose to examine these hypotheses using data from three different twin registries, having access to data already available for other research purposes. Discordance in birth weight was tested among MZ twins and corrected for birth weight discordance between DZ twins.

**Methods and Materials**

**Subjects**

**Australian Twin Registry (ATR):** Data were available from 3927 twin pairs, all born before 1965, enrolled with the volunteer ATR. This volunteer register has more than 30,000 twin pairs enrolled, about 10–20% of the estimated number of pairs in the population (18). Information was available on zygosity and birth weight. Zygosity was assigned on the basis of responses to standard items, followed up with further queries in the case of inconsistent responses, resulting in < 2% error when checked against genetic marker concordance. Data on 3909 twins were analyzed after excluding twins with missing data on zygosity and with unlikely birth weights. The sample included 512 monozygotic male (MZW), 295 dizygotic male (DZW), 1340 monozygotic female (MZF), 822 dizygotic female(DZF) and 940 dizygotic opposite sex (DZOS) twin pairs. Self-reported birth weight was obtained by mailed questionnaire from an original twin cohort of 3,808 twin pairs aged 17 to 88 years in 1980–82 and an additional smaller cohort of twins aged over 50 to 95 years in 1993–1995 (19). Birth weight was reported in pounds (lb) and ounces (oz) and converted to grams before analysis.
**Netherlands Twin Register (NTR)** (20): Twins born in 1987, 1988 or 1989 and registered on a volunteer basis at the NTR were included if birth weight of both twins was known (21). Twins are recruited via commercial organizations and population registers. This volunteer registry consists of more than 20,000 twin pairs. About 45% of the estimated number of pairs born each year are enrolled. Information on birth weight, gestational age and zygosity was extracted from the register. Birth weight and gestational age were reported by the parents in a questionnaire that they received within 6 months after delivery. Birth weight was measured by a nurse or doctor shortly after birth in the hospital and passed on to the parents. Zygosity was determined by similarity questions in other questionnaires which were completed when the twins were 5 years old (22). Data on 3059 twin pairs were analyzed after excluding twins with missing birth weights (30 pairs). The selection included 498 MZM, 518 DZM, 537 MZF, 483 DZF and 1023 DZOS twin pairs.

**The East Flanders Prospective Twin Survey (EFPTS)** (20, 23): All twins born in 1987, 1988 or 1989 in the province of East Flanders in Belgium were selected. After excluding twins with unknown zygosities (15 pairs), data on 454 twin pairs were analyzed. Birth weight was obtained from the obstetric records. Zygosity was determined through sequential analysis based on sex, fetal membranes, umbilical cord blood groups, placental alkaline phosphatase genotype and DNA fingerprints (23). The selection included 71 MZM, 80 DZM, 91 MZF, 71 DZF, 141 DZOS live born twins.

**Statistical Analysis**

For each twin pair we calculated the Relative Birth Weight Difference (RBWD). RBWD is commonly defined as the intra-pair birth weight difference expressed as a percentage of the larger twin’s birth weight \([(\text{heavier}–\text{lighter}/\text{heavier}) \times 100]\). The mean RBWD of MZ and DZSS twins within each register was tested with a student *t* test. An alternative method to test the same hypothesis is to compare the mean RBWD of MZ twins between registers. This was also tested with a student *t* test. To control for overall differences in RBWD of twins between registers, RBWD of DZ twins was included in the analysis. We expected no change in RBWD of DZ twins, but a decrease in RBWD in MZ twins when comparing historical with contemporary data or volunteer based with population based
data. Analysis of variance (ANOVA) was conducted, with RBWD as dependent and zygosity and country as independent variables, to test for interaction between zygosity and country.

Data were analyzed using SPSS.9 for Windows and SAS 6.12 computer package (SPSS INC, 1997; SAS Institute Inc, 1997). A difference was considered significant if $p < .05$.

Results

MZ monochorionic and MZ dichorionic Belgian twins were pooled as the Dutch and Australian selection did not contain information on chorionicity. MZM and MZF twins as well as DZM and DZF twins were also pooled for analysis, as there were no significant differences in mean RBWD between different sex groups within zygosities (Table 1).

Table 1. Mean Relative Birth Weight Difference (RBWD) in Percentage, According to Zygosity and Sex of Three Registers, Australia, Netherlands and Belgium. Monozygotic Males (MZM), Dizygotic Males (DZM), Monozygotic Females (MZF), Dizygotic Females (DZF), Dizygotic Opposite Sex (DZOS)

<table>
<thead>
<tr>
<th></th>
<th>Australia</th>
<th></th>
<th>Netherlands</th>
<th></th>
<th>Belgium*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>RBWD</td>
<td>SD</td>
<td>$n$</td>
<td>RBWD</td>
<td>SD</td>
</tr>
<tr>
<td>MZM</td>
<td>512</td>
<td>10.19</td>
<td>9.72</td>
<td>498</td>
<td>11.46</td>
<td>9.58</td>
</tr>
<tr>
<td>MZF</td>
<td>1340</td>
<td>10.95</td>
<td>11.08</td>
<td>537</td>
<td>11.54</td>
<td>9.94</td>
</tr>
<tr>
<td>DZM</td>
<td>295</td>
<td>12.36</td>
<td>11.15</td>
<td>518</td>
<td>11.95</td>
<td>9.14</td>
</tr>
<tr>
<td>DZF</td>
<td>822</td>
<td>12.19</td>
<td>10.78</td>
<td>483</td>
<td>11.83</td>
<td>9.75</td>
</tr>
<tr>
<td>DZOS</td>
<td>940</td>
<td>13.78</td>
<td>12.17</td>
<td>1023</td>
<td>12.57</td>
<td>9.61</td>
</tr>
</tbody>
</table>

Note: Live born twins

First we tested the hypothesis that the mean RBWD between MZ and DZSS twins differed within a time period. The RBWD of Australian MZ twins was 1.49 % lower than in DZSS twins ($p < .0001$) while RBWD in younger Dutch MZ and DZSS twins did not differ ($0.39\%; p = .36$).
Furthermore, we tested whether MZ twins have a higher RBWD in the young Dutch database compared to the historical Australian database. Between the MZ twins RBWD was higher in the Dutch than in the historical Australian database (0.76%; \( p = .060 \)). ANOVA was conducted, whereby RBWD was set as dependent and zygosity and country as independent variables, the interaction between zygosity and country was significant, \( p = .005 \) (Figure 1).

Figure 1 Relative birth weight difference (RBWD) pattern of monozygotic (MZ) versus dizygotic (DZ) Australian (1852 MZ, 2057 DZ) and Dutch (1035 MZ, 2024 DZ).

To determine the difference between volunteer based and population based twin registries, we used data from the Dutch and the Belgian register. We tested if the mean RBWD between MZ and DZSS twins within a country differed. The RBWD of Belgian MZ twins was 1.39% lower than in DZSS twins (\( p = .19 \)). To test if MZ twins have a higher RBWD in the volunteer based Dutch database compared to the population based Belgian database a student t test as well as ANOVA was conducted. Within MZ twins the RBWD of Belgian twins was 1.10% lower than of Dutch twins (\( p = .30 \)), and interaction between zygosity and country was not significant, \( p = .15 \). Both tests did not yield support for differences between volunteer and population based registers (Figure 2).
Discussion

Our hypothesis was confirmed that MZ twins are less discordant in a historical twin database. When comparing Dutch twins in the late eighties with Australian twins early in the century, intra-pair birthweight differences are influenced by many variables. Not only do health care and development of health care systems differ in both countries, genetic predisposition might also differ considerably. Most of these variables, however, are equal for MZ and DZ twins in the same country. Therefore a significant difference in RBWD between MZ and DZ twins in one database and not in another would indicate a higher survival rate of MZ twins in the latter.

This is the case comparing MZ twins with DZ twins within the Australian and the Dutch database. Australian MZ twins differed significantly in RBWD from DZSS twins whereas Dutch MZ twins did not. For this particular analysis DZOS twins were excluded to eliminate sex-dependent birth weight differences when comparing MZ twins with DZ twins. We also tested if Australian MZ twins have lower RBWD compared to Dutch MZ twins when the difference in RBWD of DZ twins is taken into account. To eliminate intercontinental differences we set the difference in RBWD among Dutch and Australian DZ twins as standard, and examined if birth weight differences between Dutch and Australian MZ twins follow the same pattern as those of DZ twins. When MZ twins are set against DZ twins any additional difference in RBWD is due to specific circumstances only affecting MZ
twins. Our data indicate that the birth weight differences between Dutch and Australian MZ twins indeed have been influenced differently compared to DZ twins. Australian MZ twins are less discordant than Dutch MZ twins and this effect is even more prominent if set against the difference between Australian and Dutch DZ twins.

MZ twins are more often born very prematurely and have less favorable neonatal outcomes than DZ twins (6, 7, 9). This perinatal mortality in MZ twins is only elevated in monochorionic pairs and not in dichorionic pairs (24). Unfortunately few twin registries have information on chorionicity. Due to the better survival chances of MZ twins over the years, this negative selection of severely discordant MZ twins would be less prominent in a contemporary database. The contribution of improved neonatal care is most probably the main reason for the increased survival of MZ twins (15, 16).

Data extracted from the Australian historical twin registry consists of more female MZ twins (72% of the MZ twins). Female MZ twins are known to have a higher intra-pair birth weight difference compared to male MZ twins (25, 26). Considering this, the Australian MZ Twins should be expected to have an even higher discordance rate than the Dutch twins, which consist of 52% females.

However we are aware that studying twin survival within one register throughout the years would be the most appropriate study design to confirm our hypothesis. A study addressing intra-pair birth weight differences within one register throughout the years is underway.

We did not find significant intra-pair birth weight differences between volunteer based twin registries and population based ones. Belgian MZ and DZSS twins did not differ significantly. RBWD between Dutch and Belgian MZ twins, when corrected for the difference between Dutch and Belgian DZ twins, did not differ significantly either. We therefore had to reject our hypothesis that in volunteer databases selection bias in relation to adverse birth outcomes is not prominent. Dutch MZ twins seem to differ even more than Belgian MZ twins regarding birth weight difference. Nevertheless, we have to take into account that sample size may have limited the power of this particular comparison.
We suggest that investigators using data from historical twin registries should bear in mind the likelihood of selective exclusion of severely discordant MZ twins. Although we tested our hypothesis in a twin setting, our findings are in accordance with findings in the literature, where an increased survival of small for gestational age singletons from the 1980s onward has been reported (15, 16, 27, 28). This has important implications for studies regarding the fetal origins hypothesis. Only the strongest and fittest babies survived low birth weight in the early 20th century. Surviving infants had to adapt to the extreme circumstances in which they were born, developing mechanisms that made the difference between life and death. These adaptive mechanisms are the result of intrauterine and neonatal programming, reprogramming or genetic predisposition, but most probably an interaction between all these factors. With the number of surviving small for gestational age babies increasing, the number of adults encountering specific health problems related to intrauterine adverse conditions may consequently increase. On the other hand these adaptive mechanisms which enabled the fittest babies to overcome life threatening conditions in their infant years, decades ago, might well be the same mechanisms which cause the diseases they encounter in later life such as diabetes. Due to modern intervention methods this selection of the fittest has partly lost its importance. Some low birth weight babies surviving today would not have survived 30 years ago. These babies do not require the adaptive mechanisms to survive today as they did decades ago. If these adaptive mechanisms are the causal connection between adult onset disease and adverse intrauterine and neonatal circumstances, infants surviving low birth weight due to modern technology today are possibly not programmed to develop the diseases of the generations before them.

Acknowledgements

We thank Professor Robert Derom, Professor Robert Vlietinck and Dr Catherine Derom for granting us access to data from The East Flanders Prospective Twin Survey (EFPTS). We also would like to thank Ruth JF Loos for helping us with the analysis of the EFPTS data.
References

SECTIO TWO

Ovarian reserve
Chapter 3

Ovarian reserve in young women with low birth weight and normal puberty: a pilot case–control study.

Sadrzadeh-Broer S, Kuijper EA, Van Weissenbruch MM, Lambalk CB.

Abstract

Aim
Studies indicate that women born small for gestational age (SGA) have impaired ovarian function. The origin of this ovarian dysfunction is still debatable. The aim of this study was to compare ovarian ageing between girls born appropriate (AGA) and small for gestational age (SGA). Therefore, we measured LH, FSH, E2, Anti-Müllerian hormone (AMH) levels and the pituitary response to endogenous GnRH in adolescent girls born small and appropriate for gestational age.

Methods
A case-controlled pilot study consisting of 7 SGA women (birth weight < 10th percentile adjusted for gestational age) and 13 AGA women with regular menstrual cycles, age 19.9 (± 0.42). Early follicular FSH, LH, E2 and AMH levels were measured. After baseline samples 100 ug GnRH was administered intravenously and at 30, 60 and 90 minutes blood samples were taken to measure gonadotropin levels and to compute the response to endogenous GnRH.

Results
Mean follicular phase LH, FSH, E2 and AMH levels did not significantly differ between young women born SGA and AGA. Furthermore, the response to endogenous GnRH showed no significant differences either.

Conclusions
We concluded against extension of this pilot study. Based on our observations it seems unlikely that limited ovarian reserve is a predominated problem in adolescent SGA.
Introduction

The effect of early growth retardation on endocrine and metabolic programming has been investigated intensively over the last decade (1). Various conditions have been linked to intra-uterine growth retardation such as variations in sexual development (2;3). It has been reported that girls born small for gestational age (SGA) have impaired ovarian development and ovarian hyposensitivity to FSH, resulting in elevated FSH levels, reduced ovarian and uterine size, reduced ovulation rates and an increased risk of developing polycystic ovary syndrome (PCOS) (4;5). Although both the hypothalamus and the ovaries are suggested to play a role, the physiological mechanism underlying these findings is still poorly understood (3). Ovarian origin of elevated gonadotropin levels can be distinguished from hypothalamic dysfunction by a GnRH challenge test. When impaired ovaries are challenged with exogenous GnRH an elevated gonadotropin response is seen due to a diminished ovarian feedback mediated by inhibin B even in patient with normal FSH (6).

In females granulosa cells of early developing follicles produce Anti-Müllerian hormone (AMH) throughout reproductive life which can be seen as a marker of the ovarian follicle pool (7). Only minimal cycle dependent fluctuations have been observed (8). Early depletion of ovarian reserve results in a decrease in AMH while other markers such as FSH remain within the normal range (9).

A study designed to compare ovarian reserve between young women born AGA and women born SGA can clarify the issue on ovarian or hypothalamic origin of impaired ovarian development in SGA women. Therefore, we measured early follicular FSH, LH, oestradiol (E2), AMH and the pituitary response to exogenous GnRH in young women with regular menstrual cycles.

Since no valid data are available on AMH levels and pituitary response to exogenous GnRH in young women with SGA and AGA this pilot study was executed to calculate an appropriate sample size and decide for further studies.

Materials and methods

Sample size calculation

Data available from other studies comparing FSH levels in SGA and AGA (10) pre puerperal
girls were used as a rough guideline to estimate an appropriate sample size. To demonstrate a difference of 3.0 IU/l in FSH concentration using a two-tailed student t-test, the minimal sample size for each group is 7, with alpha 0.05 and power 0.8 assuming a SD of 2.

**Subjects and Ethics**

The study population consists of a total of twenty healthy volunteers recruited by means of advertisement among students or participants from previous studies (11). This resulted in seven women born small for gestational age and thirteen women born appropriate for gestational age. SGA was defined as a birth weight below the tenth percentile adjusted for gestational age using the Amsterdam growth curves (12). All girls had established regular menstrual cycles and did not use any kind of hormonal contraception for at least three months prior to testing. Characteristics of the study population are described in table 1. The study was approved by the ethical committee of the institute and informed consent was obtained from all participants.

**Study design**

Information on birth weight and gestational age was retracted from birth certificates. Additional information on age of menarche, menstrual cycle, height and weight were obtained from questionnaires. FSH, LH, E2 and AMH samples were taken and GnRH tests were conducted on cycle day 2, 3 or 4. Blood was drawn from an intravenous catheter which was placed by the principal investigator or a trained research nurse. After a baseline sample was taken, 100 µg GnRH was injected intravenously via the indwelling catheter and after 30, 60 and 90 minutes additional LH, FSH and E2 samples were taken. The maximum LH, oestrogen and FSH increment was taken as a parameter for the response to endogenous GnRH.

**Hormone measurements**

LH and FSH were measured by commercially available immunometric assays (Delfia, Wallac, Finland) at the VU medical centre. Intra-assay coefficients of variation (CV) for FSH are <5% and for LH < 7%. Estradiol was measured by radio immunoassay (Sorin Biomedical,
Sallugia, Italy) with an intra-assay CV of <5%. All measurements were done in one batch. Serum AMH levels were measured by an ultra-sensitive enzyme-linked immunosorbent assay (Immunotech-Coulter, Marseilles, France) (15). The lower limit of detection, defined as negative control + 3 SD of the negative control was 0.05 μgr/l. Intra- and inter-assay coefficients of variation were <5% and <8%, respectively.

**Statistical Analyses**

Statistical analysis was performed using SPSS 16.0. The analyses used were the t test for Independent samples. Post hoc power calculations were performed using the Power Calculator provided by the University of British Columbia, Vancouver. Department of Statistics at http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html.

**Results**

The baseline characteristics of the study population are summarized in table 1. No differences were found between the SGA and AGA women for age at menarche and BMI. By definition, women born small for gestational age had a significantly lower (adjusted) birth weight compared to women born appropriate for gestational age.

<table>
<thead>
<tr>
<th></th>
<th>AGA N=13</th>
<th>SGA N=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3369±165</td>
<td>2199±269</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.8±0.7</td>
<td>37.9±1.5</td>
</tr>
<tr>
<td>Adjusted birth weight</td>
<td>84.1±3.3</td>
<td>56.9±5.3</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.7±0.3</td>
<td>12.4±0.4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.8±0.5</td>
<td>20.1±0.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.1±1.2</td>
<td>167.7±2.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1±0.9</td>
<td>23.2±1.4</td>
</tr>
</tbody>
</table>

AGA=appropriate for gestational age, SGA=small for gestational age. Adjusted birth weight=birth weight/gestational age, BMI=weight kg/height m².
Follicular phase baseline LH, FSH, E2 and AMH levels did not differ significantly between girls born SGA and AGA.

Furthermore, the response to endogenous GnRH showed no significant difference (table 2). When challenged with exogenous GnRH the FSH or LH response of SGA girls did not differ from the FSH or LH response of AGA girls (figure 1 & figure 2).

With the difference of .78 U/L in FSH levels between SGA and AGA we found in our study, the minimal sample size to achieve significance was calculated. With alpha 0.05, power 0.8 and SD 1.7 each study arm should consist of 75 participants.

Table 2. The baseline endocrine data and the maximum oestrogen and gonadotropin increment to GnRH (Δ).

<table>
<thead>
<tr>
<th></th>
<th>AGA N=13</th>
<th>SGA N=7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>Median(range)</td>
</tr>
<tr>
<td>Baseline FSH (IU/l)</td>
<td>3.8±0.5</td>
<td>3.6 (1.7–7.3)</td>
</tr>
<tr>
<td>Baseline LH (IU/l)</td>
<td>3.6±0.5</td>
<td>3.2 (.5–7.7)</td>
</tr>
<tr>
<td>Baseline E2 (IU/l)</td>
<td>103.9±27.6</td>
<td>83 (22–422)</td>
</tr>
<tr>
<td>AMH (mg/l)</td>
<td>3.1±0.3</td>
<td>2.7 (1.8–5.3)</td>
</tr>
<tr>
<td>ΔFSH response to GnRH</td>
<td>3.6±0.5</td>
<td>3.3 (1.3–6.8)</td>
</tr>
<tr>
<td>ΔLH response to GnRH</td>
<td>23.4±4.6</td>
<td>20.2 (8.5–57.3)</td>
</tr>
<tr>
<td>ΔE2 response to GnRH</td>
<td>22.0±5.7</td>
<td>16 (710 to 77)</td>
</tr>
</tbody>
</table>

AGA=appropriate for gestational age, SGA=small for gestational age, AMH=anti-müllerian hormone.
Figure 1. Baseline FSH levels and FSH response to exogenous GnRH administration after 30 min, 60 min and 90 min. Comparing adolescent girls born small for gestational age (SGA) and appropriate for gestational age (AGA). Error bars: ±1 SEM.

Figure 2. Baseline LH levels and LH response to exogenous GnRH administration after 30 min, 60 min and 90 min. Comparing adolescent girls born small for gestational age (SGA) and appropriate for gestational age (AGA). Error bars: ±1 SEM.
Discussion

To our knowledge this is the first study comparing AMH levels and the response to endogenous GnRH administration between girls born small (SGA) and girls born appropriate (AGA). In this pilot study we found no significant differences in follicular phase LH, FSH, E2 and AMH levels between SGA AGA girls for gestational age. The response to endogenous GnRH administration is also comparable between these groups. Our initial sample size calculation was based on available data on FSH differences between pre-adolescent SGA and AGA girls (10). In that study an average difference of more than 2.4 U/L was observed. We could not reproduce this result. In our study the FSH with an average of 4.61 was slightly higher in the SGA group compared to the 3.83 U/L in the control group. However in order to substantiate this difference as significant it would require a sample size of 75 subjects per study arm.

Our study showed no significant difference in AMH values. AMH values tended to be higher in the SGA group. The FSH and LH response to GnRH tended to be lower. So both these tests do not point to some indication of limitations in ovarian reserve.

Given the constellation of these pilot observations from our tests we concluded that continuation of the study to sufficiently large size to substantiate a possible statistical significant but modest higher FSH in SGA as scientifically and ethically unjustified.

Our observations are not in line with this previous study showing much higher FSH levels in SGA (10). However, based on our observations it seems unlikely that limited ovarian reserve is a predominant problem in adolescent SGA. This is particularly supported by the observation with regard to AMH, which nowadays is considered as one of the most reliable parameters of ovarian ageing (7;13).

The effect of adverse intrauterine conditions on some adult onset diseases has been established during the past decade. However, there is still a debate on the effect of intrauterine growth restriction and ovarian function. Previous findings indicating decreased oocyte numbers and consequently ovarian impairment in SGA girls (4) could not be confirmed in a larger study (14). The best documented cause of elevated FSH due to ovarian impairment is menopause (15;16). Studies investigating birth weight and age at
menopause repeatedly fail to establish a relation between these two variables (17-19). A large prospective cohort study among pre-pubertal girls showed no difference in gonadal function and ultrasonographic uterine and ovarian measurements between SGA and AGA girls (20).

Depleted ovaries when challenged with endogenous GnRH show an elevated gonadotropin response due to impaired ovarian feedback mediated by inhibin B is seen (6). The pituitary response to GnRH did not indicate ovarian impairment in our study. Demise of ovarian function is a process that develops over time and early phases may be too subtle to trace in young adults but could become more evident later in life. Future follow-up studies of the subjects that participated in our study could reveal such signs.

Acknowledgments

The authors would like to thank all the participants who volunteered for this study project. We are also grateful to Professor F.H. de Jong of the department of internal medicine Erasmus University Medical centre, Rotterdam, The Netherlands, for facilitating the Anti-Müllerian hormone assay. We also thank Ted Korsen, research nurse, who conducted the GnRH challenge tests. We thank prof. HA Delemarre-van de Waal for her critical comments.
References


Chapter 4

Premature ovarian insufficiency and perinatal parameters: A retrospective case-control study.

Sadrzadeh S, Painter RC, van Kasteren YM, Braat DD, Lambalk CB.

Maturitas. 2017 Feb;96:72-76
Abstract

Objective
The peak number of oocytes is reached during intrauterine development, after which numbers decline until reserves are depleted and woman enter menopause. In premature ovarian insufficiency (POI), the process of follicle depletion occurs at a younger age, affecting about 1% of women. In this study, we investigate whether women with POI had experienced a different perinatal milieu as reflected in their birth weight or prematurity rate.

Study design
In this retrospective case-control study, we evaluated whether women diagnosed with POI had different birth weight or prematurity rate (<37 week) compared to women with a natural age at menopause above 40. Binary logistic regression models were used to analyze the data and correct for smoking. 59 women with POI and 92 controls were recruited.

Results
13.6% of women diagnosed with POI were born pre-term compared to 6.6% of controls \( p = 0.018 \). Corrected for gestational age, women with POI did not have a different birth weight compared to women with a natural age at menopause above 40. As a consequence of the design of our study, mean age at time of interview differed significantly between groups, 37.5 yr and 46 yr respectively for women diagnosed with POI and controls. Years of oral contraception use, smoking, age at menarche, BMI and education levels were similar between groups.

Conclusion
Our findings indicate prematurity as a novel risk factor for POI. Prenatal factors contributing to the etiology of prematurity, or postnatal factors that are the result of prematurity may lead to early follicle depletion.
Introduction

Premature ovarian insufficiency (POI) is a fertility related disorder affecting 1% of women (1). POI is defined as secondary amenorrhea before the age of 40 and elevated levels of FSH indicating an early depletion of ovarian follicle reserve (2). Fertility is significantly decreased a decade before the cessation of menstrual cycles. Besides reduced fertility, POI has a distinct association with long-term health issues such as an increased cardiovascular disease risk, osteoporosis and overall mortality (3). While specific conditions such as Turner syndrome, fragile X and auto immune diseases are associated with POI, most cases have an unexplained etiology. Smoking is one of the few lifestyle factors consistently shown to reduce age of natural menopause (4).

Intrauterine deprivation due to environmental or maternal influences has been proven to affect a broad variety of anatomical structures and physiological functions, from average organ weight and number of glomeruli to insulin resistance and cardiovascular disease in the offspring (5, 6, 7). Human follicles develop solely during the fetal period and reach their maximum number of 6 million at about 20 weeks of gestation, declining rapidly thereafter by active and passive cell death to 2 million at birth (8). Intrauterine deprivation during critical gestational periods might thus affect the ovarian capacity by influencing the primary follicle production in the first trimester, limiting the initial follicle pool or mediate an accelerated follicular loss thereafter. A Dutch study showed that the ovarian follicle reserve of deceased small for gestational age infants was smaller compared to the follicle reserve of appropriate for gestational age infants (9).

Alternatively, adverse postnatal conditions or accelerated general ageing may increase postnatal oocyte attrition. POI and adverse intrauterine conditions are both associated with an increased risk of cardiovascular disease, it is conceivable that a common pathophysiological pathway was engaged in utero, resulting in both POI and increased cardio metabolic disease in later life.

Although there are several studies investigating the relation between adverse intrauterine conditions and age at menopause, the association between perinatal conditions and POI has not been previously studied. In this retrospective case control study, we
investigated whether women diagnosed with POI had different early life conditions, as reflected in their birth weight and gestational age compared to women without POI.

Patients and Methods

Ethics and study population

All cases were selected from the medical files of the department of Obstetrics and Gynecology of the VU University Medical Center, Radboud University Medical Center Nijmegen, Medical Center Alkmaar and recruited through advertising at Freya, the Dutch patient organization for patients with fertility problems. POI was defined as self-reported secondary amenorrhea for at least 4 months before the age of 40 based on a clinical diagnosis of POI.

The control group was recruited via advertising and from medical files of the department of Obstetrics and Gynecology of the VU university medical center. The control group consisted of women aged between 40 and 60 who were still menstruating or self-reported their last menstruation after the age of 40. Patients and controls that met any of the following criteria were excluded: a history of ovarian disease or surgery, abdominal radiation- or chemotherapy or diagnosed with genetic abnormalities associated with POI, use of any medication that prevents the determination of age of menopause and the presence of POI related autoimmune serology. The ethical commissions of the institutes approved the study. Patients and controls received a letter informing them about the aim of the study and signed a consent form.

Study design

We chose a retrospective case-control study design. Participants completed a written questionnaire addressing fertility and health issues. They were also asked if they could confirm their birth weight with a written record or if their birth mother was still alive to confirm the birth weight. Gestational age was reported as weeks or months of gestation. Education level was reported as low (primary school or low vocational education), middle (high school and middle vocational education) or high (higher vocational education and academic education). Smoking was reported as current smoker, former smoker or never
smoked. For analysis, current smoker and former smoker were pooled into one category. Participants also provided information on current height and weight, history of contraceptive use, hormone replacement therapy, history of gynecological treatment, age at menarche, age at menopause and if they had a twin.

Power calculation and Statistical analysis

To demonstrate a difference of 200 g in birth weight using an independent Student t-test, the minimal sample size for each group was calculated to be 60 with alpha 0.05 and power 0.8. IBM SPSS Statistics 21 was used for analysis. An independent Student t-test was used when data were continuously and normally distributed. Continuous data are presented as mean and 95% confidence interval and significance level (P value) is presented. Non-parametric data were tested with a chi² test. Binary logistic regression models were computed adjusting for categorical as well as continuous independent variables.

Flow chart 1. Participants’ inclusion and exclusion
Results

The study group consisted of 59 women diagnosed with POI and 92 women in the control group. Inclusion details are listed in flow chart 1. Baseline characteristics and study parameters, birth weight and gestational age, of continuous data are summarized in Table 1; categorical data are presented in Table 2.

As consequence of the study design age at the time of investigation differed significantly between groups. Women diagnosed with POI were significantly younger compared to the control group. Other baseline characteristics did not differ between groups. There were no twins in either groups. Difference in age at menopause was not calculated because 88% of women in the control group were still menstruating at the time of investigation.

Table 1. Comparison of continuous variables of women diagnosed with premature ovarian insufficiency (POI) and controls.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=92)</th>
<th>POI (n=59)</th>
<th>P value</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at interview (n)</td>
<td>46.0 (92)</td>
<td>37.5 (59)</td>
<td>.000</td>
<td>-10.0</td>
<td>-6.97</td>
</tr>
<tr>
<td>BMI *(n)</td>
<td>24.4 (89)</td>
<td>23.7 (59)</td>
<td>.231</td>
<td>-2.00</td>
<td>0.49</td>
</tr>
<tr>
<td>Age at menarche(n)</td>
<td>12.9 (90)</td>
<td>13.2 (59)</td>
<td>.257</td>
<td>0.29</td>
<td>-0.25</td>
</tr>
<tr>
<td>Oral contraception yr (n)</td>
<td>9.71 (86)</td>
<td>7.74 (49)</td>
<td>.163</td>
<td>-4.75</td>
<td>0.81</td>
</tr>
<tr>
<td>Birth weight g (n)</td>
<td>3427 (92)</td>
<td>3376 (59)</td>
<td>.651</td>
<td>-271.42</td>
<td>170.14</td>
</tr>
<tr>
<td>Gestational age wk (n)</td>
<td>40.0 (60)</td>
<td>39.0 (14)</td>
<td>.130</td>
<td>-268.51</td>
<td>167.23</td>
</tr>
</tbody>
</table>

Independent Student t-test was used for continuous data with a normal distribution.*BMI, body mass index defined as weight /height^2

Women reported if they were born at term, pre-mature or post mature. We categorized gestational age at birth of women who did report weeks of gestation (49%) and defined
pre-term birth as birth before 37+0 weeks of gestation, term birth between 37+0 through 40+6 weeks of gestation and post-term as over 41+0 weeks of gestation. These gestational age categories were used for statistical analysis. Using binary logistic models we adjusted for smoking.

A significantly higher percentage of women diagnosed with POI reported having been born pre-term (13.6%) compared to controls (6.6%), $p=.018$ CI=1.30-16.7 adjusted for smoking (Table 3). Birth weight did not differ significantly between women with POI and control group. To investigate a possible U shaped or threshold effect, we also analyzed birth weight in categories. We found no evidence for a difference in distribution between birth weight categories according to POI diagnosis. Odds ratio, 95% confidence interval and P value of the binary logistic models are presented in Table 3. None of the analyses revealed any associations between birth weight after adjusting for gestational age and smoking.
Table 2. Comparison of categorical variables of women diagnosed with premature ovarian insufficiency (POI) and controls.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=92)</th>
<th>POI (n=59)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-term n(%)</td>
<td>6 (6.6%)</td>
<td>8 (13.6%)</td>
<td>.016</td>
</tr>
<tr>
<td>Term n(%)</td>
<td>66 (72.5%)</td>
<td>48 (81.4%)</td>
<td></td>
</tr>
<tr>
<td>Post-term n(%)</td>
<td>19 (20.9%)</td>
<td>3 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever n(%)</td>
<td>55 (59.8%)</td>
<td>38 (64.4%)</td>
<td>.610</td>
</tr>
<tr>
<td>Never n(%)</td>
<td>37 (40.2%)</td>
<td>21 (35.6%)</td>
<td></td>
</tr>
<tr>
<td>Birthweight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.5kg n(%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>.560</td>
</tr>
<tr>
<td>&lt;2kg n(%)</td>
<td>5 (5.4%)</td>
<td>2 (3.4%)</td>
<td>.892</td>
</tr>
<tr>
<td>&lt;2.5kg n(%)</td>
<td>10 (10.9%)</td>
<td>6 (10.2%)</td>
<td></td>
</tr>
<tr>
<td>&gt;4kg n(%)</td>
<td>20 (21.7%)</td>
<td>11 (18.6%)</td>
<td>.646</td>
</tr>
<tr>
<td>Term*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-term n(%)</td>
<td>6 (6.6%)</td>
<td>8 (13.6%)</td>
<td>.016</td>
</tr>
<tr>
<td>Term n(%)</td>
<td>66 (72.5%)</td>
<td>48 (81.4%)</td>
<td></td>
</tr>
<tr>
<td>Post-term n(%)</td>
<td>19 (20.9%)</td>
<td>3 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever n(%)</td>
<td>55 (59.8%)</td>
<td>38 (64.4%)</td>
<td>.610</td>
</tr>
<tr>
<td>Never n(%)</td>
<td>37 (40.2%)</td>
<td>21 (35.6%)</td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low(%)</td>
<td>33 (37.5%)</td>
<td>18 (30.5%)</td>
<td>.134</td>
</tr>
<tr>
<td>Middle(%)</td>
<td>10 (11.4%)</td>
<td>14 (23.7%)</td>
<td></td>
</tr>
<tr>
<td>High(%)</td>
<td>45 (51.1%)</td>
<td>27 (45.8%)</td>
<td></td>
</tr>
</tbody>
</table>

chi² test was used for Categorical data
* Pre-term birth is defined as birth before 37 weeks of gestation, term birth as 38, 39, 40 weeks of gestation, post-term as above 41 weeks of gestation.
Table 3. Results of binary logistic models: comparing women diagnosed with POI to controls regarding difference in birth weight corrected for various confounders

<table>
<thead>
<tr>
<th>POI vs Control</th>
<th>Odds ratio</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight in g *</td>
<td>1.0</td>
<td>1.000-1.001</td>
<td>.272</td>
</tr>
<tr>
<td>categorical birth weight* (&gt;2kg/&lt;2kg)</td>
<td>.075</td>
<td>.002-3.068</td>
<td>.171</td>
</tr>
<tr>
<td>categorical birth weight* (&gt;2.5kg/&lt;2.5kg)</td>
<td>.460</td>
<td>.084-2.517</td>
<td>.371</td>
</tr>
<tr>
<td>categorical birth weight* (&lt;4kg/&gt;4kg)</td>
<td>2.627</td>
<td>.613-11.261</td>
<td>.193</td>
</tr>
</tbody>
</table>

* adjusted for (stepwise): gestation age categories (term =indicator), smoking category, oral contraception use (yr), age at menarche, age at time of interview

Discussion

Our findings indicate prematurity as a novel risk factor for POI. This could reflect increased follicle attrition in the immediate postpartum due to increased infectious and nutritional insults common to premature babies (10). In line with our present findings, a period of famine or impaired growth during early childhood was associated with an earlier age at menopause, in the absence of any interaction with birth weight (11-13). After mid gestation, the follicle reserve is fully established and from then onward this initial pool diminishes by apoptotic and non-apoptotic programmed cell death. While this is a relatively steady continuous process up to puberty, there is an increased perinatal rate of germ cell loss. The sudden drop of placental sex hormones after birth and the consequent follicle loss partly explains this temporary acceleration (14). In addition, many organs, including the ovary, undergo apoptotic and non-apoptotic programmed cell death after birth to adjust to postnatal nutritional stress (15).

Preterm birth has a multifactorial etiology with a strong genetic component (16, 17). So alternatively, prematurity and POI could have a shared genetic pathway or etiology. Mothers born prematurely are more likely to give birth prematurely (18). Moreover, genetic factors associated with spontaneous preterm birth point to altered autoimmune regulation (19). POI, also associated with autoimmune disease, and preterm delivery might find their joint origin in gene polymorphisms regulating autoimmunity.
As our study design did not provide information on the cause of prematurity (eg preterm pre-labor rapture of membranes, spontaneous, infectious), we were not able to differentiate between various causes of prematurity in relation to POI. So far, reports on birth weight and early menopause have yielded inconsistent results. Some studies found an association between birth weight and a younger age at menopause, (20-24) while others found no such association (12, 13).

Like most case control studies, identifying an appropriate control group is a point of concern. To eliminate any chance of POI in the control group, we selected women who were older than 40 years at inclusion; therefore, our control group was significantly older than our patient group with a mean difference of 8.5 years. Our findings could be due to the possibility that prematurely born women in the POI group had a better chance of survival having the advantage of more advanced medical care. However, regardless of term at delivery, a low birth weight also presents a higher peri-natal mortality risk (WHO 2005). If our findings, therefore, are due to an increased mortality rate of high risk infants in the control group this should also have been reflected in a more prominent difference in birth weight between groups, which was not the case.

The retrospective design of our study and self-reported data also presents the problem of recall and information bias but seems unlikely to have been different between the two groups.

To further investigate the association between premature birth and POI, future prospective studies should focus on environmental effects during gestation and childhood as well as searching for common genetic polymorphisms influencing premature birth and POI. Due to the time span between birth and (premature) menopause retrospective studies investigating etiological factors involving menopause are indispensable. To investigate the etiology, genetic and environmental influences as well as treatment protocols of relatively rare conditions such as POI, collaborative international initiatives such as the international POI registry supported by the Imperial College London are very welcome (https://poiregistry.net/)

In conclusion, our data suggest an association between pre-term birth and POI, and not between birth weight and POI. Our findings may indicate a common genetic pathway or
etiology of premature birth and decreased ovarian reserve. Alternatively, postnatal factors that are the result of prematurity may lead to early follicle depletion.

Acknowledgments
The authors would like to thank all the participants who volunteered for this study project. We also thank Ted Korsen, research nurse, who helped recruiting participants and setting up the database.
References


Chapter five

Birth weight and age at menopause in Australian female twin pairs: exploration of the fetal hypothesis.

Treloar SA, Sadrzadeh S, Do KA, Martin NG, Lambalk CB.

Abstract

In a twin sample where duration of gestation can be controlled, a specific example of the fetal origins hypothesis concerning association between low birth weight and early age at menopause is explored. The hypothesis is based on the physiologically plausible path from intrauterine growth retardation and reduced numbers of primary follicles to an earlier menopause. The sample comprised 323 Australian female twin pairs where both co-twins had reached menopause naturally and reported on their weight at birth. Regression analysis showed no linear association between the two variables ($P = 0.371, r^2 = 0.0009$). Intra-pair differences in age at menopause were investigated in the context of relative birth weight of co-twins. In 265 pairs an intra-pair birth weight difference was reported. In monozygotic (MZ) pairs ($n = 168$) this allowed for control of genetic effects as well as gestation duration. No significant differences dependent on birth weight relative to co-twin were found for age at natural menopause in either MZ or dizygotic (DZ) twin pairs, even in pairs whose birth weights differed markedly. There was some indication that twins with premature ovarian failure were heavier at birth than twins with normal or later menopausal age. We conclude that the hypothesis that lower birth weight is associated with earlier menopause is not supported by our data.
**Introduction**

There is increasing evidence that the development and function of many internal organs in later life is influenced by intrauterine conditions. The fetal origins hypothesis proposes that undernutrition during the intrauterine period, manifest in a low birth weight, programs the fetus for diseases in adult life (1). The average organ weight of infants who are small for gestational age is proven to be different compared with infants with a normal weight for gestational age (2). Animal studies have shown that intrauterine growth-retarded sheep have a smaller absolute number of glomeruli (3). Not only organ structure but also function can be impaired by intrauterine conditions. Infants who are small for gestational age seem to have a higher risk of developing cardiovascular diseases and some of its known risk factors including high blood pressure and glucose intolerance (4). Evidence supports a causal relationship between follicle depletion and menopause (5). A poor intrauterine growth in late gestation, manifest in shortness at birth, may lead to a smaller peak number of primordial follicles, which in turn may lead to an earlier menopause (6). Impairment of ovarian development observed in intrauterine growth-retarded fetuses (7) may also plausibly have implications for onset of menopause. In a twin sample where gestational length can be controlled, the fetal origins hypothesis of birth weight affecting diseases or characteristics in later life (1, 8) can be tested. We investigated the association between birth weight (and relative twin—co-twin birth weights) and age at which female twins reached natural menopause. In this model we investigated the hypothesis that lower weight at birth is associated with early menopause.

**Materials and methods**

**Sample**

Data were obtained from twins enrolled with the Australian National Health and Medical Research Council (NHMRC) Twin Registry. This volunteer register has more than 27 000 twin pairs enrolled, about 10-20% of the estimated number of pairs in the population. Two main cohorts of twins were recruited: the first between 1980 and 1982 (1979 female-female pairs aged 17-88), followed up until 1996, and a new cohort of 1628 women (aged over 50) recruited between 1993 and 1995. During the period 1980-1996 six longitudinal surveys were carried out by mailed questionnaire requesting information including age at natural menopause (9). Both
cohorts of twins in two of these surveys were asked for information on weight at birth, the first cohort in the first questionnaire mailed in 1980-1982 (10-12), and the second cohort in the questionnaire mailed in 1993-1995 (9). Twins from the first cohort who were still participating in twin research and who were aged over 50 years in 1993-1995 were also sent the latter questionnaire and were asked for their own and their co-twin’s birth weight. Overall pairwise response (including all zygosity groups) was 64% for the first questionnaire (13) and 61% for the 55 second (14). Responses were available from both members of 2460 female—female twin pairs across the two surveys. Birth weight data were provided by both members of 1761 pairs; however by using cross-twin birth weight reports (see below) this number increased to 2170 pairs. Of these pairs, we could attribute reproductive endpoints and endpoint ages for both members of 1939 pairs. In 1041 (53%) pairs both twins were still menstruating, in 94 pairs (5%) one was pre-menopausal and one had reached natural menopause, in 323 (17%, 208 MZ and 115 DZ) both had reached natural menopause and the remaining 481 pairs (25%) reported other combinations of reproductive endpoints, such as hysterectomy prior to menopause in one twin and menopause in co-twin. Therefore 323 twin pairs provided data for menopausal age and birth weight; 75 of these pairs involved a cross-twin birth weight report; and intra-pair birth weight differences were reported in 265 of the 323 pairs.

Measurements

Age at natural menopause was assessed as age at last menstrual period, where menses had ceased at least 12 months previously, and there was no report of hysterectomy, bilateral oophorectomy or hormone replacement therapy (HRT) before menopause, HRT before hysterectomy, or other reason for cessation of menstruation (9). Twins were asked to report their own birth weight, and that of their co-twin, in pounds (1b) and ounces (oz) (16 oz/lb) in both questionnaires, since the system of measurement in Australia when the twins were born was imperial rather than metric, and the imperial system was the one with which they were most familiar. Weights were converted to grams (g).
Statistical analysis

Data analyses were performed using SAS version 6.11 (SAS Institute Inc, 1997). Twins were considered both as individuals and as members of twin pairs for particular analyses. We restricted our analyses to twins in female—female monozygotic (MZ) and dizygotic (DZ) pairs. Assessment of intra-pair differences in MZ twin pairs controls for genetic or stable social factors (15) since they can only be due to specific environmental factors, whereas DZ intra-pair differences are additionally influenced by genetic factors. Statistical tests included analyses of variance, regression and correlations.

We used birth weight data from the 1980-1982 report where available, and the 1993-1995 report only if the former information was missing, given an increased probability of recall problems. However, reports of birth weight were highly correlated between these two surveys, with $r = 0.91$ for own and $r = 0.93$ for co-twin’s birth weight. The correlation between self-reported birth weight and co-twin’s cross-report of twin’s birth weight was $r = 0.95$. Consequently, where twins did not provide their own birth weight, their co-twin’s cross-report was substituted. Attribution of reproductive cycle endpoints and censoring are described fully (9, 16). We selected for analyses only those twins where there was evidence that menopause had been reached without a censoring event such as prior hysterectomy, HRT use, or other reason for cessation of menses.

Results

Mean age at natural menopause was 48.5 years ± 4.9 years (range 27-60, median 50 years). Mean birth weight was 2373 ± 695 g (range 454-5216). DZ co-twins differed in age of menopause by a mean of 4.1 ± 3.7 years and MZ co-twins by 3.2 ± 3.7 years, a significantly shorter mean interval ($P = 0.006$). These relative differences were consistent with genetic influences on timing of menopause (17). Mean birth weight differences were significantly greater for DZ co-twins than for MZ co-twins (339 g compared with 267 g, $P = 0.035$).

Association between age at natural menopause and birth weight was assessed firstly on individual twin data from co-twins in responding pairs. Regression analysis to see whether age at natural menopause varied according to birth weight showed no linear association between the two variables ($P = 0.371$, $r^2 = 0.0009$). We checked for higher-order terms in regression and other
non-linear terms to see if the relationships were linear or quadratic but none were significant. Intra-pair differences in both birth weight and age at menopause were computed. Partial correlations were performed, controlling for effects which were previously identified as significant predictors of age at menopause in a sample including this sub-sample: age, smoking, education level, age at menarche, and parity (9). Average combined income, self-rated social class, and occupation group were omitted because of their correlation with education level, and because these variables were characterized by higher levels of missing data. Length of oral contraceptive used was also omitted because it was available only for the first questionnaire respondents, and on this subset no association was observed. The partial correlation coefficient between the intra-pair differences in birth weight and age at natural menopause for 163 MZ twin pairs (where data were available for all specified predictors) was not significant (partial Pearson $r = 0.08, P = 0.320$). Controlling for predictors of natural menopause and genetic effects still left a non-significant and only very modest association between the key variables. The coefficient was even lower when both MZ and DZ pairs were pooled ($r = -0.01, n = 248$ pairs).

In order to detect possible effects of pair-wise censoring, we investigated the zygosity breakdown of twin pairs concordant and discordant for menopause. Of the 94 discordant pairs where one twin was pre-menopausal and one post-menopausal, 55 (59%) were MZ and 39 (41%) DZ pairs. This zygosity balance differed from that of the post-menopausal pairs (64% MZ, 36% DZ), where an excess of MZ pairs may have resulted in some underestimation of intra-pair differences in age at menopause. Mean ages at natural menopause were calculated for twins who were relatively heavier at birth than their co-twin and compared with the mean menopausal age of their co-twins who were lighter, irrespective of the absolute weight. All co-twins whose reported birth weights differed from each other were included in these twin pair analyses. Fifty-eight pairs where co-twins reported the same birth weight as each other were excluded. Mean intra-pair differences in birth weight and in age at menopause according to birth weight differences are shown in Table I. Finally, in order to increase sensitivity and detect any threshold effects, the analysis was restricted to those pairs who differed in birth weight by at least a given amount: 500 g, 400 g, 250 g and 100 g (see Table I). No significant differences were seen, even in a sub-set of co-twins whose birth weight difference was over 500 g. Log transformation of age at natural menopause made no difference.
Individual twins with very premature and very late natural menopause (<35 years and >56 years) differed significantly from each other in their mean birth weights (means 2774 g and 2185 g respectively, $P = 0.028$). The direction of difference was contrary to our original hypothesis, as mean birth weight was actually higher in the twins reaching menopause prematurely than in those reaching menopause after age 56 years. In 14 pairs one or both co-twins had reached natural menopause before age 40 years. In only two of the 14 pairs did the co-twin with non-premature age at natural menopause have a higher birth weight than her co-twin. In all other cases birth weight was lower than or equal to that of the co-twin ($P < 0.05$). The mean birth weight of the heavier co-twins differed significantly from the mean birth weight of the lighter co-twins in the latter group ($P = 0.05$), with a mean intra-pair difference of 307 g.

### Table I. Mean ages at natural menopause for twin pairs with non-zero intra-pair difference in birth weights

<table>
<thead>
<tr>
<th>Birthweight difference</th>
<th>MZ pairs (n=168)</th>
<th>DZ pairs (n=97)</th>
<th>Total pairs (n=265)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lighter</td>
<td>Heavier</td>
<td>$P$</td>
</tr>
<tr>
<td>Birthweight $^b$</td>
<td>2136</td>
<td>2467</td>
<td>$&lt;.001$</td>
</tr>
<tr>
<td>Age at menopause $^c$</td>
<td>48.89</td>
<td>48.49</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(4.91)</td>
<td>(4.75)</td>
<td></td>
</tr>
<tr>
<td>Birthweight difference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&gt;500g$ No.pairs</td>
<td>49.46</td>
<td>48.27</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(4.07)</td>
<td>(4.11)</td>
<td></td>
</tr>
<tr>
<td>$&gt;400g$ No.pairs</td>
<td>48.33</td>
<td>48.47</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(5.41)</td>
<td>(4.09)</td>
<td></td>
</tr>
<tr>
<td>$&gt;250g$ No.pairs</td>
<td>48.77</td>
<td>48.86</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(5.24)</td>
<td>(4.16)</td>
<td></td>
</tr>
<tr>
<td>$&gt;100g$ No.pairs</td>
<td>48.95</td>
<td>48.58</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(4.90)</td>
<td>(4.71)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD), NS = not significant

$^a$ Total pairs used for these calculations is smaller because 58 twin pairs with the same birth weights were excluded. $^b$ In grams. $^c$ In years.
There was a gap of approximately 13 years between first and second reports of birth weight for a sub-sample of 562 twins who responded to both questionnaires. The Pearson correlation coefficient between the two self-reports of birth weight at a 13 year interval was $r = 0.91$. In a smaller subsample who had reached menopause naturally ($n = 291$), $r = 0.92$. Reports by twins in the latter sub-sample on their co-twin's birth weight were also very consistent over the 13 year interval ($r = 0.93$).

**Discussion**

We found no overall association between birth weight difference and difference in age at natural menopause in this sample of Australian twin pairs. By comparing co-twins we found no evidence of a shift in age at natural menopause depending on birth weight. This applied to individual twins as well as MZ and DZ twin pairs. No significant difference in age at natural menopause was seen, even if the difference in birth weight between co-twins was over 500g. Therefore, our findings do not support the hypothesis that low birth weight results in early menopause.

Although there is still no clinical evidence, there are (mathematical) indications that ovarian oocyte reserve at birth is an important factor with respect to age at menopause (5). If so, our twin data imply that there is no relationship between limited ovarian oocyte content and low birth weight, and that possible disorders in fetal programming, as put forward by Barker (1), are not involved in reduction of oocyte reserve. It is possible, however, that ‘multiple pacemakers’ including central nervous system factors are important in determining menopausal age (18), hence numbers of follicles are not explanatory *per se*. Alternatively, it may be that there is a relationship between low birth weight and low oocyte reserve at birth, but that the absolute number of follicles has no effect on the age at natural menopause. The rate of follicle loss might be the determining factor of the age at menopause. It is also possible that we cannot observe the effect of a low oocyte reserve at birth on age at menopause in a twin population. Early age at menarche may be a relevant factor also in oocyte depletion; however, a previous analysis of age at menarche in Australian twin pairs found no significant association between menarche interval and birth weight difference in MZ co-twins (19).
We found some indication, at least in some twins, that premature menopause may be related to a higher birth weight. Finding a plausible explanation for this is challenging. A recent paper reported that in singletons, premature ovarian failure was associated with shortness at birth resulting in a high ponderal index (birth weight/length$^3$), and suggested that shortness at birth may also be an indicator of intrauterine growth deprivation (6). We had no information on length at birth with which to evaluate this finding. One mechanism involved in determining the number of oocytes in the ovary at birth is the rate of atresia, an apoptotic process which causes a reduction of the original maximum number of oocytes of about $3 \times 10^6$ at mid-gestation to $1 \times 10^6$ at birth. Theoretically, disturbances of this mechanism, based on programming defects, could lead to higher oocyte numbers at birth. If conditions like this do exist, we would expect a favorable, extended age at menopause in the lighter born twins rather than the disadvantageous premature ovarian failure in the twins who were heavier at birth.

There was a considerable mean birth weight difference of around 300 g between those twins who reached menopause before 40 years of age and their co-twins with normal menopausal age. This was also the case comparing individual twins with very late and very premature age at natural menopause. On the other hand, we did not find any difference in age at menopause in co-twins who differed substantially in birth weight. It may be that premature ovarian failure, when found in one twin and not in the co-twin, is not just an extreme of normally distributed age at menopause but a separate entity. From this particular situation it seems hazardous to speculate on possible causal relationships between certain morphometric parameters at birth and premature ovarian failure as disease in later life.

Twin studies have the advantage of controlling for gestational age and genetic factors. For example, in one study a negative correlation between birth weight and blood pressure in a group of individual twins disappeared when association between intra-pair twin differences in both variables was tested, controlling for difference in current weight (15). We were able to control for other known predictors of age at menopause (9). The female twins in our sample have been shown to be representative of the Australian population on a variety of indicators including age, general level of education and mental status (20). Twins have volunteered to participate in medical research in general and are unselected for any particular characteristics, although self-selection might introduce a bias in the target population, the direction of which is unknown. It is
possible that adult twins who are part of a registry may over-represent twins of normal rather than extremely low birth weights, due to the fact that both twins have survived and are able to complete and return survey forms.

Methodological constraints may have impeded detection of any association between birth weight and menopausal age. Pair-wise censoring is an issue arising from selection of pairs where both were post-menopausal, and may have contributed to an underestimation of the intra-pair difference in menopausal age for the sample selected. One might expect an underestimation of the difference in age at menopause if those pairs who achieved menopause closer to each other were included in the data set for statistical analysis, while others whose ages at menopause were farther apart were censored from the data set. Evidence suggests that age at menopause is more highly correlated in MZ than in DZ twins (16, 17, 21). In an earlier study we plotted Kaplan—Meier survival curves comparing MZ and DZ twins conditional on the different intervals of age of menopause in the first twin. For the co-twins of twin probands who reached menopause before age 50 years, the MZ co-twins reached menopause earlier than the DZ co-twins. However, as the age at menopause in one's twin increased, the difference between the survival probability of the MZ twins and the DZ twins became smaller (16). Underestimation may therefore have occurred, but we would not expect the effect to be large. Decreased power was a significant methodological issue. The sample size was reduced to 265 pairs when only pairs who differed in birth weight and had both reported reaching natural menopause were included in analyses. This meant that standard errors were large. Although the initial data set was large, by selecting only pairs where both had reached menopause and then only those where co-twins differed in birth weight, our power to detect significant differences was very low. Hence, we consider our study an exploration rather than a test of the fetal origins hypothesis. Mean and median ages at menopause were lower in this sub-sample than in the larger sample (9). Other limitations of the study require acknowledgement. Medical records were not sought for confirmation of birth weight and data are based on recall. Recall bias or inaccuracy of original information transmitted to twins could affect data on birth weights. However, cross-twin reporting suggested a high degree of consistency in reporting birth weight. Consistency of reporting of age at menopause over time suggests that recall bias was not a severe problem in this sample (9). There is no reason, however, why the level of one of the key
variables should be biased by the level of the other. Some questions remain about applicability of our findings to singletons, as possibly different mechanisms lead to birth weight variation in twins compared with singletons. Determinants of late gestational intrauterine growth in twins probably differ considerably from singletons (22, 23). Evidence also suggests that maternal smoking is less influential in twins than singletons for infants weighing less than 90% of mean birth weight, and of equal magnitude where infants weighed over 90% of mean birth weight (24). Despite their low mean birth weight, twins tend to have a lower blood pressure compared to singletons at ages 9 and 18 years (25). However, no differences in the probability of conception have been observed between twins and singletons (15), and mortality among twins after age 6 years is no higher than in the general population (8). It is feasible that a different situation exists for twins, and determinants involved in their late gestational growth retardation may well be uninvolved as causal factors of chronic diseases in later life (1).

In summary, our findings do not support the fetal origins hypothesis in relation to any predictive effect of birth weight on age at natural menopause in twins.

Acknowledgements

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References

Chapter 6

Early environment and age at menopause: a systematic review

S. Sadrzadeh, M. Verschuuren, L.J. Schoonmade, C.B. Lambalk, R.C. Painter

submitted
Abstract

Objectives:

When the follicle reserve, which is developed solely during the fetal period, is depleted, women enter menopause. Menopause not only marks the end of a women’s fertile lifespan but post-menopausal women are also at an increases risk of cardiovascular disease, osteoporosis and overall mortality. Intra-uterine and childhood adverse conditions might affect the ovarian capacity by influencing the primary follicle production in the first trimester, limiting the initial follicle pool or mediate an accelerated follicular loss thereafter.

Study design: The following online databases were systematically searched: PubMed, EMBASE, CINHAL (EBSCO) and Cochrane library (Wiley) with no language or date restrictions up to 1 January 2017. Cohort studies, case control studies and cross-sectional studies were eligible. Eligibility and data extraction was independently performed by two researchers. The Newcastle-Ottawa scale was used for quality assessment. The protocol of this study is registered at PROSPERO under registration number CRD42016049239.

Results: A total of 5278 studies were identified, 11 studies were deemed eligible and included. Nine were cohort studies, 1 case control study and 1 twin study. Due to the diversity of reported data and risk estimates we were unable to extract data or performed meta-analysis on pooled data. Prenatal and childhood exposure to famine was significantly associated to an earlier age at menopause in 3 studies. Mean differences in age at menopause varied from 4 months up to 1.7 years between famine exposed and unexposed women. Three studies described a significant association between a low weight at ages 1 or 2 and a younger age at natural menopause. A younger age at menopause was associated with a significantly higher weight at birth in one study and with a high ponderal index, a measure for fatness at birth in another study. None of the 9 studies reporting on low birth weight and age at natural menopause find a significant association.

Conclusion: Famine during gestation and childhood as well as an impaired childhood growth is related to a younger age at menopause. Birth weight is not associated with age at menopause.
Introduction:

Menopause occurs at an average age of 51 years (1). With increasing life expectancy, a progressively larger portion of a woman’s lifetime is postmenopausal, giving rise to new factors influencing women’s health after menopause. Menopause not only marks the end of a woman’s fertile life span, but a younger age at menopause is also associated with an increased risk of cardiovascular disease, osteoporosis and overall mortality (2). About 1% of women enter menopause before the age of 40, a condition known as premature ovarian insufficiency (POI) (3). POI can greatly hamper family planning, especially in populations that are increasingly postponing childbearing to advanced maternal ages. There are some known causes of POI: fragile X-syndrome, Turner syndrome and the autoimmune polyendocrine syndrome. However, in 90% of cases the cause is unknown, making it difficult to both predict and treat the condition(4).

One could argue that since the ovarian reserve is exhausted prematurely it is either a case of accelerated depletion or an insufficient reserve to begin with. The total number of follicles is established at around five months gestational age and is therefore finite at approximately 7 million (5, 6). When a woman reaches menarche this number has already been reduced to approximately 400.000 (7). The onset of menopause is thought to occur when the ovarian follicle count reaches approximately 1000 follicles (6). Therefore, early life could be a particularly relevant period in determining follicular endowment and age at natural menopause, as well as risk of POI. Suboptimal conditions during early development including maternal undernutrition or placental insufficiency could influence the total amount of follicles that are formed. Alternatively, adverse pre- or postnatal conditions or accelerated general ageing may increase oocyte attrition. Several studies have established that early life exposure to an adverse environment can lead to impaired adult health, including an elevated risk of cardiovascular disease and metabolic syndrome (8-11).

Here, we systematically examined the existing literature investigating the effect of adverse intrauterine conditions, early childhood growth and famine exposure on the age of natural menopause.
Material and methods:

Search strategies:

This review was conducted according to PRISMA and MOOSE guidelines. The protocol of this study is registered at PROSPERO under registration number CRD42016049239. A comprehensive search was performed in the bibliographic databases PubMed, EMBASE.com, CINAHL (via EBSCO) and Cochrane library (via Wiley) with no language or date restrictions from inception to 1 January 2017. Additionally the reference lists of relevant studies, review articles and opinion papers were checked by snowball search to identify any secondary references. Search terms included controlled terms (MeSH in Pubmed, EMtree in Embase.com), as well as free text terms. We used free text terms only in the Cochrane Library. The following terms were used (including synonyms and closely related words) as index terms or free-text words for ‘birth weight’ or ‘gestational age’ or ‘famine’ or developmental origins of health and disease’ and the outcome variable ‘menopause’ or ‘premature menopause’ with aid of a clinical librarian (LJS). Detailed information on the search strategy is listed in appendix 1.

Study selection and eligibility:

After identifying and excluding duplicate studies, two independent reviewers (SS and MV) evaluated titles and abstracts for suitability using a customized in-exclusion chart. Any disagreement between reviewers was resolved through consensus. If disagreement was not resolved, full text articles were reviewed. Studies were included if they were (i) cross-sectional, case-control, cohort or intervention studies (ii) were conducted in adult humans (iii) reported age at menopause (iv) reported birth weight, gestational age, childhood growth or famine exposure (v) the reported birth parameters and childhood influences were related to women whose menopausal age was reported. Full texts were retrieved for studies that could satisfy all selection criteria and independently reviewed by two authors (SS and MV) for eligibility using the same in-exclusion chart. Any disagreement between reviewers was resolved through consensus, if consensus could not be reached, a third author was consulted (RCP).
Quality assessment and data extraction:
Selected studies were assessed independently for methodological validity by reviewers SS and MV, using the Newcastle-Ottawa scale with a maximum of 9 stars (12). Included studies were classified as low-quality (0-4), moderate-quality (5-6) and high-quality (7-9). Any disagreement between reviewers was resolved through consensus if consensus could not be reached, a third author was consulted (RCP). A piloted data extraction form was used to extract relevant information. The form included questions on publication year, study design, baseline population, number of participants, definition of outcome variable, type of exposure and reported risk estimates.

Data synthesis and analysis:
Providing the available data were suitable they were entered in 2x2 tables, data were transformed to Review Manager (RevMan 5.3) for meta-analysis, to estimate Hazards ratios and Odds ratios.

Results
Identification of relevant studies:
The search strategy resulted in 5278 unique citations and 1 additional study through snowball search. Eleven studies were deemed eligible and included. Nine of the final 11 studies were cohort studies, 1 case control study and 1 twin study. The study described by Mishra et al. is a follow-up study of the same cohort investigated by Hardy et al. but included more participants and additional outcome variables. The selection procedure is visualized in figure 1 and characteristics of the final 11 articles are listed in table 1. Due to the diversity of reported data and risk estimates we were unable to extract data or performed meta-analysis on pooled data.
Fig 1. Flow chart of study selection process.

Records identified through database searching (n = 8450) & Additional records identified through snowball search (n = 1) → Records after duplicates removed (n = 5279)

Records screened (n = 5279) → Records excluded (n = 5249)

Full-text articles assessed for eligibility (n = 30) → Studies included in qualitative synthesis (n = 11) → Studies included in quantitative synthesis (meta-analysis) (n = 0)

Full-text articles excluded, (n=19):
- No age at menopause = 8
- Menopause not relatable to birth parameters = 4
- Article type = 3
- Posters of already included studies = 2
- Birth parameter & age at menopause not related to same person = 1
- Article still in process of publication, data not available = 1
<table>
<thead>
<tr>
<th>Lead author, date, country</th>
<th>Study type</th>
<th>Menopause definition</th>
<th>Birth weight source</th>
<th>Early life determinants</th>
<th>Primary Study population</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sadrzadeh, 2016, Netherland</td>
<td>Case-control</td>
<td>Age menopause</td>
<td>Self-reported</td>
<td>Birth weight Gestational age</td>
<td>Cases are women clinical diagnosis with POI &amp; hospital controls</td>
<td>151 post-menopausal women</td>
</tr>
<tr>
<td>Yarde, 2013, Netherland</td>
<td>Cohort study</td>
<td>Age menopause</td>
<td>Medical records</td>
<td>Famine exposure during gestation</td>
<td>Birth cohort, singletons born between 1 Feb 1945 and 31 March 1946</td>
<td>1070 men and women</td>
</tr>
<tr>
<td>Steiner, 2010, US &amp; Puerto Rico</td>
<td>Cohort study</td>
<td>Age menopause</td>
<td>Self-reported</td>
<td>Mat.Hypertension Mat. Diabetes DES exposure Breastfeeding</td>
<td>Retrospective nested study within Sister Study (breast cancer)</td>
<td>22165 women, 6659 (30%) postmenopausal at last follow-up</td>
</tr>
<tr>
<td>Tom, 2010, UK</td>
<td>Cohort study</td>
<td>No date of menopause recorded. Menopausal status at time of last follow-up recorded</td>
<td>Medical files</td>
<td>Birth weight</td>
<td>Birth cohort, women born during one week in March 1958, aged between 44-45</td>
<td>3708 women, 6% (223) postmenopausal at last follow-up</td>
</tr>
<tr>
<td>Kalichman, 2007, Central Russia</td>
<td>Cohort study</td>
<td>Age menopause</td>
<td>None</td>
<td>Famine exposure during childhood or expected maturation</td>
<td>Retrospective cohort, women recruited in a geographical region for Chuvasha Skeletal Aging Study</td>
<td>745 women, 322 of which postmenopausal</td>
</tr>
<tr>
<td>Mishra, 2007, UK</td>
<td>Cohort study</td>
<td>Age menopause</td>
<td>Medical records</td>
<td>Breastfeeding Height &amp; weight at age 2 Childhood SES Parental divorce</td>
<td>Birth cohort, women born in March 1946, age 57 at last follow-up, same cohort as Hardy et al</td>
<td>1583 post-menopausal women</td>
</tr>
<tr>
<td>Elias, 2003, Netherland</td>
<td>Cohort study</td>
<td>Estimate age menopause using interval between screening rounds</td>
<td>None</td>
<td>Famine exposure during childhood through questionnaire and place of residence</td>
<td>Nested prospective cohort of postmenopausal women aged 40-73 participating in breast screening project</td>
<td>9471 post-menopausal women</td>
</tr>
<tr>
<td>Lead author, date, country</td>
<td>Study type</td>
<td>Menopause definition</td>
<td>Birth weight source</td>
<td>Early life determinants</td>
<td>Primary Study population</td>
<td>n</td>
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<tr>
<td>Lawlor, 2003, UK</td>
<td>Cohort study</td>
<td>Age menopause</td>
<td>Self-reported</td>
<td>Childhood socio-economic status</td>
<td>Cross sectional random sample of women age 60-79 from GP lists of 23 towns</td>
<td>3513 post-menopausal women 31% (1079) reporting birth weight</td>
</tr>
<tr>
<td>Hardy, 2002, UK</td>
<td>Cohort study</td>
<td>Age menopause</td>
<td>Medical files</td>
<td>Breastfeeding Height&amp;weight at age 2 Height&amp;weight at age 7</td>
<td>Birth cohort, women born in 1946, age 53 at last follow-up, same cohort as Mishra Nested retrospective cohort of post-menopausal twins enrolled in the volunteer registry</td>
<td>1514 women</td>
</tr>
<tr>
<td>Treloar, 2000, Australia</td>
<td>Twin study</td>
<td>Age menopause</td>
<td>Self-reported</td>
<td>Birth weight</td>
<td>Birth weight</td>
<td>265 post-menopausal twin pairs</td>
</tr>
<tr>
<td>Cresswell, 1997, UK</td>
<td>Cohort study Sheffield</td>
<td>FSH levels</td>
<td>Medical files</td>
<td>Birth weight</td>
<td>Prospective birth cohort. Women born at Jessop Hospital 1952-1953. Age between 40-42</td>
<td>235 women, 5.1% (12) menopausal</td>
</tr>
<tr>
<td></td>
<td>Cohort study Hertfordshire</td>
<td>Age menopause</td>
<td>Medical files</td>
<td>Weight at age 1</td>
<td>Prospective birth cohort 1911-1948. Aged between 60-71</td>
<td>755 post-menopausal women</td>
</tr>
</tbody>
</table>
Table 2. Studies reporting age at menopause in relation to birth weight. The study of Cresswell et al. is included twice as it reports on 2 different cohorts

<table>
<thead>
<tr>
<th>Author, Pub. date,</th>
<th>n</th>
<th>Risk estimate for birth weight</th>
<th>Conclusion</th>
<th>Quality *(0-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies reporting an association between birth weight and age at natural menopause</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tom, 2010</td>
<td>2956</td>
<td>Odds ratio for age at menopause according to birth weight categories (standardized by gestational age) and being post-menopausal at 44y-45y</td>
<td>High birth weight &gt;4kg associated with early menopause</td>
<td>Moderate (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quarter 1 lowest</td>
<td>1.21 95% CI (0.75, 1.95)</td>
<td>No association between a low birth weight and age at menopause</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quarter 2</td>
<td>1.14 95% CI (0.70, 1.87)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quarter 3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quarter 4 highest</td>
<td>1.84 95% CI (1.88, 2.88)</td>
<td></td>
</tr>
<tr>
<td>Cresswell, Sheffield, 1997, cohort</td>
<td>235</td>
<td>Mean difference in ponderal index**</td>
<td>High ponderal index associated with menopausal age younger than 40-42y No association between low birthweight &amp; menopause</td>
<td>Moderate (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7 kg/m³ 95%CI (1.42, 3.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Studies reporting no association between birth weight and age at natural menopause</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sadrzadeh, 2017</td>
<td>151</td>
<td>Odds ratio 1.0 (1.0-1.001) for menopause &gt;40 y VS menopause &lt;40 y according to birthweight (gr): p=0.27</td>
<td>no association between birth weight and premature menopause</td>
<td>Moderate (6)</td>
</tr>
<tr>
<td>Yarde, 2013</td>
<td>376</td>
<td>HR*** (per y) 0.81 95%CI (0.64,1.03) for age at menopause according to birth weight (kg) adjusted for famine exposure</td>
<td>no association between birth weight and age at menopause</td>
<td>Moderate (6)</td>
</tr>
<tr>
<td>Mishra, 2007, British cohort</td>
<td>1578</td>
<td>HR 1.95 CI (0.93, 1.09) for age at menopause ≤50 y according to mean birth weight (kg) p for trend = 0.9</td>
<td>no association between birth weight and premature menopause No association between birth weight and premature menopause</td>
<td>High (7)</td>
</tr>
<tr>
<td>Hardy, 1946, born in March 2002</td>
<td>1238</td>
<td>HR (per y) for age at menopause according to birth weight categories p for trend= 0.2</td>
<td>no association between birth weight and premature menopause</td>
<td>Moderate (6)</td>
</tr>
<tr>
<td>Steiner, 2010</td>
<td>17311</td>
<td>HR (per y) for age at menopause according to birth weight categories p for trend= 0.2</td>
<td>no association between birth weight and premature menopause</td>
<td>Moderate (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;2.5 kg</td>
<td>2.501 kg: 3 kg</td>
<td>0.95 95% CI (0.57, 1.56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5 kg:3.5kg</td>
<td>0.97 95% CI (0.61, 1.54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;3.501 kg</td>
<td>1.15 95% CI (0.73, 1.82)</td>
<td></td>
</tr>
<tr>
<td>Lawlor, 2003</td>
<td>1079</td>
<td>0.04y (-0.27, 0.35) increase in mean age at menopause with 0.80 kg (1SD) increase in birthweight</td>
<td>Small effect increasing birth weight on increasing age at menopause, not significant</td>
<td>Low (4)</td>
</tr>
<tr>
<td>Treloar, 2000</td>
<td>265</td>
<td>Twin pairs</td>
<td>Lighter twin birth weight 2.170 kg</td>
<td>Heavier twin birth weight 2.527 kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Birth weight</td>
<td>48.99 y</td>
<td>48.74 y</td>
</tr>
<tr>
<td>Cresswell, Hertfordshire, 1997, cohort</td>
<td>755</td>
<td>Birth weight</td>
<td>48.99 y</td>
<td>50.2 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Menopause</td>
<td>50.2 y</td>
<td>50.0 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;2.5kg (n=36)</td>
<td>49.4 y</td>
<td>49.8 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5kg-2.9kg (n=145)</td>
<td>49.4 y</td>
<td>49.8 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 kg-3.4kg (n=261)</td>
<td>49.4 y</td>
<td>49.8 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5kg-3.9kg (n=205)</td>
<td>49.4 y</td>
<td>49.8 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;4.5kg (n=108)</td>
<td>49.4 y</td>
<td>49.8 y</td>
</tr>
</tbody>
</table>

* low-quality (0-4), moderate-quality (5-6) and high-quality (7-9)/ ** ponderal index= birth weight(kg)/height(m)^3/ ***Hazards ratio
Birth weight, neonatal adiposity and age at menopause:
From the 11 included studies, 9 (13-22) reported how birth weight was associated with age at natural menopause. None of the studies reported a statistically significant association between low birth weight and age at natural menopause. Two studies (15, 22) also reported on the association between a higher birth weight and age at natural menopause. Tom et.al found a statistically significant association, with an odds ratio (95%CI) of 1.84 (1.88,2.88) for the highest birth weight quartile to enter menopause at a younger age the other reported association was not statistical significant (Tab. 2).Cresswell et al. (Sheffield cohort) reported an association between a higher ponderal index (birth weight/height$^3$) a marker of neonatal adiposity and a menopausal age under 40-42 years

Gestational age or prematurity and age at menopause:
1 case-control study (13) reported an association between premature birth before 37 weeks of gestation, and age at natural menopause before the age of 40 with an odds ratio of 4.66 95%CI (1.3-16.7).

Exposure to famine and age at menopause:
Three studies reported on well-defined periods of famine exposure in relation to a younger age at menopause (14, 16, 18). In these studies, the age at which women had been exposed to famine varied: Yarde et.al reported on famine during gestation. Exposure to famine was associated with a 32% increase in the HR of natural menopause at any age [HR (95% CI) 1.32, (1.05,1.66)] after adjusting for smoking and birth weight. Kallichman et.al assessed the effects of famine exposure during puberty, estimating that menopause occurred 1.7 years earlier 95%CI (1.12, 2.27) compared to famine unexposed women. Elias et al. reported on the effect of famine exposure at any age with extra focus on exposure during childhood. Famine exposure at 2-7 years had the largest effect with severely famine exposed girls entering menopause 1.83 years 95% CI (-3.03, -0.63) earlier compared to unexposed women (Tab. 3).
### Table 3. Studies reporting age at menopause in relation to famine exposure.

<table>
<thead>
<tr>
<th>Author, publication date</th>
<th>n unexposed</th>
<th>n exposed</th>
<th>Adjusted for</th>
<th>Risk estimate</th>
<th>Quality* (0-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yarde 2013</td>
<td>165</td>
<td>212</td>
<td>Birth weight Smoking</td>
<td>HR (95% CI) of 1.32 (1.05, 1.66) for entering menopause at any age after gestational famine exposure.</td>
<td>Moderate (6)</td>
</tr>
<tr>
<td>Kalichman 2007</td>
<td>155</td>
<td>157</td>
<td></td>
<td>Famine exposure age 15 y = menopause 47.55 y No famine exposure = menopause 49.25 y (p for difference &lt;0.001)</td>
<td>Moderate (5)</td>
</tr>
<tr>
<td>Elias 2003</td>
<td>4606</td>
<td>3793 (moderate) 1074 (severely)</td>
<td>Smoking SES** BMI*** Parity Year of birth</td>
<td>Difference in mean age at menopause (y): Moderately exposed, any age, to famine -0.06 y 95% CI (-0.22, 0.09) Severely exposed, any age, to famine -0.36 y 95% CI (-0.6, -0.11)</td>
<td>High (7)</td>
</tr>
</tbody>
</table>

*low-quality (0-4), moderate-quality (5-6) and high-quality (7-9)/**socioeconomic status/***BMI= weight (kg)/height^2 (m)

### Table 4. Studies reporting age at menopause in relation to childhood growth.

<table>
<thead>
<tr>
<th>Author, publication date</th>
<th>n</th>
<th>Adjusted for</th>
<th>Risk estimate</th>
<th>P for trend</th>
<th>Quality* (0-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>British cohort of women born in March 1946</td>
<td>Mishra, 2007 1158</td>
<td>Smoking Infant feeding SES Parity Mothers age at menopause Birth weight</td>
<td>HR** menopause &lt;50 y according to weight at age 2 &lt;11.5 kg 1 11.51 kg-12.5 kg 0.42 95% CI (0.16, 1.06) 12.51 kg-13.4 kg 0.55 95% CI (0.23, 1.30) &gt;13.4 kg 0.41 95% CI (0.16, 1.03)</td>
<td>0.06</td>
<td>High (7)</td>
</tr>
<tr>
<td>Hardy, 2002</td>
<td>1238</td>
<td>Smoking Infant feeding SES</td>
<td>HR** for age at menopause (y) according to weight at age 2 &lt;11.5 kg 1 11.51 kg-12.5 kg 0.90 95% CI (0.65, 1.23) 12.51 kg-13.4 kg 0.76 95% CI (0.56, 1.04) &gt;13.4 kg 0.75 95% CI (0.54, 1.02)</td>
<td>0.04</td>
<td>Moderate (6)</td>
</tr>
<tr>
<td>Cresswell, 1997 Hertfordshire cohort</td>
<td>755</td>
<td>Infant feeding Smoking SES***</td>
<td>Weight categories at age 1 and corresponding ages of menopause &lt;8.2 kg 48.7 (n=74) 8.3 kg-9.1 kg 49.7 (n=208) 9.2 kg-10 kg 49.9 (n=263) 10.1 kg-10.9 kg 50.6 (n=151) &gt;11 kg 49.8 (n=59)</td>
<td>0.03</td>
<td>Low (4)</td>
</tr>
</tbody>
</table>

*low-quality (0-4), moderate-quality (5-6) and high-quality (7-9)/**Hazards ratio/***Socioeconomic status
Childhood growth and age at menopause:

Three studies detected an association between low childhood weight (at age 1 or 2 years old) and a younger age at menopause. Cresswell et.al (Hertfordshire cohort), Mishra et.al and Hardy et.al all concluded that a lower weight in infancy was associated with a younger age at menopause (Tab. 4).

Additional childhood factors:

A few of the included studies also explored other childhood exposures and their effect on the age of menopause. An association between a low socioeconomic status during childhood and an early age at menopause was reported by Lawlor et.al, \( p \) for trend <0.001; Hardy et al. \( p \) for trend 0.06 and Mishra et al. \( p \) for trend 0.03. All studies used multiple indicators for childhood socioeconomic status. Mishra et.al also reported that parental divorce was associated with a younger age at menopause. Women experiencing parental divorce during childhood had a hazards ratio (95%CI) of 6.52 (2.0,21.3) to enter menopause before the age of 50 compared to other women. Steiner et.al report an association between gestational DES exposure and a younger age at menopause, Hazards ratio 1.45 95% CI(1.27, 1.65). Hardy et.al and Mishra et.al documented that breastfed infants had a later age at menopause, HR (95%CI) and \( p \) for trend are 0.74 years (0.57,0.97) \( p=0.06 \) and 0.81 (0.65-0.99) \( p= 0.05 \) respectively.

Quality:

The score on the Newcastle-Ottawa scale for studies included in our systematic review ranged from 4 to 7. Three studies were categorized as high, 2 as low and 6 as moderate. There were no striking differences in quality of studies reporting no effect compared to studies reporting positive effects. Quality of the studies is reported in the tables 2, 3 & 4.

Discussion

Our systematic review indicates that pre- or postnatal famine exposure and slow childhood growth are both associated with a younger age at menopause. These effects were independent of birth weight. The association between birth weight, as a summary measure of poor intrauterine conditions, and age at natural menopause were largely null:
nine studies report on the association between birth weight and age at menopause but only one reports a significant association with a higher weight at birth and one of a significant association with increased adiposity at birth.

Low weight at the ages one and two as well as exposure to famine, however, were consistently associated with younger age at menopause. There is substantial evidence from animal studies and epidemiological studies supporting the hypothesis that poor intrauterine and early life nutrition cause early aging and decreased longevity (23-27). Furthermore, aging of the reproductive system precedes somatic aging (28). Our finding therefore, is most likely a reflection of a premature or accelerated senescence related to early life nutritional stress.

Increased oxidative stress has been opted as a possible pathway linking nutritional stress and cellular aging through an accelerated shortening of telomeres (29). Telomeres, a unique nucleotide repeat sequence at the distal ends of chromosomes, shorten after each mitotic division and when they reach a critical length the cell seizes to divide (30). Animal studies detect increased oxidative stress levels as well as an impaired telomere length in intrauterine nutritionally deprived rodents and non-human primates (31-33). The enzyme telomerase has the ability to elongate the telomeric DNA. Telomerase activity declines with age and there is evidence of declined activity in women with ovarian insufficiency (34, 35).

To summarize, nutritional stress during gestation and infancy could affect the ovarian germ cell population mediated through increased oxidative stress and reduced telomerase activity resulting in a diminished ovarian reserve and a younger age at menopause.

By systematically searching and reviewing all published data on this subject we minimized the chance of citation bias favoring a specific outcome. However our study has some weaknesses. Due to the differences in definition of the outcome variable and the reported risk estimates we were unable to pool data from different studies for statistical analysis. The number of studies we were able to include were also limited. Although we have no direct evidence we cannot exclude the possibility of publication bias with an under representation of smaller studies reporting no association between early life circumstances and age at natural menopause. Other famine cohorts have published on
the effects of famine on other health parameters but none of them has published on the relation between age at natural menopause and famine exposure (36-40). This might be due to lack of data on the subject but it could also reflect the fact that studies yielding negative or no associations are less likely to be submitted or to be accepted for publication. Another aspect to consider is that premature ovarian insufficiency, with menopausal ages younger than 40 years, might have a different etiology than age at natural menopause and not just be an extreme variation. While an early age at menopause might be the reflection of a premature senescence it has been postulated that a much younger menopausal age has a different pathophysiology (41, 42). Most included studies however, did not differentiate between an early age at natural menopause and premature age at menopause.

**Conclusion**

Our study shows that famine during gestation and childhood as well as impaired childhood growth is related to a younger age at menopause. This could lead to a shorter reproductive lifespan in populations with compromised early life nutrition, and may add to the increased burden of cardio-metabolic disease in postmenopausal women in populations in transition between under- and over-nutrition.

**Acknowledgments**

The authors would like to thank all authors who elaborated on their data after email contact. We also thank Peter van de Ven, consultant epidemiology and biostatistics, for advising us on the evaluation of data and calculating additional risk estimates.
References


Appendix

Pubmed
Menopauze en premature menopauze:
Birth parameters:
Premature
"Infant, Premature"[Mesh] OR prematur*[tia] OR preterm*[tia] OR pre-mature[tia]
Birthweight / birth size
Famine
Developmental origins of health and disease
dohad[tia] OR developmental origins of health and disease[tia]

Embase.com
Menopauze en premature menopauze
'menopause'/exp OR 'early menopause'/exp OR 'premature ovarian failure'/exp OR menopause:ab,ti OR 'premature menopause':ab,ti OR (premature NEAR/3 ovar* NEAR/3 failure*):ab,ti OR (premature NEAR/3 ovar* NEAR/3 insufficiency):ab,ti OR (primary NEAR/3 ovar* NEAR/3 insufficiency):ab,ti OR (premature NEAR/3 ovar* NEAR/3 syndrome):ab,ti OR 'climacterium praecox':ab,ti OR 'climacterium precox':ab,ti OR POF:ab,ti
Birth parameters:
Premature
'prematurity'/exp OR prematur*:ab,ti OR preterm*:ab,ti OR (pre NEXT/1 mature):ab,ti
Birthweight / birth size
'birth weight'/exp OR birthweight:ab,ti OR 'birth weight':ab,ti OR 'small for gestational age':ab,ti OR 'small for date':ab,ti OR sga:ab,ti OR vlbw:ab,ti OR elbw:ab,ti OR 'birth size':ab,ti OR 'neonatal underweight':ab,ti
Famine
'starvation'/exp OR 'fetal malnutrition'/exp OR starvation:ab,ti OR famine*:ab,ti OR malnutrition:ab,ti OR undernutrition:ab,ti
Developmental origins of health and disease
dohad:ab,ti OR 'developmental origins of health and disease':ab,ti
Menopause en premature menopauze:
Menopause OR “premature menopause” OR (premature NEAR/3 ovar* NEAR/3 failure*)
OR (premature NEAR/3 ovar* NEAR/3 insufficienc*) OR (primary NEAR/3 ovar* NEAR/3 insufficienc*) OR (premature NEAR/3 ovar* NEAR/3 syndrome) OR “climacterium praecox” OR “climacterium precox” OR POF
Birth parameters:
Premature
prematur* OR preterm* OR (pre NEXT/1 mature)
Birthweight / birth size
“birth weight” OR birthweight OR “small for gestational age” OR “small for date” OR sga
OR vlbw OR elbw OR “birth size” OR “neonatal underweight”
Famine
starvation OR famine* OR malnutrition OR undernutrition
Developmental origins of health and disease
dohad OR “developmental origins of health and disease”

Birth parameters:
Premature
(MH "Infant, Premature") OR (MH "Infant") OR TI (prematur* OR preterm* OR “pre-mature”) OR AB
(prematur* OR preterm* OR “pre-mature”)
Birthweight / birth size
(MH "Infant, Low Birth Weight") OR (MH "Birth Weight") OR TI (birthweight OR “birth weight” OR “small for gestational age” OR “small for date” OR sga OR vlbw OR elbw OR “birth size” OR “neonatal underweight”) OR AB (birthweight OR “birth weight” OR “small for gestational age” OR “small for date” OR sga OR vlbw OR elbw OR “birth size” OR “neonatal underweight”)
Famine
(MH "Starvation") OR (MH "Malnutrition") OR TI (starvation OR famine* OR malnutrition OR undernutrition) OR AB (starvation OR famine* OR malnutrition OR undernutrition)
Developmental origins of health and disease
ti (dohad OR “developmental origins of health and disease”) OR AB (dohad OR “developmental origins of health and disease”)
Section two

Polycystic Ovary Syndrome
Chapter 7

Heritability of Polycystic Ovary Syndrome in a Dutch Twin-Family Study.

Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI.

J Clin Endocrinol Metab. 2006 Jun;91(6):2100-4
Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age. There is evidence for a genetic component in PCOS based on familial clustering of cases.

Objective: In the present study, the heritability of PCOS was estimated.

Design/Participants: Data from 1332 monozygotic twins (genetically identical) and 1873 dizygotic twins/singleton sisters of twins who share on average 50% of their segregating genes) registered with The Netherlands Twin Register were used. PCOS was defined as less than nine menstrual cycles and acne or hirsutism in agreement with the 2003 Rotterdam consensus.

Results: Results point to a strong contribution of familial factors to PCOS. The resemblance in monozygotic twin sisters (tetrachoric correlation 0.71) for PCOS was about twice as large as in dizygotic twin and other sisters (tetrachoric correlation 0.38). Univariate analyses point to strong contributions of genetic factors to the variance in PCOS. Next, a trivariate genetic analysis of oligomenorrhea, acne, and hirsutism was carried out. This analysis confirmed that the familial component in PCOS is due to genetic factors.

Conclusions: This study demonstrated a large influence of genetic factors to the pathogenesis of PCOS, justifying the search for susceptibility genes.
Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age. The prevalence is estimated at 5–10% (1–3). In 2003 an international consensus on the definition of PCOS was published (4). PCOS is defined as at least two of the following symptoms: oligo- or anovulation, hyperandrogenism (defined as elevated androgen serum levels or hirsutism and/or acne), and polycystic ovaries on ultrasound (4). The finding of polycystic ovaries on ultrasonography was originally the hallmark for the diagnosis of the syndrome but represents a sign of a wide variety of disorders and appears to be a nonspecific finding in approximately 20% of the asymptotic women (5). The symptoms of PCOS include the consequences of excessive androgen production, anovulation, and consequently infertility (6). Early identification of women at risk for PCOS can have profound implications on prevention of PCOS-associated endocrine disorders.

The pathogenesis of PCOS has not yet been elucidated, but familial clustering suggests genetic involvement. Studies in first-degree relatives of women affected by PCOS clearly indicate genetic influences, but no clear mode of inheritance has been identified (7, 8). A polygenic multifactorial model involving multiple genes is most likely (9). To identify genes playing a role in PCOS, linkage and association analyses were carried out. For example, a study with 37 candidate genes in the known pathways for PCOS showed linkage with the follistatin gene and suggestive linkage with CYP11A (10). Other studies failed to detect any consistent association between PCOS and follistatin (11) or CYP11A (12). Other candidate genes for PCOS are genes involved in the biosynthesis and metabolism of androgens, genes involved in folliculogenesis, and the secretion and action of insulin (9, 10, 13–15).

So far, no clear estimate of the impact of genes, the heritability of PCOS, is available. Twin-family studies are commonly used for this type of investigation. Dizygotic (DZ) twins, like ordinary siblings, on average share 50% of their segregating genes, whereas monozygotic (MZ) twins share all their genes. A higher association for PCOS in MZ twins, compared with DZ twins and siblings, indicates genetic influences. Twin data allow distinguishing between the influence of genetic and environmental factors on phenotypic variation (16). Genetic influences will lead to larger MZ than DZ/sister correlations. Environmental influences can
be unique to individuals or can be shared by family members. Environmental influences shared by sisters growing up in the same family will lead to MZ, DZ, and sister-pair correlations of equal size. Unique environmental influences will not cause resemblance among sisters. Using statistical modeling techniques makes it possible to obtain a quantitative estimate of the genetic and environmental influence on PCOS. The aim of this study was to estimate the heritability of PCOS. First, a univariate model including genetic and environmental influences was fitted to data of Dutch twins and sisters. PCOS was defined as less than nine menstrual cycles a year plus acne or hirsutism. In addition, we investigated oligomenorrhea, acne, and hirsutism in a trivariate model (Fig. 1). This allowed us to study whether the three variables are indicators of a single latent unobserved trait (PCOS).

**Subjects and Methods**

**Subjects**

This study is part of an ongoing twin family study on health-related behavior in participants of The Netherlands Twin Register (17). With an interval of 2–3 yr, twins and family members receive mailed surveys. For the purpose of this study, data from the 2000 survey were used (18). Zygosity was based on (longitudinal) questionnaire data or, when available, DNA typing (n=572 females). Agreement between zygosity based on questionnaire data and zygosity based on DNA results is 96%.

In total, 4236 females participated in the 2000 survey. Spouses of male twins (n=265) and half-siblings (n=17) were excluded. When data on menstrual cycle were missing (n=726), it was not possible to classify the subject for PCOS. Data on zygosity were missing for 15 twins. The remaining data set for the univariate analyses of PCOS contained 3205 females: 1332 MZ twins, 680 DZ (same sex) twins, 474 females from dizygotic opposite sex pairs, and 719 (non-twin) sisters.

**Phenotype definition**

PCOS was defined based on questions about the number of menstrual cycles per year, when not using contraception (with answer categories more than eight, less than nine, less than six, two or less), about suffering from acne/pimples (yes or no) and suffering
from hirsutism (yes or no). PCOS was defined as less than nine natural menstrual cycles a year combined with hirsutism or acne. In addition, the survey provided information on date of birth, age at menarche, birth weight, current height and weight, having children, and smoking habits. Characteristics of participants are listed in Table 1.

Fig. 1. Diagram of the common pathway model. Ol, Oligomenorrhea (yes/no); Ac, acne (yes/no); Hi, hirsutism (yes/no); A, additive genetic influences; C, common environment; E, non-shared environment; A_s, specific additive genetic influences; D_s, specific dominant genetic influences; E_s, specific unique environment; parentheses, percentage of the total variance. The total variance for each variable is constrained at 1. For example, the total variance explained by specific additive genetic factors (72%) = factor loading of the latent PCOS construct (.07) * additive genetic influences on PCOS (79%) + specific unique environmental factors on PCOS (21%) + 0.7*0.79%+0.7*21% = 100%.
Statistical analyses

Modeling of twin data allows discrimination between phenotypic variance due to genetic factors and environmental factors (19). The additive genetic effects of contributing gene loci are expressed in the additive genetic variance reflecting the narrow-sense heritability of PCOS. Another source of genetic variation is dominance; this is the extent to which the effects of alleles at a locus do not simply add up but reflect non-additive gene action. Variance caused by shared environmental effects is reflected in common environmental variance. Environmental effects that are not shared between family members result in unique environmental variance ($e^2$). This later estimate also includes measurement error. Therefore, the unique environmental variance is always specified in the model. Phenotypic similarities in MZ twins can be due to common environmental and genetic influences. Unique environmental influences contribute to the differences between MZ twins. DZ twins, like other siblings, share approximately 50% of their genetic makeup. The correlation between their additive genetic values is 0.5, and the correlation between their non-additive genetic values is 0.25. Common environmental effects contribute similarly to similarities between DZ and MZ twins. Adding singleton sisters of twins and females from dizygotic opposite sex twin pairs to the study population enhances the statistical power for the estimation of the contribution of genetic and environmental influences (20).

Because the phenotype was a dichotomous variable, a threshold model was used (21). A categorical characteristic such as PCOS is assumed to have an underlying liability, which is continuous and normally distributed in the population. The liability to PCOS is divided into two categories, yes and no, separated by a single threshold. The threshold is obtained from the observed proportions in the two categories. Individuals falling below the threshold do not have PCOS, and those exceeding the threshold do suffer from PCOS. Information about twin resemblance in liability is given by tetrachoric correlations. Genetic models were applied to raw ordinal data using the Mx statistical program (22). First, a full model with genetic, common environmental, and unique environmental influences was fitted.
Next, the full model was reduced by excluding the genetic or common environment component. The reduced models were compared with the full model by hierarchic χ² tests. The χ² statistic was calculated by subtracting the 2log likelihood of the goodness of fit of a reduced model from the full model. If the reduced model does not describe the data significantly worse than the full model, the reduced model can be considered as the best model. Last, a multivariate model was fitted to the data on oligomenorrhea, hirsutism, and acne. To investigate whether the three variables define a single construct of PCOS, a common pathway model was applied. This model assumes that all three variables are indicators of a single latent unobserved trait (PCOS). The relative importance of the latent trait on the observed variables (oligomenorrhea, hirsutism, and acne) is indicated by the value of the loadings from the latent factor to the observed traits. The variance of the

<table>
<thead>
<tr>
<th></th>
<th>9 or more menstrual cycles (n=2947)</th>
<th>Less than 9 menstrual cycles (n=258)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ twins</td>
<td>1213</td>
<td>119 (8.9%)</td>
<td></td>
</tr>
<tr>
<td>DZ twins</td>
<td>1073</td>
<td>81 (7.0%)</td>
<td></td>
</tr>
<tr>
<td>Sisters</td>
<td>661</td>
<td>58 (8.1%)</td>
<td>0.540</td>
</tr>
<tr>
<td>Acne</td>
<td>30.0%</td>
<td>27.1%</td>
<td>0.326</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>8.5%</td>
<td>14.5%</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>29.4</td>
<td>30.1</td>
<td>0.332</td>
</tr>
<tr>
<td>Age menarche (yr)</td>
<td>13.1</td>
<td>13.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.9</td>
<td>168.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.5</td>
<td>65.8</td>
<td>0.681</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.7</td>
<td>23.2</td>
<td>0.023</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2657</td>
<td>2697</td>
<td>0.545</td>
</tr>
<tr>
<td>Having children</td>
<td>33.4%</td>
<td>37.2%</td>
<td>0.213</td>
</tr>
<tr>
<td>Current smoker</td>
<td>23.0%</td>
<td>23.0%</td>
<td>0.987</td>
</tr>
</tbody>
</table>
latent factors is decomposed into genetic and environmental components. The variance of the observed traits that is not attributable to the PCOS factor is also composed into genetic and environmental components (Fig. 1) (23).

**Results**

Characteristics of the study population are listed in Table 1. The prevalence for oligomenorrhea varied from 7.0% in singleton sisters to 8.1% in MZ twins and 8.9% in DZ twins. The prevalence were not significantly different between MZ twins, DZ twins, and singleton sisters (P=0.540). In total, 8% of the females included in this study reported to have less than nine menstrual cycles in a year. The percentage of females suffering from acne was not different for females with more or less than nine menstrual cycles. In contrast, the females with less than nine menstrual cycles report more often to suffer from hirsutism than the females with nine or more menstrual cycles a year. The two groups also differed significantly in age at menarche and height and body mass index but not in current weight and birth weight. There were 92 women (2.9%) classified as having PCOS. The prevalence was not significantly different for MZ twins, DZ twins, or singleton sisters (P=0.836).

Table 2 shows the tetrachoric correlations for MZ twin pairs and DZ twin and sister pairs. The MZ correlations are higher than the DZ and sister correlations, suggesting a large genetic influence on all variables. The pattern of correlations for acne, hirsutism, and PCOS suggest additive genetic influences (MZ correlation twice as high as the DZ correlation). For oligomenorrhea, the MZ correlation exceeds more than twice the DZ correlation, suggesting genetic dominance or epistasis.
Table 2. Twin and sibling correlations (95% CI) for oligomenorrhea (less than nine menstrual cycles in a year), acne, hirsutism, and PCOS (defined as less than nine menstrual cycles per year and acne or hirsutism)

<table>
<thead>
<tr>
<th></th>
<th>MZF r (95% CI)</th>
<th>DZF/sisters r (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligomenorrhea</td>
<td>0.67 (0.49 to 0.80)</td>
<td>0.07 (0.19 to 0.34)</td>
</tr>
<tr>
<td>Acne</td>
<td>0.78 (0.69 to 0.84)</td>
<td>0.44 (0.30 to 0.56)</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>0.86 (0.75 to 0.92)</td>
<td>0.28 (0.05 to 0.50)</td>
</tr>
<tr>
<td>PCOS</td>
<td>0.71 (0.43 to 0.88)</td>
<td>0.38 (0.00 to 0.66)</td>
</tr>
</tbody>
</table>

MZF, Monozygotic females; DZF/sisters, dizygotic females twins and non-twin sisters.

First, univariate genetic models were fitted to the PCOS data (Table 3). In the full model, 66% of the variance is explained by genetic factors (additive genetic variance), 12% by shared environment factors (common environmental variance), and 29% by unique environmental factors ($e^2$). Those results suggested that the role of shared environmental factors is small or nonsignificant. The full model could be reduced to a model including only genetic factors [$a^2 = 72\%$, 95% confidence interval (CI) 46–88%] and unique environmental factors [$e^2 = 28\%$, 95% CI 12–54%]. Next, we explored whether the shared environmental influence could be dropped (model 2) and whether both the shared environmental influence and the genetic influence could be dropped (model 3). The fit of the sub-models was compared with the fit of the full model ($\chi^2 = -2\text{LL}_{\text{full model}} - -2\text{LL}_{\text{submodel}}$ and $\Delta df = df_{\text{full model}} - df_{\text{sub-model}}$) to check whether the sub-models fit the data significantly worse. The statistical power of the analyses did not allow distinguishing between model 2 and model 3 (Table 3) because both models did not significantly worsen the fit compared to model 1 ($P = 0.05$). The best model is a model with the smallest number of parameters necessary to explain the data adequately. The Akaike’s Information Criterion (AIC = $\chi^2 - 2 \Delta df$) is a measure of the parsimony of the model and a lower value of AIC indicates a more parsimonious model. The AIC (in Table 3) indicated that the model including additive genetic influences and unique environmental factors was the most parsimonious model. In addition, a common pathway model was fitted to the data. In this model, three variables (oligomenorrhea, hirsutism, and acne) are hypothesized to be indicators of a single latent phenotype (PCOS). The variation in the latent PCOS construct is decomposed...
into additive genetic, common, and unique environmental sources. The model also allows
genetic and environmental parameters for the unique variance for each variable. Figure 1
displays the model and the estimates of the parameters. The total variance of the latent
and the observed variables (oligomenorrhea, acne, and hirsutism) is constrained to be 1.
The parameter estimates (from the best model) are depicted in Figure 1. The percentage of
the total variance is given between parentheses. This model, which has a larger statistical
power to distinguish between genetic and common environmental influences on familial
resemblance than the univariate model, clearly shows the importance of genetic factors to
PCOS. Heritability is estimated at 79%, and there is no influence of common family
environment.

**Discussion**

To our knowledge this is the first quantitative estimation of the genetic influence on the
pathogenesis of PCOS using twin data. The prevalence of PCOS was the same for MZ
twins, DZ twins, and singleton sisters. However, the prevalence in our sample was slightly
lower than reported in other population studies (1–3). It is possible that the prevalence of

### Table 3. Model-fitting results for PCOS defined as less than nine menstrual cycles and acne or

<table>
<thead>
<tr>
<th>Model</th>
<th>2LL</th>
<th>Df</th>
<th>df</th>
<th>2</th>
<th>P</th>
<th>AIC</th>
<th>a², %</th>
<th>c², %</th>
<th>e², %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ACE</td>
<td>803.116</td>
<td>3196</td>
<td></td>
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<td></td>
<td>66</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>2. AE</td>
<td>803.138</td>
<td>3197</td>
<td>1</td>
<td>0.023</td>
<td>0.880</td>
<td>1.94</td>
<td>72</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>3. CE</td>
<td>805.845</td>
<td>3197</td>
<td>1</td>
<td>2.729</td>
<td>0.099</td>
<td>0.73</td>
<td>55</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>4. E</td>
<td>827.258</td>
<td>3198</td>
<td>2</td>
<td>24.142</td>
<td>0.000</td>
<td>20.14</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A lower value of AIC indicates a more parsimonious model. A, additive genetic influences; C,
common environmental influences; E, unique environmental factors; 2LL, 2 log likelihoood; df,
degrees of freedom; df, difference in degrees of freedom; a², percent of variance explained by
additive genetic influences (heritability); c², percent of variance explained by common
environment; e², percent of variance explained by unique environmental influences.
PCOS is lower in families that produced twins. However, the prevalence in female spouses of male twins (in general not coming from twin families) is 2.3% (n = 5 of 215 subjects), which suggests that the low prevalence in our sample is not the result of selecting twin families. The prevalence is in line with a Dutch cross-sectional population based study in adolescents (being more than 3 yr after menarche), which indicated a prevalence of about 3% of oligomenorrhea combined with signs of hyperandrogenism (24). Whether the low prevalences are due to lifestyle differences in The Netherlands, compared with other countries, or to other factors, is unknown.

We did not include ultrasound criteria in the definition of PCOS but relied on self-reported items on oligomenorrhea, hirsutism, and acne. This is very much analogous to the link between oligomenorrhea and cardiovascular diseases from the Nurse’s Health Study (25). Epidemiological studies have shown that oligomenorrhea, hirsutism, and acne are very good indicators of PCOS in the general population (14).

Our study points to a strong contribution of genetic factors to PCOS and indicates that a model including additive genetic factors and unique environmental factors is the most parsimonious. In this model the variance in the pathogenesis of PCOS is for 72% due to genetic influences. The high heritability was confirmed by the results from the multivariate genetic analysis, which has a larger power to detect genetic influences (26).

There are few data regarding PCOS in twins. Jahanfar et al. (27) studied 34 twin pairs (19 MZ and 15 DZ) from an original group of 500 twin pairs that volunteered for PCOS related ultra-sonographic, clinical, and biochemical evaluation. Of the 68 individuals, 33 had PCO ovaries on ultrasound scan, 19 had acne, 12 were hirsute, and seven had oligo/amenorrhea. Eleven pairs were discordant for PCO ovaries on ultrasound scan (five were MZ and six were DZ twins). From this small study they concluded that PCOS is not the result of a single autosomal genetic defect but that PCOS may be the result of polygenic factors or that environmental factors are involved in the pathogenesis of this disorder (27).

Several other studies showed that there appears to be evidence for a genetic component in PCOS based on familial clustering of cases (7, 8, 15). In most family studies, the number of participants is small and PCOS was defined in different ways. In accordance with the
Rotterdam consensus (4), we defined PCOS as less than nine natural menstrual cycles a year combined with hirsutism or acne. No ultrasound data were available. The twin correlations for oligomenorrhea, hirsutism, and acne showed that these variables are largely influenced by genetic factors because the MZ twin correlation was (more than) twice the DZ/sister correlation. Noteworthy, the DZ correlation for oligomenorrhea is less than twice the MZ correlation, indicating that non-additive genetic influences play an important role. The results are in accordance with other studies. For example, in 2002 Bataille et al. (28) showed that 81% of the variance in acne was attributable to additive genetic effects, whereas the remaining 19% were attributed to unique environmental factors. Previous studies also showed that androgen levels and androgen production rates in humans are under genetic control (29).

In a further step, we modeled the three variables, oligomenorrhea, acne, and hirsutism, in an independent pathway model. This model confirmed our finding with the univariate analyses: the latent variable (PCOS) was highly influenced by genetic variance (79%) and unique environmental influence (21%). Shared environmental influence did not contribute to the latent variable. The results point to the importance of genetic involvement in PCOS and justify the continuous effort to trace the responsible genes. This has been relatively unsuccessful so far, probably due to a number of reasons. One major problem remains: the definition of the phenotype. For example, the most recently developed guideline allows for no less than four possibly distinct phenotypes (3). Another problem is the underlying complexity of the disorder. The metabolic nature of PCOS with combined dysregulation of carbohydrate and fat metabolism and abnormal steroid hormone secretion (9) has led to the suggestion of numerous candidate genes. For a detailed update on this, we referred to several recently published overviews (10–12). Additional difficulties are the lack of a clear male phenotype and PCOS being a major cause of female infertility (9). Finally, environmental factors are also of importance. Environmental factors, *i.e.* weight gain, may trigger the development of PCOS in predisposed women (10–12). The environmental factors may vary between populations and may actually themselves include a genetic component (30).
Currently a prevailing view is that genetic compounds account for disturbed regulation of ovarian androgenic activity and that environmental circumstances that are of influence on glucose/insulin household predominantly act by aggravating the syndrome through hyperinsulinism and insulin resistance (10–12).

In summary, a number of studies have shown familial aggregation for PCOS, but less was known about the magnitude of a genetic effect, and the putative PCOS genes have not yet been identified. The present study points to a strong contribution of genetic factors to the pathogenesis of PCOS, justifying further search for these susceptibility genes.

Acknowledgments

We thank Dr. R. Homburg for his contribution to the manuscript.
References


Birth weight and age at menarche in patients with polycystic ovary syndrome or diminished ovarian reserve, in a retrospective cohort.

Sadrzadeh S, Klip WA, Broekmans FJ, Schats R, Willemsen WN, Burger CW, Van Leeuwen FE, Lambalk CB; OMEGA Project group

Abstract

BACKGROUND: Few studies have investigated the association between subfertility in women and factors in early life such as birth weight and age at menarche, and most have produced contradictory results. In the present study, this association was investigated among women undergoing artificial reproductive techniques (ART), including IVF for reason of polycystic ovary syndrome (PCOS) or diminished ovarian reserve. Herein, PCOS included oligomenorrhea and at least one additional symptom such as hyperandrogenism, hirsutism or polycystic ovaries on ultrasound. In most patients this was concomitant with elevated serum LH levels. Diminished ovarian reserve was defined as receiving a donated oocyte or having a low response to ovarian hyperstimulation. METHODS: Among a retrospective cohort of 26 428 women diagnosed with subfertility between 1980 and 1995, three study groups and one reference group were defined using data from medical records. Women were included in the first group if diagnosed as having PCOS (n = 265). In order to define diminished ovarian reserve capacity, two groups were selected: (i) women receiving a donated oocyte (n = 98); and (ii) women having a low response (three follicles or less) to ovarian hyperstimulation in both their first and second IVF cycles (n = 351). Women with tubal obstruction formed the reference group (n = 957). In a logistic regression model, the effect of birth weight and age at menarche was examined. Information on both variables was obtained from mailed questionnaires. RESULTS: Birth weight did not differ significantly between the study groups and the reference group. However, PCOS patients were significantly older at menarche [OR 3.31 (2.18-5.04)]. Women receiving a donated oocyte and low responders were significantly younger at menarche [OR 2.67 (1.35-5.29) and OR 2.01 (1.26-3.20) respectively]. CONCLUSION: The fetal origins hypothesis, the association between intrauterine growth retardation and disease in adult life, could not be confirmed, though a relationship between timing of menarche and PCOS and a diminished ovarian reserve was identified. Further investigation of the effect of birth weight on fertility outcome in a prospective setting is strongly advised.
Introduction

In recent years, infertility treatment has become one of the most pressing issues of modern health care. In the United States, up to 43% of women aged between 15 and 44 years have been estimated as having impaired fecundity, and 24% of them benefit from some kind of artificial reproductive technology (ART) (1). Polycystic ovary syndrome (PCOS) and a diminished ovarian reserve contribute to subfertility among women. In order to identify women at risk of developing fertility-related problems, at a stage when more therapeutic options are open, early risk factors such as age at menarche and birth weight are being explored. Although the effect of age of menarche on PCOS is still unclear, PCOS is found in relation to premature pubarche, which in girls is defined as the appearance of pubic hair before the age of 8 years (2-4). Contradictory findings have been reported with regard to age at menopause, the final stage of ovarian follicle pool depletion. A young age (5, 6), as well as an older age at menarche(7-9) have been associated with a premature or young age at menopause. Most investigators however have failed to confirm any direct relationship between age at menarche and age at menopause (10-13).

Birth weight, while being a crude indicator for intrauterine adverse conditions, has become a major factor to consider when investigating the aetiology of adult-onset disease. Over the past two decades a considerable amount of convincing evidence has been brought forward associating adverse intrauterine conditions with adult-onset disease, including high blood pressure and glucose intolerance (14). Impaired fetal growth as well as a higher weight at birth have both been reported as a factor in the aetiology of PCOS, as well as a diminished ovarian reserve (15-17).

The aim of the present study was to investigate the association between birth weight, age at menarche and participation in ART for reasons of PCOS or diminished ovarian reserve. For this purpose, access was acquired to a unique set of data from a retrospective cohort consisting of all women undergoing fertility treatment in the Netherlands between 1980 and 1995.
Materials and methods
The cohort

Information for this study was extracted from data available from the OMEGA Project, a nationwide cohort of 26,428 women diagnosed with subfertility in all 12 IVF clinics in The Netherlands between January 1980 and January 1995. The main aim of the OMEGA study was to investigate the late health effects of hormonal stimulation due to ART (18). Medical information on subfertility diagnosis and subfertility treatment was obtained by trained research assistants from medical records which were provided by all the participating IVF centres. Additional information on self-reported risk factors was extracted from the questionnaires mailed to all women who were alive and traceable in January 1997. Of 25,323 questionnaires posted to these women, a total of 16,284 was completed and returned. Detailed information on the cohort and methods of data collection has been described elsewhere (18).

Selection of study groups

For the purpose of the present study, three patient groups and one reference group were selected from women enrolled in the OMEGA database with completed questionnaires. Information from the medical records was used for primary selection. Women were included in the first patient group if diagnosed as having PCOS (n = 265), the criteria for which varied from centre to centre. However, in all centres diagnostic criteria for PCOS included oligomenorrhoea and at least one additional symptom such as hyperandrogenism, hirsutism or polycystic ovaries on ultrasound. In most patients this situation was concomitant with elevated serum LH levels.

Two different patient groups were defined with regard to diminished ovarian reserve capacity. The first group comprised all women who had received an embryo conceived by donated oocytes (n = 98). The second group comprised women who had a low response to adequate ovarian hyper-stimulation in both their first and second IVF cycles (n = 351). A low response was defined as three or less follicles at oocyte retrieval, or cancellation of the stimulation cycle due to low response. The reference group consisted of women whose only cause of subfertility was tubal obstruction, assuming that intrauterine growth
retardation and hormonal factors do not play a prominent role in this specific cause of subfertility (n = 957).

Women diagnosed with any additional cause of subfertility, other than the causes which were considered for inclusion, were excluded. After the primary selection, the following women were excluded: those diagnosed with cancer; those treated with radiation therapy or chemotherapy; those diagnosed with any chromosomal or genetic defect such as fragile X or Turner syndrome (19-21); or those who were exposed to diethylstilbestrol (DES) in utero. Furthermore, all women with infections or surgery on the reproductive organs were excluded, with the exception of the tubal operations as carried out in the reference group. Information on causes of subfertility, IVF treatment and date of first attending a fertility clinic was obtained from the medical records. Information on whether participants were part of a multiple gestation, their mother's age at the time of birth, weight at birth, date of birth, birth order, country of birth, age at menarche, current height and weight, smoking habits and education level were taken from the mailed questionnaires. Participants were asked to report age at menarche as the exact calendar year of first menstruation, or exact age at first menstruation, or, if they could not recall the precise age or year, to choose between the categories under 12 years, 12-14 years, or over 14 years. The questionnaires provided no information on the gestational age of the participants.

Statistical analysis

Logistic regression analysis was performed to estimate the difference in birth weight and age at menarche between the individual groups, simultaneously correcting for relevant confounders. Potential confounders were added to the logistic regression model and then subsequently removed from the model in order of least significance. The significance level of Wald statistics to include a variable as confounder was set at P = 0.2. Odds ratios (OR) were considered significant if the P-value of the Wald statistics was < 0.05 and the 95% confidence interval (CI) of the OR did not include the value 1. Individual cases with missing data on any of the items included in the logistic regression model were automatically excluded from the analysis. Logistic regression analysis was also performed to estimate OR
for the characteristic of having a missing value for birth weight between individual groups and the reference group. Data were analyzed using SPSS.9 for Windows.

The effect of birth weight was examined as a continuous as well as a categorical variable. In the latter case, birth weight was categorized as lighter or heavier than 2.5 kg (low birth weight) or as lighter or heavier than 1.5 kg (very low birth weight). These categories were analyzed separately.

In order to distinguish between extremes in age at menarche, a categorical variable was used for analysis. Education level was used as an indicator for socioeconomic status. Participants could choose between seven levels of increasing education levels ranking from no education up to university. These categories were pooled into three categories of: no education and primary school; high school and vocational training; or college, university and higher. Country of birth was categorized as Europe and North America or other. For the analysis, a categorical variable on smoking was used: never smoked; or ever smoked. Current height and body weight were used to calculate the body mass index (BMI) as: body weight (kg)/height$^2$ (cm).

Results

The general characteristics of the study population are listed in Table I. The reference group had a mean birth weight of 3323 g, while the mean birth weights of PCOS patients, women receiving an embryo from a donated oocyte and low responders were 3311 g, 3318 g and 3293 g respectively. The mean birth weight of girls born during the early 1970s in The Netherlands was 3352 g (22). Of all participants, 27% failed to report a birth weight. There were no statistical differences between study groups and the reference group in availability of self-reported birth weights (Table II).
Table I. General characteristics of the study population selected from the OMEGA study cohort with completed questionnaires by subfertility diagnosis. Participants with more than one subfertility diagnosis were excluded

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference group (n = 957)</th>
<th>PCOS (n = 265)</th>
<th>Oocyte donation (n = 98)</th>
<th>Low responders (n = 351)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3323 ± 669</td>
<td>3311 ± 677</td>
<td>3318 ± 675</td>
<td>3293 ± 658</td>
</tr>
<tr>
<td>Age visit fertility clinic (years)(^a)</td>
<td>31 ± 4.7</td>
<td>29 ± 3.9</td>
<td>33 ± 4.5</td>
<td>33 ± 4.8</td>
</tr>
<tr>
<td>Age at 1st IVF (years)(^b)</td>
<td>32 ± 3.8</td>
<td>-</td>
<td>-</td>
<td>35 ± 3.9</td>
</tr>
<tr>
<td>Age at time of study (years)</td>
<td>38 ± 4.9</td>
<td>35 ± 4.5</td>
<td>38 ± 4.4</td>
<td>40 ± 4.3</td>
</tr>
<tr>
<td>BMI at time of study (kg/m(^2))</td>
<td>23.8 ± 3.8</td>
<td>25.8 ± 5.3</td>
<td>24.1 ± 3.8</td>
<td>24.6 ± 4.7</td>
</tr>
<tr>
<td>Mother's age at delivery (years)</td>
<td>29 ± 6.1</td>
<td>29 ± 6.5</td>
<td>29 ± 6.4</td>
<td>30 ± 6.3</td>
</tr>
<tr>
<td>Mother's age time of study (years)</td>
<td>67 ± 8.2</td>
<td>64 ± 8.4</td>
<td>67 ± 8.0</td>
<td>70 ± 8.0</td>
</tr>
<tr>
<td>Age at menarche (%) &lt;11 years</td>
<td>12 (115)</td>
<td>15.5 (41)</td>
<td>20.9 (18)</td>
<td>19.0 (65)</td>
</tr>
<tr>
<td>12-14 years</td>
<td>74 (709)</td>
<td>58.5 (155)</td>
<td>58.1 (50)</td>
<td>70.6 (242)</td>
</tr>
<tr>
<td>&gt;15 years</td>
<td>13.8 (132)</td>
<td>26 (69)</td>
<td>20.9 (18)</td>
<td>10.5 (36)</td>
</tr>
<tr>
<td>Country of birth (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe or N. America</td>
<td>92.8 (883)</td>
<td>90.5 (237)</td>
<td>92.6 (75)</td>
<td>92.3 (322)</td>
</tr>
<tr>
<td>Other</td>
<td>7.2 (69)</td>
<td>9.5 (25)</td>
<td>7.4 (6)</td>
<td>7.7 (27)</td>
</tr>
<tr>
<td>Education (%) Low</td>
<td>27.2 (260)</td>
<td>40.3 (106)</td>
<td>37 (30)</td>
<td>32.5 (114)</td>
</tr>
<tr>
<td>Middle</td>
<td>51.9 (495)</td>
<td>47.1 (124)</td>
<td>46.9 (38)</td>
<td>45.5 (160)</td>
</tr>
<tr>
<td>High</td>
<td>20.9 (119)</td>
<td>12.5 (33)</td>
<td>16 (13)</td>
<td>21.9 (77)</td>
</tr>
<tr>
<td>Birth order (%) First child</td>
<td>30.2 (281)</td>
<td>31.7 (82)</td>
<td>44.9 (44)</td>
<td>31.6 (108)</td>
</tr>
</tbody>
</table>

Values are mean ±SD; Values in parentheses are numbers of patients
\(^a\)Mean age at first visit to fertility clinic; \(^b\)For the comparison of low responders with the reference group, the mean age at time of first IVF cycle was used instead of age at first visit to fertility clinic.
BMI = body mass index
Table II. Results of the logistic regression analysis estimating odds ratios (OR) for the variable missing birth weight between study groups

<table>
<thead>
<tr>
<th>Study group</th>
<th>Not reporting birth weight</th>
<th>OR(^a) (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PCOS</td>
<td>0.84 (0.58-1.22)</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Oocyte donation</td>
<td>1.05 (0.60-1.83)</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Low responders</td>
<td>1.01 (0.74-1.37)</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Adjusted for age at menarche, education level, age at time of study, country of birth and birth order.

There were no statistical differences between study groups and the reference group in availability of self-reported birth weights. Women with PCOS were younger at their first visit to a fertility clinic and had a higher BMI compared to the reference group. The differences of 2 years in age at first visit and 2.0 points in BMI were both significant (P < 0.0001) and were included in the final logistic regression model. Furthermore, women receiving an embryo from oocyte donation and low responders were more likely to have a low education level. This effect was significant, and hence educational level was included in the final logistic regression model. No significant association was found between PCOS and birth weight. The OR (95% CI) of PCOS associated with mean birth weight was 0.91 (0.71-1.17). In the reference group, 711 women reported a birth weight, 92 had a low birth weight, and nine had a very low birth weight. From the 200 reported birth weights in the PCOS group, 27 were low and none was very low. The OR (95% CI) for PCOS associated with a low birth weight, defined as <2500 g, was 0.88 (0.53-1.48).

However, patients diagnosed with PCOS had a significantly older age at menarche. At the time of menarche, 26% of the PCOS patients versus 13.8% in the reference group were aged over 14 years. The OR associated with an age at menarche over 14 years compared with 12-14 years was 3.31 (2.18-5.04).

A diminished ovarian reserve was not significantly associated with birth weight. Sixty women receiving an embryo from a donated oocyte reported a birth weight. With birth
weight as a continuous variable, the OR (95% CI) for receiving an embryo from a donated oocyte was 1.02 (0.67-1.55). For low birth weight (n = 6) and very low birth weight (n = 1) these values were 0.75 (0.31-1.85) and 1.15 (0.14-9.66) respectively. For the low responders (243 reported birth weights), the OR (95% CI) associated with mean birth weight was 0.93 (0.73-1.19). The OR associated with low birth weight (n = 39) and very low birth weight (n = 2) were 1.12 (0.70-1.80) and 0.44 (0.08-2.32) respectively.

Women with a diminished ovarian reserve were significantly younger at the time of their menarche. In women receiving a donated oocyte, the OR associated with a menarchal age of 11 years and younger was 2.67 (1.35-5.29) compared with 12-14 years. In the low responders group, the OR was 2.01 (1.26- 3.20). At the time of menarche, 12% of the reference group versus 20.9% of women receiving a donated embryo and 19% of the low responders were aged less than 11 years.

The results of the logistic regression analyses of the main determinants and other variables in the final logistic regression model are listed in Table III.
Table III. Results of the logistic regression analysis examining the effect of birth weight and age at menarche between the patient groups and the reference group being tubal obstruction. Confounders which reached the significance level of $P = 0.2$ are included in the model.

<table>
<thead>
<tr>
<th>Study group</th>
<th>PCOS OR (95% CI)</th>
<th>PCOS P</th>
<th>Oocyte donation OR (95% CI)</th>
<th>Oocyte donation P</th>
<th>Low responders OR (95% CI)</th>
<th>Low responders P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean birth weight</td>
<td>0.91 (0.71-1.17)</td>
<td>0.46</td>
<td>1.02 (0.67-1.55)</td>
<td>0.92 (NS)</td>
<td>0.93 (0.73-1.19)</td>
<td>0.57 (NS)</td>
</tr>
<tr>
<td>LBW$^1$</td>
<td>0.88 (0.53-1.48)</td>
<td>0.64</td>
<td>0.75 (0.31-1.85)</td>
<td>0.54 (NS)</td>
<td>1.12 (0.70-1.80)</td>
<td>0.61 (NS)</td>
</tr>
<tr>
<td>VLBW$^2$</td>
<td>-a</td>
<td>(NS)</td>
<td>1.15 (0.14-9.66)</td>
<td>0.54 (NS)</td>
<td>2.01 (1.26-3.20)</td>
<td>0.004 (NS)</td>
</tr>
<tr>
<td>Menarche$^3$&lt;11 years</td>
<td>0.99 (0.59-1.70)</td>
<td>0.99</td>
<td>2.67 (1.35-5.29)</td>
<td>0.005</td>
<td>2.01 (1.26-3.20)</td>
<td>0.004 (NS)</td>
</tr>
<tr>
<td>12-14 years</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&gt;15 years</td>
<td>3.31 (2.18-5.04)</td>
<td>&lt;0.0001</td>
<td>1.65 (0.75-3.61)</td>
<td>0.21 (NS)</td>
<td>0.87 (0.50-1.50)</td>
<td>0.61 (NS)</td>
</tr>
<tr>
<td>Education$^4$</td>
<td>-b</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Low</td>
<td>-b</td>
<td>0.43 (0.23-0.80)</td>
<td>0.008</td>
<td>0.69 (0.49-1.04)</td>
<td>0.08 (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.38 (0.17-0.89)</td>
<td>0.025</td>
<td>0.53 (0.33-0.88)</td>
<td>0.01 (NS)</td>
<td></td>
</tr>
<tr>
<td>Mean age$^5$</td>
<td>0.90 (0.86-0.94)</td>
<td>&lt;0.0001</td>
<td>1.05 (0.99-1.13)</td>
<td>0.08 (NS)</td>
<td>1.22 (1.16-1.29)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>1.10 (1.05-1.13)</td>
<td>&lt;0.0001</td>
<td>-b</td>
<td>1.05 (1.01-1.10)</td>
<td>0.06 (NS)</td>
<td>0.06 (NS)</td>
</tr>
<tr>
<td>Country of birth$^6$</td>
<td>0.47 (0.13-1.63)</td>
<td>0.23</td>
<td>2.34 (0.81-6.69)</td>
<td>0.12 (NS)</td>
<td>-b</td>
<td>-b</td>
</tr>
<tr>
<td>Birth order$^7$</td>
<td>-b</td>
<td>1.52 (0.85-2.72)</td>
<td>0.15 (NS)</td>
<td>-b</td>
<td>-b</td>
<td>-b</td>
</tr>
</tbody>
</table>

$^1$Low birth weight (LBW) = <2500 g; reference category birth weight >2500 g. $^2$Very low birth weight (VLBW) = <1500 g; reference category birth weight >1500 g. $^3$12-14 years reference category. $^4$Low education level is reference category. $^5$For low responders, mean age at first IVF treatment; for all others, mean age at first visit fertility clinic. $^6$Europe and North America as reference category. $^7$First child versus not first child as reference category. *In the PCOS group no one had a birth weight lower than 1500 g. *These variables did not reach the significance level of $P = 0.2$ and were therefore not included in the final model.
Discussion

In a subfertile population seeking fertility treatment, birth weight was not related to the risk of PCOS or to diminishing ovarian reserve. An older age at menarche, however, was related to a greater risk of PCOS, whereas a young age at menarche was significantly associated with strong indicators of a diminished ovarian reserve.

The present finding that birth weight was unrelated to PCOS seems to be at variance with previously published findings. However, studies reporting a positive association between low birth weight and PCOS were conducted in a specific group of young girls who had not only a low birth weight but also premature pubarche and hyperandrogenism (3). It is very likely that this may be a highly selected subgroup of patients with PCOS features, which is quite different from the majority of PCOS patients in a subfertile population. The association with birth weight might be restricted to this specific subpopulation. In contrast, given the retrospective nature of the present study and the international disagreement on the definition of PCOS, patient selection herein might have been too heterogeneous to draw conclusions as to the effect of birth weight on PCOS.

In the present study population, diminished ovarian reserve was not related to birth weight, and this was not in accordance with the present authors' previous findings. In a twin study, a positive association was found between a high birth weight and a premature menopause before the age of 40 years (16). In that study, twin sisters with premature menopause were significantly heavier compared to their non-affected twin sisters. the mean difference in birth weight being 307 g (P = 0.05).

Some limitations of the current analysis should be considered. One potential source of bias was self-reported birth weights. Many women in the Netherlands choose to deliver at home with the aid of qualified midwives; hence, there is a high level of dependency on self-reported birth weights. International studies have shown that self-reported birth weights in women aged less than 40 years correlate highly with birth-certificate values, with coefficients between 0.75 and 0.83 (23, 24). The present authors' experience with a similar study population is that among 140 interviewed women in the age range of 25-55 years, 85% reported the correct birth weight (data unpublished). Thus, reported birth weights can be considered as fairly accurate. Moreover, it can be assumed that there is no
difference in recollection between the reference group and the case groups as they were all selected from the same cohort of women who visited a clinic with fertility-related problems.

Another limitation of the present study was that no information was available on gestational age, which is another potential confounding variable. Infants born prematurely often have lower birth weights compared with infants born at term, although this low birth weight could be appropriate for the given gestational age. On the other hand, infants with a birth weight in the normal range might be small for their gestational age.

A strong association was found between a diagnosis of PCOS and an experience of first menstruation after the age of 14 years. Although premature pubarche has been shown to be related to PCOS, the effect of age at menarche remains unclear (2-4, 25). The present findings may, however, have been affected by recall bias. Very irregular menstruation with a long time span between two successive menstrual cycles in adolescence might affect the precise recollection of first menstruation. However, given the impact of the first menstruation in a young girl's life, it can be assumed that the effect of recall bias is minimal, particularly for women with a very early or very late age at menarche. As very few data on age at menarche were missing (1.0%), there was not expected to be any significant effect of missing data on age at menarche. Extreme cases of oligomenorrhea, however, might present themselves as primary amenorrhea or late-onset menarche with an otherwise completed sexual development (26).

It was also shown that the receipt of an embryo conceived by donated oocytes, as well as having a low response to ovarian stimulation, were significantly linked to a younger age at menarche. The association between age at menarche and age at menopause, the final stage of ovarian depletion, remains controversial. A young age at menarche, as well as an older age, have been reported as risk factors for early menopause (5-9). However, in many cases investigators found no association between these two fertility parameters (10-13, 27). The present data suggest that women with a menarchal age of less than 12 years are 2-fold more likely to respond inadequately to stimulation, and 2.6-fold more likely to have subfertility problems requiring an oocyte donation. The discrepancy between the present findings and those of previous reports might be due to the chosen definition of ovarian
depletion. Age at menopause may be an inaccurate estimator of depletion of ovarian reserve. Age at menopause is a retrospective diagnosis, whilst menopausal transition is a gradual process that takes 10-15 years. Hence, pinpointing a date can be extremely difficult, especially when it is also taken into account that many women use oral contraceptives or hormone replacement therapy during this period of life. A low response to stimulation in two successive IVF cycles as well as a lack of any response, however, are considered direct estimates of diminished ovarian reserve (28). The prospective follow-up of the OMEGA study population has shown that, indeed, women with a low response to ovarian hyperstimulation are more likely to become post-menopausal at a younger age (29), and this finding has been supported by other recent studies (30).

Assuming that inadequate responses to hyperstimulation and menopause have the same underlying mechanisms, the present findings would indicate that an ‘early start’ leads to an ‘early end’.

The present data indicate that a low education level is significantly associated with fertility treatment due to a diminished ovarian reserve. This is in accordance with previously published findings, where a low educational level was seen to be frequently correlated with subfertility (31, 32).

In summary, the results of this large analysis suggest that there is no clear close relationship between birth weight and the fertility disorders PCOS and limited ovarian reserve. Possible minor effects, if present, may have been obscured due to the retrospective nature of the study. However, in order to reveal subtle effects of birth weight on fertility, then additional accurate prospective studies are required.
Acknowledgements

The authors thank the participants of the OMEGA Project. They also thank the medical registries of the participating clinics for making patient selection possible, and all attending physicians for providing access to their patients' medical files. The OMEGA Project Group includes: M.Kortman and E.R.te Velde (University Medical Center, Utrecht); N.Macklon (Academic Hospital Dijkzigt, Rotterdam); C.A.M.Jansen (Diaconessenhuis, Voorburg); R.A.Leerentveld (Isala Clinics, Zwolle); W.N.P.Willemsen (Academic Hospital Nijmegen, St Radboud); R.Schats (Academic Hospital Free University, Amsterdam); N.Naaktgeboren and F.M.Helmerhorst (Leiden University Medical Center); R.S.G.M.Bots (St Elisabeth Hospital, Tilburg); A.H.M.Simons (Academic Hospital, Groningen); H.V.Hogerzeil (Academic Medical Center, Amsterdam); J.L.H.Evers (Academic Hospital, Maastricht); and P.A.van Dop (Catharina Hospital, Eindhoven).
References

Chapter 9

Developmental origins of PCOS, a case control study comparing birth weight in women with PCOS and controls.

Sadrzadeh S, Painter RC, Lambalk CB.

Abstract:
Evidence from various epidemiological studies and experimental animal studies has linked adverse intrauterine circumstances with health problems in adult life. This field of investigation is known as Developmental Origins of Health and Disease (DOHaD). Both low birth weight as well as a relatively high weight at birth has been associated with polycystic ovary syndrome (PCOS). In this retrospective case-control study, we evaluated whether women diagnosed with PCOS had lower birth weight compared to women with a regular menstrual cycle (controls). Binary logistic regression models were used to analyze the data and correct for known confounders. 65 women with PCOS and 96 controls were recruited for this purpose. The average birth weight of PCOS women (3357 g) did not differ from the average birth weight of controls (3409g). PCOS women had a significantly older age at menarche compared to controls, 13.7 and 12.8 P=.006 respectively. In conclusion, we could not confirm the effect of adverse intrauterine conditions, reflected in birth weight, on developing PCOS.
Introduction

Evidence from epidemiological studies and experimental animal studies has linked adverse intrauterine circumstances with health problems in adult life, recognized as Developmental Origins of Health and Disease (DOHaD) [1]. From an evolutionary biological point of view, a fetus growing in unfavorable intrauterine circumstances is known to permanently alter its’ organ growth and function possibly to adapt to its future environment [2]. A fetus prepared for the future by a lean intrauterine environment due to maternal or temporary environmental factors is ill fit to cope with our abundant lifestyle [3;4]. Intrauterine growth restriction is known to affect organ structure and average organ weight in animal and human offspring [5;6;7;8;9;10].

Polycystic ovary syndrome (PCOS) is a common fertility related disorder associated with high levels of luteinizing hormone (LH) and subfertility with an estimated prevalence of up to 20% depending on the diagnostic criteria [11]. PCOS is often associated with metabolic disorders including insulin resistance, obesity and cardiovascular disease [12]. Insulin resistance and cardiovascular disease are also among the well documented sequelae of intrauterine growth restriction [13]. Animal studies suggest that elevated prenatal testosterone levels during critical periods of gestation can cause a PCOS like phenotype as well as intrauterine growth restriction mediated through impaired placental function [14]. PCOS and insulin resistance have also been suggested to find their joint origin in fetal growth restriction mediated by excessive serine phosphorylation [15].

A Brazilian prospective study indicates that the prevalence of PCOS among women born small for gestational age (SGA) is twice as high as women born appropriate for gestational age (AGA) [16]. In adolescent girls with a history of premature pubarche, impaired fetal growth has also been reported as a factor in the etiology of polycystic ovary syndrome [17]. On the other hand, in a large Danish study, high birth weight was significantly associated with PCOS in adult life [18]. In addition, a lower ponderal index and high birth weight have each been associated with an increase in PCOS related symptoms [19].

There are few studies investigating the relation between birth weight and fertility parameters taking impaired fertility as a starting point. In this retrospective case control
study, we investigate if women with manifested impaired fertility due to PCOS indeed had a lower weight at birth compared to women showing no symptoms of PCOS.

**Patients and Methods**

**Study population**

Subjects were patients from the department of Obstetrics and Gynecology VU Medical Centre in Amsterdam. All patients were clinically diagnosed with PCOS, according to the Rotterdam criteria [20] based on the presence of at least two of the following clinical features: oligo/amenorrhea, polycystic ovarian morphology and hyperandrogenism. Controls were recruited via advertising and from medical files of the department of Obstetrics and Gynecology of VU Medical Centre. All controls had self-reported spontaneous regular menstrual cycles and no history of sub/infertility. Patients and controls meeting any of the following criteria were excluded: history of ovarian disease or operation, abdominal radiation- or chemotherapy or diagnosed with genetic abnormalities.

The ethical commission of the institute approved the study. Patients and controls received a letter informing them about the aim of the study and signed a consent form.

**Study design**

We used a retrospective case-control study design. Participants completed a written questionnaire addressing fertility and health issues. They were also asked to confirm their birth weight with a written record or if their birth mother was still alive to confirm the birth weight. Gestational age was reported as weeks or months of gestation. Education level was reported as: low (primary school or low vocational education), middle (high school or middle vocational education) or high (higher vocational education or academic education). Smoking was categorized as current smoker, former smoker or never smoked. Participants provided information on current height and weight, history of contraceptive use, hormone replacement therapy, history of gynecological treatment, age at menarche and age at menopause. We defined pre-term birth as birth before 37 weeks of gestation,
and term birth as 38, 39, 40 weeks of gestation and post-term as above 41 weeks of gestation.

Statistics
Data available from other studies relating birth parameters to adverse fertility outcome were used as a guideline to estimate an appropriate sample size [21]. To demonstrate a clinically relevant difference of 200 g in birth weight using an independent Student t-test, the minimal sample size for each group was calculated to be 60 with alpha 0.05 and power 0.8.

IBM SPSS Statistics 21 was used for all statistical analyses. An independent Student t-test was used when data were continuously and normally distributed. Continuous data are presented as mean and 95% confidence interval and significance level (P value) is presented. Non-parametric data were tested with a chi² test. Binary logistic regression models were computed adjusting for categorical as well as continues independent variables.

Results
The study group consisted of 65 women diagnosed with PCOS according to the Rotterdam criteria. 96 women with regular menstrual cycles were included in the control group.
Baseline characteristics and study parameters of continuous data are summarized in Table I, categorical data are presented in Table II.
Birth weight, gestational age, premature or post-term delivery did not differ significantly between women with PCOS and women with a regular menstrual cycle. To investigate a possible U shaped or threshold effect, we also analyzed birth weight in categories. We found no evidence for a difference in distribution between the birth weight categories according to PCOS diagnosis.
Table I. Comparison of continuous variables of women diagnosed with polycystic ovarian syndrome (PCOS) and control group. An independent Student t-test was used for continuous data with a normal distribution

<table>
<thead>
<tr>
<th></th>
<th>Control Mean (n=96)</th>
<th>PCOS Mean (n=65)</th>
<th>P value</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age interview, years (n)</td>
<td>45.2 (96)</td>
<td>39.8 (65)</td>
<td>.000</td>
<td>3.67</td>
<td>7.05</td>
</tr>
<tr>
<td>BMI, kg/m² * (n)</td>
<td>24.5 (92)</td>
<td>26.4 (63)</td>
<td>.021</td>
<td>-3.55</td>
<td>-.297</td>
</tr>
<tr>
<td>Age at menarche, years (n)</td>
<td>12.8 (94)</td>
<td>13.7 (62)</td>
<td>.006</td>
<td>-1.57</td>
<td>-.265</td>
</tr>
<tr>
<td>Birth weight g (n)</td>
<td>3409 (96)</td>
<td>3357 (65)</td>
<td>.618</td>
<td>-154</td>
<td>253</td>
</tr>
<tr>
<td>Gestational age wk (n)</td>
<td>40 (64)</td>
<td>39 (21)</td>
<td>.337</td>
<td>-1.542</td>
<td>1.56</td>
</tr>
</tbody>
</table>

*BMI, body mass index defined as weight (kg)/height (m)²

Age at the time of investigation, age at menarche, BMI and education level however differed significantly between groups. Women diagnosed with PCOS were significantly younger, had an older age at menarche, a higher BMI and lower education levels compared to the control group. Using binary logistic models, we investigated whether the fact that we failed to find an association between birth weight and PCOS in our dataset was due to these differences in baseline characteristics. Confounders were added in a step forward manner. Odds ratio, 95% confidence interval and P value of the final stage of the models are presented in Table III. None of the analyses revealed any associations between early life determinants, including birth weight and gestational age at birth, after adjusting for confounders. A higher BMI and a later age of menarche, remained positively associated with PCOS (data not shown).
Table II. Comparison of categorical variables of women diagnosed with polycystic ovarian syndrome (PCOS) and control group. chi² test was used for categorical data.

<table>
<thead>
<tr>
<th></th>
<th>Control Mean (n=96)</th>
<th>PCOS Mean (n=65)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight categories</td>
<td>n=96</td>
<td>n=65</td>
<td></td>
</tr>
<tr>
<td>&lt;1.5kg n (%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>-</td>
</tr>
<tr>
<td>&lt;2kg n (%)</td>
<td>5 (5.2%)</td>
<td>1 (1.5%)</td>
<td>.403</td>
</tr>
<tr>
<td>&lt;2.5kg n (%)</td>
<td>11 (11.5%)</td>
<td>8 (12.3%)</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt;4kg n (%)</td>
<td>20 (20.8%)</td>
<td>9 (13.8%)</td>
<td>.301</td>
</tr>
<tr>
<td>Term*</td>
<td>n=95</td>
<td>n=62</td>
<td></td>
</tr>
<tr>
<td>Preterm n (%)</td>
<td>8 (8.4%)</td>
<td>6 (9.7%)</td>
<td>.426</td>
</tr>
<tr>
<td>Term n (%)</td>
<td>67 (70.5%)</td>
<td>48 (77.4%)</td>
<td></td>
</tr>
<tr>
<td>Post-term n (%)</td>
<td>20 (21.1%)</td>
<td>8 (12.9%)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>n=96</td>
<td>n=65</td>
<td></td>
</tr>
<tr>
<td>Former or current n(%)</td>
<td>56 (58.3%)</td>
<td>42 (64.6%)</td>
<td>.511</td>
</tr>
<tr>
<td>Never n (%)</td>
<td>40 (41.7%)</td>
<td>23 (35.4%)</td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td>n=92</td>
<td>n=65</td>
<td></td>
</tr>
<tr>
<td>Low (%)</td>
<td>35 (38%)</td>
<td>38 (58.5%)</td>
<td></td>
</tr>
<tr>
<td>Middle (%)</td>
<td>11 (12%)</td>
<td>11 (16.9%)</td>
<td>.006</td>
</tr>
<tr>
<td>High (%)</td>
<td>46 (50%)</td>
<td>16 (24.6%)</td>
<td></td>
</tr>
</tbody>
</table>

* Pre-term birth is defined as birth before 37 weeks of gestation, term birth as 38, 39, 40 weeks of gestation and post-term as above 41 weeks of gestation.
Table III. Results of binary logistic models: comparing women diagnosed with PCOS to controls regarding difference in birth weight corrected for various confounders

<table>
<thead>
<tr>
<th>PCOS vs Control</th>
<th>Odds ratio</th>
<th>95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)*</td>
<td>1.0</td>
<td>.999-1.00</td>
<td>.78</td>
</tr>
<tr>
<td>Categorical birth weight( &gt;2kg/&lt;2kg)*</td>
<td>.514</td>
<td>.046-5.77</td>
<td>.59</td>
</tr>
<tr>
<td>Categorical birth weight ( &gt;2.5kg/&lt;2.5kg)*</td>
<td>1.12</td>
<td>.286-4.41</td>
<td>.87</td>
</tr>
<tr>
<td>Categorical birth weight(&lt;4kg/&gt;4kg)*</td>
<td>1.24</td>
<td>.373-4.10</td>
<td>.73</td>
</tr>
</tbody>
</table>

*Corrected for (stepwise): gestation age categories, age at menarche, BMI, smoking categories, education categories, age at time of interview.

**Discussion**

We found no relation between PCOS and a low birth weight or any other perinatal determinant.

Literature on this subject is inconsistent. Some studies report an association between a low birth weight and PCOS [17;16]. However, these studies seem to have described a specific phenotype of women with small for gestational age (SGA), an early age at puberty, hyperandrogenism and PCOS. Furthermore, impaired catch up growth, small adult stature and a low adult BMI were hallmarks of the phenotype described by Ibanez et al in contrast to the majority of women with PCOS described in other studies, who have an average height with a higher BMI and a later age at menarche [22]. There might therefore be a specific group of women in which being SGA is the offset or the first manifestation of endocrine dysfunction or that in these cases SGA is an added symptom in a more complex disease system. In other studies, low birth weight was not associated with PCOS [21;18;23]. A large Danish study, by Mumm et al, found that women whose birth weight was in the highest percentiles were more likely to develop PCOS, as were women born to mothers with gestational diabetes. As gestational diabetes and macrosomic offspring are more common in women with PCOS while PCOS also has a high heritability these findings more likely indicates a genetic common pathway [24;25]. It seems that low and high birth
weight per se, as parameters for possible intrauterine nutritional disorders are not relevant for PCOS.

Investigations in the field of DOHaD shift from size at birth to focusing on the timing of malnutrition during pregnancy. An increasing body of evidence from experimental animal studies indicates that the timing of malnutrition during and even before gestation has a profound effect on adult health while not resulting in a low birth weight [26;27;28]. The Dutch hunger winter presented an exceptional situation in history to study the effect of malnutrition in different gestational periods [29]. Adults affected by famine during their early gestation had a higher incidence of coronary heart disease and a higher BMI. Their birth weight however, did not differ significantly [30;31]. The highest peak of oocyte production as well as oocyte apoptosis in humans is in early, up to mid gestation. One, therefore, would not expect adverse environmental influences late in gestation, affecting birth weight, negatively influencing the oocyte reserve. On the other hand, in humans temporary placental dysfunction or temporary food restriction besides hyperemesis gravidarum is extremely rare.

Primate animal models prenatally exposed to exogenous androgen develop a PCOS-like reproductive and metabolic phenotype in adulthood. Primate animal models, unlike rodent and sheep animal models, show no signs of intrauterine growth restriction [32]. To our knowledge there are no studies experimenting with maternal nutritional or placental function during different periods of gestation in PCOS animal models to investigate the effect of intrauterine growth restriction in PCOS predisposed animals on PCOS severity or birth weight.

Obviously, our study also has a few shortcomings. First of all the retrospective design of our study presents a potential recall bias. The possibility of recall bias cannot be excluded, but we see no reason why women with PCOS would be more or less likely to be in possession of a written document confirming their birth weight. The fact that all information, other than PCOS diagnosis in the patient group, is self-reported can also introduce an additional information bias. Another issue is that the control group was significantly older than the patients were. Although there is no evidence that the prevalence of PCOS changed over the years [11],
we eliminated any potential confounding effects by adding age to the binary logistic models.

The PCOS patients in our study had a significantly lower education level compared to the control group. Even though we did not find any correlation between PCOS and education level in the literature, this might explain the difference in BMI between groups. A lower social economic level is indeed related to a higher BMI [4]. Though, when correcting BMI for education level, BMI remained significantly related to PCOS.

In conclusion, our study findings were not consistent with the hypothesis that intrauterine growth is a determinant of PCOS. Meanwhile other studies point in the direction of adverse environmental conditions acting only in specific developmental windows during gestation and negative lifestyle effects during infancy and puberty. Animal studies focusing on environmental effects during different stages of gestation, the effect of hyperemesis gravidarum and lifestyle intervention during infancy and puberty should shed more light on these issues while.

**Acknowledgments**

Authors would like to thank all the participants who volunteered for this study project. We also thank Ted Korsen, research nurse, who helped recruiting participants and setting up the database.
References

Chapter 10

Birth weight and the development of PCOS: a systematic review and meta-analysis.


Human Reproduction Open, Volume 2017, Issue 2, 12 July 2017, hox010,
Abstract

Study question: Are intrauterine conditions, reflected in birth weight, associated with the development of polycystic ovary syndrome (PCOS)?

Summary answer: We could not confirm the association between adverse intrauterine conditions, reflected in birth weight and PCOS.

What is known already: The etiology of PCOS is still largely unknown. Besides subfertility women diagnosed with PCOS have an increased risk of chronic health issues. PCOS has been linked to adverse pre-natal conditions.

Study design: A systematic search and meta-analysis on pooled data was undertaken, according to PRISMA and MOOSE guidelines.

Material & methods: The following online databases were systematically searched: PubMed, EMBASE, CINAHL(via EBSCO) and Cochrane library with no language or date restrictions up to 1 October 2016.

Main results: A total of 1354 unique studies were identified, 15 studies were included, 13 of which provided data for meta-analysis. The exposure variable birth weight was either analyzed as a categorical variable with birth weight categories being <2.5kg, 2.5-4kg and >4 kg, or as a continuous variable. We composed a birth weight category consisting of birth weights <2.5 kg plus birth weights >4 kg, reflecting extreme birth weights. We compared this category to birth weights between 2.5kg and 4kg. For the latter analysis we were able include 528521 women (I²=60%), women born with extreme birth weights had an odds ratio [95% CI] of 1.21 [0.92,1.59] for PCOS compared to women born with an average birth weight.

Wider implications of the findings: Birth weight as a summary measure of intrauterine environment is not a considerable factor in the etiology of PCOS.
**Introduction:**

With a documented prevalence of up to 20% depending on diagnostic criteria, Polycystic ovary syndrome (PCOS) is one of the most common causes for reduced fertility in women (1). The diagnostic criteria for PCOS have been subject to change over the last decades. The NIH criteria published in 1990, require the presence of hyperandrogenism and menstrual dysfunction to diagnose PCOS (2). The Rotterdam criteria first published in 2003 require 2 out of 3 of the following features; oligo-anovulation, hyperandrogenism and polycystic ovaries (PCO) by ultrasound (3). In 2006 the Androgen Excess Society published new diagnostic criteria which required the presence of clinical or biochemical hyperandrogenism in the presence of either PCO or menstrual dysfunction to diagnose PCOS (4). The majority of women diagnosed with PCOS, present with fertility issues. However an increased risk of obesity, insulin resistance, cardiovascular disease and overall mortality are also highly associated with PCOS (5)(6)(7).

Whilst the etiology of PCOS remains largely unclear, the clinical manifestations of PCOS often present themselves during childhood or puberty suggesting early life or prenatal influences. A “two –hit” hypothesis has been suggested were two insults during specific developmental windows are required for the development of PCOS. The first hit being prenatal or early childhood influences such as hyperandrogenism due to genetic or environmental factors (8, 9). Animal studies in non-human primates indicate that elevated prenatal testosterone levels during critical periods of gestation can cause a PCOS like phenotype, as well as intrauterine growth restriction (10) (11).

In the last decades, epidemiological and animal studies have also provided compelling evidence for the association between adverse perinatal conditions, often using birth weight as a maker, and an increased risk of cardiovascular disease and insulin resistance (12).

As PCOS and adverse perinatal conditions both are established risk factors for insulin resistance and cardiovascular disease the question remains if these two risk factors are related. With adverse perinatal conditions being the starting point of the pathogenesis of PCOS followed by metabolic syndrome or an additional risk factor for developing PCOS as well as metabolic syndrome.
This review seeks to establish, through systematic examination of the available literature and meta-analysis of pooled data, the association of intrauterine conditions reflected in birth weight with the development of polycystic ovary syndrome.

**Material and methods:**

Search strategies:

This review was conducted according to PRISMA and MOOSE guidelines. The protocol of this study is registered at PROSPERO under registration number CRD42016048972. A comprehensive search was performed in the bibliographic databases PubMed, EMBASE.com, CINAHL (via EBSCO) and Cochrane library (via Wiley) with no language or date restrictions from inception to 1 October 2016. Additionally the reference lists of relevant studies, review articles and opinion papers were checked by snowball search to identify any secondary references. Search terms included controlled terms (MeSH in Pubmed, EMtree in Embase.com), as well as free text terms. We used free text terms only in the Cochrane Library. The following terms were used (including synonyms and closely related words) as index terms or free-text words for ‘birth weight’ or ‘gestational age’ and the outcome variable ‘Polycystic ovary syndrome’ with aid of a clinical librarian (LJS). Detailed information on the search strategy is listed in appendix 1.

Study selection and eligibility:

After identifying and excluding duplicate studies, two independent reviewers (SS and EVHH) evaluated titles and abstracts for suitability using a customized in-exclusion chart. Any disagreement between reviewers was resolved through consensus if consensus could not be reached the article was added to the full-text selection. Studies were included if they were (i) cross sectional, case control, cohort or intervention studies (ii) were conducted in adult humans (iii) reported cases with PCOS diagnosis and diagnostic criteria for PCOS (iv) described a control group of women not diagnosed with PCOS, self-reported or after medical examination (v) reported birth weight and/or gestational age, self-reported or extracted from medical files (vi) the reported birth parameters were related to women who were diagnosed with PCOS. Full texts were retrieved for studies that satisfied all selection criteria and independently reviewed by two authors (SS and EVHH)
for eligibility using the same in-exclusion chart.

Quality assessment and data extraction:
Selected studies were assessed independently for methodological validity by 2 reviewers using the Newcastle-Ottawa scale with a maximum of 9 stars (13). Any disagreement between reviewers was resolved through consensus if consensus could not be reached a third reviewer (CBL) was consulted.
A piloted data extraction form was used to extract relevant information. The form included questions on publication year, study design, baseline population, number of participants, definition of outcome variable, type of exposure and reported risk estimates. In case of multiple publications on identical populations, only the most recent publication was included.

Data synthesis and analysis:
After data extraction and methodological quality assessment data were transferred to Review Manager (RevMan 5.3) for analysis. We performed meta-analysis of pooled data when data were sufficiently homogeneous. We assessed heterogeneity by eyeball test and the I^2 statistic, the later was distinguished as high heterogeneity, ergo not fit to be pooled, when I^2 ≥ 75-100% (14). We reported the results of our meta-analysis using the random effect model as we still expected some heterogeneity in our data due to the difference in diagnostic criteria for PCOS between studies.
The exposure variable birth weights reported in pounds were converted into kilograms and was either analyzed as a categorical or a continuous variable. Four comparisons were examined in meta-analyses: 1) birth weight was dichotomized at 2,5 kg to compare the risk of developing PCOS above and below this value. 2) similarly, birth weights were dichotomized to compare those above and below 4 kg. 3) To investigate a possible non-linear U shaped relation between birth weight and PCOS, we composed a birth weight category (birth weight extremes) consisting of birth weights less than 2,5 kg plus birth weights above 4 kg comparing it to birth weights between 2,5kg and 4kg (normal birth weight). 4) Birth weight was also analyzed as a continuous variable, comparing mean birth
weights of women diagnosed with PCOS and controls. Publication bias was evaluated through a funnel plot, plotting log odds ratio against odds ratio. To investigate bias through study design a subset analysis was performed differentiating between cohort studies and case control studies.

Results
Identification of relevant studies:
The search strategy identified 1349 unique citations and 5 additional studies were included through the snowball search. Screening on title and abstract resulted in 30 eligible studies. Full text articles of these studies were retrieved for further evaluation. 15 studies were included in the final selection, 13 of which provided data for statistical analysis of pooled data. The selection process is visualized in Fig 1 and characteristics of the final 15 articles are listed in table 1.

Characteristics of included studies
Eight of the final 15 studies were case control studies, 6 cohort studies and one cross-sectional study. De Melo (15), described their study design as a prospective cohort, while the original design was indeed a prospective birth cohort, the data selection for this article met the criteria of a nested case-control study. Characteristics of the primary study populations are listed in table 1. We could not extract data from Michelmore (16) and Davies (17). Michelmore et al. reported on women with PCO subsequently and categorized them according to severity of additional PCOS symptoms in a previous publication (18). We were not able to directly link the reported birth weights to any given PCOS category. Davies et al categorized women according to NIH as well as Rotterdam criteria an reported relative risks. We could however not to extract data for pooled analysis.
Fig 1. Flow chart of study selection process.

Identification

- Records identified through database searching (n = 2467)
- Additional records identified through snowball search (n = 5)

Records after duplicates removed (n = 1354)

Screening

- Records screened (n = 1354)
- Records excluded (n = 1324)

Eligibility

- Full-text articles assessed for eligibility (n = 30)
  - Full-text articles excluded, (n = 15)
    - No PCOS = 4
    - Age participants < 18 = 4
    - Article type = 3
    - Posters of already included studies = 2
    - No birth weight = 1
    - No control group = 1

- Studies included in qualitative synthesis (n = 15)

Included

- Studies included in quantitative synthesis (meta-analysis) (n = 13)
<table>
<thead>
<tr>
<th>Lead author, publication date, country</th>
<th>Study type</th>
<th>PCOS definition</th>
<th>Birth weight source</th>
<th>Primary Study population</th>
<th>Total nr</th>
<th>Quality max.</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minooee, 2016, Iran</td>
<td>Case-control</td>
<td>Rotterdam criteria</td>
<td>Unknown</td>
<td>PCOS out patients and hospital controls</td>
<td>140</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Seddighi, 2016, Netherlands</td>
<td>Case-control</td>
<td>Rotterdam criteria</td>
<td>Self-reported</td>
<td>PCOS out patients and hospital controls or advertisement</td>
<td>161</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Paschou, 2015, Greece</td>
<td>Case-control</td>
<td>NIH criteria</td>
<td>Self-reported</td>
<td>PCOS out patients, students or hospital staff as controls</td>
<td>344</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Minooee, 2015, Iran</td>
<td>Case-control</td>
<td>Rotterdam criteria</td>
<td>Unknown</td>
<td>PCOS out patients and hospital controls</td>
<td>140</td>
<td>5</td>
<td>Study in original language</td>
</tr>
<tr>
<td>Legro, 2010, USA</td>
<td>Case-control</td>
<td>NIH criteria</td>
<td>Self-reported</td>
<td>All women born SGA between 1-6-1976 and 31-5-1979 in Ribeirao Preto and random selected controls (Out of 3) out of the same cohort</td>
<td>553</td>
<td>3</td>
<td>Part of a larger study</td>
</tr>
<tr>
<td>De Melo, 2010, Brazil</td>
<td>Nested case-control</td>
<td>Rotterdam criteria</td>
<td>Medical files</td>
<td>Randomly recruited women born with a birth weight &lt;5000 from regional records and next full term singleton as control</td>
<td>165</td>
<td>8</td>
<td>Authors describe study as a cohort study but design for this particular manuscript meets the criteria of a nested case control study</td>
</tr>
<tr>
<td>Pandolfi, 2008, Italy</td>
<td>Case-control</td>
<td>NIH criteria</td>
<td>Medical files</td>
<td>Women diagnosed with PCOS and controls from hospital records or advertisement</td>
<td>70</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Sverrissson, 2008, Sweden</td>
<td>Case-control</td>
<td>Rotterdam criteria</td>
<td>Birth register</td>
<td>Women diagnosed with PCOS and controls from hospital records or advertisement</td>
<td>38</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Munn, 2013, Denmark</td>
<td>Cohort</td>
<td>Discharges diagnostic code PCOS, excluding other related diagnosis with similar symptoms</td>
<td>Birth register</td>
<td>Linkage individual data from national register with national patient register. Women born between 1973-1991</td>
<td>521757</td>
<td>7</td>
<td>The prevalence of PCOS in this study is 0.6%</td>
</tr>
<tr>
<td>Davies, 2012, Australia</td>
<td>Cohort</td>
<td>Rotterdam criteria and NIH criteria both listed</td>
<td>Hospital records</td>
<td>Female births between 1973-1975 at one hospital</td>
<td>948</td>
<td>6</td>
<td>Unable to extract data for meta-analysis</td>
</tr>
<tr>
<td>Ibanez, 2008, Spain</td>
<td>Cohort</td>
<td>Rotterdam criteria</td>
<td>Medical records</td>
<td>Cohort of women seen between 2005-2006 all clinically diagnosed with hyperandrogenism</td>
<td>85</td>
<td>5</td>
<td>Data only includes in the meta-analysis with birth weight as a continuous variable</td>
</tr>
<tr>
<td>Laakso, 2009, Finland</td>
<td>Cohort</td>
<td>Rotterdam criteria</td>
<td>Birth register</td>
<td>1964 birth cohort</td>
<td>2007</td>
<td>6</td>
<td>Self-reported signs of hyperandrogenism and oligomenorrhea</td>
</tr>
<tr>
<td>Sadeghi, 2013, Netherlands</td>
<td>Cohort</td>
<td>Clinical PCOS diagnosis</td>
<td>Self-reported</td>
<td>Women receiving IVF between 1980-1995 in one of the 12 IVF centers in the Netherlands</td>
<td>911</td>
<td>7</td>
<td>Diagnostic criteria differ between centers</td>
</tr>
<tr>
<td>Cresswell, 1997, UK</td>
<td>Cohort</td>
<td>Polycystic ovary</td>
<td>Medical records</td>
<td>Birth cohort of women born between 1952 and 1953 at Jessop Hospital, Sheffield</td>
<td>235</td>
<td>8</td>
<td>Authors selection criteria was PCO but all women with PCO had clinically elevated testosterone levels, meeting Rotterdam criteria</td>
</tr>
</tbody>
</table>
Exposure variable birth weight:
The primary exposure variable was birth weight. In 8 out of 15 studies birth weights were extracted from birth registers or medical records, 5 studies listed self-reported birth weights and 2 studies did not provide information on how birth weights were obtained.

Outcome variable Polycystic ovary syndrome:
Studies used various criteria for the outcome variable PCOS. 7 studies defined PCOS according to the Rotterdam criteria, 3 according to NIH criteria, 1 study described cases according to both criteria, 2 studies had PCO as outcome and 2 studies used hospital discharge diagnosis which is a combination of different criteria. Even though Cresswell (19) selected PCO as their outcome variable, the article provided sufficient information on clinical features such as hyperandrogenism and oligomenorrhea to reallocate the PCO population as PCOS according to Rotterdam criteria. In all other cases, we used the author’s definition for PCOS. To prevent an overestimation of PCOS in the Danish population Mumm (20), used the discharges diagnostic code PCOS while excluding related other diagnosis with similar symptoms. This resulted in a remarkably low prevalence of PCOS; 0.6% in the total population.

Characteristics of the control populations:
Six case-control studies compared birth weights between PCOS patients (cases) and controls while 2 case-control studies investigated the prevalence of PCOS in women born SGA or with a low birth weight (cases) and women born AGA or with an average birth weight (controls).

Minooee (21) (22), Paschou (23), Legro (24) and Sverrisdottir (25) defined cases as PCOS patients and included controls using following criteria: (i) no polycystic ovaries on ultrasonography, performed by the investigators (ii) no signs of hirsutism, examined by a physician (iii) no ovulatory dysfunction, using information from standardized questionnaires. In addition Sverrisdottir et al. matched for age and weight while Legro et.al also evaluated hyperandrogenism through blood tests. Sadrzadeh (26) ruled out
ovulatory dysfunction by using information from standardized questionnaires on gynecological history.

The following 2 case-control studies defined cases based on a low birth weight. De Melo et al. selected every third women born AGA from their original cohort as controls. Pandolfi (27) selected the next full term singleton born after the selected low birth weight women (case) from medical records as controls.

Birth cohorts were used by following authors: Mumm et.al, Davies et.al, Laitinen (28) and Cresswell et.al. The cohort of Ibanez (29) consisted of women clinically diagnosed with hyperandrogenism between 2005-2006 in a single hospital and Sadrzadeh (30) described all women receiving IVF between 1980 and 1995 in Netherlands.

Statistical relation between a low birth weight and PCOS:
12 studies reported on birth weights lower than 2.5 kg in relation to the diagnosis PCOS. Heterogeneity of the data was assessed as moderate, $I^2=48\%$, and a total of 528,521 women were included in the pooled analysis. Pooled odds ratio [95%CI] for PCOS was 1.17 [0.89,1.53] among women who had a birth weight lower than 2.5kg compared to women with a birth weight above 2.5 kg (Fig 2).

Statistical relation between a high birth weight and PCOS:
To investigate the association between a higher than average birth weight and developing PCOS data from 7 studies were pooled. Women born with birth weights above 4 kg had an odds ratio [95% CI] of 1.15 [0.75, 1.76] for PCOS compared to women born with birth weights less than 4 kg after pooling data on 528521 women for meta-analysis($I^2=45\%$) (Fig 3).
Fig. 2 Odds ratio for PCOS; comparison between women born with low birth weights, less than 2.5 kg and women born with birth weights above 2.5 kg

*None of the participants had a birth weight less than 2.5 kg

Fig. 3 Odds ratio for PCOS; comparison between women born with birth weights above 4 kg and women born with birth weights less than 4 kg
U shaped relation of birth weight and PCOS:

To investigate a U shaped association between birth weight and PCOS we created a birth weight category of extreme birth weights, combining women born with a low birth weight less than 2.5 kg and women with high birth weights above 4 kg. When comparing this birth weight category with women born with an average birth weight, between 2.5 kg and 4 kg, we were able to include 528566 women from 12 studies ($I^2=60\%$) for meta-analysis. Women born with extreme birth weights had an odds ratio [95% CI] of 1.21 [0.92,1.59] for PCOS compared to women born with an average birth weight. The forest plot is visualized in figure 4 (Fig 4).

Fig.4 Odds ratio for PCOS among women with extreme birth weights, <2,5kg and >4kg, compared to women with birth weights between 2.5 kg and 4 kg.

<table>
<thead>
<tr>
<th>Total nr participants</th>
<th>weight</th>
<th>OR[CI 95%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legro et al. 2010</td>
<td>553</td>
<td>15.3%</td>
</tr>
<tr>
<td>Pandolfi et al. 2008</td>
<td>70</td>
<td>.9%</td>
</tr>
<tr>
<td>Paschou et al. 2015</td>
<td>344</td>
<td>12.7%</td>
</tr>
<tr>
<td>Melo et al. 2010</td>
<td>165</td>
<td>3.6%</td>
</tr>
<tr>
<td>Minooee et al. 2015</td>
<td>140</td>
<td>9.0%</td>
</tr>
<tr>
<td>Minooee et al. 2016</td>
<td>140</td>
<td>8.4%</td>
</tr>
<tr>
<td>Sadrzadeh et al. 2016</td>
<td>161</td>
<td>6.1%</td>
</tr>
<tr>
<td>Stracquadanio et al. 2017</td>
<td>373</td>
<td>10.5%</td>
</tr>
<tr>
<td>Sverristottir et al. 2008</td>
<td>38</td>
<td>0.9%</td>
</tr>
<tr>
<td>Mumm et al. 2013</td>
<td>523757</td>
<td>15.5%</td>
</tr>
<tr>
<td>Sadrzadeh et al. 2003</td>
<td>911</td>
<td>7.7%</td>
</tr>
<tr>
<td>Laitinen et al. 2003</td>
<td>2007</td>
<td>5.8%</td>
</tr>
<tr>
<td>Cresswell et al. 1997</td>
<td>235</td>
<td>1.38%</td>
</tr>
<tr>
<td>Total number</td>
<td>528892</td>
<td>100%</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>$I^2= 66%$</td>
<td></td>
</tr>
</tbody>
</table>
Birth weight analyzed as a continuous variable:
When comparing women diagnosed with PCOS with controls the mean difference [95%CI] in birth weight was calculated. Seven studies, reporting mean birth weights of 2628 women could be included for meta-analysis \(I^2=40\)% resulting in a 38.51 gram higher birth weight [-44, 121] among women diagnosed with PCOS.

Subset analysis:
To investigate bias through study design we conducted a subset analysis distinguishing between case-control and cohort studies testing the U shaped association between birth weight and consequential PCOS diagnosis. When data of the 8 case-control studies were pooled, we calculated an odds ratio [95%CI] of 1.57 [0.92,2.68] including 1611 women. The pooled odds ratio [95%CI] of the 4 cohort studies was 1.00 [0.91,1.09] (Fig 5).

Publication bias:
The funnel plot presented is from the analysis were most of the studies could be included, investigating a U shaped association between birth weight and PCOS (Fig 6). The skewed funnel plot indicates a publication bias. Studies reporting a positive association between birth weight and consequential PCOS diagnosis and large studies seem to be overrepresented, while smaller studies reporting negative or no association seem to be missing in published literature.
Fig. 5  Subset analysis differentiating between case-control and cohort studies: Odds ratio for PCOS among women with extreme birth weights, <2.5 kg and >4 kg, compared to women with birth weights between 2.5 kg and 4 kg.

### 5.1 Case-control studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Total nr participants</th>
<th>weight</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legro et al. 2010</td>
<td>553</td>
<td>16.7%</td>
<td>0.7 [0.4, 1.22]</td>
</tr>
<tr>
<td>Melo et al. 2010</td>
<td>165</td>
<td>13.8%</td>
<td>2.68 [1.17, 6.13]</td>
</tr>
<tr>
<td>Minooe et al. 2015</td>
<td>140</td>
<td>14.2%</td>
<td>3.03 [1.38, 6.66]</td>
</tr>
<tr>
<td>Minooe et al. 2016</td>
<td>140</td>
<td>15.2%</td>
<td>1.37 [0.68, 2.75]</td>
</tr>
<tr>
<td>Pandolfi et al. 2008</td>
<td>70</td>
<td>7.4%</td>
<td>11 [2.27, 53.37]</td>
</tr>
<tr>
<td>Paschou et al. 2015</td>
<td>344</td>
<td>14.6%</td>
<td>1.33 [0.63, 2.83]</td>
</tr>
<tr>
<td>Sadrzadeh et al. 2016</td>
<td>161</td>
<td>15.2%</td>
<td>0.74 [0.37, 1.49]</td>
</tr>
<tr>
<td>Sverristottir et al. 2008</td>
<td>38</td>
<td>3%</td>
<td>0.89 [0.05, 15.44]</td>
</tr>
</tbody>
</table>

Total number: 1611  
Heterogeneity: $I^2 = 68\%$

### 5.2 Cohort studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Total nr participants</th>
<th>weight</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cresswell et al. 1997</td>
<td>235</td>
<td>1.2%</td>
<td>1.52 [0.66, 3.52]</td>
</tr>
<tr>
<td>Laitinen et al. 2003</td>
<td>207</td>
<td>2.5%</td>
<td>0.74 [0.42, 1.33]</td>
</tr>
<tr>
<td>Mummm et al. 2013</td>
<td>523757</td>
<td>92.2%</td>
<td>1 [0.91, 1.1]</td>
</tr>
<tr>
<td>Sadrzadeh et al. 2003</td>
<td>1001</td>
<td>4.0%</td>
<td>0.94 [0.6, 1.49]</td>
</tr>
</tbody>
</table>

Total number: 526910  
Heterogeneity: $I^2 = 0\%$

Odds Ratio  
M-H, Random, 95% CI
Discussion:
To our knowledge, this is the first systematic review on the relationship between PCOS and birth weight. After systematically reviewing and meta-analyzing published data, we could not confirm the association between adverse prenatal conditions, reflected in birth weight, and subsequent PCOS diagnosis in the general population, nor did we find evidence for any effect of extremes in birth weight affecting subsequent PCOS diagnosis. We did find evidence of publication bias indicated by the funnel plot and subset analysis. Birth weight is a crude summary measure of intrauterine conditions. Our systematic review was not able to systematically appraise the evidence for two specific in utero exposures that may affect subsequent PCOS: high androgen levels, and maternal hyperinsulinism. Animal studies in non-human primates suggest that fetal exposure to androgens primes the hypothalamic-pituitary-ovary axis in a way that it sets off a reaction leading to intrauterine growth retardation, metabolic dysfunction and ovarian morphology similar to PCOS (31, 32). However the human fetus is protected from excess maternal
androgens by Sex Hormone Binding Globulin (SHBG) and placental aromatase, which converts androgens into estrogens. Therefore, in this condition a fetal origin of androgen, either of ovarian or adrenal origin, is presumed (33).

Pregnant women previously diagnosed with PCOS have an increased risk of developing (gestational) diabetes resulting in large for gestational age offspring (34). Due to the high heritability of PCOS (35), large for gestational age daughters born to mothers with PCOS have an increased risk of developing PCOS explaining the association between a relatively high birth weight and an increased risk for PCOS. Although our review was unable to discern a statistically significant effect of high birth weight on PCOS later in life, the hyperinsulinemic state of pregnant women with diabetes and the consequential placental aromatase inhibition resulting in elevated fetal androgen levels could induce PCOS (36).

Mumm et al show that large for gestational age women born to diabetic mothers have an increased risk to develop PCOS, with the relatively leanest ones amongst them having the highest risk.

With pooled data on a total of 528521 women this is by far the largest statistical analysis on the relation between birth weight and PCOS. By systematically searching and reviewing all published data on this subject we minimized the chance of citation bias favoring a specific outcome.

Our study has some weaknesses. While cohort studies can ultimately detect rare exposures, due to the long follow up period between exposure and outcome there are currently only a few reporting on this subject and none of them was designed to prospectively investigate the relation between perinatal conditions and PCOS. By including case-control as well as cohort studies we increased to the power of our analysis. However, observational case-control studies are generally less comparable because of differences and challenges in choosing an appropriate control group. The definition of the case group also differed significantly between studies. While some studies selected cases on birth weight and investigated the associating with PCOS others selected on PCOS and compared birth weights between groups. Additionally, case-control studies by design cannot address the causality of any associations they find.
Remarkably Mumm et al., with the largest included cohort study, reported a PCOS prevalence of 0.6%. As this is much lower than international reports on the prevalence of PCOS, we assume an underestimation but cannot predict how this might affect the association between birth weight and PCOS.

The skewed funnel plot suggests publication bias but as in all systematic reviews it is debatable if the skewed funnel plot can be explained by an underrepresentation of small studies yielding negative results or other sources of heterogeneity (37).

Finally a point of concern is that most studies did not provide enough information on gestational age to distinguish between women born with a low birth weight but appropriate for gestational age and women born small for gestational age. Future prospective studies collecting perinatal information, maternal endocrine status, as well as fetal androgen levels while distinguishing between low birth weight, high birth weight, small and large for gestational age should clarify the relation between birth conditions and PCOS, and to what extend insulin and androgens are key players in this process.

In conclusion, we cannot statistically confirm the association between adverse intrauterine conditions reflected in birth weight and PCOS.
References


Appendix

Pubmed
polycystic ovary syndrome:

Birth parameters:

Embase.com
polycystic ovary syndrome:
'ovary polycystic disease'/exp OR pco:ab,ti OR pcos:ab,ti OR (stein:ab,ti AND leventhal:ab,ti) OR ((polycystic*:ab,ti OR sclerocystic:ab,ti OR micropolycystic:ab,ti OR (poly:ab,ti AND cystic*:ab,ti)) AND (ovary:ab,ti OR ovaries:ab,ti OR ovarian:ab,ti OR ovarium:ab,ti OR ovaria:ab,ti OR ovaric:ab,ti OR ovarii:ab,ti))

Birth parameters:
'prematurity'/exp OR prematur*:ab,ti OR preterm*:ab,ti OR 'pre mature':ab,ti OR 'birth weight'/exp OR birthweight:ab,ti OR 'birth weight':ab,ti OR 'small for gestational age':ab,ti OR 'small for date':ab,ti OR sga:ab,ti OR vlbw:ab,ti OR elbw:ab,ti OR 'birth size':ab,ti OR 'neonatal underweight':ab,ti OR 'starvation'/exp OR 'fetal malnutrition'/exp OR starvation:ab,ti OR famine*:ab,ti OR malnutrition:ab,ti OR undernutrition:ab,ti OR dohad:ab,ti OR 'developmental origins of health and disease':ab,ti

Cochrane Library (Wiley)
polycystic ovary syndrome:
“ovary polycystic disease” OR pco OR pcos OR (stein AND leventhal) OR ((polycystic* OR sclerocystic OR micropolycystic OR (poly AND cystic*)) AND (ovary OR ovaries OR ovarian OR ovarium OR ovaria OR ovaric OR ovarii))

Birth parameters:
prematur* OR preterm* OR (pre NEXT/1 mature) OR “birth weight” OR birthweight OR “small for gestational age” OR “small for date” OR sga OR vlbw OR elbw OR “birth size” OR “neonatal underweight” OR starvation OR famine* OR malnutrition OR undernutrition OR dohad OR “developmental origins of health and disease”
Cinahl (EBSCO)

polycystic ovary syndrome:
(MH "Polycystic Ovary Syndrome") OR TI ( pco OR pcos OR (stein AND leventhal) OR ((polycystic* OR sclerocystic OR micropolycystic OR (poly AND cystic*)) AND (ovary OR ovaries OR ovarian OR ovarium OR ovaria OR ovarici OR ovarii)) ) OR AB ( pco OR pcos OR (stein AND leventhal) OR ((polycystic* OR sclerocystic OR micropolycystic OR (poly AND cystic*)) AND (ovary OR ovaries OR ovarian OR ovarium OR ovaria OR ovarici OR ovarii)) )

Birth parameters:
(MH "Infant, Premature") OR (MH "Infant, Low Birth Weight+") OR (MH "Birth Weight") OR (MH "Starvation") OR (MH "Malnutrition") OR TI (prematur* OR preterm* OR “pre-mature” OR birthweight OR “birth weight” OR “small for gestational age” OR “small for date” OR sga OR vlbw OR elbw OR “birth size” OR “neonatal underweight” OR starvation OR famine* OR malnutrition OR undernutrition OR dohad OR “developmental origins of health and disease”) OR AB (prematur* OR preterm* OR “pre-mature” OR birthweight OR “birth weight” OR “small for gestational age” OR “small for date” OR sga OR vlbw OR elbw OR “birth size” OR “neonatal underweight” OR starvation OR famine* OR malnutrition OR undernutrition OR dohad OR “developmental origins of health and disease”)
Conclusion
Chapter eleven

Summary, discussion and future perspectives
Summary, discussion and future perspectives

This thesis is built in three sections. The first is an introductory section. In section two we used different methods and outcome variables searching for the answer if adverse early life conditions affect ovarian reserve. The third section concentrates on the effect of perinatal conditions on one of the most common causes of female infertility, Polycystic Ovary Syndrome (PCOS).

Summary of main findings

Section one: Introduction

In chapter 1 we lay out the concept of developmental origins of health and disease (DOHaD) and discuss this concept in an evolutionary perspective. We also give an overview of intrauterine oocyte development, follicle formation and hormonal regulation as well as programmed cell death. Finally we elaborate the concept of ovarian reserve and the definition for PCOS.

By comparing birth weight differences between historical databases and contemporary databases using twin data in chapter 2, we showed that monozygotic (MZ) twins born before 1980 were less discordant than twins born after 1980, indicating that these older cohorts may be more susceptible to selection bias by selective survival of only fitter cohort members. Due to improvements in neonatal care, more at-risk babies survive the crucial pre- and perinatal period and reach maturity (1, 2). The survival of increasing numbers of low birth weight and preterm infants may, by virtue of case selection and altered postnatal environment, lead to a different spectrum of long term sequelae when compared to historical peers. These babies do not require the adaptive mechanisms to survive today as they did decades ago. If these adaptive mechanisms are the causal connection between adverse peri-natal circumstances and adult onset disease they are possibly not programmed to develop the diseases of the generations before them. Future research should take into account that selective survival may play a role in explaining differences in findings between cohorts from different historical eras. We found no evidence that voluntary versus mandatory enrollment was prone to selection bias.
Section two: Ovarian reserve

In chapter 3 we quantified ovarian reserve in a group of healthy adolescent volunteers born small for gestational age (SGA) or appropriate for gestational age (AGA) in a pilot study. For this purpose we measured LH, FSH, E2, AMH levels and the pituitary response to exogenous GnRH, all of which are established markers for ovarian reserve (3, 4). AMH in particular is documented as the most effective marker of ovarian reserve (5). Because we were unable to detect a clinically relevant or statistical significant difference in ovarian reserve between these young women born SGA and AGA, the results of this pilot study did not lead to continuation of this line of research. Our findings are in line with other published literature on this subject (6). A definite marker of ovarian depletion is natural menopause. We performed a case-control study investigating the influence of early environment on age at menopause before the age of 40 (POI) (chapter 4). We concluded that, in our study population, a preterm birth was significantly associated with menopause before the age of 40. Birth weight, however, was not related to the onset of menopause before the age of 40.

When quantifying the influence of birth weight on health and disease in later life, the effect of gestational age on birth weight is a considerable issue, due to the fact that gestational age is frequently not precisely known, particularly in historical cohorts. Even when gestational age is reported, the best way to correct for gestational age is still is a matter of debate as reference values for birth weight and gestational age are not available for all populations or ethnicities. By investigating the intra-twin differences in birth weight and their relationship with age at natural menopause in chapter 5 we found a solution for the confounding effect of gestational age regardless of ethnicity. Twins discordant for birth weight did not differ in age of onset of natural menopause. Mean age at menopause in this study was 48.5 y which is lower than the average of 51 y reported in international publications (7) but in agreement with other twin studies reporting age at menopause (8-10). While our study could not establish an association between age at menopause and birth weight, our findings may have limited generalizability due to the relatively young age at menopause in twins, which might in part be due to prematurity common in twins (11).

Chapter 6, the final chapter of this section is the first systematic review, conducted
according to PRISMA and MOOSE principles, on the effect of adverse intrauterine conditions, early childhood growth and famine exposure on the age at menopause. The final selection of this study included 11 published articles. Due to the differences in the definition of outcome variable and the reported risk estimates we were unable to pool data for statistical analysis. Nevertheless, we could conclude that famine during gestation and childhood as well as slow childhood growth is related to a younger age at menopause. Birth weight on the other hand, had no effect on age at menopause.

To summarize the second section, we conclude that birth weight is not related to accelerated ovarian depletion. Premature birth and impaired childhood growth as well as famine exposure however, seem to result in an earlier age at menopause.

**Section three: Polycystic Ovary Syndrome**

Before investigating the role of specific early environmental influences on the etiology of PCOS, we quantified the genetic influences on the etiology of PCOS by computing the heritability of PCOS using twin data in chapter 7. We concluded that up to 79% of the pathogenesis of PCOS is caused by genetic variance. However 21% of the pathogenesis of PCOS can be attributed to environmental factors. Further understanding of non-genetic etiological factors such as perinatal conditions, could be of benefit for an early detections of women at risk of developing PCOS and the prevention of PCOS as well as PCOS related co-morbidity. In chapter 8 we investigated the effect of birth weight and age at menarche on PCOS and diminished ovarian reserve in a large retrospective cohort of all women undergoing IVF in The Netherlands between 1980 and 1995 (OMEGA cohort). In this cohort, self-reported birth weight was not related to PCOS or a diminished ovarian reserve. An older age at menarche, however, was associated with PCOS. A younger age at menarche was associated with indicators of diminished ovarian reserve. The case-control study in chapter 9, comparing markers of early life environment between women diagnosed with PCOS and a control group, could not establish a relation between self-reported birth weight or gestational age and PCOS. Chapter 10, the final chapter of this section contains the first systematic review conducted according to PRISMA and MOOSE principles on the effect of birth weight on PCOS. By pooling and analyzing data on 528,521
women from 13 studies, we conclude that birth weight is not related to the development of PCOS.

To conclude the third section we state that birth weight as a summary measure for gestational adverse conditions, is not a considerable factor in the etiology of PCOS.

Discussion:
The summary finding of this thesis is that PCOS and age at menopause, indicators of female fertility, do not seem to be grossly affected by intrauterine and early childhood influences, this in stark contrast to the effects described on cardio-metabolic outcomes. Our findings support the notion that female reproductive fitness is a trait that has been conserved throughout evolution, where health or lifespan may be more easily traded off when faced with early life adversity. Due to intrauterine and early childhood environmental cues, the phenotype of an individual is altered accordingly (12). These adaptations often serve to increase reproductive fitness and to keep the individual alive and healthy long enough to reproduce. We did find exposure to famine, slow childhood growth and premature birth to be associated with decreased age at menopause, possibly due to early depletion of ovarian reserve during nutritional stress. The consequences of this relatively early depletion manifest themselves long after our biological reproductive maturation. Our direct ancestor had a life expectancy at birth of approximately 30 years (13). In evolutionary terms an early menopause at the age of 40 is collateral damage and of no consequence for the fitness of the species, although some authors have suggested that menopause itself does present benefits for the survival of the (grand) offspring owing to a surplus of non-reproductively active female carers (14).

Periods of decreased food availability, malnutrition and starvation are likely to have been frequent enough to have presented an evolutionary pressure. Evolutionary novel challenges such as obesity and nutritional abundance are more likely to strain developmental plasticity, one of the mechanisms ensuring reproductive fitness during adverse conditions (15). Maternal obesity is independently related to preterm birth, perinatal mortality, offspring asthma and obesity, and offspring early mortality (16-20). From an evolutionary perspective, the impact of maternal obesity could be much more
profound than periods of famine which was a relatively natural environment for our ancestors.

We show that high-risk, SGA and preterm infants born after the 1980s have a greater chance of survival when compared to high-risk infants born in the decades before. Studies investigating the effect of adverse intrauterine conditions are mainly conducted in adults born before 1980. The coping mechanisms ensuring survival of these individuals might be the same mechanisms causing cardiovascular and metabolic diseases in later life. It will be interesting to see if the generations born after 1980, surviving adverse perinatal conditions, will see an increase or decrease in cardiovascular and metabolic disease compared to the generations before them. As discussed in the introduction, adaptive mechanisms that arise in response to an adverse early environment may be subject to evolutionary selection if they optimize reproductive fitness. Infants at high risk of perinatal mortality born after 1980 are likely to have survived to adulthood due to interventions of modern neonatal care, which may result in a different spectrum of alterations in physiology than previous historical cohorts. The association between PCOS and low birth weight documented in younger populations might be due to this effect.

Although fertility seems to be well preserved, we show that adverse perinatal and early childhood circumstances are related to an earlier age at menopause, which may lead to an increase in morbidity associated with early menopause including osteoporosis and cardiovascular morbidity. In the modern western society, the life expectancy of women estimated at birth is more than 80 years (21). A young age at menopause consequently exposes these women to the increased health risks associated with menopause such as obesity, depression and cardiovascular disease (22-24). With women worldwide delaying child bearing until their thirties, a young age at menopause and the inevitable decade of subfertility preceding, also causes a significant amount of emotional distress and relational problems as well as an increasing demand on medically assisted reproductive treatments.

**Preventive medicine:**

In the past centuries, the main focus of western medicine has been curing disease and handling complications after the onset of disease. The shift from infectious disease to chronic diseases, and increasing prosperity, as well as the growing awareness of personal
responsibilities and lifestyle choices has made the need for evidence-based preventive medicine a pressing issue. There is ample evidence on the relation between early life and even pre-conception conditions on later mental and physical health. Early life adverse influences predispose an individual to disease, the first hit, and influences later in life cause the onset of disease, the second hit (25, 26). This two-hit-theory provides a novel framework for preventive approaches to non-communicable disease (27). Although preventive medicine has gained interest in the last decades the focus is still mainly on secondary prevention or as you could say preventing the second hit. In prevention of diabetes and cardiovascular disease, for example, a great focus has been put on preventing obesity and encouraging a healthy lifestyle in midlife. The most effort, ergo resources, has been spent on patients already at risk because of a history of cardiovascular problems or early stages of insulin resistance. Although these efforts can have a great influence on individual wellbeing, community based primary intervention addressing the first hit will have a much more profound effect on the health status of the community and in consequence the individual. It is also much easier, and in the long run, more effective to learn healthy behavior at a young age, rather than change established behavior in adult life. By educating parents (to be) and investing in preventive public health measures we can intervene at the time of the first hit ensuring the future generation to be at a lower risk of developing non-communicable disease. Studies looking at the introduction of smoking bans resulting in a decrease of low birth weight infants demonstrate this concept (28, 29).
Figure 1. First and second hit theory of non-communicable disease and the influencing factors

- Individual environment influencing the first or second hit, examples: genetic predisposition, eating and drinking habits, smoking, elevated cortisol levels due to stress, breast feeding etc.

- Group environment influencing the first or second hit, examples: poverty, ethnic background, geographical influences such as proximity to polluting industries, community acceptance and support for a healthy lifestyle and breast feeding, social norms that value girls and women etc.

- Governmental and social factors influencing the first or second hit, examples: Programs and funds for community development, equal educational opportunities, non-discriminatory employment opportunities, accessible health care and preventive programs, immunization policy, regulating (healthy) food prices and accessibility etc.
The following, however, should be considered. As fetal and childhood environment inevitably is associated with motherhood, an unfortunate side effect of communicating preventive recommendations to reduce the effect of the first hit can be mother-blaming or community-blaming, holding maternal choices or even physical stature during and before pregnancy as well as the effect of her direct community responsible for the misfortunes of the offspring (30). By putting the heavy load of responsibility on the shoulders of mothers (to be) or stigmatizing her direct social environment, preventive measures will not reach those needing them the most. Individuals and social/ethnic groups are part of and affected by a larger community and ill-positioned to influence health determinants on a larger scale. Figure 1 visualizes different individual and social factors influencing health and disease.

**The Dutch primary preventive healthcare system**

The Dutch preventive youth healthcare system (JGZ) reaches up to a 100% of children between the ages of 0-4 (31). JGZ provides evidence-based information, promoting a healthy lifestyle, as well as supporting and coaching parents in achieving healthy lifestyle goals for their children and themselves. Healthcare systems, including the JGZ, traditionally focus on the needs and risk factors of the individual. However as shown in figure 1, there are many factors influencing non-communicable disease which transcend individual responsibility and action range and fall under the concept of public health. JGZ has a good view on health determinants affecting specific populations in defined geographic regions. By combining this information with evidence-based preventive methods we can advise and guide local and national governments to translate this information into health care policy. When promoting preventive medicine, an obstacles of the Dutch health care system one should be aware of, is that preventive and curative healthcare are funded differently. The curative sector is mainly financed by insurance funds while preventive medicine is the responsibility of local governments. In the long run, preventive medicine is much more cost effective compared to the curative sector (32, 33), but because of this separate funding the local governments are reluctant to finance preventive measures as they see no financial gain and insurance companies don’t feel
responsible as they traditionally are more concerned with curative measures. Researchers should therefore focus more on the financial aspects and cost effectiveness of early intervention in relation to curative medicine and collaborate with insurance funds on this matter. There is no point of proving that an intervention works in a research setting if there is no one to pay for it in real life.

By providing evidence-based preventive (youth) healthcare measures and also emphasizing the financial benefits of early intervention, we can ensure an optimal developmental environment for future generations while also reducing the burden on the health care system.

**Strength and limitations**

One of the strengths of this thesis is that different epidemiological designs were used to explore the influence of perinatal deprivation on ovarian reserve and PCOS. We tested our hypothesis in case-control studies, a retrospective cohort, twin studies and finally a systematic review of all published literature. Furthermore, we used various angles to explore ovarian reserve starting with hormonal status in young adolescents, ovarian depletion in women seeking fertility treatment, premature menopause and age at natural menopause. When examining the relation between birth parameters and PCOS, in contrast to some other investigators, we chose to only include post adolescent women. Many girls experience transitional PCOS like symptoms including irregular menses and hirsutism in the first years after menarche, which naturally evolve into normal menstrual patterns and fertility during maturation (34, 35).

The most prominent methodological weakness of this thesis is that all studies have a retrospective nature. Due to the long time span between exposure (early environment), and the outcome (ovarian depletion and PCOS), the data available were mainly retrospective. This is a common problem in this field of investigation. Although some investigators use data from birth cohorts, where birth weight and other birth parameters are gathered prospectively, most determinants and confounders are collected retrospectively through questionnaires or reviewing medical charts. Another limitation of this specific line of investigation, being the effect of early life influences on reproductive
issues, is that most cohorts investigating the interaction between birth parameters and health and disease in adult life mainly focus on cardiovascular and metabolic diseases, information on reproductive outcome is often collected as a confounder or an additional determinant. Given this study design, assessment of reproductive diagnoses are rarely conducted according to validated measures or questionnaires.

Another issue we were confronted with is that, even though data on birth weight in general can be accurate and verifiable even decades after birth, gestational age is more often prone to recall bias. The recollection of gestational age reduces with age, birth number and is less reliable in uncomplicated pregnancies (36, 37). Excluding studies using data from birth cohorts, most studies, including our own studies, fail to collect or verify reliable data on gestational age. Using intra-twin variability in reproductive parameters is a method to eliminate this problem. The downside of using inter-twin data is that the effect of gestational age itself cannot be tested if gestational age is unknown.

Another limitation when investigating PCOS is the variability in diagnostic criteria, a continuing methodological frustration many authors have remarked on (38). When including patients for studies conducted for the purpose of this thesis we consistently used the Rotterdam criteria. For the systematic review and in our retrospective follow-up cohort of women treated for infertility in the IVF centers in The Netherlands between 1980-1995 we used the definitions of the authors or clinical criteria of the various IVF centers.

Another aspect to consider is that premature ovarian insufficiency, with menopausal ages younger than 40 years, might have a different etiology than age at natural menopause and not just be an extreme variation. While an early age at menopause might be the reflection of a premature senescence it has been postulated that a much younger menopausal age has a different pathophysiology (39, 40). Most included studies however, did not differentiate between an early age at natural menopause and premature age at menopause.

**Directions for further research**

Future studies should focus on collecting data on periconceptional, prenatal and postnatal nutritional status of mother and child in a prospective setting. Fertility related issues
should be considered when designing the study. Birth weight has been the main focus of studies investigating DOHaD as an indicator for prenatal adverse conditions. We advise to put more emphasis on gestational age and early childhood growth when investigating fertility related issues.

It is also of great interest to see if children surviving adverse prenatal conditions after the 1980s display different health related risk factors compared to high-risk infants surviving in to adulthood before 1980.

Another interesting field of investigation, already pursued in many western countries, is the effect of nutritional abundance and the overload of high carbohydrate and high protein nutrients in our modern diet on the development of our fertility, which seem to lead to a self-perpetuating transgenerational cycle of obesity and metabolic disease. The impact of these issues on fertility are currently not well understood, but is likely to be detrimental.

To translate these findings into healthcare policy, cost-benefit analysis in collaboration with insurance funds and local governments should also take a more prominent place in the field of preventive medicine.
References


Chapter twelve
Samenvatting
Dutch summary
Deel 1: Introductie

In het eerste hoofdstuk belichten we het concept van ongunstige perinatale omstandigheden en aandoeningen op latere leeftijd en bespreken we hoe dit concept binnen de evolutieleer past. In dit hoofdstuk zetten we ook de intra-uteriene ovariële ontwikkeling, intra-uteriene follikelformatie, intra-uteriene ontwikkeling van de hypothalamus–hypofyse-as en de geprogrammeerde celdood uiteen en leggen we uit wat we onder ovariële reserve en het polycysteus ovarium syndroom (PCOS) verstaan.

In hoofdstuk twee van het proefschrift vergelijken we geboortegewichtverschillen tussen tweelingenregisters. Door het vergelijken van geboortegewichtverschillen tussen historische databases, met tweelingparen geboren vóór 1980 en jongere databases met tweelingparen geboren na 1980 tonen we aan dat eeneliige (MZ) tweelingen geboren vóór 1980 minder discordant zijn dan tweelingen geboren na 1980. Dit impliceert dat selectiebias een grotere rol speelt bij oudere cohorten door selectieve overleving van fittere cohortleden. Als gevolg van de verbeterde prenatale en neonatale zorg overleven meer hoog-risiconeonaten de cruciale pre- en perinatale periode en bereiken de volwassen leeftijd (1, 2). Hoog-risiconeonaten die vóór 1980 zijn geboren en de volwassen leeftijd bereiken, beschikken wellicht over adaptieve mechanismen die hoog-risiconeonaten geboren ná 1980 niet meer nodig hebben om te overleven. Als deze adaptieve mechanismen bijdragen aan het effect van ongunstige perinatale omstandigheden op het ontstaan van ziektes op latere leeftijd, zou het ziekteprofiel van de jongere generatie er anders uitzien dan de huidige generatie. Wij adviseren dan ook dat toekomstig onderzoek op dit gebied rekening houdt met de verschillen in overlevingskans tussen historische en jongere cohorten. We vinden geen bewijs dat vrijwillige versus verplichte deelname aan een tweelingenregister bijdraagt aan selectiebias.

Deel 2: Ovariële reserve

In deel twee van het proefschrift laten we doormiddel van verschillende methoden en uitkomstmaten voor ovariële reserve, zien hoe ongunstige perinatale omstandigheden van invloed zijn op de ovariële reserve en leeftijd menopauze. In hoofdstuk 3 kwantificeren wij
de ovariële reserve van een groep gezonde adolescente vrijwilligers met een laag (*smaal for gestational age, SGA*) of een normaal geboortegewicht (*appropriate for gestational age, AGA*) voor de zwangerschapsduur. Alle vrijwilligers hadden een regelmatig menstruatiepatroon en gebruikten geen hormonale anticonceptie. Om de ovariële reserve te bepalen hebben wij de volgende hormonspiegels in het bloed van de onderzoeksgroepen bepaald: LH, FSH, E2, AMH en de reactie van de hypofyse op exogene GnRH getest. Deze hormonen zijn bewezen markers van de ovariële reserve (3, 4). Dit pilot onderzoek kon de hypothesen dat SGA-meisjes een lagere ovariële reserve hebben vergeleken met AGA-meisjes niet bevestigen. Deze bevinding is in overeenstemming met bevindingen van andere onderzoekers (5).

Een absolute marker voor ovariële uitputting is de leeftijd waarop een vrouw in de overgang raakt, de leeftijd menopauze. **Hoofdstuk 4** beschrijft een patiënt-controleonderzoek waarbij de patiënten bestaan uit vrouwen die tijdens de overgang jonger waren dan 40 jaar. In dit onderzoek vergelijken we de perinatale omstandigheden van deze vrouwen met vrouwen die ouder waren dan 40 jaar toen ze in de overgang rakten, de controlegroep. In onze onderzoekspopulatie was een menopauzeleeftijd van jonger dan 40 jaar gerelateerd aan een zwangerschapsduur korter dan 37 weken. Geboortegewicht, laag of hoog, was niet gerelateerd aan de leeftijd menopauze.

Bij onderzoek naar de relatie tussen geboortestandrijkmomenten en ziekte op latere leeftijd is het corrigeren voor zwangerschapsduur een bekend struikelblok. Veel retrospectieve cohorten bevatten geen gegevens over zwangerschapsduur. Zelfs als deze bekend zijn is er geen overeenstemming over hoe men het beste voor zwangerschapsduur kan corrigeren mede omdat voor veel populaties en etniciteiten geen referentiewaarden voor zwangerschapsduur en geboortegewicht zijn gedefinieerd. Door het onderzoeken van het verschil in menopauzeleeftijd tussen tweelingenparen hebben we in **hoofdstuk 5** dit probleem omzeild. Tweelingenparen die discordant waren voor geboortegewicht verschilden echter niet in de leeftijd waarop ze in de menopauze traden. Met 48,5 jaar was de gemiddelde leeftijd van menopauze in onze onderzoekspopulatie jonger dan de gemiddeld leeftijd van de overgang bij eenlingen, te weten 51 jaar (6). Deze relatief jonge leeftijd van overgang is wel vergelijkbaar met andere studies waar de leeftijd menopauze
van tweelingen gerapporteerd wordt (7-9). Het feit dat tweelingen over het algemeen prematuur geboren worden (10) kan gerelateerd zijn aan de jonge leeftijd waarop tweelingen in de menopauze treden. **Hoofdstuk 6**, het laatste hoofdstuk van dit deel van het proefschrift is een systematisch uitgevoerde review volgens de principes van PRISMA en MOOSE waarin alle tot 1 januari 2017 gepubliceerde artikelen meegenomen zijn. Onze vraagstelling was of intra-uteriene omstandigheden, groei in de eerste levensjaren en blootstelling aan hongersnood de leeftijd van menopauze beïnvloedt. De uiteindelijke selecte bevatte 11 studies. Gezien de verschillen in gerapporteerde uitkomstmaten en variabelen was het niet mogelijk om de data te poolen een meta-analyse te verrichten. Wel kunnen we concluderen dat blootstelling aan hongersnood tijdens zwangerschap of op kinderleeftijd evenals een vertraagde groei in de eerste 2 levensjaren gerelateerd is aan een jongere leeftijd menopauze. Geboortegewicht was niet gerelateerd aan de leeftijd menopauze.

Dit deel van het proefschrift samenvattend trekken we de **conclusie** dat geboortegewicht, als maat voor intra-uteriene deprivatie, geen invloed heeft op de ovariële reserve. Prematuriteit en groei in de eerste levensjaren daarentegen lijkt tot een versnelde depletie van de ovariële reserve te leiden.

**Deel 3: Polycysteuze ovaria**

Het **derde deel** van het proefschrift richt zich op het effect van perinatale omstandigheden op een van de meest voorkomende oorzaken van vrouwelijke subfertilititeit, PCOS (11). In **hoofdstuk 7** schatten we aan de hand van een tweelingenonderzoek in, welk deel van de pathogenese van PCOS toe te schrijven is aan genetische factoren en welk deel aan omgevingsfactoren. Uit ons onderzoek blijkt dat ongeveer 79% van de pathogenese van PCOS verklaard kan worden door genetische factoren terwijl omgevingsfactoren 21% van de pathogenese verklaren. Om PCOS in de toekomst beter te diagnosticeren en eventuele preventieve maatregelen mogelijk te maken is het van belang om omgevingsfactoren te belichten die aan de pathogenese van PCOS bijdragen. Perinatale omstandigheden vormen een van deze omgevingsfactoren. In **Hoofdstuk 8** onderzoeken wij de relatie tussen geboortegewicht en leeftijd menarche op
het ontwikkelen van PCOS of een verminderde ovariële reserve. Hiervoor gebruiken we data van het OMEGA-cohort, een cohort van alle vrouwen die tussen 1980 en 1995 in een van de 13 IVF-centra in Nederland in behandeling zijn geweest. In dit cohort was geboortegewicht, laag of hoog, niet gerelateerd aan PCOS of een verminderd ovariële reserve. Een oudere leeftijd van menarche was wel gerelateerd aan PCOS, een jonge leeftijd van menarche was daarentegen gerelateerd aan een verminderd ovariële reserve. De patiënt-controleonderzoek in hoofdstuk 9 waar we het geboortegewicht en zwangerschapsduur van vrouwen met PCOS vergelijken met een controlegroep laat geen verschil tussen beide groepen zien. Het laatste hoofdstuk van dit deel is een systematische review en een meta-analyse volgens de principes van PRISMA en MOOSE. In dit artikel analyseren we de data uit 13 artikelen en includeren we 528.521 vrouwen in de meta-analyse. De uitkomst van de meta-analyse is dat geboortegewicht geen bepalende factor is in het ontwikkelen van PCOS op latere leeftijd.

Aan het einde van het derde deel van het proefschrift komen we tot de conclusie dat geboortegewicht, als maat voor intra-uteriene deprivatie, geen belangrijke factor is in de etiologie van PCOS.

De conclusie van dit proefschrift is dat PCOS en leeftijd menopauze, determinenten van de vrouwelijke fertilititeit, vrijwel niet wordt beïnvloed door nadelige omstandigheden tijdens de zwangerschap of kinderleeftijd. Onze bevindingen bevestigen de gedachte dat vrouwelijke fertilititeit een evolutionair sterk geconserveerde eigenschap is. Waar gezondheid en levensduur onder invloed van periodes van ongunstige (perinatale) omstandigheden opgeofferd worden ten gunste van overleving, investeert het biologische organisme, in dit geval de mens, in het beschermen van voortplanting.

We vinden wel een relatie tussen vroeggeboorte, vertraagde groei in de eerste 2 levensjaren en blootstelling aan hongersnood, en een vroege leeftijd menopauze. Aangezien deze relatief vroege leeftijd menopauze buiten de vruchtbare leeftijdperiode van de vrouw valt heeft dit evolutionaire gezien geen consequentie voor het voortbestaan van de soort. Echter, met de toegenomen levensverwachting van de mens worden vrouwen die vervroegd in de overgang treden ook op een jongere leeftijd bloot gesteld aan de nadelige gezondheidseffecten die samen gaan met menopauze, zoals
botontalking en hart- en vaatziekte. Daarbij komt dat 10 jaar vóór het intreden van de overgang de vruchtbaarheid beduidend afneemt. Met de huidige gemiddelde leeftijd van 30 jaar waarop veel vrouwen in westerse landen voor het eerst moeder worden kan een vroege overgang tot ongewenste kinderloosheid leiden.
Referenties

Appendix

(Zwarte blz nu 201)

Dankwoord

Curriculum Vitae

Abbreviations list
Dankwoord

In de eerste plaats wil ik alle proefpersonen bedanken die zich belangeloos hebben laten prikken, vragenlijsten hebben ingevuld en in kelders en op zolders op zoek zijn gegaan naar ‘iets’ waar hun geboortegewicht op staat.

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Anne, naast dat je mijn vriendin en meditatieleraar bent, heb je ook de kaft van dit proefschrift ontworpen. Ik vond het een bijzonder proces om samen met je door te maken. Ik hoop nog vele uren tegenover je op het kussen te mogen zitten.


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Dear Tante, thank you for your generosity and your fantastic culinary creations.

Father, you left us to soon. You helped me realize that life is too short to be afraid.

Dear mother, you are one of the most courageous and kind women I have ever met. Your adventurous nature, love for life and your immense capacity to accept, respect and love without judgment is a great and rare gift. You soften my ego, thank you for life and all thereafter.

Shole en Shahram, my sister and brother. I cannot imagine a life without you two. We all traveled pretty weird, unconventional and utterly different paths in our lives but even despite huge geographical distances we never for a moment lost our connection. When I think of fun, love and appreciating life I think of you. You are the only people with whom I can literally LOL.
Guus, paranimf en vriendin voor het leven, zonder jou was er letterlijk geen promotie! Je was de aanzet tot de reboot van het proefschrift en ik ben je immens dankbaar daarvoor. Je bent een van de moedigste en grappigste mensen die ik ken. Kijk uit naar nog heel veel kopjes koffie en wijn, bij jou kan ik altijd mezelf zijn.

Lana, paranimf, lieve dochter, my Happy Lama. Vanaf het moment dat je mij als moeder koos heb je mijn leven veranderd, verlicht en gefocust. Je grote doorzettingsvermogen en motivatie in combinatie met je open en liefdevolle levenshouding maken je een bijzonder mens. Hoe het je lukt om naast het harde werken zo veel te feesten is mij een raadsel. Dat je vandaag naast mij staat, maakt van deze dag de meest bijzondere dag van mijn leven. ‘Je geeft me vertrouwen’ werkt twee kanten uit. Volgende keer andersom en thanks for the English editing. World Wide Underground, here we come.

Guus, mijn film- en sportmaatje, lieve zoon. Dat je op jouw leeftijd altijd de essentie van het leven kan zien en bewaken is een enorm talent. Als ik er even niet uit kom, en dat is regelmatig, weet je met een kritische blik de goede vragen te stellen en op een empathische manier precies het goede te zeggen om me verder te helpen. De wereld wacht op jou en gelukkig zijn er genoeg dure hotels.

Jaap je bent mijn rots in de branding. In een wereld waar iedere ademteug zal branden, iedere stap zal breken geef je me moed. With you I am fearless. Een fijne bijkomstigheid is je gevoel voor orde, de chaos in mijn hoofd woont ook in mijn huis, dank voor al het opruimen, ordenen, regelen en wassen, wassen en nog eens wassen. Ik wens ons nog vele jaren samen.
Curriculum vitae

Sheda Sadrzadeh was born on the 12th of May 1967 in Bielefeld, Germany and returned to Iran with her family in the 1970s. In 1984 after the Iranian revolution, at the age of seventeen, she left her family behind to finish high school at the scholengemeenschap Holten in the Netherlands while living with a foster family. In 1993 she finished the first phase of her medical education in Groningen, The Netherlands, at the Rijksuniversiteit Groningen. During the year waiting to start her internship she worked as a student researcher at the VU Medical Centre with professor Lambalk which resulted in her first publication. In 1997 she received her medical doctorate at Vrije Universiteit Amsterdam. After her graduation she worked as a junior researcher at the Dutch Twin Register and subsequently as a junior researcher (AIO) at the department of obstetrics and gynecology, division of reproductive medicine, VU Medical Centre up to September 2002. In 2001 she also received her masters degree in Epidemiology. From 2002 to 2004 she worked at the department of medical genetics at the VU Medical Centre (AGNIO). In 2004 she switched to a carrier in preventive youth healthcare and is now working at the Municipal Health service Kennemerland (GGD Kennemerland) as a youth health care physician. In 2009 she finished the first phase (Jeugdart KNMG) of the medical specialization for community medicine (arts Maatschappij en Gezondheid) and is currently completing the second phase.
# Abbreviations list

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA</td>
<td>Appropriate for gestational age</td>
</tr>
<tr>
<td>AMH</td>
<td>Anti-Müllerian hormone</td>
</tr>
<tr>
<td>ASKI</td>
<td>apoptosis-regulating signal kinase I</td>
</tr>
<tr>
<td>DNA</td>
<td>Desoxyribo Nucleic Acid</td>
</tr>
<tr>
<td>DOHaD</td>
<td>Developmental Origins of Health and Disease</td>
</tr>
<tr>
<td>E2</td>
<td>Estrogen</td>
</tr>
<tr>
<td>ETC</td>
<td>electron transport chain</td>
</tr>
<tr>
<td>Evo-devo</td>
<td>evolutionary development</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle-stimulating hormone</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>IVF</td>
<td>In vitro fertilization</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>MOF</td>
<td>Multi oocyte follicles</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>$O_2^-$</td>
<td>superoxide</td>
</tr>
<tr>
<td>ONOO$^-$</td>
<td>oxidant peroxynitrite</td>
</tr>
<tr>
<td>PCO</td>
<td>Polycystic ovary</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
</tr>
<tr>
<td>PGC</td>
<td>Primordial germ cells</td>
</tr>
<tr>
<td>POF</td>
<td>Premature ovarian failure</td>
</tr>
<tr>
<td>POI</td>
<td>Premature ovarian insufficiency</td>
</tr>
<tr>
<td>RA</td>
<td>Retinonic acid</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
</tbody>
</table>
The most outrageaous thing we can do
is to accept what happens and fly with it

Sakyong Mipham