During the past decades, in vitro fertilization (IVF) has become a routine procedure in reproductive medicine to overcome subfertility problems in couples all over the world. Since the first IVF birth in 1978, an estimated three million children have been born worldwide after IVF or related assisted reproductive technologies. Nowadays, there is a substantial body of evidence that IVF children are at increased risk for adverse perinatal outcome. In addition, various studies suggested an increased incidence of congenital abnormalities and rare imprinting diseases among IVF children. Nevertheless, several studies indicated that IVF children do not differ from spontaneously conceived controls in terms of mental and psychomotor development. There is still no consensus on whether observed health problems are related to the IVF procedure itself, the underlying subfertility problems of the parents, or a combination. Due to the lack of systematic follow-up of these children during the past years, it is largely unclear whether IVF treatment in humans is associated with substantial developmental consequences in later stages of life in conceived offspring. The present thesis addressed a broad scale of developmental aspects which are important during childhood and adolescence in IVF singletons and spontaneously conceived controls born to subfertile parents. Various cardiovascular measures were examined in view of the "developmental origins of health and disease" hypothesis.
Growth and Development of Children born after IVF Treatment
Cover design by Anouk Ceelen: Lotus flower – a symbol of fertility, creation, and purity.

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Growth and Development of Children born after IVF Treatment

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ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
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in het openbaar te verdedigen
ten overstaan van de promotiecommissie
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TANDEM FIT SURCULUS ARBOR

Eens wordt de stek een boom

ANON.
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Aims, design and outline of the thesis
At 11:47 p.m. on July 25, 1978, an apparently healthy five-pound 12-ounce baby girl, named Louise Joy Brown, was born in Britain (Figure 1). Louise’s parents had tried for nine years to conceive naturally before turning to the pioneering in vitro fertilization (IVF) treatment being developed by Edwards and Steptoe. The world’s first test-tube birth was considered a revolutionary breakthrough indicating the beginning of a new era in reproductive medicine. Approximately three decades after the first IVF birth, IVF has become a routine treatment utilized to overcome subfertility problems in couples all over the world. It is estimated that worldwide more than three million children have been born after IVF and ~2.3% of the current births in the Netherlands are established following IVF or other assisted reproductive technologies.

Figure 1  Announcement of the world’s first IVF baby
Source:  http://z.about.com/d/golondon/1/0/P/4/~/~/19-test_tube_baby.jpg

Even though shortly after the birth of Louise Brown serious medical concerns were raised, the introduction of IVF has not been accompanied by a formal health evaluation. By contrast, research in the field of reproductive medicine initially focused on the technical aspects of the IVF procedure in order to improve implantation and pregnancy rates. Since several years the importance to closely monitor the short- and long-term consequences of IVF for both the mother and the child is increasingly acknowledged.
During an IVF treatment cycle, controlled ovarian stimulation by gonadotrophic hormones is used to promote multiple follicular development. When follicle stimulation is considered sufficient, based on hormone tests and serial ultrasound examinations, human chorionic gonadotropin (hCG) is administered to complete oocyte maturation. Prior to the expected ovulation, the oocytes are collected using needle aspiration of the ovarian follicles. In the laboratory the oocytes are inseminated with a prepared semen sample. Generally, two to three days after oocyte retrieval the embryos consisting of 2–8 cells are transferred into the uterine cavity. Each of these phases of an IVF treatment cycle is substantially different from natural conception.

The course and outcome of IVF pregnancies have been extensively studied over the last decades, although the methodology and inferences of the early studies have been criticized. For a long time, the increased incidence of multiple pregnancies has been held responsible for poor perinatal outcome after IVF. To date, the number of meta-analyses and other well-designed studies investigating the potential side effects of IVF treatment among offspring are steadily increasing. There is substantial evidence that IVF-conceived children, including singletons, are at increased risk for low birth weight, preterm birth and perinatal death. In addition, various studies suggested an increased incidence of congenital abnormalities and rare imprinting diseases among IVF children. It is still unclear whether observed health problems originate from the IVF procedure itself and/or the underlying subfertility problems of the parents.

**Scope of this thesis**

Current knowledge regarding postnatal growth and development of children born after IVF treatment is still limited. However, the need for such investigations is underscored by various epidemiological studies which showed that adverse prenatal conditions including maternal undernutrition and other environmental factors are associated with an increased susceptibility to disease in later life. It has been suggested that events during early prenatal life can lead to persistent changes in the development of organs and their function. Specific critical periods in prenatal development have been identified. The periconceptional period represents a susceptible phase as early embryos are adaptive to the environment they encounter during development. Retrospective data from the Dutch Hunger Winter study have demonstrated that those who were exposed to famine in early gestation appeared to be at increased risk for coronary heart disease, raised lipids, altered clotting and obesity in adult life. Likewise, increasing numbers of experimental studies report associations between harmful conditions during the periconceptional period and perturbed body systems with serious pre- and postnatal developmental consequences. Additionally, various animal studies have shown that distinct phases of assisted reproductive techniques may interfere with normal programming of early development. Intriguingly, embryo culture conditions during the preimplantation period in mice were demonstrated to have detrimental postnatal effects on blood pressure, enzymatic regulators of cardiovascular and...
metabolic physiology, body mass and adiposity in progeny \cite{29,32}. Consequently, concerns have been expressed that the manipulation of gametes and embryos inherent to ART may also affect postnatal functioning in humans \cite{11,22}.

The research performed in this thesis aimed to investigate postnatal growth and development of 8- to 18-year-old IVF-conceived individuals and age- and gender-matched spontaneously conceived controls born to subfertile parents. The majority of the approached IVF and control children were selected using data of the OMEGA study, which was primarily designed to examine long-term effects of ovarian stimulation in subfertile women. Between 2003–2006, 246 of the 354 approached IVF children (69\%) and 233 of the 454 approached controls (51\%) participated in our study. Shortly before visiting the VU University medical center in Amsterdam, parents were sent a questionnaire to gather information on various demographic, lifestyle and medical factors. During the hospital visit, a structured interview was conducted to obtain information regarding the medical condition of the child from birth up to follow-up (e.g. neonatal period, occurrence of hospital admissions and/or surgeries, use of medication), school performance and other important developmental aspects. Subsequently, anthropometric measurements like height, weight, skinfold thickness, waist and hip circumference, blood pressure and pubertal stage were collected. In addition, the parents were asked to bring the original postnatal growth certificate of their child. Secondly, a pubertal subpopulation was invited for additional research including a fasting blood withdrawal, DXA scans of the lumbar spine, hip and total body followed by an X-ray of the wrist. These tests were used to further examine body composition, postnatal growth and pubertal development.

**Outline of the thesis**

A critical overview of the currently available literature concerning growth and development of children born after IVF treatment is given in Chapter 2. This chapter includes perinatal outcome of IVF pregnancies, the occurrence of congenital abnormalities, rare imprinting diseases and malignancies among IVF children and postnatal growth of IVF children. In addition, recently proposed mechanisms for specific health problems in IVF children are discussed. In analogy to the “developmental origins hypothesis”, stating that detrimental insults during critical phases of prenatal development are associated with several cardiovascular risk factors in later life, blood pressure (Chapter 3), fasting glucose and insulin (Chapter 3) and body composition (Chapter 4) were studied in IVF and control children. In Chapter 5, our results regarding pubertal Tanner stage, bone age and hormonal levels of IVF and control children are presented. Chapter 6 describes the growth of IVF children and controls during infancy and early childhood in relation to their blood pressure and body fat measures at follow-up. In Chapter 7, IVF children and controls were compared on measures of education level, general cognitive ability, school performance and rates of learning and developmental disorders. Chapter 8 presents a summary in English and the
general discussion, including methodological reflections, pathophysiological considerations, current implications and recommendations for future research. Finally, a summary in Dutch concludes this doctoral thesis.

All chapters in this thesis have been published (Chapter 2–7).
References


Aims, design and outline of the thesis


Growth and development of children born after in vitro fertilization

Manon Ceelen, Mirjam M. van Weissenbruch, Jan P. W. Vermeiden, Flora E. van Leeuwen, Henriette A. Delemarre-van de Waal

Fertility and Sterility, 2008, 90: 1662-1673
Abstract

Background: Over the past two decades, considerable interest has been focused on the growth and development of children born after IVF treatment.

Methods: Literature review.

Results: At present, there is substantial evidence that children born after IVF are at increased risk for adverse perinatal outcome, congenital malformations, and rare epigenetic defects. It is still unclear whether observed health problems originate from the IVF procedure itself and/or the underlying subfertility problems of the parents. Current follow-up studies regarding postnatal growth and morbidity rates are scarce with conflicting results and other areas of long-term research in children born after IVF are still in its infancy.

Conclusions: The importance of the worldwide continuing monitoring of children born after IVF to investigate potential long-term consequences including the development of cardiovascular diseases is highlighted.
In vitro fertilization (IVF) used to overcome reproductive problems in humans is considered to be one of the most spectacular medical discoveries of the 20th century. Steptoe and Edwards have been actively working on finding an alternative solution for conception since 1966, although the idea of IVF had first been put forward as early as the 1930s. It was not until the 1970s when it became possible to fertilize a human oocyte outside the female’s body. Finally, on July 25, 1978, the world’s first “test-tube” baby, named Louise Joy Brown, was born in Great Britain. Although this birth was interpreted as a technological miracle, the introduction of this new medical technology raised also various ethical and moral questions. However, for many years, research in the field of assisted reproductive technologies (ART) has primarily concentrated on the technical aspects of the IVF procedure to improve pregnancy rates.

During an IVF treatment cycle, the ovaries are stimulated with gonadotropic hormones to promote development of several follicles. When stimulation of the follicles is considered sufficient based on hormone tests and serial ultrasound examinations, hCG is administered to complete the maturation of the oocytes. Before the expected ovulation, the oocytes are recovered using needle aspiration of the ovarian follicles. In the laboratory, the oocytes are inseminated with a prepared sample of sperm. Two to 5 days after oocyte retrieval the embryos are transferred into the uterus by passing a thin embryo transfer catheter through the cervix to the top of the uterus. Each phase of the IVF procedure, including stimulation of multiple folliculogenesis, the process of oocyte retrieval and spermatozoa preparation, in vitro fertilization, culture of embryos in medium for several days, and embryo transfer into the uterus instead of the oviduct, is substantially different from natural conception. It has been suggested that these distinct aspects might have tremendous effects on the developing conceptus. Many epidemiological studies have demonstrated that prenatal events can lead to persistent changes in the development of organs and their function and therefore may cause diseases later in life. In addition, various animal experiments have shown that fetal growth of offspring generated by techniques related to ART used in humans can be affected.

Application of IVF has rapidly increased since its introduction in 1978. Although IVF has initially been developed to overcome fertility problems due to blocked fallopian tubes, at present medical indications for IVF have been expanded by a wide spectrum of subfertility causes. Approximately 1.6% of the current births in the Netherlands are established after ART and it is estimated that worldwide more than 1 million children have been born after assisted conception. The number of children born after IVF will continue to increase. Worldwide increasing delayed childbearing and the availability of new technologies, such as preimplantation genetic diagnosis to prevent transmission of severe or lethal diseases to offspring, will contribute to the increasing demand for IVF. Therefore, the need to evaluate the potential effects of fertility treatments is steadily growing.

Fortunately, for several years attempts to closely monitor the short- and long-term consequences of ART for both the mother and the child are increasing. This review will summarize current knowledge regarding the health of children born after IVF, including perinatal outcome after IVF,
the incidence of congenital malformations, postnatal growth, and the occurrence of malignancies and imprinting disorders in children born after IVF.

_The prenatal period and environmental influences_

Human development from conception to birth is a complex physiological process. The prenatal period can be divided into three main periods: the germinal (0–2 weeks of gestation), the embryonic (3–8 weeks of gestation), and the fetal period (9th week of gestation until birth). The germinal period includes the development of the zygote, cell division, and attachment of the blastocyst to the uterine wall. During the second period of prenatal development, known as the embryonic stage, differentiation and development of the major organs and body systems occur. Growth and development continue dramatically during the fetal period, including further differentiation and functional maturation of organs and tissues, as well as significant increases in organ size. Prenatal life is not only a time of major developmental changes, but also represents one of the most vulnerable periods of the life course. During this critical period, the developing conceptus (embryo or fetus) is responsive to influences from both intrinsic and external conditions. Disruption of organogenesis during the embryonic development can cause irreversible structural anomalies, whereas disruption during the fetal period often affects fetal growth and size or function of specific organs. The first days after conception also represent a susceptible phase as early embryos are adaptive to the environment they encounter during development. The sensitivity of preimplantation embryos to environmental influences can lead to altered fetal development with both prenatal and postnatal consequences. Likewise, many animal studies demonstrated that fertilization in vitro and culture systems used during preimplantation stages of ART can alter normal development.

Adaptations of the conceptus to adverse conditions during one of the critical windows in the prenatal period can have far-reaching, permanent effects on structure, physiology, and metabolism of an individual. Many adult diseases are thought to be the long-term consequences of prenatal developmental defects. Therefore, the health consequences caused by prenatal environmental conditions, in this review especially concentrating on the conception period, should not be minimized.

_Perinatal outcome IVF pregnancies_

During the past two decades considerable interest has been focused on the perinatal health outcome of IVF pregnancies. Pregnancies after IVF have been reported to be at increased risk for adverse perinatal outcome, including preterm birth, low birth weight, and perinatal death. This has often
been attributed to the increased incidence of multiple pregnancies after IVF and to confounding by maternal characteristics. For example, a 20-fold increased risk of twins and a 400-fold increased risk of higher order pregnancies have previously been demonstrated in women undergoing IVF. This is directly related to the practice of replacing multiple embryos at embryo transfer. Multiple births are associated with serious adverse infant and maternal outcomes. In an attempt to reduce the frequency of multiple gestations with its related obstetric and perinatal complications in ART programs, the embryo transfer policy and especially the number of embryos transferred is currently reconsidered worldwide. Similarly, perinatal problems reported after IVF have been ascribed to differences in maternal characteristics. Comparison of perinatal outcomes of IVF pregnancies and spontaneous conceptions is hampered by differences between patients undergoing IVF and the general obstetric population. The clinical and scientific experience is that women undergoing IVF treatment are more often primiparous and of older age. It is generally accepted that advanced maternal age and primiparity itself are associated with perinatal complications. In addition, IVF patients and the general population differ with respect to many other potentially confounding factors, including maternal toxin exposure and nutrition. Finally, many studies have investigated perinatal outcome in relatively small cohorts of women and appropriate data for comparison was lacking, which limit validity and interpretation of findings.

**Figure 1** Biological factors known to influence prenatal growth and development
Recently, several thorough systematic reviews of the existing literature on perinatal outcome after IVF have been published. Methodological limitations of individual studies were obviated in these reviews by excluding studies where methods were considered inadequate, by conducting meta-analyses using data from methodologically sound studies only and by examining singletons separately. Helmerhorst et al. compared the perinatal outcome between natural and assisted conception using relevant studies published in the period of 1985–2002. Use of an appropriate control group from the same population as the IVF pregnancies and separate analyses for singleton and multiple pregnancies were requirements for inclusion in the meta-analysis. In total, 17 of the 25 included studies had matched their controls for maternal age, parity and other confounding factors. The meta-analysis conducted by Jackson et al. included studies investigating the outcome of singleton IVF pregnancies that controlled for maternal age and parity in either the study design or statistical analysis. An extensive search for relevant literature published between 1978 and 2002 identified 15 studies comprising 12,283 IVF and 1.9 million spontaneously conceived infants. The meta-analyses of McDonald et al. were based on relevant studies examining perinatal outcome after IVF published between 1978 and 2003, which controlled for maternal age (singleton analysis: 14 case control studies and 4 cohort studies; twin analysis: 11 case control studies). All meta-analyses demonstrated that singletons conceived after IVF are at increased risk for preterm birth, low birth weight, being small for gestational age, perinatal mortality, and other adverse perinatal health outcomes, after correction for maternal age and parity (Table 1). Meta-analysis of the studies of twin gestations by Helmerhorst et al. showed less profound differences between the natural and the assisted conception population, except for a strikingly decreased perinatal mortality risk in favor of the assisted conception population. The lower rate of monochorionic pregnancies has been suggested to be responsible for the lower risk of perinatal health problems in IVF twins. On the contrary, McDonald et al. reported increased rates of preterm birth and cesarean delivery among IVF twins compared with spontaneously conceived twins who were matched for maternal age.

Several other well-conducted studies published since these meta-analyses confirmed the higher rate of adverse perinatal outcome in IVF singletons. An American population-based study, including 42,463 infants born after ART in 1996 and 1997 and all infants born in 1997 in the US after natural conception (3,389,098 infants), demonstrated that singletons conceived with ART were at increased risk for low birth weight, especially those who were born at term (standardized incidence ratio [SIR] = 2.6; 95% confidence interval [CI] 2.4, 2.7). Known differences between the two populations, including maternal age, maternal parity, and gestational age at delivery could not explain these increased risks. A Hungarian cohort study, comparing 230 pregnancies after IVF with 230 spontaneously conceived pregnancies after elaborate matching for maternal age, parity, gravidity, and previous obstetric outcome, reported an increased premature birth rate of singleton pregnancies after fertility treatment.
Table 1  Adverse perinatal risk estimates of ART pregnancies: meta-analyses by Helmerhorst, Jackson, and McDonald

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Helmerhorst et al., 2004</th>
<th>Jackson et al., 2004</th>
<th>McDonald et al., 2005</th>
<th>McDonald et al., 2005</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95%)</td>
<td>OR (95%)</td>
</tr>
<tr>
<td></td>
<td>Single births</td>
<td>Twin births</td>
<td>Single births</td>
<td>Single births</td>
</tr>
<tr>
<td>Very preterm birth (&lt; 32 wk)</td>
<td>3.27 (2.03, 5.28)</td>
<td>0.95 (0.78, 1.15)</td>
<td>3.10 (2.00, 4.80)</td>
<td>2.99 (1.54, 5.80)</td>
</tr>
<tr>
<td>Preterm birth (&lt; 37 wk)</td>
<td>2.04 (1.80, 2.32)</td>
<td>1.07 (1.00, 1.14)</td>
<td>1.95 (1.73, 2.20)</td>
<td>1.93 (1.36, 2.74)</td>
</tr>
<tr>
<td>Very low birth weight (&lt; 1,500 g)</td>
<td>3.00 (2.07, 4.36)</td>
<td>0.89 (0.74, 1.07)</td>
<td>2.70 (2.31, 3.14)</td>
<td>3.78 (2.49, 5.75)</td>
</tr>
<tr>
<td>Low birth weight (&lt; 2,500 g)</td>
<td>1.70 (1.50, 1.92)</td>
<td>1.03 (0.99, 1.08)</td>
<td>1.77 (1.40, 2.22)</td>
<td>1.40 (1.01, 1.95)</td>
</tr>
<tr>
<td>Small for gestational age (&lt; 10th percentile)</td>
<td>1.40 (1.15, 1.71)</td>
<td>1.27 (0.97, 1.65)</td>
<td>1.60 (1.25, 2.04)</td>
<td>1.59 (1.20, 2.11)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>1.54 (1.44, 1.66)</td>
<td>1.21 (1.11, 1.32)</td>
<td>2.13 (1.72, 2.63)</td>
<td>1.81 (1.41, 2.32)</td>
</tr>
<tr>
<td>Admission neonatal intensive care unit</td>
<td>1.27 (1.16, 1.40)</td>
<td>1.05 (1.01, 1.09)</td>
<td>1.60 (1.30, 1.96)</td>
<td>1.36 (1.20, 1.54)</td>
</tr>
<tr>
<td>Perinatal mortality</td>
<td>1.68 (1.11, 2.55)</td>
<td>0.58 (0.44, 0.77)</td>
<td>2.19 (1.61, 2.98)</td>
<td>2.40 (1.59, 3.63)</td>
</tr>
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</table>

Note: see text for abbreviations.
* Based on data of matched studies only; n = 17.
* Based on data of case control studies only; n = 11.
* Based on data of case control studies only; n = 8.
* Very preterm birth has been defined as delivery before 33 completed weeks.
Chapter 2

**Origins adverse perinatal outcome after IVF**

Despite the persuasive body of evidence indicating an increased risk of perinatal complications after IVF, there is still no consensus about the etiology of these health problems. Knowledge of any potentially avoidable or treatable factors in the genesis of obstetric complications after ART is important as it may influence the planning and delivery of maternity care for affected couples, as has been acknowledged by Thomson et al. A number of factors have been suggested to be involved, including lower thresholds for obstetric monitoring and intervention in women with fertility problems, underlying causes of infertility that necessitated ART, and aspects of the IVF procedure itself. To unravel the true effects of each of these factors, randomized clinical trials comparing perinatal outcome of IVF pregnancies conceived in subfertile women with the outcome of IVF pregnancies in fertile women are warranted. Likewise, from a scientific point of view randomization of IVF treatment versus no IVF treatment for subfertile couples would be valuable. However, as these types of randomized clinical trials are ethically impossible, clues can only be obtained from observational studies investigating the relation between untreated subfertility, treated subfertility, and pregnancy outcome, as well as studies that compared perinatal complications after distinct types of infertility treatments.

Several studies have suggested that subfertility is associated with the development of pre-eclampsia, low birth weight, preterm delivery, and perinatal death independently of treatment. Thomson et al. found no differences in the frequency of prematurity and low birth weight between untreated and treated subfertile women. A large Australian population study recently examined perinatal outcome after ART according to type of infertility problems (female factor infertility vs. male factor infertility) and type of IVF treatment (transfer of fresh embryos vs. transfer of cryopreserved embryos). Female factor infertility was found to independently increase the likelihood of preterm birth and low birth weight for ART singletons and twins. Pregnancy-related complications, including defective uteroplacental interaction, hypertension, and bleeding, were suggested to be related to the female fertility problems leading to reduced birth weight and gestation. According to the investigators, the increased risks of low birth weight and preterm birth observed after fresh embryo transfer was related to the embryo selection process used before cryopreservation resulting in the freezing of high-quality embryos. In addition, another possible selection bias has been proposed based on the relation between good response to ovulation induction therapy and excess embryos available for freezing.

Although these studies suggest that fertility problems are associated with adverse perinatal outcome, the influence of the IVF procedure itself on fetal growth and development continues to be a hot topic of debate. A recent population-based cohort study among subfertile women showed that increased adverse perinatal risks after IVF cannot be explained by subfertility. Furthermore, Schieve et al. demonstrated that ART infants conceived with presumably healthy gametes (oocytes from an egg donor and sperm from a partner without the diagnosis of male factor fertility) or
carried by a presumably healthy woman (no female infertility diagnosis reported; ART used because of male factor infertility) are also at increased risk of low birth weight and very low birth weight. Likewise, lower birth weights were found among children born from subfertile women conceived after superovulation as compared with children born from subfertile women after spontaneous conception. Finally, prospective comparison of perinatal outcome in a cohort of previously subfertile women who conceived after ART treatment and spontaneous conception showed a shorter pregnancy span and a lower mean birth weight among IVF singletons. Unfortunately, the use of most observational studies comparing perinatal outcome after different types of ART techniques to estimate the influence of the IVF procedure on fetal growth and development in humans is rather limited, as distinct subtypes of ART are commonly used for different types of subfertility. As a consequence, effects of fertility problems versus effects of treatment often cannot be disentangled. Especially prospective studies designed to examine perinatal outcome of IVF pregnancies and spontaneous pregnancies similar with regard to subfertility cause, duration of subfertility, and other relevant obstetric characteristics could make an important contribution to the unraveling of the origins of adverse perinatal IVF outcome.

**Congenital anomalies**

The term congenital anomaly refers to a broad spectrum of structural defects, which are apparent at birth or detected shortly after birth. In addition to genetic factors, various environmental conditions contribute to the etiology of congenital anomalies. Environmental factors known to cause congenital defects include infectious agents, maternal illness and deficiency states, physical agents such as radiation and hyperthermia, and alcohol and drug use. The relation between folic acid deficiency and the development of neural tube defects, as well as the importance of the use of periconceptional folic acid supplementation to prevent these birth defects, is widely recognized. Fetal developmental defects are greatly dependent on the timing, intensity and nature of exposure, the nature of the teratogens, and genetic susceptibility.

Since the study of Lancaster, published in 1987, describing a possible increase in the incidence of neural tube defects and transposition of the great vessels among IVF children, the association between IVF and congenital anomalies has been extensively investigated and debated. Although various studies reported an increased risk of birth defects after IVF, others found that congenital abnormality rates among children born after IVF were not increased. Several of these reports dealt with limited numbers of pregnancies and have methodological limitations such as the lack of an appropriate comparison group and failure to take into account potentially confounding variables. Other common methodological pitfalls involve the use of different methods of birth defect assessment in IVF-exposed and -unexposed children, as well as inconsistent criteria to classify anomalies.
To unravel the complex and conflicting literature regarding congenital anomalies and IVF, Hansen et al. \(^{42}\) conducted a systematic review based on all articles published by March 2003 to assess the risk of birth defects after ART. Only 7 of the 25 studies originally selected met the methodological quality requirements and were included in a meta-analysis. The majority of these studies were population-based with a clear definition of a birth defect and had a large sample size. Birth defects were ascertained without knowledge of conception status in all seven studies. The pooled overall odds ratio (OR) was 1.40 (95% CI 1.28, 1.53), indicating a statistically significant 40% increased risk of birth defects in children born after ART (Figure 2). The results of subgroup analyses, which pooled estimates from studies including only major birth defects, only singleton infants, or children conceived after only IVF, or only intracytoplasmic sperm injection (ICSI), showed that the pattern of increased risk of birth defects in ART infants remains, regardless of the way in which these data are grouped. When the meta-analysis was restricted to those studies examining birth defects in children conceived by IVF only (n = 3), the pooled OR was 1.90 (95% CI 1.41, 2.54).

![Figure 2](image.png)

**Figure 2** Estimates of congenital malformation risk (pooled odds ratios) in children born after ART

Published by Hansen et al. 2005

In accordance with the results of Hansen et al. \(^{42}\), several studies recently reported increased risks of major birth defects among children conceived by IVF \(^{51,58,86}\). In addition, the population-based study by Klemetti et al. \(^{58}\) revealed that the risk was especially increased among singleton IVF boys (OR = 1.63; 95% CI 1.23, 2.15), whereas the risk among multiple IVF girls was obviously decreased (OR = 0.45; 95% CI 0.22, 0.93). Furthermore, Kallen et al. \(^{51}\) suggested that the observed increased risk for congenital malformations in children born after IVF is most likely related to parental characteristics like infertility, as after correction for years of involuntary childlessness the increased risk disappeared nearly completely (OR = 1.05; 95% CI 0.95, 1.16).
Although larger data sets are needed to detect specific risk increases for special malformations, certain organs systems have been suggested to be affected more often among children born after IVF including neural tube defects, gastrointestinal defects, orofacial defects, hypospadias and other genitourinary defects, cardiovascular defects, musculoskeletal defects, and chromosomal defects. In view of multiple comparisons, careful interpretation of these results, due to increased risks of chance findings, is of great importance. It is still unclear whether the slightly increased risk of congenital malformations observed among infants born after IVF is inherent to factors associated with the underlying causes of infertility and/or factors associated with the IVF procedure. The need for further prospective surveillance and collection of detailed and accurate information on the duration and causes of infertility, exact information regarding maternal drug exposure, and other parental background characteristics has been recently underscored. Those countries that have cross-discipline population registries for ART, obstetric care, and birth defects, which enable record linkage research that is cost effective and minimizes losses to follow-up, can make an important contribution to the elucidation of biological pathways and interactions related to ART birth defects.

**Childhood cancer**

Distinct events during prenatal life are known to contribute to the initiation of carcinogenesis. Intrauterine exposure to carcinogenic agents has been suggested to predispose toward the development of pediatric malignancies. One of the most widely recognized carcinogens is diethylstilbestrol, which was widely used in the years 1940–1975 to prevent spontaneous and habitual abortions. Women who were exposed to this synthetic estrogen in utero were found to have strongly increased risk for developing cancer of the vagina and cervix at an unusually young age. Furthermore, a relation between prenatal X-ray exposure and childhood malignancies, such as leukemia, has been described.

During the past decade, several case control studies described a significantly increased risk of embryonal tumors, specifically neuroblastoma and leukemia, among infants who were prenatally exposed to sex hormones. Various case series reports suggested a possibly increased incidence of embryonal tumors in children born after assisted conception. In addition, an approximately fivefold increased risk for retinoblastoma among children born after IVF was documented recently based on five cases of retinoblastoma. Therefore, there might be an association between fertility treatment and the development of childhood cancer.

However, recently published cohort studies on childhood cancer incidence in children born after ART did not show increased overall risks (Table 2). Doyle et al. compared records from the register of 2,507 children born after assisted reproduction in Britain between 1978 en 1991 with the National Registry of Childhood Tumours. Only 2 cases of cancer were identified compared with 3.5 cancers expected (SIR = 0.6; 95% CI 0.7, 2.1). In an Australian cohort, data on 5,249 children born...
Table 2  Cohort studies regarding the occurrence of childhood cancer in children born after IVF

<table>
<thead>
<tr>
<th>Authors, year (country)</th>
<th>Study period</th>
<th>Cohort Size</th>
<th>Average follow-up</th>
<th>Incidence</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Observed cancers</td>
<td>Expected cancers</td>
</tr>
<tr>
<td>Doyle et al., 1998 (Britain)</td>
<td>1978–1991</td>
<td>2,507</td>
<td>8.6 y</td>
<td>2 cases</td>
<td>3.5 cases</td>
</tr>
<tr>
<td>Bergh et al., 1999 (Sweden)</td>
<td>1982–1995</td>
<td>5,856</td>
<td>Not mentioned</td>
<td>4 cases</td>
<td>3.6 cases</td>
</tr>
<tr>
<td>Bruinsma et al., 2000 (Australia)</td>
<td>1979–1995</td>
<td>5,249</td>
<td>3.75 y</td>
<td>6 cases</td>
<td>4.3 cases</td>
</tr>
<tr>
<td>Lerner-Geva et al., 2000 (Israel)</td>
<td>1981–1994</td>
<td>332</td>
<td>4.3 y</td>
<td>0 cases</td>
<td>1.7 cases</td>
</tr>
<tr>
<td>Klip et al., 2001 (The Netherlands)</td>
<td>1980–1995</td>
<td>9,050</td>
<td>4.6 y</td>
<td>6 cases</td>
<td>6.8 cases</td>
</tr>
<tr>
<td>Kallen et al., 2005 (Sweden)</td>
<td>1982–2001</td>
<td>16,280</td>
<td>5.5 y</td>
<td>29 cases</td>
<td>21 cases</td>
</tr>
</tbody>
</table>

Note: see text for abbreviations. Confidence intervals (CI) calculated by the investigators are shown; in case no confidence intervals have been calculated by the investigators, exact 95% Poisson confidence intervals are presented.
after IVF were linked with a population-based cancer registry to determine the number of cancer cases that occurred during follow-up. A total of 4.3 cases of cancer were expected, whereas 6 were observed, giving a nonsignificantly increased risk of cancer in the IVF group as compared with the general population (SIR = 1.4; 95% CI 0.6, 3.1). A small Israeli study evaluated the cancer incidence rate among a cohort of 332 children conceived after IVF between 1981 and 1994. No cancer cases were observed among the study cohort as compared to 1.7 cases that were expected. Klip et al. determined the cancer incidence in a Dutch cohort consisting of 9,484 children born after ART and 7,532 spontaneously conceived children born from subfertile parents. In total, 16 children developed cancer during an average follow-up period of 6.0 years, whereas 15.5 were expected (SIR = 1.0; 95% CI 0.6, 1.7). A direct comparison between children born after ART and naturally conceived children revealed no increased risk for childhood malignancies (relative risk [RR] = 0.8; 95% CI 0.3, 2.3). The largest study yet on cancer occurrence after ART has recently been published concerning all children born after IVF in Sweden between 1982 and 2001 (n = 16,280). No increase in overall cancer risk was seen as 29 children with cancer were observed, whereas 21.4 cancer cases were expected (SIR = 1.4; 95% CI 0.9, 2.0). Although the probability of a chance finding due to the multiple comparisons performed cannot be ruled out, unexpectedly many children with histiocytosis were noted (SIR = 5.6; 95% CI 1.8, 13).

Although those findings provide some reassurance, definite conclusions regarding the cancer risk and IVF cannot be drawn yet as most relevant studies deal with methodological limitations. Case reports are rather useful in suggesting possible adverse effects of a treatment and generating hypotheses for further investigation than that they are suitable to constitute proof of a causal relationship. In addition, the majority of the described case control studies and cohort studies concern relatively small numbers of children born after IVF and a short follow-up time. Furthermore, with the current number of IVF cancer cases available it is not possible to estimate risks for specific tumor locations or to correct for confounding variables. Because childhood cancer is a rare disease, a sample size of at least 20,000 children would be required to observe doubling of the risk of cancer in the cohort. Therefore, children born after IVF treatment should be followed worldwide to establish a large enough cohort that will enable assessment of the long-term safety of this procedure.

**Growth and physical development**

During the past years, numerous studies on short-term outcome after IVF have reported increased rates of preterm birth, perinatal deaths, intrauterine growth retardation, and congenital malformations. Although preterm birth and low birth weight are known to be associated with childhood and adult morbidity and mortality, few well-designed studies have addressed postnatal growth and physical development of children born after IVF. Generally, differences in follow-up time, types of control groups, or control for confounding and possible selection bias due to selective infant participation hampered comparison of several studies and the drawing of valid conclusions.
Studies investigating growth in children born after IVF between birth and 18 months of age \(^{111}\), at the ages of 12–45 months \(^{12}\), at 5 years of age \(^{10}\) and between 6 to 13 years of age \(^{65}\) showed no major pathological features concerning growth and physical development. Contrary to these findings, a Finnish population-based cohort study reported dissimilarities in the growth patterns concerning weight and height among children born after IVF during the first 3 years of life \(^{62}\). The risk of low weight and height, below the lowest quartile, at 1 year of age (OR = 1.5 and 1.6, respectively) and at 2 years of age (OR = 1.6 and 1.7, respectively) was significantly higher among children born after IVF as compared with spontaneously conceived matched controls. It was suggested that poor perinatal outcome affected the growth during the first years of childhood.

The prospective multicenter cohort study performed by Bonduelle et al. \(^{10}\) showed that 5-year-old IVF singletons were more likely to have had a significant childhood illness, to have had a surgical operation, to require medical therapy, and to be admitted to hospital than naturally conceived children. Likewise, among IVF singletons as compared with spontaneously conceived infants, an increased cumulative incidence of different diseases diagnosed in outpatient or inpatient care was found during the 3-year follow-up period, especially regarding respiratory diseases and diarrhea \(^{62}\). Although Kallen et al. \(^{52}\) recently confirmed the increased rates of respiratory tract infections, other studies did not find any indications of increased morbidity rates or increased use of medical health care resources among children born after IVF \(^{90, 91, 111}\). A possible explanation is that children born after IVF treatment might be more susceptible to morbidity, given the increased risk of perinatal complications among IVF infants. On the other hand, due to excessive parental concern IVF parents may seek medical help more often, or IVF children could be more easily referred to specialized pediatric care. Increased hospitalization rates among at term born IVF children up to an age of 6 years were demonstrated to be related to length of involuntary childlessness \(^{33}\).

A large population-based registry study assessing the development of neurological sequelae in 5,680 children born after IVF, aged 18 months to 14 years, and 11,360 matched controls noted that IVF children have an almost fourfold increased risk of cerebral palsy and suspected developmental delay as compared with matched controls \(^{104}\). When singletons were studied separately and after correction for strong risk factors, like low birth weight, low gestational age, and sex, IVF still affected on the risks of these two neurological outcomes, although this was not significant. Further evidence of neurological problems after IVF has been provided by Ericson et al. \(^{33}\), describing statistically significantly increased risks of hospitalization for cerebral palsy (1.7) and epilepsy (1.5) among children born after IVF. Recently, this Swedish study has been extended to nearly twice the cohort size and a maximum follow-up time of 16 years \(^{52}\). The observed increased risks for cerebral palsy and epilepsy were related to the duration of unwanted childlessness. An increased risk of convulsions among children born after IVF was also shown, although independent of gestational age and subfertility.
Metabolic and endocrine profiles of 51 IVF prepubertal singletons born at term and 56 naturally conceived control children have been recently investigated. Consistent with the taller stature after correction for midparental height, children born after IVF were found to have higher serum levels of growth-stimulating hormones such as insulin-like growth factor I (IGF-I), IGF-binding protein-3, and IGF-II as compared with the naturally conceived infants. Furthermore, lower body mass index (BMI) values and a more favorable lipid profile with lower HDL cholesterol ratio and lower triglyceride levels were found among the children born after IVF. The investigators speculated that the phenotype and endocrine changes are due to alterations in imprinting of genes in the growth and metabolic axis.

Furthermore, Rojas-Marcos et al. highlighted the need for monitoring children born after IVF throughout childhood and into adolescence and adulthood to investigate whether pubertal development and fertility are influenced by in utero exposure to elevated sex steroids levels which have been found in ART pregnancies. In their case series, seven children between the ages of 5 and 21 months, who were conceived by ART, were referred for endocrine evaluation of possible precocious puberty.

In summary, because current follow-up studies regarding postnatal growth and morbidity rates are scarce with conflicting results and other areas of long-term research are still in its infancy, there is an urgent need for long-term follow-up of children born after IVF.

**Epigenetic defects**

Recently a biological mechanism called genomic imprinting and its potential link to IVF-related health problems has become a topic of major interest. Genomic imprinting, an inherited epigenetic form of gene regulation, has been increasingly recognized as one of the key determinants for normal intrauterine development. A significant number of imprinted genes appear to have important roles in embryonic/fetal growth and placental function. At imprinted loci, only one of the parental alleles is active, transcription of the inactive allele is repressed due to epigenetic marks by histone modification and/or cytosine methylation according to parental origin. A variety of control mechanisms promote imprint erasure in the primordial germ cells followed by remethylation and maintenance of imprints during gametogenesis and early embryonic development. Deregulation of imprinted genes has profound effects on fetal growth and development, varying from embryonic death to excessive, defective, or impaired growth. In addition, several human genetic syndromes, such as Beckwith-Wiedemann syndrome, Prader-Willi syndrome, and Angelman syndrome, are known to be caused by the disruption of genomic imprinting.
Recently published studies revealed a possible increased incidence of genomic imprinting disorders such as Beckwith-Wiedemann syndrome and Angelman syndrome among children conceived after ART. Cox et al. 21 and Orstavik et al. 88 reported three children who were conceived by ICSI and subsequently developed Angelman syndrome due to a sporadic imprinting defect on the maternal chromosome (Figure 3). Angelman syndrome, which is associated with severe mental retardation, motor defects, lack of speech, and happy disposition, is very rarely caused by such an imprinting defect (<5% of the Angelman syndrome cases) 21. Another classic imprinting defect called Beckwith-Wiedemann syndrome is characterized by a wide spectrum of symptoms including somatic overgrowth, congenital malformations and a predisposition to embryonic neoplasia. In only 50–60% of Beckwith-Wiedemann syndrome cases is an epigenetic defect rather than a mutation in the gene involved. During the past years, three Beckwith-Wiedemann syndrome registry studies demonstrated three- to sixfold increases in the frequency of ART infants in cohorts of individuals with Beckwith-Wiedemann syndrome 26, 40, 73 (Figure 3). The majority of those Beckwith-Wiedemann syndrome cases were linked to a loss of methylation. Subsequently, an Australian case control study identified IVF as the method of conception in 4 of the 37 Beckwith-Wiedemann syndrome cases (10.81%) and in 1 of the 148 matched controls (0.67%) (OR = 17.8, 95% CI 1.8, 432.9; P = 0.006) 41. By analyzing the proportions of Beckwith-Wiedemann syndrome diagnoses among the children born in the general population and the children born after IVF between 1983 and 2003, they estimated that children born after IVF are nine times more likely to have Beckwith-Wiedemann syndrome. These findings are not confirmed by Lidegaard et al. 68, who assessed the incidence rate of imprinting diseases and related disorders in IVF singletons (n = 6,052) and spontaneously conceived children (n = 442,349)
born in Denmark between 1995 and 2001. No specific imprinting diseases were reported among the children born after IVF. However, low numbers of imprinting diseases were also detected in the general population. For instance, no case of Beckwith-Wiedemann syndrome was detected among the naturally conceived children. A limitation of the study was the lack of specific diagnosis codes used by the national register for several imprinting diseases, including Angelman syndrome. Furthermore, according to the investigators, it is likely that many children with imprinting diseases were not recorded with the appropriate specific diagnosis code due to difficulties to diagnose an imprinting disease during infancy. The association between a third imprinting disorder known as Prader-Willi syndrome and ART has been recently studied. Although an increased frequency of ART in children with Beckwith-Wiedemann syndrome was confirmed, there was no significant association with Prader-Willi syndrome. However, Prader-Willi syndrome is mainly caused by paternal allele deletion and maternal uniparental disomy, whereas loss of methylation at a critical imprinting control region is suggested to be the molecular mechanism underlying the association between ART and imprinting defect such as Beckwith-Wiedemann syndrome and Angelman syndrome.

**Origins of imprinting abnormalities after IVF**

There is growing evidence that ART procedures could perturb the important epigenetic processes during the preimplantation period leading to altered growth and development. In livestock, the large offspring syndrome frequently observed after in vitro culture has been found to be associated with aberrant methylation and expression of the imprinted Igf2r gene. Large offspring syndrome has substantial phenotypical similarities with the Beckwith-Wiedemann syndrome in humans. Additional imprinted genes are likely to be implicated in the pathogenesis of large offspring syndrome. Shi and Haaf found increased rates of abnormal methylation patterns in mice embryos after superovulation and after the use of certain embryo culture media. In addition, several other studies using mouse models have demonstrated that genomic imprinting in preimplantation embryos can be disturbed by specific culture conditions, some with developmental consequences. For instance, the presence of fetal calf serum in culture medium not only affected the expression of imprinted genes at the blastocyst stage, but also resulted in postnatal overgrowth and behavioral alterations.

In view of the associations between superovulation, embryo culture, and imprinting defects in animal studies, the increased frequency of imprint perturbations observed among children born after IVF have been proposed to originate from artificial aspects of the IVF procedure. First, because the primary imprinting process occurs at a relatively late stage in oogenesis, gonadotropic hormones used during superovulation to mature many oocytes simultaneously may harmfully affect imprint acquisition in oocytes. Alternatively, oocytes that have not completed the imprinting process might be prematurely released, or oocytes of poor quality that would not have ovulated without treatment might mature. Second, the use of the different culture media in clinical practice,
as well as prolonged embryo culture to blastocyst stage on human embryos, might affect the imprinting process during the preimplantation period. Chang et al. 20 recently started unraveling this issue by questioning whether culture media can be implicated as a major determinant among individuals with Beckwith-Wiedemann syndrome conceived after ART. Definitive conclusions cannot yet be drawn and the need for large epidemiological studies to systematically assess the potential risk factors associated with imprinting defects after IVF has been expressed.

However, in addition to potential environmentally induced epigenetic alterations among children born after IVF, the possibility that genetic predisposition underlying subfertility also results in an increased frequency of imprinting defects among offspring cannot be ruled out 71. Clinical findings supportive to this hypothesis were recently provided by Ludwig et al. 70 who examined the correlation between infertility treatment and imprinting defects. The risk in untreated couples with time to pregnancy exceeding 2 years was identical to that of those treated by ICSI or by hormonal stimulation alone (RR = 6.25; 95% CI 0.70, 22.57). It was twice as high in couples who had received treatment and also had time to pregnancy >2 years (RR = 12.5; 95% CI 1.40, 45.13). They concluded that imprinting defects and subfertility might have a common cause and that superovulation rather than ICSI may further increase the risk of conceiving a child with an imprinting defect.

**Current implications**

An accumulating body of evidence indicates that children born after IVF are at increased risk for several types of health problems. Although the influence of underlying fertility problems of the parents is not clear yet, health problems observed after IVF might (partially) originate from adaptations of the developing conceptus to the IVF procedure. The exposure of a gamete or an embryo to the different phases of an IVF treatment (e.g. fertility drugs, in vitro culture) during a critical period of development could have long-lasting developmental consequences. It is of great importance that during the following decades postnatal developmental and health aspects of children born after IVF will continue to be monitored worldwide. Likewise, in view of the fetal origin hypothesis postulating that prenatal events irreversibly program metabolic processes in the fetus, which causes cardiovascular disease and type 2 diabetes in adult life, specific investigation of the long-term effects after IVF with regard to the development of these chronic diseases and related risk factors will be necessary in the near future.

In agreement with the “tip of the iceberg” theory, previously formulated by Maher et al. 72, perhaps the most important implication of the increased frequency of epigenetic disturbances observed among children born after IVF is that additional epigenetic alterations not yet being recognized to be associated with IVF might occur. In addition, given the essential function of imprinted genes in embryonic and placental growth and the major role of epigenetic changes in the pathogenesis of many pediatric diseases, it is tempting to speculate that adverse health outcomes observed
after IVF might have an epigenetic origin as well. To elucidate whether children born after IVF are at increased risk for environmentally induced epigenetic modifications during early prenatal development with long-lasting consequences in postnatal life and perhaps adult life, causal pathways between IVF-related health problems and early prenatal epigenetic programming should be investigated and unraveled. Finally, as transgerational inheritance of epigenetic alterations is possible when epigenetic modifications occurs shortly after fertilization but before specification of the germ line, a complete safety evaluation might even require studies from a two-generation perspective.
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Cardiometabolic differences in children born after in vitro fertilization: follow-up study

Manon Ceelen, Mirjam M. van Weissenbruch, Jan P.W. Vermeiden, Flora E. van Leeuwen, Henriette A. Delemarre-van de Waal
Abstract

Background: Increasing evidence suggests that adverse conditions during early prenatal life are associated with cardiometabolic dysfunction in postnatal life. In vitro fertilization (IVF) conception may be an early prenatal life event with long-term health consequences. Our objective was to investigate several cardiometabolic measures in 8- to 18-yr-old IVF singletons and spontaneously conceived controls born from subfertile parents.

Methods: This follow-up study was conducted at the VU University medical center, Amsterdam, The Netherlands. Blood pressure was examined in 225 IVF-conceived children and 225 age- and gender-matched spontaneously conceived control children. Several indicators of insulin resistance were studied in a pubertal subpopulation (131 IVF children and 131 controls).

Results: Systolic and diastolic blood pressure levels were higher in IVF children than controls (109 ± 11 vs. 105 ± 10 mm Hg, P < 0.001; and 61 ± 7 vs. 59 ± 7 mm Hg, P < 0.001, respectively). Children born after IVF were also more likely to be in the highest systolic and diastolic blood pressure quartiles (odds ratio = 2.1, 95% confidence interval 1.4, 3.3; odds ratio = 1.9, 95% confidence interval 1.2, 3.0, respectively). Furthermore, higher fasting glucose levels were observed in pubertal IVF children (5.0 ± 0.4 vs. 4.8 ± 0.4 mmol/liter in controls, P = 0.005). Blood pressure and fasting glucose differences could not be explained by current body size, birth weight, and other early life factors or by parental characteristics, including subfertility cause.

Conclusions: These findings highlight the importance of continued cardiometabolic monitoring of IVF-conceived children and might contribute to current knowledge about periconceptional influences and their consequences in later life.
Introduction

According to the "developmental origins of adult disease" hypothesis, adaptive responses to environmental stimuli during critical or sensitive periods in early life may have long-lasting consequences, due to permanent reprogramming of physiological, metabolic, and endocrine key systems. Specific critical windows in prenatal development for long-term programming of cardiovascular and metabolic dysfunction have been identified. In rats and sheep, maternal undernourishment solely during either the periconceptional or pre-implantation period induced irreversible programming of hypertension and cardiovascular dysfunction among offspring. Maternal undernutrition during the periconceptional period has also been associated with altered fetal metabolism in sheep. Furthermore, animal studies have shown that conditions during assisted reproductive technologies may interfere with normal programming of early development with subsequent postnatal developmental consequences, including aberrant cardiovascular physiology.

In humans, little is known about the effects of poor periconceptional and/or pre-implantation environment on postnatal cardiovascular and metabolic functioning. Concerns have recently been raised about the children born after subfertility treatment. Accumulating evidence suggests that in vitro fertilization (IVF) singletons are at increased risk for adverse perinatal outcome. It is still unclear whether the IVF process in humans could affect the vulnerable processes occurring during early embryonic development with long-term health consequences. Therefore, we studied postnatal growth and development in 8- to 18-yr-old children born from subfertile parents who were either successfully treated with IVF or conceived spontaneously. The main objective of the present study was to investigate blood pressure and indicators of insulin resistance in IVF and control children.

Subjects and methods

Study population

The OMEGA-study is a Dutch retrospective cohort study aimed to examine long-term health effects of hormone stimulation. The cohort consists of 26,428 women diagnosed with subfertility problems in one of the 12 IVF clinics between 1980 and 1995; 19,840 women received IVF treatment, and 6,588 women did not. Eligible women had not achieved conception after at least 1-yr frequent unprotected intercourse at their first visit to the fertility clinic. Risk factor questionnaires to the women and detailed data collection from the medical records provided information on the children born from the OMEGA participants up to 1996–1997. The questionnaire response rate was 73% among subfertile women with children. The present study was restricted to IVF and spontaneously conceived children born from OMEGA participants who were treated for subfertility in the VU University medical center (VUmc). IVF children born from women treated in the VUmc who did not participate in the OMEGA-study were also eligible for recruitment.
Approach of study subjects

From the 553 eligible singletons born after standard IVF treatment, we invited 95% of IVF children born between 1986 and 1991, 74% of IVF children born between 1992 and 1993, and 41% of IVF children born between 1994 and 1995 to achieve equal representation of all 1-yr age categories. For each participating IVF child, we searched one control child of the same gender and similar age (≤ 3-month age difference) born after spontaneous conception from subfertile parents. In case an approached control child did not want to participate, the control recruitment process was repeated until an appropriate control child was found that did agree to participate. Between 2003 and 2006, 354 IVF and 454 control children and their parents were informed by letter about our study on growth and development of IVF children (Figure 1). Finally, 69% of the approached IVF children and 51% of the approached controls agreed to participate, resulting in 233 matched pairs. Subsequently, pubertal children were recruited for additional research, including the collection of a fasting blood sample (female criteria of puberty: ≥ stage 2 breast development, male criteria: ≥ stage 2 genital development and/or testis volume ≥4 ml \(^{29}\)). Additional research was restricted to pubertal children only to avoid low participation rates, especially among the youngest children, due to the invasive character of these tests. In total, 80% of the pubertal children underwent a blood withdrawal. All children and their parents gave written informed consent to participate in the study.

Families who refused to participate in the study received a single questionnaire regarding health, education, and other characteristics of the respective child (n = 283). A total of 179 families (63%) returned the questionnaire. Nonparticipation analysis yielded no significant differences between participants and non-participants regarding children’s current height, weight, and body mass index (BMI). Nonparticipating children were older (12.9 ± 2.6 vs. 12.0 ± 2.6 yr, P = 0.002), and their mothers were less often highly educated (26 vs. 37%, P = 0.015), but these findings were observed in both the IVF and control population.

The study protocol was approved by the ethics committee of the VUmc and the National Medical Ethics Committee known as the “Centrale Commissie Mensgebonden Onderzoek” located in The Hague, The Netherlands.

Data collection

Systolic and diastolic blood pressure was measured twice at the nondominant arm in the sitting position using an automatic device with appropriate cuff size (Dinamap PRO 100; Criticon, Munich, Germany). The first measurement was performed after a 30- to 45-min interview and the second measurement within a few minutes after the first one. The mean of these two readings was used in analyses. Body weight and height were assessed to the nearest 0.1 kg and 0.1 cm using an electronic scale (SECA, Hanover, MD) and a stadiometer (Holtain Ltd., Crymych, Dyfed, UK),
respectively. Skinfold thickness measurements (triceps, biceps, subscapular, and suprailiac) were collected by a Harpenden caliper (Harpenden, West Sussex, UK). Other body fat measures have been reported elsewhere.

Blood samples were drawn between 0900 and 1000 h after an overnight fast. Fasting glucose and insulin were determined using the Gluco-quant Glucose/HK, Roche assay kit (Roche Diagnostics GmbH, Mannheim, Germany) and Bayer/ACS Centaur immunoassay (Bayer Diagnostics, Mijdrecht, The Netherlands), respectively. The glucose to insulin ratio and the homeostasis assessment model (HOMA) were chosen as measures of insulin sensitivity. HOMA insulin resistance and β-cell function were calculated according the original formula \(^2\). Laboratory measurements were performed at the Department of Clinical Chemistry of the VUmc.
Before the follow-up visit in the VUmc, a questionnaire was sent to the parents to gather information on various demographical, lifestyle, and medical factors, including the cause of subfertility, parental education level, maternal smoking during pregnancy, and birth weight and gestational age of the respective child. Maternal BMI and highest level of education completed by either parent were used as indicators of socioeconomic conditions. Information about drug use of the child and family history of disease in terms of diabetes type 2, cardiovascular disease, and hypertension among parents and grandparents was obtained by an interview. None of the children used medication that could have affected blood pressure. Birth weight, either extracted from VUmc birth certificates (49%) or outpatient clinic reports (38%), or self-reported by the parents (13%), was expressed as the SD score (SDS) to correct for gestational age and gender.

Statistical analysis

After exclusion of eight matched pairs due to missing blood pressure measurements, data of 225 IVF-control pairs and data of the subsequent pubertal subset consisting of 131 unmatched IVF children and 131 controls were analyzed (Figure 1). Differences between IVF-control pairs were tested using the paired t test for continuous variables and McNemar’s test for dichotomous variables. Metabolic data of pubertal IVF and control children were compared after correction for age and gender. Logistic regression analyses were performed to estimate crude odds ratios for being in the highest quartile of several outcome parameters associated with IVF conception. Furthermore, potential confounders of the association between blood pressure and indicators of insulin sensitivity on the one hand and IVF conception on the other hand were examined separately by regression analysis (e.g. gender, current weight, birth weight, gestational age, parity, maternal smoking during pregnancy, parental education, parental age, maternal BMI, subfertility cause, and family history of disease). Factors that changed the crude difference in outcome between IVF and control children with more than 10% were considered as confounders and included in the final regression model. Reported P values were based on two-sided tests of significance.

Results

Perinatal and follow-up characteristics of the study population are shown in Table 1. Birth weight, birth weight SDS, and gestational age were significantly lower in children conceived by IVF compared with controls. Age at follow-up of IVF and control children was 12.3 ± 2.6 yr. Both systolic and diastolic blood pressures were higher in IVF children (109 ± 11 vs. 105 ± 10 mm Hg in controls, P < 0.001; and 61 ± 7 vs. 59 ± 7 mm Hg in controls, P < 0.001, respectively). Furthermore, IVF children were 2.1 times more likely to be in the highest systolic blood pressure quartile (≥ 114.5 mm Hg) and 1.9 times more likely to be in the highest diastolic blood pressure quartile (≥ 65.5 mm Hg) than controls (highest quartile vs. lowest three quartiles: 95% confidence interval [CI] 1.4, 3.3; 95% CI 1.2, 3.0, respectively).
Table 1  Perinatal and follow-up characteristics of the study population

<table>
<thead>
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<th>Controls</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>225</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.22 ± 0.63</td>
<td>3.44 ± 0.54</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birth weight SDS (a)</td>
<td>−0.16 ± 1.00</td>
<td>0.09 ± 1.07</td>
<td>0.02</td>
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<tr>
<td>Gestational age (wks)</td>
<td>38.9 ± 2.5</td>
<td>39.6 ± 1.8</td>
<td>0.002</td>
</tr>
<tr>
<td>No. of preterm infants (%)(b)</td>
<td>29 (13%)</td>
<td>13 (6%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

| Anthropometry and blood pressure |         |
| No. of subjects                 | 225      |
| Age (yr)                        | 12.3 ± 2.6 |
| Gender (% male)                 | 49       |
| Height (cm)                     | 156.4 ± 15.0 |
| Body weight (kg)                | 47.8 ± 16.0 |
| BMI (kg/m²) \(c\)              | 19.1 ± 3.6 |
| Sum of skinfolds (mm)           | 40.5 ± 20.4 |
| Systolic blood pressure (mm Hg) | 109 ± 11  |
| Systolic blood pressure: quartiles |        |
| ≤ 99.5 mm Hg                    | 44 (20%) | 72 (32%) | 0.001 |
| 100.0–106.0 mm Hg               | 50 (22%) | 59 (26%) |
| 106.5–114.0 mm Hg               | 59 (26%) | 53 (24%) |
| ≥ 114.5 mm Hg                   | 72 (32%) | 41 (18%) |
| Diastolic blood pressure (mm Hg)| 61 ± 7   |
| Diastolic blood pressure: quartiles |       |
| ≤ 54.5 mm Hg                    | 42 (19%) | 63 (28%) | 0.01  |
| 55.0–59.5 mm Hg                 | 58 (26%) | 65 (29%) |
| 60.0–65.0 mm Hg                 | 56 (25%) | 55 (24%) |
| ≥ 65.5 mm Hg                    | 69 (31%) | 42 (19%) |
| Heart rate (beats per minute)   | 74 ± 11  |

| Fasting glucose and insulin    |         |
| No. of subjects \(d\)         | 131      |
| Fasting glucose (mmol/l)       | 5.0 ± 0.4 |
| Fasting glucose: quartiles     |         |
| ≤ 4.6 mmol/l                   | 21 (16%) | 39 (30%) | 0.05  |
| 4.7–4.9 mmol/l                 | 43 (33%) | 36 (28%) |
| 5.0–5.1 mmol/l                 | 25 (19%) | 26 (20%) |
| ≥ 5.2 mmol/l                   | 41 (32%) | 30 (23%) |
| Fasting insulin (pmol/l) \(e\) | 47.5 (33.0–69.2) | 47.2 (34.2–63.6) | 0.58  |
| Glucose-insulin ratio \(f\)    | 0.10 (0.07–0.15) | 0.11 (0.08–0.14) | 0.88  |
| HOMA-insulin resistance \(g\)  | 1.8 (1.2–2.6)  | 1.8 (1.2–2.3)  | 0.35  |
| HOMA-beta cell function \(h\)  | 110.4 (77.6–151.4) | 117.3 (81.7–164.2) | 0.24  |

Data represent mean ± SD, percentages or \(\#\) median (25th–75th percentile).

\(a\) Birth weight SDS is a measure of birth weight corrected for gestational age and gender using a reference population \(24\).

\(b\) Premature birth was defined as birth occurring before 37-wk gestation.

\(c\) BMI was defined as weight divided by height squared.

\(d\) Metabolic data were only available for children who participated in the pubertal substudy; these unmatched data were corrected for age and gender.
Table 2  Differences in blood pressure (mm Hg) and fasting glucose (mmol/l) between IVF children and control children after adjustment for current risks factors, early life factors, and parental characteristics

<table>
<thead>
<tr>
<th>Model adjustment for the following potential confounders</th>
<th>Systolic blood pressure</th>
<th></th>
<th></th>
<th>Diastolic blood pressure</th>
<th></th>
<th></th>
<th>Fasting glucose</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference (mm Hg)</td>
<td>95% CI</td>
<td>P-value</td>
<td>Difference (mm Hg)</td>
<td>95% CI</td>
<td>P-value</td>
<td>Difference (mmol/l)</td>
<td>95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>4.2</td>
<td>2.2, 6.2</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.1–3.6</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.04–0.22</td>
<td>0.005</td>
</tr>
<tr>
<td>Gender</td>
<td>4.2</td>
<td>2.2–6.2</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.1, 3.6</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.005</td>
</tr>
<tr>
<td>Current risk indicators</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current weight (kg)</td>
<td>3.9</td>
<td>2.0, 5.7</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.0, 3.6</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.006</td>
</tr>
<tr>
<td>Current height (cm)</td>
<td>4.2</td>
<td>2.3, 6.0</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.1, 3.6</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>4.0</td>
<td>2.1, 5.9</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.0, 3.6</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.007</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td>3.7</td>
<td>1.8, 5.7</td>
<td>&lt; 0.001</td>
<td>2.1</td>
<td>0.8, 3.4</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.007</td>
</tr>
<tr>
<td>Early life factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.5</td>
<td>1.5, 5.5</td>
<td>0.001</td>
<td>2.1</td>
<td>0.8, 3.4</td>
<td>0.002</td>
<td>0.13</td>
<td>0.03, 0.22</td>
<td>0.007</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>3.6</td>
<td>1.6, 5.6</td>
<td>&lt; 0.001</td>
<td>2.1</td>
<td>0.8, 3.4</td>
<td>0.002</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.007</td>
</tr>
<tr>
<td>Primiparity (Y/N)</td>
<td>3.8</td>
<td>1.7, 5.9</td>
<td>0.001</td>
<td>1.8</td>
<td>0.5, 3.2</td>
<td>0.009</td>
<td>0.13</td>
<td>0.03, 0.23</td>
<td>0.010</td>
</tr>
<tr>
<td>Maternal smoking during pregnancy (Y/N)</td>
<td>3.8</td>
<td>1.8, 5.8</td>
<td>&lt; 0.001</td>
<td>2.2</td>
<td>0.9, 3.5</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.006</td>
</tr>
<tr>
<td>Parental characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subfertility cause a</td>
<td>3.9</td>
<td>1.8, 6.0</td>
<td>&lt; 0.001</td>
<td>2.2</td>
<td>0.9, 3.6</td>
<td>0.001</td>
<td>0.11</td>
<td>0.02, 0.21</td>
<td>0.022</td>
</tr>
<tr>
<td>Parental educational level b</td>
<td>4.1</td>
<td>2.1, 6.1</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.0, 3.6</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.03, 0.22</td>
<td>0.008</td>
</tr>
<tr>
<td>Maternal age at follow-up (yr)</td>
<td>4.1</td>
<td>2.2, 6.1</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.1, 3.6</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.04, 0.23</td>
<td>0.005</td>
</tr>
<tr>
<td>Paternal age at follow-up (yr)</td>
<td>4.2</td>
<td>2.2, 6.2</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.0, 3.6</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.005</td>
</tr>
<tr>
<td>Maternal BMI at follow-up (kg/m²)</td>
<td>4.0</td>
<td>2.0, 6.0</td>
<td>&lt; 0.001</td>
<td>2.2</td>
<td>0.9, 3.5</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.006</td>
</tr>
<tr>
<td>Family history of diabetes type 2 (Y/N) c</td>
<td>4.3</td>
<td>2.3, 6.3</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.0, 3.6</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04, 0.22</td>
<td>0.006</td>
</tr>
<tr>
<td>Family history of hypertension (Y/N) c</td>
<td>4.4</td>
<td>2.4, 6.5</td>
<td>&lt; 0.001</td>
<td>2.4</td>
<td>1.0, 3.7</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.04, 0.23</td>
<td>0.004</td>
</tr>
<tr>
<td>Family history of cardiovascular disease (Y/N) c</td>
<td>4.3</td>
<td>2.3, 6.4</td>
<td>&lt; 0.001</td>
<td>2.3</td>
<td>1.0, 3.6</td>
<td>0.001</td>
<td>0.13</td>
<td>0.04, 0.23</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Each row represents a separate regression analysis. N, No; Y, yes.

* Subfertility was categorized as female subfertility (tubal factors, endometriosis, ovarian disorders, cervical factors, and uterine abnormalities), male subfertility, subfertility caused by both parents, or unexplained subfertility. In 14 cases the cause of subfertility was missing. * Highest level of education completed by either parent was categorized as low (primary school, low occupational training), medium (high school, medium occupational training) and high (high occupational training, university). * Family history of diabetes type 2, hypertension, and cardiovascular disease was considered positive if any of the parents or grandparents was reported to suffer from this type of disease. Data on the family history of disease of 14 children were missing.
Table 3 Differences in blood pressure (mm Hg) and fasting glucose (mmol/l) between IVF children and control children after adjustment for confounders: multivariate analysis

<table>
<thead>
<tr>
<th>Multivariate models</th>
<th>B</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP difference (mm Hg) after adjustment for birth weight, gestational age, and sum of skinfolds</td>
<td>3.0</td>
<td>1.1, 5.0</td>
<td>0.003</td>
</tr>
<tr>
<td>DBP difference (mm Hg) after adjustment for birth weight, gestational age, parity, and sum of skinfolds</td>
<td>1.4</td>
<td>0.03, 2.8</td>
<td>0.046</td>
</tr>
<tr>
<td>Glucose difference (mmol/l) after adjustment for subfertility cause</td>
<td>0.11</td>
<td>0.02, 0.21</td>
<td>0.02</td>
</tr>
</tbody>
</table>

DBP, Diastolic blood pressure; SBP, systolic blood pressure.

IVF children had a significantly higher sum of skinfolds compared with controls (40.5 ± 20.4 vs. 36.9 ± 17.5 mm, P = 0.04). In addition, higher fasting glucose levels were observed in IVF children (5.0 ± 0.4 vs. 4.8 ± 0.4 mmol/liter in controls, P = 0.005). IVF-conceived children were 2.5 times more likely to be in the highest fasting glucose quartile (≥ 5.2 mmol/liter) than controls (highest quartile vs. lowest quartile: 95% CI: 1.2, 5.2). No significant differences in fasting insulin concentrations, insulin resistance measures, height, weight, and BMI were found between both study groups.

Influences of potentially confounding factors on the difference in blood pressure and fasting glucose levels between IVF children and controls are shown in Table 2. The systolic blood pressure difference was predominantly affected by birth weight, gestational age, and sum of skinfolds, whereas the diastolic blood pressure difference was also influenced by parity. By contrast, subfertility cause was the main factor that substantially changed the fasting glucose difference between IVF and control children. Multivariate regression analysis demonstrated that blood pressure and fasting glucose levels in IVF children remained significantly increased after controlling for the relevant confounding factors simultaneously (Table 3).

Discussion

This is the first follow-up study investigating blood pressure levels and several indicators of insulin resistance in 8- to 18-yr-old IVF and control children born from subfertile parents. Significant differences in both systolic and diastolic blood pressure, as well as in fasting glucose levels were found among IVF children compared with controls. These differences could neither be explained by current risk indicators, early life factors, nor by parental characteristics, including subfertility cause.

In clinical practice, the 3- to 4-mm Hg higher systolic blood pressure and the 1- to 2-mm Hg higher diastolic blood pressure in IVF children may seem like small increases, but at a population level, these differences might have a major impact on public health. A slight increase in blood pressure is associated with a remarkably increased risk of developing cardiovascular disease. For instance,
lowering mean systolic blood pressure in adults by 2 mm Hg corresponds to an 8% reduction in the risk of stroke. Furthermore, it cannot be excluded that increased blood pressure after IVF may be amplified throughout life because blood pressure is known to track from childhood into adult life.

Over the past years, cardiovascular developmental consequences and potentially underlying mechanisms after environmental manipulation during early prenatal development have been documented in both human and animal studies. The Dutch famine study demonstrated that exposure to malnutrition during early pregnancy is associated with an increased risk of coronary heart disease in adult life. Periconceptional undernutrition has been associated with the precocious activation of the hypothalamo-pituitary-adrenal (HPA) axis. Gardner et al. recently reported minor influences on HPA axis function in young adult sheep after periconceptional undernutrition. It has been suggested that the early activation of the HPA axis may not only lead to inappropriate elevation of prostaglandin levels and early birth but may also be associated with further programming effects due to inappropriate exposure of the fetus to glucocorticoids. Other targets, like the renin-angiotensin system and the sympathoadrenal axis, have also been associated with developmental origins of nutritional or other influences on cardiovascular function. Due to the complexity of the cardiovascular system, it is unlikely that the relation between periconceptional insults and postnatal cardiovascular dysfunction originates from one single cause. Early prenatal developmental plasticity in relation to environmental stimuli has been reported to lead to changes in fetal development through changes in imprinted gene expression, nutrient and stress-related signaling pathways, or cell cycle and apoptotic rates. Further research is necessary to investigate the role of these pathways in the development of cardiovascular dysfunction after periconceptional insults. In addition, in view of the present study, it remains to be elucidated whether increased blood pressure among IVF children originates from early prenatal life adaptations mediated through neuroendocrine pathways related to the HPA axis and/or through one of the unidentified mechanisms. However, increased blood pressure levels in IVF children were to a large extent independent of birth weight, suggesting that the underlying mechanisms can modify the cardiovascular system even without affecting size at birth. This is in line with previous studies examining associations between early life factors and blood pressure among offspring. It is important to realize that birth weight is just a proxy for fetal growth. Prenatal environmental insults that may affect embryonic and/or fetal growth trajectories can result in altered postnatal physiology without an effect on birth weight. Similarly, exposure to early prenatal life effects may even induce developmental adaptations in organ development and function that are not accompanied by changes in fetal growth characteristics.

Although exposure to adverse prenatal conditions, especially during late gestation, has been linked to decreased glucose tolerance in adults, it is unclear whether early prenatal insults in humans can influence postnatal glucose metabolism. Fasting glucose levels studied in the present study are within the normal range, and the difference in fasting glucose levels between pubertal IVF children and controls is small, not accompanied by differences in fasting insulin levels and other related measures.
However, in view of the observed differences in blood pressure and body fat composition between IVF children and controls, considerable research is necessary to investigate the hypothesis that further changes in glucose metabolism might manifest in later life. Gold-standard assessments, i.e. euglycemic hyperinsulinemic clamp tests and hyperglycemic clamp tests, will be useful to additionally investigate insulin sensitivity and β-cell capacity in IVF-conceived offspring.

When interpreting our results, the strengths and limitations of our study need to be considered. The strengths of our study include the relatively large study size and the comparison group consisting of spontaneously conceived children born from subfertile parents. Furthermore, we collected various variables related to blood pressure and metabolism that provided the opportunity to examine blood pressure and fasting glucose differences between IVF children and controls while adjusting for these potentially confounding factors. It is possible that we have underestimated the true association between IVF and outcome parameters by adjusting for intermediate factors (e.g. sum of skinfolds in case of evaluated blood pressure). In addition, the slight attenuation of the blood pressure differences by adjusting for birth weight and gestational age was to be expected. IVF is known to be associated with lower birth weight and shorter gestational age 12, 13, whereas these factors themselves have been found to increase blood pressure. Another limitation is potential selection bias because our study was based on 56% (n = 450) of the total number of subjects approached (n = 808). However, nonparticipation analysis yielded no significant differences between participants and nonparticipants in anthropometric measures. The main reason for nonparticipation was unwillingness of the child to participate. In addition, it cannot be excluded that the degree of subfertility in those who conceived after IVF was more severe compared with those who conceived spontaneously and that this difference in subfertility contributed to the physiological abnormalities observed in IVF offspring. Nevertheless, most control mothers who participated in the present study were diagnosed with subfertility in an era (1982–1990) when IVF was not a routine procedure and was not ethically acceptable to many women. Moreover, estimated differences in blood pressure between IVF and control children were hardly affected by adjustment for parental subfertility causes, rendering residual confounding very unlikely. Similarly, the fasting glucose difference between IVF and control children remained statistically significant after adjustment for subfertility cause.

In conclusion, increased blood pressure and fasting glucose levels among IVF children could not be explained by current risk indicators, early life factors, and parental characteristics. Although underlying mechanisms are largely elusive, the periconceptional period of IVF-conceived children might be a critical time window during which cardiometabolic function can be perturbed. Before definitive conclusions can be drawn, our results need to be reproduced by other prospective follow-up studies. Nevertheless, our findings underscore the importance of the continuing worldwide monitoring of postnatal development of IVF children and contribute to the current understanding of periconceptional exposure effects with regard to the development of both short- and long-term consequences in humans.
References


Body composition in children and adolescents born after in vitro fertilization or spontaneous conception

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Flora E. van Leeuwen, Henriette A. Delemarre-van de Waal

Abstract

Background: Increasing evidence suggests that adverse conditions during prenatal life are associated with the development of chronic diseases in adult life. It is still unclear whether in vitro fertilization (IVF) could affect the vulnerable developmental processes in humans occurring during early prenatal development with long-term perturbations of developmental pathways. Our objective was to examine body composition in 8- to 18-yr-old IVF singletons and spontaneously conceived controls born from subfertile parents.

Methods: This follow-up study was conducted at the VU University medical center in Amsterdam, The Netherlands. Body composition measures were assessed by anthropometry in 233 IVF children and 233 age- and gender-matched control children. Dual energy X-ray absorptiometry (DXA) was used in a pubertal subpopulation (139 IVF children and 143 controls).

Results: IVF children had a significantly lower subscapular-triceps skinfold ratio and a significantly higher sum of peripheral skinfolds, peripheral body mass, and percentage of peripheral body fat as compared with controls. Although not reaching statistical significance, both DXA and skinfold measurements suggested that total body fat in IVF children is increased. Neither current and early risk factors nor parental factors such as subfertility cause could explain the differences in peripheral fat assessed by anthropometry between IVF children and controls. No differences in bone mineral composition between IVF children and controls were found.

Conclusions: Our observations indicate that body fat composition in IVF children is disturbed. Follow-up of IVF children to monitor body fat pattern and potentially related health problems from adolescence into adulthood is of great importance.
Body composition in IVF children

Introduction

In view of the rising public health problems with regard to obesity and osteoporosis in adult life, the need to elicit determinants of body fat composition and skeletal architecture is compelling. Accumulating evidence indicates that environmental influences during prenatal life are associated with changes in adult body composition including altered fat distribution and low bone mineral content (BMC) \textsuperscript{22, 30, 31}. These associations are thought to be the consequence of programming of physiological, metabolic, and endocrine key systems whereby adaptive responses to environmental stimuli during critical or sensitive periods in early life may have long-lasting consequences \textsuperscript{1}.

The period around fertilization appears to be one of the critical time windows during which the developing conceptus is susceptible to environmentally induced changes. It has been demonstrated in animal models that poor periconceptional and preimplantational conditions can disturb both prenatal and postnatal developmental potential \textsuperscript{23}. It is still largely unclear whether developmental trajectories such as skeletal development and adipogenesis can irreversibly be perturbed by periconceptional insults. Nevertheless, the Dutch famine study demonstrated that exposure to undernutrition in early pregnancy is associated with an increased risk for obesity in adult life \textsuperscript{27, 28}. Periconceptional undernutrition was also found to increase fetal adiposity in sheep twins, suggesting the importance of environmental influences during early prenatal life for adipose tissue development \textsuperscript{6}. The number of children born after in vitro fertilization (IVF) treatment is steadily growing as nowadays approximately 1–3\% of the current births in developed countries are established after IVF \textsuperscript{20}. Growing and convincing evidence suggests that IVF children are at increased risk of low birth weight, preterm birth, and perinatal death \textsuperscript{13, 15}. Several animal studies demonstrated that embryo manipulation techniques are linked to long-term alterations in the characteristics of fetal and postnatal growth and development \textsuperscript{7, 19}. Intriguingly, embryo culture conditions during the preimplantation period in mice were found to have detrimental influences on body mass and adiposity in adult progeny \textsuperscript{32}. It is still unclear whether the IVF process in humans could affect the vulnerable developmental processes occurring during early prenatal development with long-term perturbations of developmental pathways. Therefore, we investigated postnatal growth and development in 8- to 18-yr-old children born from subfertile parents who were either successfully treated with IVF or conceived spontaneously after all. In the current study, we examined whether postnatal body composition is influenced by method of conception by measuring bone mass, bone density, body fat mass, and lean mass using dual energy X-ray absorptiometry (DXA) and anthropometry in IVF children and control children. Based on the above-mentioned studies regarding prenatal programming of body composition, we hypothesized that IVF children may be at increased risk for elevated body fat mass and low bone mass.
Subjects and methods

Study population

The OMEGA-study is a Dutch retrospective cohort study aimed to examine long-term health effects of hormone stimulation. The cohort consists of 26,428 women diagnosed with subfertility problems in one of the 12 IVF clinics between 1980 and 1995; 19,840 women received IVF treatment and 6,588 women did not. Eligible women had not achieved conception after at least 1 year of frequent unprotected intercourse at the time of their first visit to the fertility clinic. Risk factor questionnaires to the women and detailed data collection from the medical records provided information on the children born from the OMEGA-participants up to 1996–1997. The questionnaire response rate was 73% among subfertile women with children. The present study was restricted to IVF and spontaneously conceived children born from OMEGA-participants who were treated for subfertility in the VU medical center (VUmc). IVF children born from women treated in the VUmc who did not participate in the OMEGA-study were also eligible for recruitment.

From the 553 eligible singletons born after standard IVF treatment, we invited 95% of IVF children born between 1986–1991, 74% of IVF children born between 1992–1993, and 41% of IVF children born between 1994–1995 to achieve equal representation of all 1-yr age categories. For each participating IVF child, one spontaneously conceived child of similar gender and age (≤ 3 months age difference) born from subfertile parents was searched. In case this control child did not want to participate, the control recruitment process was repeated until an appropriate control child was found who agreed to participate. The study protocol was approved by the ethics committee of the VUmc and by the National Medical Ethics Committee known as the “Centrale Commissie Mensgebonden Onderzoek” located in The Hague, The Netherlands.

Approach of eligible study subjects

Between March 2003 and March 2006, children and their parents were informed by letter about our study on growth and development of IVF children (n = 354 IVF children and n = 454 control children). By means of a reply form and a pre-stamped envelope, parents were able to inform us whether they were willing to participate in our study. Address information of the families was checked and/or obtained using extensive tracing techniques. After 4–8 wk, nonresponders were approached by telephone. Inclusion results are summarized in Figure 1. In total, 72% of the IVF responders (n = 246) and 55% of the control-responders (n = 233) agreed to participate, resulting in 233 matched pairs. Children and their parents gave written informed consent to participate in the study. Those children who were in the pubertal stage were recruited for additional research including DXA measurements (female criteria for puberty: ≥ stage 2 of breast development, male criteria: ≥ stage 2 of genital development and/or testis volume ≥ 4 ml, assessed according to Tanner et al. 34). In total, 85% of the pubertal children underwent DXA scanning.
Figure 1  Overview of the inclusion process and study population

Families who refused to participate in the study received a single questionnaire regarding health, education, and other characteristics of the respective child (n = 283). Nonparticipation analysis yielded no significant differences between participants and nonparticipants regarding children's current height, weight, and body mass index (BMI). On average, non-participating children were significantly older (12.9 ± 2.6 vs. 12.0 ± 2.6 yr, P = 0.002) and their mothers were less often highly educated (26 vs. 37%, P = 0.015), but these differences were similar in the IVF and control population.

Data collection and measurements

Height to the nearest 0.1 cm and body weight to the nearest 0.1 kg were measured using a stadiometer with children dressed only in underwear. From these measurements, the BMI was calculated as weight divided by height squared (kilograms per square meter). SD scores (SDS) for weight, height, and BMI were calculated using a Dutch reference population 9, 10. Skinfold thickness measurements (triceps, biceps, subscapular, and suprailiac) were collected in triplicate on the nondominant side of the body by means of a Harpenden caliper. Coefficients of variation for repeated skinfold measurements at the different locations were 3.4, 4.3, 2.6, and 3.8%, respectively.
The sum of the triceps and biceps skinfold was used as a measure of peripheral adiposity, the sum of the subscapular and supra-iliac as an index of truncal adiposity, and the subscapular to triceps skinfold ratio was calculated as an measure of truncal to peripheral adiposity. The sum of the four measured skinfold thicknesses was used as an index of total adiposity. Waist circumference was measured using a tape measure. Pubertal maturity was assessed using breast developmental stages or genital developmental stages according to Tanner. The majority (94%) of the anthropometric measurements were performed by one observer (MC).

BMC (grams) and bone mineral density (BMD, grams per square centimeter) of the L1-L4 region of the lumbar spine, the nondominant side of the femur (femoral neck, femoral trochanter, and femoral intertrochanter, separately, and combined as total hip) and the total body were determined using the Hologic QDR-4500 bone densitometer operated in the fan beam mode (Hologic Inc., Waltham, MA). Body fat and lean mass measures were estimated from the total body scan to investigate body fat patterning. Body regions were delineated with the use of specific anatomical landmarks. All scans were analyzed using Hologic software version 12.3 and were subsequently evaluated by a single blinded investigator (JB).

Information regarding various demographic, lifestyle, and medical factors was obtained by questionnaire. Birth weight was either extracted from birth certificates of the VUmc (49%), or outpatient clinic reports (37%), or self-reported by the parents (14%) and were expressed as SDS to correct for gestational age and gender. Socioeconomic status was defined as the highest level of education completed by either parent, categorized as low (primary school, low occupational training), medium (high school, medium occupational training), and high (university, high occupational training). Other relevant outcomes, such as blood pressure levels, have been reported elsewhere.

**Statistical analysis**

In the present study, characteristics of 233 matched IVF control pairs were compared using the paired t-test for continuous variables and the McNemar test for dichotomous variables (SPSS version 12.0; SPSS Inc., Chicago, Illinois). Differences in DXA measures between the unmatched pubertal subpopulations, consisting of 139 IVF children and 143 control subjects, were compared after correction for age and gender. Odds ratios associated with method of conception for being in the highest quartile of sum of peripheral skinfolds and in the lowest quartile of subscapular-triceps skinfold ratio were estimated by logistic regression analysis. Furthermore, linear regression analysis was carried out to explore the relation between method of conception and postnatal body composition measures after correction for current risk factors (age, sex, pubertal stage, and height), early life factors (maternal smoking during pregnancy, birth weight, and gestational age), and parental factors (parental education, maternal BMI at follow-up, and subfertility cause). The square root of height was used to adjust for body size as suggested by Van Itallie et al. Skewed-distributed variables were log-transformed before analysis. P-value of < 0.05 was considered to be statistically significant, based on two-sided testing.
Results

Birth weight, birth weight SDS, and gestational age were significantly lower in children conceived by IVF compared with controls (3.2 ± 0.6 vs. 3.4 ± 0.6 kg, P < 0.001; –0.15 ± 1.00 vs. 0.08 ± 1.08, P = 0.025; 38.9 ± 2.5 vs. 39.5 ± 1.8 wks, P = 0.004, respectively). Age at follow-up of IVF children and controls was 12.2 ± 2.6 years. Table 1 presents various fat and lean mass measures of the IVF children and controls. IVF children had a significantly higher sum of peripheral skinfolds (21.9 ± 10.4 vs. 19.7 ± 8.9 mm, P = 0.014) and a significantly lower subscapular-triceps skinfold ratio as compared with control children (0.72 ± 0.22 vs. 0.77 ± 0.25, P = 0.010). Likewise, children born after IVF treatment were 1.8 times more likely to be in the highest quartile of peripheral skinfold sum (≥ 25 mm) than control children (95% CI: 1.1, 3.1). Similarly, IVF-conceived children were 1.9 times more likely to be in the lowest subscapular-triceps skinfold ratio quartile (≤ 0.57) as compared with controls (95% CI: 1.2, 3.3). Total sum of skinfolds appeared to be higher in IVF children, although this observation did not reach statistical significance (40.3 ± 20.3 vs. 37.1 ± 17.5 mm, P = 0.054). No differences in other anthropometric measurements such as height, weight, BMI, pubertal Tanner stage, and central fat measures between IVF children and control children were found.

Comparison of fat and lean mass measures assessed by DXA in the pubertal subset of children demonstrated that peripheral body fat mass and percentage of peripheral body fat were significantly higher in IVF children (7.59 ± 4.22 vs. 6.69 ± 3.15 kg, P = 0.039; 27.5 ± 9.0 vs. 25.8 ± 8.3%, P = 0.030, respectively) (Table 2). Absolute peripheral lean mass did not differ between IVF children and controls, whereas percentage of peripheral lean mass was significantly lower among IVF children (69.0 ± 8.7 vs. 70.5 ± 8.0 %, P = 0.023). No differences in central fat and lean mass measures were found between IVF and control children. Although not statistically significant, IVF children had a different total body fat mass, percentage of total body fat, and percentage of total body lean mass compared with controls. Linear regression analysis was used to investigate whether differences in body fat composition between IVF children and controls could be explained by current risk factors, early life factors and/or parental factors (Table 3). After correction for these potential confounding factors, differences in subscapular-triceps skinfold ratio and sum of peripheral skinfolds between IVF children and controls remained statistically significant. Total sum of skinfolds and peripheral and total body fat mass assessed by DXA seemed to be increased in IVF children after correction for potentially confounding variables, although these differences did not reach statistical significance. Variation in central body fat measures was predominantly explained by maternal smoking during pregnancy, gender, and/or height. Pearson correlation analyses demonstrated that skinfold measurements and DXA were highly correlated (sum of peripheral skinfolds and DXA peripheral body fat: r = 0.868, P < 0.001; sum of central skinfolds and DXA central body fat: r = 0.927, P < 0.001; and total sum of skinfolds and DXA total body fat: r = 0.921, P < 0.001).
Table 1  Body fat and lean mass measurements assessed by anthropometry at follow-up in IVF-conceived subjects and control subjects

<table>
<thead>
<tr>
<th></th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>233</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>Age at follow-up (yr)</td>
<td>12.2 ± 2.6</td>
<td>12.2 ± 2.6</td>
<td>0.323</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>49</td>
<td>49</td>
<td>1.000</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.1 ± 15.0</td>
<td>155.4 ± 15.6</td>
<td>0.297</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.17 ± 1.02</td>
<td>0.06 ± 1.01</td>
<td>0.232</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>47.5 ± 15.9</td>
<td>46.3 ± 14.7</td>
<td>0.158</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.28 ± 1.08</td>
<td>0.14 ± 1.05</td>
<td>0.162</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.0 ± 3.6</td>
<td>18.7 ± 3.2</td>
<td>0.238</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.28 ± 1.04</td>
<td>0.15 ± 1.09</td>
<td>0.234</td>
</tr>
<tr>
<td>Pubertal stage 1</td>
<td>62 (27%)</td>
<td>67 (29%)</td>
<td>0.628</td>
</tr>
<tr>
<td>Pubertal stage 2</td>
<td>52 (23%)</td>
<td>48 (21%)</td>
<td></td>
</tr>
<tr>
<td>Pubertal stage 3</td>
<td>25 (11%)</td>
<td>28 (12%)</td>
<td></td>
</tr>
<tr>
<td>Pubertal stage 4</td>
<td>43 (19%)</td>
<td>47 (20%)</td>
<td></td>
</tr>
<tr>
<td>Pubertal stage 5</td>
<td>49 (21%)</td>
<td>41 (18%)</td>
<td></td>
</tr>
<tr>
<td><strong>Peripheral measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of peripheral skinfolds (mm)</td>
<td>21.9 ± 10.4</td>
<td>19.7 ± 8.9</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Central measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of truncal skinfolds (mm)</td>
<td>18.5 ± 10.7</td>
<td>17.3 ± 9.6</td>
<td>0.190</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>66.6 ± 9.0</td>
<td>66.1 ± 8.8</td>
<td>0.449</td>
</tr>
<tr>
<td><strong>Central-peripheral measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscapular-triceps skinfold ratio</td>
<td>0.72 ± 0.22</td>
<td>0.77 ± 0.25</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Total body measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sum skinfolds (mm)</td>
<td>40.4 ± 20.3</td>
<td>37.1 ± 17.5</td>
<td>0.054</td>
</tr>
</tbody>
</table>

Data represent mean ± SD unless indicated otherwise.

* Continuous variables were analyzed using paired t-test; dichotomous variables were analyzed using McNemar test.
Table 2  Body fat and lean mass measurements assessed by DXA at follow-up in pubertal IVF-conceived subjects and control subjects

<table>
<thead>
<tr>
<th></th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>136</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Age at follow-up (yr)</td>
<td>13.7 ± 2.1</td>
<td>13.5 ± 2.1</td>
<td>0.634</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>48</td>
<td>48</td>
<td>0.913</td>
</tr>
<tr>
<td>Pubertal stage 2</td>
<td>34 (25%)</td>
<td>36 (25%)</td>
<td>0.557</td>
</tr>
<tr>
<td>Pubertal stage 3</td>
<td>19 (14%)</td>
<td>27 (19%)</td>
<td></td>
</tr>
<tr>
<td>Pubertal stage 4</td>
<td>39 (29%)</td>
<td>43 (30%)</td>
<td></td>
</tr>
<tr>
<td>Pubertal stage 5</td>
<td>44 (32%)</td>
<td>37 (26%)</td>
<td></td>
</tr>
<tr>
<td>Peripheral measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral fat mass (kg)</td>
<td>7.59 ± 4.22</td>
<td>6.69 ± 3.15</td>
<td>0.039</td>
</tr>
<tr>
<td>Peripheral fat percentage</td>
<td>27.5 ± 9.0</td>
<td>25.8 ± 8.3</td>
<td>0.030</td>
</tr>
<tr>
<td>Peripheral lean mass (kg)</td>
<td>18.27 ± 5.17</td>
<td>17.85 ± 4.87</td>
<td>0.679</td>
</tr>
<tr>
<td>Peripheral lean percentage</td>
<td>69.0 ± 8.7</td>
<td>70.5 ± 8.0</td>
<td>0.023</td>
</tr>
<tr>
<td>Central measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central fat mass (kg)</td>
<td>4.80 ± 3.19</td>
<td>4.34 ± 2.71</td>
<td>0.166</td>
</tr>
<tr>
<td>Central fat percentage</td>
<td>18.6 ± 7.7</td>
<td>17.7 ± 7.2</td>
<td>0.180</td>
</tr>
<tr>
<td>Central lean mass (kg)</td>
<td>19.11 ± 4.97</td>
<td>18.62 ± 4.88</td>
<td>0.501</td>
</tr>
<tr>
<td>Central lean percentage</td>
<td>79.5 ± 7.5</td>
<td>80.4 ± 7.0</td>
<td>0.123</td>
</tr>
<tr>
<td>Total body measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body fat mass (kg)</td>
<td>13.26 ± 7.37</td>
<td>11.89 ± 5.74</td>
<td>0.075</td>
</tr>
<tr>
<td>Total body fat percentage</td>
<td>23.1 ± 7.6</td>
<td>21.8 ± 7.0</td>
<td>0.078</td>
</tr>
<tr>
<td>Total body lean mass (kg)</td>
<td>40.42 ± 10.22</td>
<td>39.46 ± 9.91</td>
<td>0.555</td>
</tr>
<tr>
<td>Total body lean percentage</td>
<td>73.7 ± 7.3</td>
<td>74.9 ± 6.7</td>
<td>0.060</td>
</tr>
<tr>
<td>Total body weight (kg)</td>
<td>55.48 ± 15.43</td>
<td>53.08 ± 13.82</td>
<td>0.167</td>
</tr>
</tbody>
</table>

Data represent mean ± SD unless indicated otherwise; three children did not undergo a total body scan.

* Body composition measures were corrected for age and gender.
### Table 3  Associations between method of conception and peripheral and total body fat measures adjusted for potential confounders

<table>
<thead>
<tr>
<th></th>
<th>Association with IVF after adjusting for current risk factors</th>
<th>Association with IVF after adjusting for current risk and early life factors</th>
<th>Association with IVF after adjusting for current risk, early life and parental factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td>Peripheral body fat measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscapular-triceps skinfold ratio</td>
<td>-0.114</td>
<td>0.007</td>
<td>-0.151</td>
</tr>
<tr>
<td>Sum of peripheral skinfolds (mm)</td>
<td>0.125</td>
<td>0.005</td>
<td>0.119</td>
</tr>
<tr>
<td>DXA peripheral fat mass (kg)</td>
<td>0.105</td>
<td>0.037</td>
<td>0.089</td>
</tr>
<tr>
<td>Total body fat measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sum of skinfolds (mm)</td>
<td>0.100</td>
<td>0.021</td>
<td>0.085</td>
</tr>
<tr>
<td>DXA total body fat mass (kg)</td>
<td>0.091</td>
<td>0.073</td>
<td>-</td>
</tr>
</tbody>
</table>

Data represent standardized regression coefficients (β) describing the association between IVF conception and several body fat composition measures after correction for the following variables when appropriate: current risk factors (age, sex, height, and pubertal stage), early life factors (maternal smoking during pregnancy, birth weight, and gestational age), and parental factors (parental education, maternal BMI, and subfertility cause).

*Not significant; P ≥ 0.100.
Comparison of BMC and BMD between the pubertal IVF subjects and the control subjects revealed no statistically significant differences in skeletal status (Table 4). However, there seemed to be a trend toward a higher total body BMD in IVF children (0.97 ± 0.11 vs. 0.95 ± 0.11 g/cm², P = 0.064). Linear regression analysis was performed to examine whether confounding variables obscured a potential association between method of conception and bone composition. After correction for sex, age, height, pubertal stage, birth weight SDS, and parental education, method of conception appeared to have no significant influence on bone mineral composition. Variation in BMC and BMD was predominantly caused by age, height, and gender.

Table 4  Bone mineral measurements at various skeletal regions in adolescents according to method of conception

<table>
<thead>
<tr>
<th></th>
<th>IVF children (n = 139)</th>
<th>Control children (n = 143)</th>
<th>P-value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>45.67 ± 14.81</td>
<td>44.30 ± 15.41</td>
<td>0.436</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.82 ± 0.16</td>
<td>0.80 ± 0.17</td>
<td>0.156</td>
</tr>
<tr>
<td>Femoral neck</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>3.97 ± 0.93</td>
<td>3.87 ± 0.94</td>
<td>0.504</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.78 ± 0.13</td>
<td>0.78 ± 0.13</td>
<td>0.796</td>
</tr>
<tr>
<td>Femoral trochanter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>6.98 ± 2.74</td>
<td>6.89 ± 2.69</td>
<td>0.864</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.69 ± 0.13</td>
<td>0.69 ± 0.14</td>
<td>0.787</td>
</tr>
<tr>
<td>Femoral intertrochanter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>18.79 ± 6.31</td>
<td>18.31 ± 6.47</td>
<td>0.583</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.99 ± 0.18</td>
<td>0.96 ± 0.18</td>
<td>0.195</td>
</tr>
<tr>
<td>Total hip</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>29.86 ± 9.51</td>
<td>29.08 ± 9.75</td>
<td>0.691</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.87 ± 0.14</td>
<td>0.86 ± 0.15</td>
<td>0.444</td>
</tr>
<tr>
<td>Total body</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g)</td>
<td>1790.2 ± 456.1</td>
<td>1723.9 ± 485.1</td>
<td>0.199</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.97 ± 0.11</td>
<td>0.95 ± 0.11</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Data represent mean ± SD.

* BMC and BMD are corrected for age and gender.

Discussion

In the present study, we compared body composition among 8- to 18-yr-old IVF singletons and spontaneously conceived controls born from subfertile parents. Peripheral adipose tissue mass assessed by skinfold measurements and DXA was significantly higher in IVF children compared with controls. Conversely, a significant lower percentage of peripheral lean tissue was observed among IVF children. Although not statistically significant, both DXA and skinfold measurements suggested that total body fat is higher in IVF children compared with controls. Differences in peripheral body fat assessed by anthropometry could not be explained either by current and early
life factors or by parental factors such as subfertility cause and socioeconomic status. These observations suggest that the IVF procedure unfavorably affects body fat composition of children conceived by IVF, irrespective of potentially confounding variables.

Concerns about potential significant lifelong implications for health in IVF children have recently been expressed. Many epidemiologic studies demonstrated that prenatal events can permanently program metabolic processes in the fetus which cause chronic diseases in adult life. Because body fatness is recognized as a major risk indicator of cardiovascular disease, our data demonstrating a disturbed body fat composition in IVF children confirm the importance of long-term research after IVF conception. It has to be taken into account that especially peripheral adipose tissue was increased in IVF children, whereas increased risk for cardiovascular health problems has been suggested to be primarily associated with a central body fat deposition. On the other hand, in view of our other findings demonstrating elevated blood pressure and fasting glucose levels in IVF children compared with controls (paper under review), continued body fat monitoring in IVF children is of great importance. In addition, current knowledge regarding fat patterning development in childhood and adolescence as well as their relative contributions to the development of diseases in later life is fairly limited. There are indications that central fat deposition is increasing from adolescence into adulthood, in particular in males, due to an increase in truncal fat mass and a decrease in peripheral fat mass.

It has been proposed that developmental plasticity after exposure to early prenatal insults can lead to irreversible changes in imprinted gene expression, nutrient and stress-related signaling pathways, and/or cell cycle and apoptotic rates. Especially the role of epigenetics in developmental plasticity is increasingly recognized. During the periconceptional period, important events including the transition of maternal to embryonic control and epigenetic reprogramming of the genome make the developing embryo highly susceptible to environmentally induced changes. Increasing evidence suggests that manipulation of gametes and embryos inherent to assisted reproductive technologies can perturb these important epigenetic processes causing altered expression of important genes related to fetal growth and development with both pre- and postnatal consequences. Additional research is necessary to investigate whether similar epigenetic mechanisms contribute to the disturbed body fat composition observed among IVF children in the present study.

With regard to skeletal status, no differences in bone mineral composition between IVF children and control children were observed in the present study. Method of conception was demonstrated to have no significant influence on BMC and BMD. There is growing evidence that environmental influences during prenatal life have long-term consequences on bone composition, which influences fracture risk in later life. For instance, a relation between maternal diet during pregnancy and bone mass in childhood has been found. Despite the lack
of evidence of disturbed skeletal development in IVF children in the present study, investigation
of other important processes, including the amount of attained peak bone mass during early
adulthood and subsequent rate of bone loss in late adult life are warranted.

When interpreting our results, potential limitations need to be considered. First, our study was based
on 58% (n = 466) of the total number of subjects approached (n = 808). However, no differences
in anthropometric measures such as height, weight, and BMI were found between the participants
and nonparticipants who returned the questionnaire. Second, it must be acknowledged that both
DXA and skinfold thickness measurements do not distinguish between different types of adipose
tissue. Especially visceral fat has been shown to be related to several metabolic disease risk factors
33. However, skinfold measurements, which measures only subcutaneous body fat, and body fat
measured by DXA, which comprises all internal and subcutaneous body fat, were highly correlated
in the present study. These findings are in line with other studies, which suggested that during
childhood and adolescence, most of the total body fat is deposited subcutaneously 8,35.

In conclusion, this is the first study addressing body composition in IVF children and adolescents
and controls using DXA and anthropometric measures. Although underlying mechanisms
remain to be identified, the periconceptional period might represent a critical time window in
humans during which environmental influences can perturb developmental pathways leading to
aberrant fat distribution in postnatal life. It is of great importance that follow-up of IVF children
is continued to monitor physiological changes in fat distribution from adolescence into adulthood
and to address potential related health problems.

Acknowledgements

The authors would like to thank Jos Burcksen for evaluating the DXA scans.
References


Pubertal development in children and adolescents born after IVF and spontaneous conception

Manon Ceelen, Mirjam M. van Weissenbruch, Jan P.W. Vermeiden, Flora E. van Leeuwen, Henriette A. Delemarre-van de Waal

Human Reproduction, 2008, 23: 2791-2798
Abstract

Background: Previous studies demonstrated a link between adverse conditions during prenatal life and the development of diseases in adult life. It is still unclear whether IVF conception could permanently affect early prenatal development in humans, with postnatal health consequences. The objective of the present study is to examine pubertal development in 8- to 18-year-old IVF singletons and spontaneously conceived controls born from subfertile parents.

Methods: Pubertal stage by Tanner’s classification, age at menarche and menstrual cycle characteristics were studied in the total population (n = 466: 115 IVF-conceived boys and 118 IVF-conceived girls, each with age-matched comparison groups). Bone age and sex hormone levels were examined in two distinct pubertal subpopulations.

Results: Pubertal stage and age at menarche were not significantly different between IVF and control children. In the pubertal subpopulation, a higher bone age-chronological age (BA-CA) ratio and a larger BA-CA difference were observed in IVF-conceived girls compared with controls (1.04 ± 0.07 vs. 1.02 ± 0.08, P = 0.022 and 0.54 ± 0.82 vs. 0.18 ± 1.00 yr, P = 0.021, respectively). Furthermore, dehydroepiandrosterone sulphate (DHEAS) and LH levels were significantly higher in IVF-conceived girls than in control subjects (2.5 µmol/l vs. 1.9 µmol/l, P = 0.017 and 1.5 U/l vs. 0.6 U/l, P = 0.031, respectively).

Conclusions: Bone age appeared to be advanced in pubertal IVF-conceived girls, but not in boys, compared with controls. Increased DHEAS and LH concentrations were found among IVF girls.
Introduction

Application of IVF has rapidly increased since the first IVF birth in 1978. Approximately 1.6% of the current births in the Netherlands are established after assisted reproduction technologies (ART) \(^ {24}\), and it is estimated that worldwide over a million children have been born after assisted conception \(^ {34}\). There is an accumulating body of evidence that IVF children are at increased risk for adverse perinatal outcome, including low birth weight, preterm birth and other complications \(^ {14,19}\). In addition, concerns about potential lifelong health implications after IVF conception in humans have recently been expressed \(^ {16,26,29}\). The manipulation of gametes and embryos inherent to ART has been suggested to influence developmental pathways with postnatal consequences \(^ {20}\). Recently, higher blood pressure and fasting glucose, and altered body fat composition have been reported among 8–18-year-old IVF children when compared with controls born to subfertile parents \(^ {3,4}\). These findings highlight the importance of the continuing worldwide monitoring of postnatal development of IVF offspring.

Despite the fact that the number of adolescents born after IVF treatment is steadily increasing, sexual maturation in IVF children has not yet been examined. Rojas-Marcos et al. \(^ {33}\) highlighted the need for monitoring IVF children throughout childhood into adolescence to investigate pubertal development. Although epidemiological data are scarce and inconclusive, an association between prenatal development and timing and progression of puberty in humans has been suggested over the last decade \(^ {15,40}\). Furthermore, in view of the early embryonic programming influences on several body systems which have been described \(^ {7,25,35}\), it can be questioned whether developmental processes related to the hypothalamic-pituitary-gonadal axis can also be impaired by periconceptional events, such as IVF, resulting in a disturbed pubertal development. Hence, we investigated anthropometric, radiological and biochemical characteristics of pubertal development in IVF children and spontaneously conceived control children born from subfertile couples.

Subjects and methods

Study population

The OMEGA-study is a Dutch retrospective cohort study aimed at examining the long-term health effects of hormone stimulation. The cohort consists of 26,428 women diagnosed with subfertility problems in one of the 12 IVF clinics between 1980 and 1995; 19,840 women received IVF treatment and 6,588 women did not \(^ {6,22,23}\). Eligible women had not achieved conception after at least 1 year of frequent unprotected intercourse at the time of their first visit to the fertility clinic. Risk factor questionnaires to the women and detailed data collection from the medical records provided information on the children born from the OMEGA-participants up to 1996–1997. The questionnaire response rate was 73% among subfertile women with children. The present study
was restricted to IVF and spontaneously conceived children born from OMEGA-participants who visited the fertility clinic of the VU University medical center (VUmc). Mothers of the spontaneously conceived children did not receive hormonal stimulation prior to conception. IVF children born from women treated in the VUmc who did not participate in the OMEGA-study were also eligible for recruitment.

From the 553 eligible singletons born after standard IVF treatment, we invited 95% of IVF children born between 1986 and 1991, 74% of IVF children born between 1992 and 1993, and 41% of IVF children born between 1994 and 1995 in order to achieve equal representation of all age categories. For each participating IVF child, one spontaneously conceived child of similar gender and age (≤ 3 months age difference) born from subfertile parents was searched. In case this control child did not want to participate, the control recruitment process was repeated until an appropriate control child was found which agreed to participate. The study protocol was approved by the ethics committee of the VUmc and by the National Medical Ethics Committee known as the “Centrale Commissie Mensgebonden Onderzoek” located in The Hague, the Netherlands.

![Figure 1](image_url)  
**Figure 1**  Overview of the inclusion process and study population

**Approach of eligible study subjects**

Between March 2003 and March 2006, children and their parents were informed by letter about our study on growth and development of IVF children (n = 354 IVF children and n = 454 control...
children). By means of a reply form and a pre-stamped envelope, parents were able to inform us whether they were willing to participate in our study. Address information of the families was checked and/or obtained using extensive tracing techniques. After 4–8 weeks non-responders were approached by telephone. Inclusion results are summarized in Figure 1. In total, 69% of the IVF-responders (n = 246) and 51% of the control-responders (n = 233) agreed to participate, resulting in 233 matched pairs.

Anthropometric measurements of all participating children were obtained. Pubertal children (as assessed according to Tanner criteria 36) were recruited for additional research including the assessment of skeletal maturation and a fasting blood test. Hormonal concentrations were determined in pubertal premenarcheal girls (B2–3 Tanner stage) and pubertal boys (G3–5 Tanner stage). All children and their parents gave written informed consent to participate in the study.

Families who refused to participate in the study received a single questionnaire regarding health, education and other characteristics of the respective child (n = 283). Non-participation analysis yielded no significant differences between participants and non-participants regarding children's current height, weight and body mass index (BMI). On average, non-participating children were significantly older (12.9 ± 2.6 vs. 12.0 ± 2.6 year, P = 0.002) and their mothers were less often highly educated (26 vs. 37%, P = 0.015), but these differences were observed in both the IVF and control population.

Data collection and measurements

Pubertal development using breast or genital developmental stages and pubic hair growth were recorded according to Tanner and Whitehouse 36. In boys, testicular volume was determined by means of a Prader orchidometer. Body weight and height were assessed to the nearest 0.1 kg and 0.1 cm using an electronic scale (SECA) and a stadiometer (Holtain Ltd, Crymych, Dyfed, UK), respectively, with children dressed only in underwear. From these measurements, BMI was calculated as kg/m². Height was expressed as standard deviation score (SDS) for chronological age and bone age using Dutch references 11. Target height was calculated as midparental height corrected for sex and Dutch secular trend 9 and was expressed as SDS using Dutch references 11. Corrected height SDS was defined as height SDS minus target height SDS. Skinfold thickness measurements (triceps, biceps, subscapular and supra-iliac) were collected in triplicate on the non-dominant side of the body by means of a Harpenden caliper. A tape measure was used to measure waist and hip circumference and waist-hip ratio was calculated. The majority (94%) of the anthropometric measurements were performed by one observer (MC).

Bone age was estimated from left-hand radiographs using the Greulich and Pyle standards, by one observer (MC) 13. To validate accuracy, skeletal age of 45 randomly selected children was determined by an experienced paediatric endocrinologist as well. The Spearman correlation
coefficient between the measurements was 0.987 (P < 0.001). The difference between bone age and chronological age and the bone age-chronological age (BA-CA) ratio were calculated.

Blood samples were drawn between 9:00 and 10:00 am. Plasma levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined by immunofluorometric assays (Delfia, Wallac, Turku, Finland). For the LH assay, the detection limit was 0.3 U/l and for the FSH assay, the limit of detection was 0.5 U/l. Serum estradiol (E$_2$) concentrations were measured with the use of a double-antibody radioimmunoassay (Diasorin, Saluggia, Italy). The detection limit was 18 pmol/l. Serum concentrations of testosterone and dehydroepiandrosterone sulphate (DHEAS) were analysed by the Coat-A-Count radioimmunoassay (DPC, Los Angeles, USA). For the testosterone assay, the detection limit was 1 nmol/l. For the DHEAS assay, the limit of detection was 0.2 µmol/l. All methods had intra- and interassay coefficients of variation from 3 to 20% within the relevant concentration ranges. All laboratory measurements were performed in the endocrinological laboratory at the Department of Clinical Chemistry of the VUMc.

Prior to the follow-up visit in the VUMc, a questionnaire was sent to the parents in order to gather information on various demographic, lifestyle and medical factors including cause of subfertility, duration, parental education level, maternal smoking during pregnancy and birth weight and gestational age of the respective child. Birth weight was expressed as standard deviation score (SDS) to correct for gestational age and gender. Information regarding girls’ menstrual cycle pattern was obtained during the visit by means of an interview. An average length of the menstrual cycle between 22 and 41 days was classified as “regular”. Other relevant outcomes, such as blood pressure levels and body composition, have been reported elsewhere.

Statistical analysis

Characteristics of 233 matched IVF-control pairs were compared using the paired t-test for continuous variables and the McNemar test for dichotomous variables (Statistical Package for the Social Sciences version 12.0; SPSS Inc., Chicago, IL, USA). Girls with Tanner breast stage ≥ B2 and boys with Tanner genital stage ≥ G2 and/or a testis volume ≥ 4ml were classified as pubertal children. The proportion of pubertal IVF and control children and the proportion of IVF and control children in the distinct Tanner stages were tested. Furthermore, per Tanner stage mean age of IVF and control children was calculated and compared. Differences in skeletal maturation and sex hormone levels between the pubertal IVF and control subjects were tested by using Student’s t-test or Mann-Whitney U-test when appropriate. P-value of < 0.05 was considered to be statistically significant, based on two-sided testing.
Results

Perinatal outcome and anthropometry at follow-up

Birth weight, birth weight SDS and gestational age were significantly lower in children conceived by IVF when compared with controls (3.2 ± 0.6 vs. 3.4 ± 0.6 kg, P < 0.001; –0.15 ± 1.00 vs. 0.08 ± 1.08, P = 0.025; 38.9 ± 2.5 vs. 39.5 ± 1.8 weeks, P = 0.004, respectively). Anthropometric characteristics of the study subjects are listed in Table 1. Age at follow-up of IVF children and controls was 12.2 ± 2.6 years. Total sum of skinfold thickness of IVF children tended to be higher when compared with controls (40.4 ± 20.3 vs. 37.1 ± 17.5 mm; P = 0.054). No significant differences in anthropometric measurements, such as height, weight, and BMI between IVF children and control children were found. Also height SDS, including the supposed difference shown between IVF boys and control boys, was not statistically significant between the IVF and control population.

Table 1  Anthropometrical characteristics of IVF-conceived and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IVF children</td>
<td>Controls</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>115</td>
<td>115</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>12.0 ± 2.6</td>
<td>12.0 ± 2.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>156.8 ± 16.2</td>
<td>155.4 ± 17.1</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.22 ± 1.01</td>
<td>–0.01 ± 1.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>47.4 ± 16.6</td>
<td>46.2 ± 15.6</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.34 ± 1.10</td>
<td>0.17 ± 1.17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.7 ± 3.2</td>
<td>18.6 ± 3.3</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.33 ± 1.00</td>
<td>0.22 ± 1.20</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
<td>35.4 ± 18.3</td>
<td>32.5 ± 16.8</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>67.2 ± 9.1</td>
<td>67.0 ± 9.6</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>80.3 ± 11.0</td>
<td>79.3 ± 10.8</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.84 ± 0.04</td>
<td>0.85 ± 0.05</td>
</tr>
</tbody>
</table>

Data represent mean ± SD unless indicated otherwise. SDS, Standard deviation score.

Physical signs of puberty

Although the proportion of pubertal IVF boys and control boys was similar (65 vs. 67%, respectively, P = 0.69), a trend towards a higher proportion of pubertal IVF girls was demonstrated (80 vs. 73%
Figure 2  Proportion of children in distinct pubertal stages (A) and age distribution per pubertal stage of IVF children and controls (B). Pubertal stage was classified as prepubertal (Tanner 1), early pubertal (Tanner 2), midpubertal (Tanner 3+4), late pubertal (boys; Tanner 5) and postmenarcheal (girls); no significant differences in pubertal stage between IVF and control children were found. TV, Testicular volume.
in controls, \( P = 0.064 \). On the other hand, comparison of the proportion of children in the distinct Tanner stages revealed no significant differences between IVF and control population (Figure 2a). Furthermore, mean age of IVF children and controls in the distinct pubertal stages was comparable (Figure 2b). In IVF subjects, mean age of boys in G2 stage and boys with testicular volume of 4 ml was 11.0 ± 1.3 and 12.0 ± 1.5 year, respectively, whereas mean age of girls in B2 stage was 10.4 ± 1.2 year. In control subjects, mean age of boys in G2 stage and boys with testicular volume of 4 ml was 11.1 ± 1.1 and 12.2 ± 1.2 year, respectively, whereas mean age of girls in B2 stage was 10.6 ± 0.8 year. Height, weight and BMI at onset of puberty did not differ between the IVF and control population (data not shown). On average, testicular volume of IVF-conceived boys tended to be higher when compared with controls (8.0 ± 7.6 vs. 7.1 ± 6.4 ml, \( P = 0.059 \)). No difference was found with respect to the proportion of children with pubic hair development (69 of IVF girls vs. 66% of control girls, \( P = 0.69 \); 48 of IVF boys vs. 45% of control boys, \( P = 1.0 \)).

Table 2  Menstrual characteristics of post-menarcheal IVF and control girls

<table>
<thead>
<tr>
<th></th>
<th>IVF children</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of post-menarcheal girls</td>
<td>49 (42%)</td>
<td>49 (42%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Age at follow-up (yr)</td>
<td>14.9 ± 1.5</td>
<td>14.8 ± 1.7</td>
<td>0.58</td>
</tr>
<tr>
<td>Reported age at menarche (yr)</td>
<td>12.5 ± 1.2</td>
<td>12.6 ± 1.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Actual menstrual cycle pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 6 months after menarche</td>
<td>6 (12%)</td>
<td>11 (22%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Regular menstrual cycles</td>
<td>22 (45%)</td>
<td>24 (49%)</td>
<td></td>
</tr>
<tr>
<td>Irregular menstrual cycles</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td>17 (35%)</td>
<td>13 (27%)</td>
<td></td>
</tr>
<tr>
<td>Bleeding time (days)</td>
<td>5.2 ± 1.5</td>
<td>5.1 ± 1.3</td>
<td>0.80</td>
</tr>
<tr>
<td>Dysmenorrhoea</td>
<td>16 (33%)</td>
<td>13 (27%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Maternal age at menarche (yr)</td>
<td>13.1 ± 1.5</td>
<td>12.7 ± 1.6</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data represent mean ± SD unless indicated otherwise.

Menses

The same proportion of IVF girls and control girls had reached menarche (42 vs. 42%, \( P = 1.0 \)). Menstrual cycle characteristics of these girls are presented in Table 2. Reported age at menarche of IVF girls and control girls was 12.5 ± 1.2 and 12.6 ± 1.2 year, respectively (\( P = 0.53 \)). The difference in reported age at menarche of IVF mothers and their daughters did not reach statistical significance (\( P = 0.054 \)). No differences were found between IVF and control girls were found in terms of actual menstrual cycle pattern, bleeding time and prevalence of dysmenorrhoea. In
addition, height SDS, weight SDS and BMI SDS of menarcheal IVF and control girls did not differ (data not shown).

**Bone age**

In the pubertal subpopulation, a higher bone age-chronological age (BA-CA) ratio and a larger BA-CA difference were found among IVF-conceived girls compared with control girls (1.04 ± 0.07 vs. 1.02 ± 0.08, P = 0.022; 0.54 ± 0.82 vs. 0.18 ± 1.00 year, P = 0.021, respectively) (Table 3). Height and weight were significantly correlated with chronological age (r = 0.734, P < 0.001; r = 0.672, P < 0.001, respectively) and bone age (r = 0.762, P < 0.001; r = 0.764, P < 0.001, respectively). No differences in corrected height measures between the two study groups were noted.

**Table 3** Skeletal maturation and corrected height measures in pubertal IVF-conceived subjects and controls

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IVF children</td>
<td>Controls</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>67</td>
<td>68</td>
</tr>
<tr>
<td>Age at follow-up (yr)</td>
<td>13.6 ± 2.0</td>
<td>13.6 ± 2.0</td>
</tr>
<tr>
<td>Bone age (yr)</td>
<td>13.9 ± 2.2</td>
<td>13.7 ± 2.4</td>
</tr>
<tr>
<td>BA-CA ratio</td>
<td>1.02 ± 0.08</td>
<td>1.01 ± 0.08</td>
</tr>
<tr>
<td>BA-CA difference (yr)</td>
<td>0.22 ± 1.06</td>
<td>0.10 ± 1.09</td>
</tr>
<tr>
<td>Height SDS a</td>
<td>0.21 ± 1.14</td>
<td>−0.01 ± 1.05</td>
</tr>
<tr>
<td>Height SDS for bone age a</td>
<td>0.07 ± 1.01</td>
<td>−0.05 ± 0.84</td>
</tr>
<tr>
<td>Corrected height SDS b</td>
<td>−0.12 ± 0.91</td>
<td>−0.30 ± 0.96</td>
</tr>
</tbody>
</table>

Data represent mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
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<td>IVF children</td>
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</tr>
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<td>68</td>
</tr>
<tr>
<td>Age at follow-up (yr)</td>
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<td>13.6 ± 2.0</td>
</tr>
<tr>
<td>Bone age (yr)</td>
<td>13.9 ± 2.2</td>
<td>13.7 ± 2.4</td>
</tr>
<tr>
<td>BA-CA ratio</td>
<td>1.02 ± 0.08</td>
<td>1.01 ± 0.08</td>
</tr>
<tr>
<td>BA-CA difference (yr)</td>
<td>0.22 ± 1.06</td>
<td>0.10 ± 1.09</td>
</tr>
<tr>
<td>Height SDS a</td>
<td>0.21 ± 1.14</td>
<td>−0.01 ± 1.05</td>
</tr>
<tr>
<td>Height SDS for bone age a</td>
<td>0.07 ± 1.01</td>
<td>−0.05 ± 0.84</td>
</tr>
<tr>
<td>Corrected height SDS b</td>
<td>−0.12 ± 0.91</td>
<td>−0.30 ± 0.96</td>
</tr>
</tbody>
</table>

Data represent mean ± SD.

a Height was expressed as SDS for chronological age (height SDS) and bone age (height SDS for bone age).

b Corrected height SDS was defined as height SDS minus target height SDS.

c P = 0.022, versus IVF girls.

d P = 0.021, versus IVF girls.

BA-CA, Bone age-chronological age.

**Sex hormones**

Table 4 presents sex hormone concentrations in pubertal IVF-conceived subjects and control subjects. IVF-conceived girls showed significantly higher DHEAS concentrations than controls (2.5 [2.0–2.9] vs. 1.9 [1.2–2.2] µmol/l, P = 0.017). In addition, LH levels were significantly increased in IVF girls when compared with control girls (1.5 ± 1.6 vs. 0.6 ± 0.7 U/l, P = 0.031). No significant differences in FSH and E2 were found between IVF girls and controls. Similar levels of LH, FSH,
DHEAS and testosterone were found in IVF and control boys. DHEAS levels were not correlated with birth weight, birth weight SDS, gestational age and sum of skinfolds. A weak but significant correlation was found between DHEAS concentrations and BA-CA ratio and BA-CA difference (r = 0.257, P = 0.006; r = 0.317, P = 0.001, respectively).

**Table 4** Hormonal concentrations in pubertal IVF-conceived subjects and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th>Girls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IVF</td>
<td>Controls</td>
<td>IVF</td>
<td>Controls</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>40</td>
<td>35</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Age at follow-up (yr)</td>
<td>14.7 ± 1.6</td>
<td>15.0 ± 1.5</td>
<td>11.4 ± 0.9</td>
<td>11.2 ± 0.9</td>
</tr>
<tr>
<td>Bone age (yr)</td>
<td>15.0 ± 1.8</td>
<td>15.1 ± 2.0</td>
<td>12.0 ± 0.7</td>
<td>11.2 ± 1.2 b</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.27 ± 1.08</td>
<td>−0.05 ± 0.95</td>
<td>−0.005 ± 0.86</td>
<td>0.11 ± 1.19</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.49 ± 1.18</td>
<td>0.09 ± 1.14</td>
<td>0.10 ± 0.84</td>
<td>−0.14 ± 0.86</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.48 ± 0.99</td>
<td>0.12 ± 1.23</td>
<td>0.13 ± 0.98</td>
<td>−0.19 ± 0.93</td>
</tr>
<tr>
<td>LH (U/l)</td>
<td>2.6 ± 1.0</td>
<td>2.9 ± 1.9</td>
<td>1.5 ± 1.6</td>
<td>0.6 ± 0.7 c</td>
</tr>
<tr>
<td>FSH (U/l) a</td>
<td>2.7 (2.3–4.4)</td>
<td>2.9 (1.9–4.5)</td>
<td>4.0 (2.3–6.9)</td>
<td>3.0 (1.7–4.5)</td>
</tr>
<tr>
<td>DHEAS (µmol/l) a</td>
<td>3.9 (3.1–6.0)</td>
<td>4.1 (2.7–5.8)</td>
<td>2.5 (2.0–2.9)</td>
<td>1.9 (1.2–2.2) d</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>-</td>
<td>-</td>
<td>80.5 ± 41.4</td>
<td>57.9 ± 41.1</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>13.9 ± 7.0</td>
<td>15.2 ± 6.8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data represent mean ± SD or * median (25th–75th percentile).

b P = 0.015, versus IVF girls.
a P = 0.031, versus IVF girls.
d P = 0.017, versus IVF girls.
DHEAS, Dehydroepiandrosterone sulphate.

**Discussion**

This is the first report that assessed clinical signs related to pubertal development, adrenarche, skeletal maturation and sex hormone levels in IVF children compared with controls. Despite a trend towards a higher proportion of pubertal IVF girls, chronological age at important pubertal milestones, including age at menarche, appeared to be similar in IVF and control girls. Similarly, the proportion of post-menarcheal IVF and control girls and their menstrual cycle characteristics did not differ. Between IVF and control boys, no differences in chronological age at distinct genital stages and subsequent categories of testicular volume were found. Furthermore, the proportion of pubertal IVF and control boys was similar. In contrast, mean testicular volume tended to be higher in IVF boys. Testicular volume has been suggested to be a more finely graduated indicator of development than other
measures. On the other hand, others stated that testicular volume is not without its own measurement issues, as systematic overestimation of testicular volume has been reported and testicular volume measurement is subject to great inter-observer variation. In the current study, the vast majority of the boys were examined by a single investigator, which reduces misclassification bias.

Bone age of IVF girls was advanced when compared with control girls. In the literature, accelerated skeletal maturation has been associated with compromised adult stature due to premature closure of epiphyseal junctions. Nevertheless, height SDS of menarchal IVF and control girls did not differ. As post-menarcheal growth in height is limited, it is therefore unlikely that final height is substantially reduced in IVF girls.

Among pubertal IVF girls, higher DHEAS levels were found when compared with controls. In accordance with these findings, some studies showed higher serum DHEAS levels in girls born following prenatal growth restraint, which may be linked to an exaggerated adrenarche. Increased DHEAS levels have been reported in asymptomatic non-obese, post-menarcheal girls born small for gestational age (SGA). Ghirri et al. demonstrated that DHEAS levels were significantly higher in SGA girls than in control subjects despite the lack of differences in clinical signs of puberty. In addition, elevated DHEAS levels have also been found in low birth weight boys. Although several other studies did not find such an association, these described increased DHEAS levels in children born SGA suggest that birth weight is an important factor that has to be taken into account when investigating DHEAS levels in children. Nonetheless, in the present study increased DHEAS concentrations found in IVF girls could not be explained by the observed difference in birth weight.

It is important, however, to realize that birth weight is just an indicator of fetal growth and that developmental adaptations to prenatal environmental insults are not necessarily reflected in birth weight. Likewise, other studies demonstrated that serum DHEAS levels are positively correlated with weight, particularly weight gain and adiposity. In the current study, no correlations were found between DHEAS levels and body fat measures including BMI and sum of skinfolds. A possible explanation might be the low number of obese children in our study population.

The cause of the increased LH levels observed in pubertal IVF girls is not yet clear. Previous studies have shown that during puberty nocturnal increases in LH pulse amplitude and frequency are followed by a diurnal pattern of LH pulsatility. It cannot be excluded that comparison of LH concentrations between midpubertal IVF and control girls is hindered due the large variation in LH secretory activity.

Growing evidence has emerged on the relation between prenatal growth restraint, premature adrenarche, early puberty and polycystic ovary syndrome (PCOS). PCOS is characterized by hyperandrogenaemia, elevated plasma LH concentrations, insulin resistance, menstrual abnormalities with anovulation, obesity and ultrasonographic evidence of polycystic ovaries. The clinical relevance of the higher DHEAS concentrations and the increased LH levels found in pubertal IVF girls in comparison with control children has to be further established.
In order to be able to adequately examine postnatal growth and development in IVF children, an appropriate comparison group of unexposed children was needed. It is generally known that IVF parents differ from the general reproductive population with regard to age, parity and other important characteristics. To avoid confounding due to these known differences, comparison with children born to subfertile parents after spontaneous conception was preferred. Our study was based on 58% (n = 466) of the total number of subjects approached (n = 808). No differences in anthropometric measures, such as height, weight and BMI, were found between the participants and non-participants who returned the questionnaire. On the other hand, there appeared to be a significant difference in maternal education between the non-participating and participating children. This phenomenon was found in both the IVF and the control population. Therefore, it did not confound the comparisons performed between IVF and control children in the present study, but it might have implications for the generalizability of our results. Selection bias could have occurred when the relationships between parental education and studied outcome variables are different among children who participated and those who did not participate. It would be interesting to follow the current study population prospectively to further investigate sexual development and subsequent reproductive functioning of IVF offspring. Longitudinal series can be used to characterize the various pubertal events in more detail than cross-sectional series 21. For logistical reasons, it was not possible in the present study to plan the blood withdrawal of menarcheal girls within a specific phase of their menstrual cycle. Therefore, we did not measure hormonal levels in these girls, as variation in hormone levels due to differences in menstrual cycle would hamper the comparison between IVF and control subjects. Nevertheless, it would be worthwhile to examine hormonal levels, including LH and DHEAS concentrations, during late adolescence. It remains to be elucidated whether the slight differences in DHEAS and LH levels between IVF and control girls will persist during pubertal development.

In conclusion, in IVF girls bone age was advanced and increased LH and DHEAS concentrations were found. Conversely, no differences in pubertal stage between IVF and control children were observed. Differences in LH levels between IVF and control girls might be the consequence of the major variation in pulsatile LH secretion. However, it is important to realize that these findings did not follow from a specific hypothesis being tested and that multiple significance testing was performed which can lead to chance findings. Before definitive conclusions can be drawn, our findings need to be reproduced by other prospective follow-up studies.
References


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Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8–18 years of IVF children and spontaneously conceived controls born to subfertile parents

Manon Ceelen, Mirjam M. van Weissenbruch, Janneke Prein, Judith J. Smit, Jan P. W. Vermeiden, Marieke Spreeuwenberg, Flora E. van Leeuwen, Henriette A. Delemarre-van de Waal
Abstract

Background: Little is known about postnatal growth in IVF offspring and the effects of rates of early postnatal growth on blood pressure and body fat composition during childhood and adolescence.

Methods: The follow-up study comprised 233 IVF children aged 8–18 years and 233 spontaneously conceived controls born to subfertile parents. Growth data from birth to 4 years of age, available for 392 children (n = 193 IVF; n = 199 control), were used to study early postnatal growth. Furthermore, early postnatal growth velocity (weight gain) was related to blood pressure and skinfold measurements at follow-up.

Results: We found significantly lower weight, height and BMI standard deviation scores (SDSs) at 3 months and weight SDS at 6 months of age in IVF children compared with controls. Likewise, IVF children demonstrated a greater gain in weight SDS (P < 0.001), height SDS (P = 0.013) and BMI SDS (P = 0.029) during late infancy (3 months to 1 year) versus controls. Weight gain during early childhood (1–3 years) was related to blood pressure in IVF children (P = 0.014 systolic, 0.04 diastolic) but not in controls. Growth during late infancy was not related to skinfold thickness in IVF children, unlike controls (P = 0.002 peripheral sum, 0.003 total sum). Growth during early childhood was related to skinfold thickness in both IVF children and controls (P = 0.005 and 0.01 peripheral sum and P=0.003 and 0.005 total sum, respectively).

Conclusions: Late infancy growth velocity of IVF children was significantly higher compared with controls. Nevertheless, early childhood growth instead of infancy growth seemed to predict cardiovascular risk factors in IVF children. Further research is needed to confirm these findings and to follow-up growth and development of IVF children into adulthood.
**Introduction**

According to the “developmental origins of adult disease hypothesis”, many adult diseases are thought to be the long-term consequence of programming during early life \(^1,^4\). Exposure to environmental insults at critical windows during various stages of prenatal development may induce structural and functional adaptations. It is well recognized that these adaptations may provide a short-term survival benefit, but eventually lead to an increased risk of chronic diseases, including type 2 diabetes and cardiovascular disease, in later life \(^2\).

Over the last years, numerous epidemiological studies indicated that the link between impaired prenatal development and cardiovascular morbidity in adult life is substantially modified by early postnatal growth \(^1^4,^1^6,^2^3\). Individuals exposed to adverse prenatal conditions seem to be more susceptible to cardiovascular disease and type 2 diabetes if they “catch-up” in weight during early postnatal life. Associations between rapid early postnatal growth and several cardiovascular risk factors, like blood pressure and fat mass, have also been described in children and adolescents \(^1^3,^2^2,^3^3\).

Today, reproductive technologies including IVF are used all over the world to treat subfertility. The number of IVF-conceived children is steadily growing with approximately 1–3% of the current births in developed countries being established after IVF \(^3^0\). However, concerns that IVF conception may influence prenatal development with longlasting consequences have been increasingly expressed \(^3^6\). Numerous studies have reported increased risks of low birth weight and preterm birth among IVF pregnancies \(^1^9,^2^4\). Furthermore, we previously demonstrated that IVF-conceived children and adolescents are at increased risk of higher blood pressure levels and altered body composition \(^1^0,^1^1\). Little is known, however, about postnatal growth among IVF offspring and the relationships between early postnatal growth and blood pressure and body fat composition are also still elusive.

Therefore, in the present study, we addressed several early postnatal growth parameters of IVF and spontaneously conceived control children from subfertile parents from birth to 4 years of age. Furthermore, we investigated the associations of growth velocity during infancy and/or early childhood in relation to blood pressure and body fat composition in 8–18-year-old IVF children and controls. In line with numerous studies examining the relationship between postnatal growth and blood pressure and body fat, weight gain was used as an early postnatal growth velocity parameter \(^1^5,^2^0,^2^2,^2^9,^3^3\).
Subjects and methods

Study population

This study is part of a follow-up study investigating postnatal growth and development in children and adolescents aged 8–18 years old born from subfertile parents who were either successfully treated with IVF or conceived spontaneously, as described previously. Families with a singleton child born after IVF treatment performed in the VU University medical center (VUmc) in Amsterdam, the Netherlands, were invited by mail to participate in the study. Spontaneously conceived children born from parents who previously visited the Department of Gynecology of the VUmc with fertility problems (i.e. no conception after at least 1 year of frequent unprotected intercourse at the time of their first visit to the fertility clinic) were used as controls. For each participating IVF child, a control child of same gender and similar age (≤ 3 month's age difference) was identified. If a matched control child did not want to participate, the control recruitment process was repeated until an appropriate control child was found that did agree to participate. Between March 2003 and March 2006, 69% of the 354 IVF children and 51% of the 454 controls who were approached agreed to participate, resulting in 233 matched IVF-control pairs.

Data collection

Anthropometric measurements of all participating children were obtained during a visit to the VUmc to evaluate their growth and development. Shortly before the hospital visit, a questionnaire was sent to the parents to gather information on various demographic, lifestyle and medical factors including cause of subfertility, parental education level and birth weight and gestational age of the respective child. In addition, parents were asked to bring the original postnatal growth chart of their child to the hospital visit. In the Netherlands, virtually all children undergo regular periodic health examinations by health professionals of the Municipal Health Services during the first years of life. Some parents fulfilled the request to send a copy in case they forgot to bring the growth chart. In total, postnatal growth charts of 394 children were provided by the parents. Postnatal growth data were only used for statistical analysis when the child had been measured more than three times between birth and the age of 4 years. No differences in birth weight or socioeconomic status were found between children with (n = 392 across age groups) versus without (sufficient) postnatal growth data (n = 74). Children without sufficient data were more often born preterm (16 vs. 8%, P = 0.04), but these findings were observed in both the IVF and control population. Postnatal growth measurements were expressed as standard deviation score (SDS) using the 1997 Dutch growth standards.

During the hospital visit at follow-up in the VUmc, blood pressure was measured twice at the non-dominant arm in the sitting position using an automatic device with appropriate cuff size (Dinamap PRO 100, Criticon, Munich, Germany). Skinfold thickness measurements (triceps,
biceps, subscapular, and supra-iliac) were collected in triplicate by means of a Harpenden caliper. Birth weight, either extracted from VUmc birth certificates (49%) or outpatient clinic reports (38%), or self-reported by the parents (13%), was expressed as SDS to correct for gestational age and gender. Gestational age was obtained using parental recall. Mean age (±SD) at follow-up of IVF children and controls was 12.2 ± 2.6 years. No differences in pubertal stage according to Tanner were found between IVF and control children (prepubertal: 27 vs. 29%; postpubertal: 21 vs. 18%, respectively). Twins were not eligible to participate in the study. The study protocol was approved by the ethics committee of the VUmc and by the National Medical Ethics Committee known as the “Centrale Commissie Mensgebonden Onderzoek” located in The Hague, the Netherlands. All participating children and their parents gave written informed consent.

Statistical analysis

Postnatal growth from shortly after birth up to 4 years of age of IVF children and controls was compared by means of general estimation equation (GEE) analyses. This regression technique adjusts for dependency of several measurements within one individual and is capable of dealing with missing data. Postnatal growth of children was also cross-sectionally analyzed at 3 months, at 6 months, at 1 year of age and during the second and third year of life. Weight gain during late infancy (weight SDS at 1 year of age minus weight SDS at 3 months) and during early childhood (weight SDS at 3 years of age minus weight SDS at 1 year) were calculated. In addition to these calculations using the Dutch growth standards, weight gain during early infancy was examined (weight SDS at 3 months minus birth weight SDS). Subsequently, for each of the three periods under examination, weight gain was divided into tertiles of the distribution, as done by Oren et al. The lowest, middle and highest tertiles of weight gain were used as measures for slow (“decelerated”), constant and rapid (“exaggerated”) growth during early life. Differences in blood pressure and body fat measures (cardiovascular parameters) at follow-up between IVF children with exaggerated growth and IVF children with decelerated growth were compared. To disentangle the effects of perinatal outcome and early postnatal growth on these cardiovascular parameters, additional analyses were performed to correct for birth weight, gestational age and body size at follow-up. The square root of height was used as a measure of body size as suggested by VanItallie et al. Similar analyses were performed for the control population. Total sum of skinfolds and sum of peripheral skinfolds were not normally distributed and therefore were logtransformed before analysis. A P-value < 0.05 was considered to be statistically significant.
Results

Growth characteristics of the study population

Birth weight, birth weight SDS and gestational age were significantly lower in children conceived by IVF than in controls (3.2 ± 0.6 vs. 3.4 ± 0.6 kg, \( P < 0.001 \); −0.15 ± 1.00 vs. 0.08 ± 1.08, \( P = 0.025 \); 38.9 ± 2.5 vs. 39.5 ± 1.8 weeks, \( P = 0.004 \), respectively). Likewise, significantly more IVF children were born prematurely, i.e. < 37 weeks of gestation (13 vs. 6% in the control group, \( P = 0.015 \)) and had a low birth weight, i.e. < 2500 g (11 vs. 3.5% in the control group, \( P = 0.004 \)). IVF children had an average of 16 ± 4 growth measurements shortly after birth up to the fourth year of life compared with 14 ± 4 growth measurements in controls (\( P = 0.002 \)). GEE analyses revealed significant differences in postnatal growth parameters between IVF and control children during the first months of life (Figure 1). Weight SDS and height SDS was significantly lower in IVF children than in controls during the first 6 months of life (0–3 months: weight SDS difference: \( P = 0.001 \); height SDS difference: \( P = 0.039 \); 3–6 months: weight SDS difference: \( P = 0.005 \); height SDS difference: \( P = 0.028 \)). BMI SDS was significantly lower among IVF children compared with control children during the period shortly after birth and 3 months (\( P = 0.004 \)).

Comparison of growth measurements at the different cross-sectional moments demonstrated significant differences in weight SDS, height SDS and BMI SDS at 3 months, and weight SDS at 6 months of age between IVF and control children (Table 1). Gain in weight, height and BMI during late infancy was significantly higher in IVF children as compared with controls (respectively \( P < 0.001 \), \( P = 0.013 \) and \( P = 0.029 \)). No significant differences were found in weight gain during early infancy and weight, height or BMI gain during early childhood between IVF and control children.

Subsequently, we related weight gain during early infancy, late infancy and early childhood to blood pressure levels and skinfold thicknesses measurements at follow-up in IVF and control children (Table 2 and 3). It was demonstrated that IVF children with rapid weight gain during late infancy did not differ with regard to blood pressure and sum of skinfolds at follow-up from IVF children with slow weight gain during late infancy. In contrast, rapid weight gain among controls in late infancy was associated with significantly increased skinfold thickness compared with controls with slow growth. Furthermore, rapid weight gain during early childhood in IVF children appeared to be related to higher blood pressure levels at follow-up, independently of birth weight, gestational age and height at follow-up, but not in controls. Weight gain during early infancy was not related to blood pressure or skinfold thickness in IVF children and controls. In both IVF and control children, rapid growth during early childhood was related to significantly higher sum of skinfolds at follow-up.
Figure 1 Postnatal measurements of weight (n = 5380; Figure 1A), height (n = 4559; Figure 1B) and BMI (n = 4540; Figure 1C) of 193 IVF and 199 control children.
Table 1  Growth during infancy and early childhood of 193 IVF children and 199 controls, all born to subfertile parents: cross-sectional data

<table>
<thead>
<tr>
<th>Weight</th>
<th>N</th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight SDS 3 mo</td>
<td>388</td>
<td>–0.18 ± 1.17</td>
<td>0.17 ± 1.05</td>
<td>0.002</td>
</tr>
<tr>
<td>Weight SDS 6 mo</td>
<td>384</td>
<td>–0.13 ± 0.98</td>
<td>0.08 ± 0.86</td>
<td>0.027</td>
</tr>
<tr>
<td>Weight SDS 1 yr</td>
<td>382</td>
<td>–0.03 ± 0.93</td>
<td>–0.03 ± 0.86</td>
<td>1.0</td>
</tr>
<tr>
<td>Weight SDS 2 yrs</td>
<td>308</td>
<td>0.06 ± 0.90</td>
<td>0.07 ± 0.95</td>
<td>0.9</td>
</tr>
<tr>
<td>Weight SDS 3 yrs</td>
<td>289</td>
<td>0.13 ± 0.91</td>
<td>0.02 ± 0.92</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Height</th>
<th>N</th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height SDS 3 mo</td>
<td>381</td>
<td>–0.06 ± 1.09</td>
<td>0.17 ± 1.17</td>
<td>0.045</td>
</tr>
<tr>
<td>Height SDS 6 mo</td>
<td>379</td>
<td>0.02 ± 1.08</td>
<td>0.20 ± 0.97</td>
<td>0.095</td>
</tr>
<tr>
<td>Height SDS 1 yr</td>
<td>380</td>
<td>0.07 ± 1.06</td>
<td>0.08 ± 0.97</td>
<td>1.0</td>
</tr>
<tr>
<td>Height SDS 2 yrs</td>
<td>297</td>
<td>0.11 ± 1.01</td>
<td>0.22 ± 1.04</td>
<td>0.3</td>
</tr>
<tr>
<td>Height SDS 3 yrs</td>
<td>284</td>
<td>0.16 ± 1.05</td>
<td>0.06 ± 0.99</td>
<td>0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI</th>
<th>N</th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI SDS 3 mo</td>
<td>355</td>
<td>–0.22 ± 1.08</td>
<td>0.01 ± 1.02</td>
<td>0.041</td>
</tr>
<tr>
<td>BMI SDS 6 mo</td>
<td>373</td>
<td>–0.24 ± 0.98</td>
<td>–0.12 ± 0.95</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI SDS 1 yr</td>
<td>376</td>
<td>–0.02 ± 0.96</td>
<td>–0.03 ± 0.94</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI SDS 2 yrs</td>
<td>292</td>
<td>–0.01 ± 0.93</td>
<td>–0.08 ± 1.05</td>
<td>0.5</td>
</tr>
<tr>
<td>BMI SDS 3 yrs</td>
<td>280</td>
<td>–0.003 ± 0.87</td>
<td>–0.05 ± 0.95</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight gain</th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ weight SDS 0–0.25 yr</td>
<td>388</td>
<td>–0.04 ± 1.15</td>
<td>0.15 ± 1.06</td>
</tr>
<tr>
<td>Δ weight SDS 0.25–1 yr</td>
<td>379</td>
<td>0.15 ± 0.94</td>
<td>–0.20 ± 0.86</td>
</tr>
<tr>
<td>Δ weight SDS 1–3 yrs</td>
<td>285</td>
<td>0.15 ± 0.72</td>
<td>0.11 ± 0.67</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Height gain</th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ height SDS 0.25–1 yr</td>
<td>371</td>
<td>0.15 ± 0.84</td>
<td>–0.07 ± 0.82</td>
</tr>
<tr>
<td>Δ height SDS 1–3 yrs</td>
<td>280</td>
<td>0.10 ± 0.79</td>
<td>0.01 ± 0.78</td>
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</table>

<table>
<thead>
<tr>
<th>BMI gain</th>
<th>IVF population</th>
<th>Control population</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ BMI SDS 0.25–1 yr</td>
<td>343</td>
<td>0.21 ± 1.03</td>
<td>–0.04 ± 1.02</td>
</tr>
<tr>
<td>Δ BMI SDS 1–3 yrs</td>
<td>280</td>
<td>0.02 ± 0.92</td>
<td>0.03 ± 0.99</td>
</tr>
</tbody>
</table>

Continuous variables were analyzed using Student t-test. SDS, Standard deviation score; 0–0.25 yr, early infancy; 0.25–1 yr, late infancy; 1–3 yrs, early childhood.
Table 2  Weight gain during early infancy (Δ weight SDS 0–0.25 year), late infancy (Δ weight SDS 0.25–1 year) and early childhood (Δ weight SDS 1–3 years) in relation to blood pressure at follow-up in IVF and control children, all born to subfertile parents

<table>
<thead>
<tr>
<th>Δ weight SDS 0–0.25yr</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Diastolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IVF children</td>
<td>P-value</td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>109 ± 11</td>
<td>0.8</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>108 ± 11</td>
<td></td>
</tr>
<tr>
<td>Highest tertile</td>
<td>111 ± 11</td>
<td></td>
</tr>
<tr>
<td>Δ weight SDS 0.25–1yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>110 ± 13</td>
<td>0.4</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>106 ± 10</td>
<td></td>
</tr>
<tr>
<td>Highest tertile</td>
<td>111 ± 10</td>
<td></td>
</tr>
<tr>
<td>Δ weight SDS 1–3yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>106 ± 11</td>
<td>0.014</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>109 ± 9</td>
<td></td>
</tr>
<tr>
<td>Highest tertile</td>
<td>112 ± 13</td>
<td></td>
</tr>
</tbody>
</table>

a Lowest versus highest tertile after adjustment for birth weight, gestational age, gender, age at follow-up and height at follow-up.
Table 3  Weight gain during early infancy (Δ weight SDS0–0.25 year), late infancy (Δ weight SDS 0.25–1 year) and early childhood (Δ weight SDS 1–3 years) in relation to skinfold thickness at follow-up in IVF and control children, all born to subfertile parents

<table>
<thead>
<tr>
<th>Δ weight SDS 0–0.25 yr</th>
<th>Peripheral sum of skinfolds (mm)</th>
<th>Total sum of skinfolds (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IVF children</td>
<td>Controls</td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>20.4 ± 9.5</td>
<td>21.1 ± 10.3</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>23.4 ± 11.2</td>
<td>19.0 ± 8.2</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>20.9 ± 9.3</td>
<td>19.2 ± 8.0</td>
</tr>
<tr>
<td>Δ weight SDS 0.25–1yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>20.3 ± 8.1</td>
<td>18.1 ± 8.2</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>21.5 ± 11.5</td>
<td>19.7 ± 9.7</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>22.6 ± 10.4</td>
<td>22.1 ± 8.4</td>
</tr>
<tr>
<td>Δ weight SDS 1–3yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>18.9 ± 7.6</td>
<td>17.3 ± 6.1</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>19.7 ± 8.5</td>
<td>17.9 ± 7.0</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>24.9 ± 12.9</td>
<td>22.8 ± 12.0</td>
</tr>
</tbody>
</table>

* Lowest versus highest tertile after adjustment for birth weight, gestational age, gender, age at follow-up and height at follow-up.
Discussion

This study is the first to examine growth velocity during early postnatal life in relation to cardiovascular risk factors in 8–18-year-old IVF and control children. IVF children had lower birth weight and showed a significantly greater gain in weight SDS, height SDS and BMI SDS during late infancy as compared with controls. Interestingly, exaggerated weight gain during late infancy was associated with increased skinfold thickness at follow-up in controls but not in IVF children. However, rapid weight gain during early childhood was related to higher blood pressure levels at follow-up, independent of birth weight, gestational age and body size at follow-up, among IVF children in contradiction to controls. Early childhood weight gain appeared to correlate with follow-up skinfold thickness in both IVF and control children.

In the present study, weight, height and BMI of IVF children were significantly lower shortly after birth compared with controls. Approximately 6 months after birth, the anthropometric differences observed between the IVF and control newborns were no longer present. Our findings are in line with several other studies during the past years that have addressed postnatal growth of IVF children. Normal weight and height parameters in IVF children ranging from 1 to 13 years were reported previously 8, 9, 26, 32, 37, 39, 45. Only one Finnish population-based cohort study reported that growth in IVF singletons was still behind the controls at the age of 3 years despite a catch-up growth during the first year of life 28.

We hypothesize that the exaggerated growth of IVF children during infancy, specifically observed between 3 and 12 months after birth, is a physiological and compensatory process to promote the restoration of the infants’ genetic growth trajectory after a period of prenatal growth restraint due unfavorable environmental conditions. This concept is supported by a recent study examining postnatal changes in body fat measures in infants who previously experienced fetal growth retardation 6. Catch-up growth appeared to correlate to the fetal growth pattern itself, irrespective of birth weight, and not to hyperphagia during infancy. Furthermore, growth velocity returned to physiological values when body composition was restored. An alternative explanation could be that the catch-up growth in IVF children during late infancy is primarily dependent upon postnatal nutritional environment. Adaptations to adverse conditions during early prenatal life may predispose to rapid postnatal weight gain in a more favorable postnatal environment. Furthermore, it should be explored why IVF children seem to catch-up in weight during late infancy and not directly after birth.

To our knowledge, it is currently unknown during which phase of prenatal life growth retardation in IVF children is caused and, importantly, which factors are responsible. As longitudinal information on fetal growth is rarely available, for most studies, including ours, birth weight is often the only feasible measure. Nevertheless, it is important to emphasize that environmental stimuli may affect embryonic and/or fetal growth trajectories without an effect on birth weight 7. It remains to be
established if parental characteristics, technical aspects of IVF treatment or a combination of these are involved in the induction of these aberrant prenatal growth patterns and subsequent accelerated postnatal growth.

Currently, there is still debate as to whether there are critical time windows during early postnatal life that are important in determining later blood pressure and body composition. Infancy is often hypothesized to represent an important period, because it is a time of extremely rapid growth, particularly for those infants who experienced fetal growth restriction. In the present study, rapid weight gain during late infancy was associated with higher body fat measures in controls. These findings are in accordance with studies linking rapid weight gain during infancy to an increased risk for obesity in childhood and young adulthood. Several cohort studies demonstrated a positive association between weight gain in the first year of life and later blood pressure. However, despite the significantly faster growth during late infancy observed among IVF children as compared with controls, weight gain during infancy was not associated with blood pressure or skinfold measurement at ages 8–18 years in IVF children. Our data emphasize that the catch-up growth during infancy is not accompanied by detrimental consequences on blood pressure and body composition during late childhood and adolescence in IVF offspring. IVF children may follow an optimal pathway of healthy catch-up growth during infancy: such pathways have recently been suggested, although they are considered to be rare or narrow in view of the accumulating body of evidence for detrimental effects of rapid catch-up growth in general. A recent study supporting this concept showed that rapid weight gain in fetal growth restricted infants promoted the restoration of body size and fat stores without detrimental consequences at 1 year of age on body composition or metabolic profile. On the other hand, rapid weight gain was not related to blood pressure in our controls, and this lack of association has been reported by others. Conflicting results regarding the relationship between rapid weight gain in infancy and blood pressure could occur as a result of differences in characteristics of the study subjects or method of analysis.

Our findings highlight the critical influence of early childhood, rather than infancy, on weight gain in IVF children. Significant associations between childhood weight gain and systolic blood pressure at follow-up were found in IVF children, irrespectively of perinatal outcome and body size at follow-up. Such associations were not found in the control children. Our findings indicate that particularly growth during early childhood programs later systolic blood pressure in IVF offspring. We propose that IVF children could show different types of rapid growth during early postnatal life, with distinct health effects. Different processes may be involved during rapid weight gain in infancy and early childhood. It is important to realize that catch-up growth is not always similar to weight gain. Catch-up growth indicates weight gain appropriate for height gain, whereas excessive weight gain as such does not necessarily mirror changes in height. This distinction could explain some discordant results on the relationship between growth measurements during early postnatal life and subsequent metabolic risk. The mechanisms linking early childhood growth to later cardiovascular risk factors are not yet known, and will likely include interactions between
antenatal growth restraint, postnatal nutrition and genetic factors. Further research is necessary to find out whether these aspects could explain the association between rapid weight gain during early childhood and higher blood pressure at age 8–18 years in IVF children, or whether other mechanisms are involved.

One of the strengths of this study lies in the selection of the control children, which were born from parents previously diagnosed with subfertility. To adequately examine postnatal growth and development in IVF children, an appropriate comparison group of children who were conceived without use of assisted reproduction technology was needed. It is generally known that IVF parents differ from the general reproductive population in terms of age, parity and other important characteristics. Specifically, there are several studies indicating that subfertility itself, independent from the mode of conception, is a risk factor for adverse neonatal outcome and pregnancy complications. Studies which compare growth and development of IVF children to spontaneously conceived children from fertile couples should always keep in mind a potential effect of the underlying subfertility in the IVF group. To avoid confounding related to these known differences, comparison with children born to subfertile parents after spontaneous conception was preferred. Our study sample, based on availability of sufficient early growth measurements and follow-up measurements, did not differ regarding birth weight from the larger study cohort. However, there appeared to be a significant difference in the number of children born preterm. This phenomenon was found in both the IVF and the control population. Therefore, it is unlikely that the comparisons performed between IVF and control children in the present study have been confounded, but it might have implications for the generalizability of our findings.

Body fat composition differs between prepubertal and postpubertal children, and between boys and girls. One could argue whether the combined analysis of pre- and postpubertal children as well as boys and girls is appropriate. Our previous study demonstrated that differences in body fat composition between IVF and control children could not be explained by pubertal stage or gender. Therefore, data regarding blood pressure and body fat of children aged 8–18 years were combined in the present study. As previously discussed, it has to be taken into account that especially peripheral adipose tissue was increased in IVF children, although increased risk for cardiovascular health problems has been indicated to be primarily linked to a central body fat deposition. However, in view of our other findings demonstrating higher blood pressure and fasting glucose levels in IVF children compared with controls, continued body fat monitoring in IVF offspring is of great importance. Lastly, we might have underestimated the true link between early postnatal growth and cardiovascular risk factors at follow-up among IVF children by correcting for birth weight, gestational age and body size at follow-up. IVF is known to be associated with lower birth weight and shorter gestational age, although these factors themselves have been found to relate to early postnatal growth, blood pressure and body fat composition.

In conclusion, late infancy growth velocity of IVF children was significantly higher compared with controls. However, growth during this period has not been associated with an adverse cardiovascular
profile at follow-up in IVF children. In contrast, weight gain during early childhood seems to predict cardiovascular risk factors in IVF children. These results suggest that caution in promoting excessive early childhood growth among IVF children is necessary. Preventive health strategies might be useful in the future to moderate early postnatal growth of IVF children by taking into account the possible benefits of early growth, such as improved cognition, as well as the potential harms to cardiovascular and metabolic health. However, as this study is the first to examine the associations of early postnatal growth in relation to blood pressure and body fat composition in IVF and control children, our results first need to be reproduced by other prospective follow-up studies.
References


37. Place I and Englert Y. *A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilization*. Fertil Steril 2003;80:1388-1397.


School functioning in 8- to 18-year-old children born after in vitro fertilization

Karin Wagenaar, Manon Ceelen, Mirjam M. van Weissenbruch, Dirk L. Knol, Henriette A. Delemarre-van de Waal and Jaap Huisman

Abstract

Background: Only a limited number of studies have evaluated the school performance of in vitro fertilization (IVF) children. The aim of this study was to examine the school functioning of 8- to 18-year-old children born after IVF.

Methods: Two hundred thirty-three children born after IVF were compared with 233 matched control children born spontaneously from parents with fertility problems on measures of education level, general cognitive ability, school performance (need for extra help, repeating a grade, special education), and rates of learning and developmental disorders.

Results: No differences were found between IVF and control children on these measures of school functioning. More than 60% of adolescents at secondary school attended high academic levels (with access to high school or university). They do not experience any more educational limitations than the naturally conceived children and adolescents of the control group.

Conclusions: Children and adolescents born after IVF show good academic achievement and general cognitive ability. The tendency of reassuring school functioning already found in younger IVF children has been shown to continue at secondary school age.
Introduction

The first birth after in vitro fertilization (IVF) was reported in 1978. Since then, the numbers of newborns conceived by this technology have been growing rapidly and, today, IVF is part of the modern management of infertility worldwide. Approximately 1–3% of the children in developed countries have been born after IVF conception and a considerable number of these children meanwhile have reached adolescence or young adulthood.

Shortly after the first IVF births, clinicians and researchers became aware of possible increased physical and psychological risk associated with IVF, which resulted in the evaluation of the children born from it. From the psychological point of view, mental and psychomotor development in the early years was an important focus. Although IVF pregnancies appear to be associated with an increased risk of multiple pregnancy, preterm delivery, low birth weight, cesarean sections, and transfer to a neonatal intensive care unit, studies on mental and psychomotor development, in general, did not show any differences between IVF and naturally conceived children in the first 3 years of life. Developmental and neurological problems that were found at this young age were mainly related to prematurity, low birth weight, or multiple births. Also, intellectual development around the age of 5 years was investigated and found not to be different in children conceived by IVF compared to naturally conceived children.

However, from an age of about 6 years, when school functioning becomes more important and cognitive demands increase, only a limited number of studies have evaluated the school performance of IVF children. Although the data from these three studies indicate normal intellectual and school functioning, it can be questioned as to whether one may be conclusive yet on the overall educational outcome of IVF children. No control groups were used and the children were a maximum of 13 years old, or the study was conducted on a relatively small number of children. Most importantly, no single study has described IVF children's school functioning at secondary school yet.

At the VU University Medical Center (VUMc) in the Netherlands, IVF has been conducted since the early 1980, with the first birth occurring in 1986. Since a considerable group of these IVF children have now reached (pre)pubertal years, we performed a large study on the growth, health, and psychological functioning of IVF children born between 1986 and 1995. In this study, IVF children were matched and compared to children born spontaneously from parents with fertility problems. The selection of such a control group gives us the opportunity to equalize important parental differences (such as age of the mothers at birth, desirability of and involvement with the child, and the educational level of the parents) and evaluate the role of IVF as such on the child's development. Considering the mentioned literature on academic performance, the aim of the study described here was to evaluate the school functioning of IVF children and adolescents who are at the end of primary school and at secondary school. This paper describes the education
level, general cognitive ability, school performance (need for extra help, repeating a grade, special education), and rates of learning or developmental disorders in our cohort of IVF children aged 8 to 18 years, compared to that of the matched control group.

**Materials and methods**

**Population and participants**

The data described in this paper were derived from a large study performed between March 2003 and March 2006 on the growth, health, and psychological functioning of IVF children born between 1986 and 1995 (see for more details of the study population Ceelen et al. 5). Families with a singleton child conceived by IVF conception in the VUmc, Amsterdam, the Netherlands, were invited by mail to participate in the study. Twins and children born from other reproductive techniques, such as intracytoplasmic sperm injection (ICSI), were excluded. Of the 354 invited IVF families, 12 children were not reachable because they moved or did not respond. Of the 342 families who could be traced, 96 parents and/or children themselves refused to participate, whereas 246 agreed to take part in the study (representing a response rate of 69%).

A control group of children spontaneously born between 1986 and 1995 from parents who previously visited the Department of Gynecology of the VUmc with fertility problems was used. Children were selected and matched one-to-one on sex and age (± 3 months) with the IVF children. Initially, 454 control families were invited, of which, 31 families were not reachable and three children were deceased. Of the 420 control families who could be traced, 233 children and their parents wanted to participate in the study (response rate 51%). This means that for 13 IVF children, no matched control child was found. These 13 IVF children were excluded from the analysis and 233 matched couples were included.

Families who refused to participate in the study received a questionnaire regarding growth, health, and education level of both parents and the child, and other child characteristics. The participating children were significantly younger than non-participants (12.2 ± 2.6 vs. 12.7 ± 2.4 years, P < 0.01) and mothers of participating children more often had a high education level (37 vs. 24% in mothers of non-participating children, P < 0.01). Differences were similar for the IVF and control populations.

**Procedure and measurements**

The study protocol was approved by the Medical Ethics Committee of the VUmc and the Central Committee of Human Research (CCMO) in The Hague. Parents, and children from 12 years of age, signed informed consent forms prior to participation.
Children who participated in the study were seen at the VUmc for evaluation of their physical and developmental history and present growth, health, and psychological functioning. Shortly before the hospital visit, parents were sent a questionnaire to fill in at home, to make an inventory of their fertility problems and treatment, the course of the pregnancy, and socio-demographic characteristics. For the current paper, the following data were used.

**Perinatal and socio-demographic data.** With respect to the pregnancy, the gestational age and birth weight of the children were inquired. Gestational age was obtained by parents’ self-report and the birth weight data were obtained from the hospitals’ birth certificates (49%), outpatient clinic reports (37%), or self-reporting by the parents (14%). From the parents, the age of the mother at delivery and the current age of the parents were calculated by using the child’s and parents’ birth dates and the date of the hospital visit for participation in the study. Parental education level was scored as low (elementary school or lower level of secondary school and vocational training), middle (medium and higher level of secondary school or medium level of vocational training), or high (university or higher level of vocational training), and was evaluated in three ways: educational level of the mother, educational level of the father, and the highest educational level of one of either parent. Furthermore, it was registered whether the mother was a first-time mother (primipara) or not.

**Education level of the child.** In general, data were obtained about the current education level of the child according to the levels of the Dutch school system. Subsequently, secondary school levels were grouped into low (lower level of secondary school), middle (medium level of secondary school), or high (higher level of secondary school, e.g., school levels with access to high school or university).

**General cognitive ability.** To screen whether there are differences in the general cognitive ability between the groups, the results of a national test of educational achievement (CITO) administered around the age of 12 in the last year of primary school were used. From the children in the study group who took the CITO test, permission was sought to use their score. In the Netherlands, this test is almost generally used to determine high school entrance level. The CITO scores correlate with IQ measures of 0.63 at 12 years of age.

**School performance and learning and developmental disorders.** School performance was evaluated by the rate of children who needed extra lessons, repeated a grade, or who had attended special education during their school career so far. Moreover, the existence of learning or developmental disorders diagnosis, such as dyslexia, attention deficit hyperactivity disorder (ADHD), or autism, as reported by the parents, was registered.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS) Windows version 12.0 and Stata 8.0, a program for statistical data analysis, were used for the analyses. Perinatal and socio-demographic data were analyzed by SPSS using a paired sample t-test for continuous data, McNemar test for dichotomous data, and Wilcoxon signed rank test for non-dichotomous data. Perinatal and socio-demographic
data that differed significantly between the groups were entered into further statistical analyses as covariates. Group comparisons for school performance and learning and developmental disorders were performed with Stata by using a random-effects logistic regression. Group comparison for CITO scores was done in SPSS using multiple linear regression.

**Results**

*Perinatal and socio-demographic data*

In Table 1, the characteristics of the 233 IVF and 233 age- and sex-matched control children and their parents are summarized. The mean age of the children was 12.20 years (SD ± 2.61) in the IVF group and 12.21 years (SD ± 2.59) for the control children (the age range and distribution within both groups are presented in Figure 1). On average, the IVF children had a significantly shorter gestational age than children in the control group (38.91 vs. 39.48 weeks, t[232]= −2.88, p = 0.004). There was also a significant difference in the proportion of children born prematurely, i.e., < 37 weeks (13% in the IVF vs. 6% in the control group, χ²[1, n = 233]= 5.92, P = 0.015). Birth weight was significantly lower for IVF children than for controls (3,217.7 vs. 3,427.6 grams, t[232]= −3.85, P = < 0.001) and more IVF children had birth weights below 2,500 grams (11% in the IVF vs. 3.5% in the control group, χ²[1, n = 232]= 8.26, P = 0.004).

![Figure 1](image-url)  
*Figure 1*  Age range and distribution within the in vitro fertilization (IVF) and control group
Table 1  Child and parental characteristics of the in vitro fertilization (IVF) and control group

<table>
<thead>
<tr>
<th></th>
<th>IVF children (n = 233)</th>
<th>Controls (n = 233)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (boys/girls)</td>
<td>115 (49%)/118 (51%)</td>
<td>115 (49%)/118 (51%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>Mean ± SD 12.20 ± 2.61</td>
<td>12.21 ± 2.59</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Range 8–18</td>
<td>8–18</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td>Mean ± SD 38.91 ± 2.48</td>
<td>39.48 ± 1.85</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Range 27–44</td>
<td>33–43</td>
<td></td>
</tr>
<tr>
<td>Premature *</td>
<td>30 (13%)</td>
<td>14 (6%)</td>
<td>0.015</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>Mean ± SD 3,217.7 ± 626.2</td>
<td>3,427.6 ± 554.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Range 870–5,000</td>
<td>1,450–5,110</td>
<td></td>
</tr>
<tr>
<td>LBW b</td>
<td>26 (11%)</td>
<td>8 (3.5%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Mothers’ age at delivery (yr)</td>
<td>Mean ± SD 34.62 ± 3.77</td>
<td>34.46 ± 4.01</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Range 24.83–42.15</td>
<td>23.76–43.26</td>
<td></td>
</tr>
<tr>
<td>Mothers’ age at follow-up (yr)</td>
<td>Mean ± SD 46.82 ± 4.50</td>
<td>46.66 ± 4.27</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Range 35.11–58.04</td>
<td>36.15–57.91</td>
<td></td>
</tr>
<tr>
<td>Fathers’ age at follow-up (yr)</td>
<td>Mean ± SD 49.26 ± 5.51</td>
<td>49.32 ± 5.23</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Range 36.25–71.79</td>
<td>36.92–65.29</td>
<td></td>
</tr>
<tr>
<td>Primipara</td>
<td>186 (80%)</td>
<td>107 (46%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Maternal education level *</td>
<td>Low 50 (21.5%)</td>
<td>35 (15%)</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Middle 106 (45.5%)</td>
<td>99 (42.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High 76 (33%)</td>
<td>99 (42.5%)</td>
<td></td>
</tr>
<tr>
<td>Paternal education level *</td>
<td>Low 45 (19.5%)</td>
<td>35 (15%)</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>Middle 103 (44.5%)</td>
<td>96 (41.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High 83 (36%)</td>
<td>101 (43.5%)</td>
<td></td>
</tr>
<tr>
<td>Parental highest education level *</td>
<td>Low 21 (9%)</td>
<td>19 (8%)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Middle 101 (43.5%)</td>
<td>88 (38%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High 110 (47.5%)</td>
<td>126 (54%)</td>
<td></td>
</tr>
</tbody>
</table>

Paired t-tests were used to compare the group means, dichotomous data were analyzed using McNemar tests (* Wilcoxon signed rank test).

* Premature = born < 37 weeks of gestation.

b LBW (low birth weight) = birth weight < 2,500 grams.
n.s., Not significant.
Mothers’ age at delivery and mothers’ and fathers’ age at assessment did not differ significantly between the groups. In the IVF children, the mothers were significantly more often primipara at their birth than the mothers of the children from the control group (80% in the IVF vs. 46% in the control group, \( \chi^2[1, n = 466] = 46.44, P < 0.001 \)). Mothers (\( Z = -2.66, P = 0.008 \)) and, to a lesser extent, fathers (\( Z = -1.96, P = 0.05 \)) of IVF children more often had low or middle and less often had a high educational level than parents of children from the control group (Table 1).

The variables on which the groups differed (gestational age, birth weight, parity, and mothers’ educational level) were entered into further statistical analyses as covariates.

**Education level and general cognitive ability**

Table 2 demonstrates that, at the time of evaluation, 53% of the children in both groups were in primary school, and 44% of the IVF and control children attended secondary school. About 2.5% of the children already attended vocational education or university, had left school, or had a job.

At secondary school, a majority of both the IVF and control children (61% in the IVF and 67% in the control group, respectively) attended a high school level (with access to high school or university). Compared to the non-participants, at secondary school, participants more often had a high school level and less often had a low school level. This was the case in IVF as well as in the control children.

CITO test scores were used to screen the level of general cognitive ability in both groups. Of the total group of 233 IVF and 233 control children, 101 IVF children (43%) and 92 control children (40%) did take a CITO test. Of the remaining children, 107 children in the IVF (46%) and 112 children in the control groups (48%) did not have a CITO score available because they had not taken the CITO test already, and 25 IVF children (11%) and 29 control children (12%) undertook another test in their last year to determine their advice for secondary school.

CITO test scores of 74 IVF children (73%) and 66 control children (72%) were really available for analyses. From the remaining children who did take a CITO test, the score was not received, even after repeated requests to the parents (no significant difference in the response rate between IVF and controls). As shown in Table 2, no significant differences were found for CITO scores in the IVF vs. the control groups. In the statistical analysis, the gestational age, birth weight, parity, and maternal education level were entered as covariates. In both groups, the scores are above the overall Dutch population score (mean score 535) and above the score for children from urban districts (mean 538; http://www.cito.nl).
Table 2  Education level and general cognitive ability in the IVF and control group

<table>
<thead>
<tr>
<th></th>
<th>IVF children (n = 233)</th>
<th>Controls (n = 233)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary school</td>
<td>123 (52.8%)</td>
<td>124 (53.2%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Secondary school</td>
<td>104 (44.6%)</td>
<td>103 (44.2%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Secondary school level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>7 (6.7%)</td>
<td>5 (4.9%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Middle</td>
<td>34 (32.7%)</td>
<td>29 (28.2%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>63 (60.6%)</td>
<td>69 (66.9%)</td>
<td></td>
</tr>
<tr>
<td>Vocational education/university</td>
<td>5 (2.1%)</td>
<td>4 (1.7%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Left school and/or job</td>
<td>1 (0.4%)</td>
<td>2 (0.9%)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>IVF children (n = 74)</th>
<th>Controls (n = 66)</th>
<th>Adjusted difference* (IVF–control)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTTO test score</td>
<td>539.43 (± 9.5)</td>
<td>540.70 (± 7.9)</td>
<td>−1.37</td>
<td>0.377</td>
</tr>
</tbody>
</table>

*With covariates parity, maternal education level, gestational age, and birth weight in the univariate linear model
n.s., Not significant
Table 3  Extra lessons, repeated a grade, special education, and the existence of learning or developmental disorders in the IVF and control group

<table>
<thead>
<tr>
<th></th>
<th>IVF children (n = 233)</th>
<th>Controls (n = 233)</th>
<th>Adjusted odds ratio *</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra lessons</td>
<td>88 (37.8%)</td>
<td>91 (39.1%)</td>
<td>1.02</td>
<td>0.927</td>
</tr>
<tr>
<td>Repeated a grade</td>
<td>48 (20.6%)</td>
<td>52 (22.3%)</td>
<td>0.94</td>
<td>0.800</td>
</tr>
<tr>
<td>Nursery school</td>
<td>17 (6.9%)</td>
<td>14 (6.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>21 (8.5%)</td>
<td>20 (8.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>6 (2.4%)</td>
<td>5 (2.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than once</td>
<td>1 (0.4%)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeated a grade followed by special education</td>
<td>11 (4.5%)</td>
<td>16 (6.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Special education</td>
<td>15 (6.4%)</td>
<td>16 (6.9%)</td>
<td>0.90</td>
<td>0.791</td>
</tr>
<tr>
<td>Learning or developmental disorder</td>
<td>32 (13.7%)</td>
<td>42 (18.0%)</td>
<td>0.73</td>
<td>0.310</td>
</tr>
<tr>
<td>Dyslexia</td>
<td>14 (6.0%)</td>
<td>18 (7.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-verbal learning disorder</td>
<td>1 (0.4%)</td>
<td>5 (2.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Learning disabled</td>
<td>4 (1.7%)</td>
<td>5 (2.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD</td>
<td>2 (0.9%)</td>
<td>3 (1.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autistic disorder</td>
<td>1 (0.4%)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor coordination problems/cerebral palsy</td>
<td>3 (1.3%)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td>7 (3.0%)</td>
<td>11 (4.7%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* With covariates parity, maternal education level, gestational age, and birth weight in the random-effects logistic regression.
School performance and learning or developmental disorders

As shown in Table 3, no differences were found between the IVF and control groups on the need for extra lessons, repeating a grade, or attending special education. Gestational age, birth weight, parity, and maternal education level were entered in the statistical analysis as covariates. A total of 38% of the IVF children and 39% of the control children needed extra lessons and 21 vs. 22% repeated a grade, most children at nursery and primary school. In both the IVF and control groups, 6% of the children attended special education (primary and/or secondary school). According to the parents, in 14% of the IVF children and 18% of the control children, a learning or developmental disorder has been diagnosed. Also, this difference does not reach statistical significance. Dyslexia is the most common disorder in both groups (about 7%). Also, 3% of the IVF and 5% of the control children have a combination of diagnoses, such as ADHD and an autistic disorder or dyslexia and ADHD. In the IVF group, but not in the control group, motor coordination disorders due to cerebral palsy are present (n = 3).

Excluding children with learning or developmental disorders from the analyses did not lead to significant differences between the groups. Rather, a somewhat lower proportion of children in both the IVF and control groups then needed extra lessons, repeated a grade, or attended special education (results not shown in the table).

Discussion

In this paper, we described the school functioning of a large cohort of IVF singletons born between 1986 and 1995 after conception in the VUmc in the Netherlands. All children, 8–18 years of age, were at the end of primary school or in secondary school, school years on which no conclusive data were yet available. Our findings in IVF children were compared to that of matched control children born spontaneously after a period of subfertility in their parents. The use of this control group gave us the opportunity to equalize as much as possible important parental differences and evaluate the role of IVF as such on the child's development.

We found no indications for educational limitations in IVF children at the end of primary and in secondary school. As many IVF as control children needed extra lessons, repeated a grade, or attended special education. IVF children did not have learning and developmental disorders more often than children from the control group. Also, on general cognitive ability, no differences were found between IVF and control children, and their scores were above the overall Dutch population score and the score for children from urban districts. The distribution of low, middle, or high education level in the children at secondary school did not differ between the groups and was comparable to the distribution found in the parents. More than 60% of adolescents at secondary school attended a high academic level (with access to high school or university).
Our findings are reassuring and in line with the recent studies in young IVF children\textsuperscript{2, 4, 6, 7, 9, 10, 13, 18-22, 24, 25} and at primary school age\textsuperscript{11, 16, 17}. Olivennes et al.\textsuperscript{16} found, in a follow-up study of 422 IVF children aged 6 to 13 years, according to the French school system, that 92.2\% of the children had at least average school achievement. In 1- to 9-year-old IVF children conceived from cryopreserved embryos, the same authors found comparable results for scholastic performance in the school-age children\textsuperscript{17}. Levy-Shiff et al.\textsuperscript{11} examined 9- and 10-year-old IVF children in comparison with naturally conceived children recruited from the IVF children's schools and found that intelligence did not differ between the groups. In addition, our findings show also that the tendency of good educational outcome in IVF children continues at secondary school.

However, before drawing any definite conclusions, some aspects have to be considered. With the selected control group in our study, we tried to equalize important parental factors. While most aspects were similar, unfortunately, there were some differences in the education level of the parents. Parents of control participants appeared to be more often higher educated than IVF parents. By using the maternal education level as a covariate, we tried to correct for this difference, as we did also for differences in parity, gestational age, and birth weight. In addition, in our study population, the participants were more highly educated than non-participants. This could have led to an underestimation of the number of children having extra lessons, repeated a grade, attending special education, or having a learning or developmental disorder, and the enhancement of the mean values of the CITO score. Therefore, the precise proportions have to be considered with some caution. Although education level differences were similar in IVF and control (non-)participants, we stress the importance of including lower educated IVF children in future studies.

Notwithstanding the above, we conclude that the tendency of reassuring school functioning already found in younger IVF children has been shown to continue at secondary school age.
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Summary and general discussion
Summary

During the past decades, in vitro fertilization (IVF) has become a routine procedure in reproductive medicine to overcome subfertility problems in couples all over the world. Since the first IVF birth in 1978, an estimated three million children have been born worldwide after IVF or related assisted reproductive technologies (ART). In 2005, ~2.3% of all Dutch babies were born after assisted reproduction. Nowadays, it is increasingly acknowledged that the IVF procedure may affect the vulnerable processes occurring during conception and early embryonic development. There is a substantial body of evidence that IVF children are at increased risk for adverse perinatal outcome. In addition, various studies suggested an increased incidence of congenital abnormalities and rare imprinting diseases among IVF children. However, due to the lack of systematic follow-up of these children during the past years, it is largely unclear whether IVF treatment in humans is associated with substantial developmental consequences in later stages of life in conceived offspring. Therefore, the present thesis addressed several aspects of postnatal growth and development in 246 8- to 18-year-old IVF singletons and 233 spontaneously conceived controls born to subfertile parents. The aims and outline of the thesis were described in Chapter 1.

Chapter 2 presented an extensive review of the current literature about growth and development of children born after IVF treatment. Perinatal outcome of IVF pregnancies, the occurrence of congenital anomalies, imprinting disorders, malignancies and postnatal growth characteristics in IVF offspring were thoroughly discussed. Several meta-analyses and other well-designed studies provided substantial evidence that IVF children are at increased risk for adverse perinatal outcome, congenital malformations and rare epigenetic defects. There is still no consensus on whether observed health problems are related to the IVF procedure itself and/or the underlying subfertility problems of the parents. Studies examining postnatal growth, development and morbidity rates are scarce with conflicting results and other areas of long-term research in IVF children are still in its infancy.

The objective of Chapter 3 was to investigate several cardiometabolic measures in 8- to 18-year-old IVF singletons and spontaneously conceived controls born from subfertile parents. Previous studies have demonstrated that adverse conditions during early prenatal life are associated with cardiometabolic dysfunction in postnatal life. Blood pressure was examined in 225 IVF children and 225 age- and gender-matched spontaneously conceived control children. Several indicators of insulin resistance were studied in a pubertal subpopulation. Systolic and diastolic blood pressure levels were higher in IVF children than in controls (109 ± 11 vs. 105 ± 10 mm Hg, P < 0.001; 61 ± 7 vs. 59 ± 7 mm Hg, P < 0.001, respectively). IVF children appeared ~2 times more likely to be in the highest systolic and diastolic blood pressure quartiles. Moreover, significantly higher fasting glucose levels were found in pubertal IVF children (5.0 ± 0.4 vs. 4.8 ± 0.4 mmol/l in controls, P = 0.005). Differences in blood pressure and fasting glucose could neither be explained by current body size, birth weight and other early life factors nor by parental characteristics including
cause of subfertility. Therefore, we hypothesized that the IVF procedure might contribute to the programming of cardiometabolic physiology and function in conceived offspring. The findings described in this chapter highlight the importance of continued cardiometabolic monitoring of IVF-conceived children in order to evaluate the clinical consequences in later life.

In Chapter 4, body composition in 233 IVF singletons and 233 spontaneously conceived controls born from subfertile parents was examined by anthropometry. In addition, body composition was also assessed in a pubertal subpopulation by means of dual energy X-ray absorptiometry (DXA). Other studies indicated a link between adverse stimuli during periconception versus disturbed adipose tissue development and obesity in postnatal life. Children born after IVF had a significantly lower subscapular-triceps skinfold ratio and a significantly higher sum of peripheral skinfolds, peripheral body mass, and percentage of peripheral body fat compared with controls. Although not reaching statistical significance, both DXA and skinfold measurements indicated that total body fat in IVF children is increased. The differences in peripheral fat assessed by anthropology between IVF and control children could neither be explained by current and early risk factors nor parental factors like cause of subfertility. No differences in bone mineral composition between IVF children and controls were demonstrated. Since these observations suggest that body fat composition in IVF children is disturbed, follow-up of IVF children to monitor body fat pattern and potentially related health problems from adolescence into adulthood is of great importance.

Chapter 5 focused on several anthropometric, radiological and biochemical characteristics of pubertal development in IVF children and spontaneously conceived control children. Pubertal stage by Tanner’s classification, age at menarche and menstrual cycle characteristics were examined in 115 IVF-conceived boys and 118 IVF-conceived girls, each with age-matched comparison groups. Bone age and sex hormone levels were studied in two distinct pubertal subpopulations. Pubertal stage and age at menarche were not significantly different between IVF and control children. Advanced bone age and higher DHEAS and LH levels were observed in pubertal IVF girls in comparison to controls. However, since these findings did not follow from a specific hypothesis being tested, multiple statistical significance testing could have led to chance findings.

Numerous studies have demonstrated a relationship between early postnatal growth and blood pressure and body fat in children and adolescents. Since little is known about postnatal growth in IVF offspring and its effects on blood pressure and body fat composition during childhood and adolescence, these aspects were studied in Chapter 6. Data from original growth charts up to four years of age were used to study early postnatal growth in 193 IVF and 199 control children born to subfertile parents. Significantly lower weight, height and BMI standard deviation scores (SDSs) at 3 months and weight SDS at 6 months of age were found in IVF children compared with controls. Likewise, IVF children demonstrated a greater gain in weight SDS, height SDS and BMI SDS during late infancy (3 months to 1 year) versus controls. Weight gain during early childhood (1–3 years) was related to blood pressure in IVF children but not in controls. Growth during late
infancy was not related to skinfold thickness in IVF children, unlike controls. Growth during early childhood was associated with skinfold thickness in both IVF and controls. It was concluded that growth during early childhood is related to several cardiovascular risk factors in IVF offspring during late childhood and adolescence.

The aim of the study in Chapter 7 was to examine the school functioning of 8- to 18-year-old IVF children and spontaneously conceived controls born to subfertile parents. Measures of education level, school performance (need for extra help, repeating a grade, special education), and rates of learning and developmental disorders were compared in 233 IVF children and 233 controls. General cognitive ability was examined in a pubertal subpopulation. None of the measures concerning school functioning differed between IVF and control children. Children and adolescents born after IVF demonstrated good academic achievement and general cognitive ability. Furthermore, they did not experience more educational limitations than spontaneously conceived children and adolescents. These results suggest that school functioning of children and adolescents born after IVF treatment appears to be normal.

General discussion

The goal of the general discussion is to address methodological issues, pathophysiological considerations, potential implications of our findings and recommendations for future research.

Methodological considerations

The methodological issues of the studies presented in this thesis have been discussed in the separate chapters. The strengths of our study include the relatively large study size and the comparison group consisting of spontaneously conceived children born from subfertile parents. Furthermore, the IVF singletons and controls were carefully matched according to age and gender. All children were examined in a standardized manner and data regarding a wide range of developmental aspects were collected. In the following section, methodological considerations regarding the comparison group used, the potential for selection bias, the assessment of outcome variables, and confounding and the statistical analyses are described in more detail.

Comparison group

In order to be able to adequately examine postnatal growth and development in IVF children, an appropriate comparison group of unexposed children is needed. Ideally, the unexposed group resembles the exposed group with respect to all factors related to the outcomes of interest, except for the exposure under investigation. Although from a scientific point of view randomization of
IVF treatment versus no IVF treatment for subfertile couples would be valuable, such randomized clinical trials are ethically not feasible. As a consequence, clues can only be obtained from observational studies which investigate postnatal growth and development in IVF offspring. However, it is generally known that IVF parents differ from the general reproductive population with regard to age, parity and other important characteristics, of which some are known to be associated with perinatal complications. To avoid confounding due to these known differences, a comparison group consisting of children born to subfertile parents after spontaneous conception was preferred in the present follow-up study. Children born after spontaneous conception to couples who originally visited the VU medical center due to subfertility problems, and who subsequently participated in the OMEGA study, were eligible to participate in the study as control children. Age and gender were used as matching criteria with a maximum difference in the age of the IVF child and the matched control of three months. Unfortunately, additional matching on parental subfertility characteristics was not possible due to the relatively small numbers of eligible children. It cannot be excluded that the type and the severity of subfertility differed between parents who conceived after IVF and those who conceived spontaneously. Such differences may have produced bias in the comparison of outcome variables in IVF and control children. Nevertheless, most control mothers who participated in the present study were diagnosed with subfertility in an era (1982–1990) when IVF was not a routine procedure, it was ethically controversial and it was not accessible to many women. In addition, observed differences in blood pressure and fasting glucose between IVF and control children were hardly affected by adjustment for parental subfertility causes, rendering residual confounding very unlikely. Likewise, in assisted reproduction in animals, which shares many similarities with IVF used in humans, neither the animals donating the gametes nor the recipients suffer from infertility. However, the produced offspring is also at increased risk for developmental abnormalities, suggesting that assisted reproduction techniques as such contribute to the induction of adverse outcomes. Lastly, parental educational level of the participating IVF children and controls appeared to differ. Nonetheless, adjustment for these factors did not change our outcome parameters. Other important parental characteristics like parental age and BMI did not differ between the IVF and control group.

Selection bias

Selection bias typically arises when study participants are not representative of the population in question. Ideally, the subjects in a study should be very similar to the larger population from which they are drawn. If there are important differences between the study sample and the total population, the results of the study may not be valid. This is especially the case if the response rates and characteristics of the non-participants differ between those with and without the study exposure of interest. The overall participation rate of our follow-up study was 59%. Approximately 69% of the approached IVF children and 51% of the approached controls (children of subfertile parents, not conceived by IVF) participated in our study. The main reason for non-participation appeared to be unwillingness of the invited child to participate. Non-participation analysis yielded
no significant differences between participating children and non-participating children in anthropometric measures. This suggests that those who participated are a representative sample of the entire cohort. On the other hand, mothers of non-participating children less often appeared to be highly educated compared with mothers of participating children (26 vs. 37%, $P = 0.015$). However, this phenomenon was observed in both the IVF and the control population. Therefore, confounding of the comparisons performed between IVF and control children is unlikely, but it might have implications for the generalisability of our results. Furthermore, our observation is consistent with a large amount of evidence stating that persons with higher socioeconomic status are more likely to participate in scientific studies.\textsuperscript{14}

\textit{Assessment of outcome}

As our study is one of the first to investigate postnatal growth of 8- to 18-year-old IVF-conceived offspring and matched controls, it has a rather explorative character and a broad scale of developmental aspects which are important during childhood and adolescence has been studied. Blood pressure, glucose/insulin metabolism and body composition were examined as these outcome variables are particularly relevant in view of the “developmental origins of health and disease” hypothesis. The majority (94%) of the anthropometric measurements were performed by one observer (MC), indicating that the measurements were unaffected by interobserver variation. In the following paragraphs, the way the outcome variables were measured will be discussed in more detail.

Blood pressure was measured twice in a standardized manner at the non-dominant arm in the sitting position using an automatic device with appropriate cuff size (Chapter 3). The first measurement was performed in rest after a 30–45 minute interview and the second measurement within a few minutes after the first one. It is well recognized that the predictive power of multiple blood pressure determinations is much greater than a single office reading.\textsuperscript{29} In the statistical analyses, we used the mean of two adequate readings. The potential advantages of automated measurements are the elimination of observer error and the minimization of the white coat effect.\textsuperscript{29} Furthermore, systolic and diastolic blood pressure readings by means of the type of device used in the present study have been found to strongly correlate with intra-arterial readings.\textsuperscript{28}

The hyperinsulinemic euglycemic glucose clamp technique method is considered to be the golden standard for the measurement of insulin sensitivity in humans because it directly measures metabolic actions of insulin under steady state conditions.\textsuperscript{31} However, this technique is difficult to perform in large-scale studies as it is complex, invasive and time-consuming. Several alternative methods for the assessment of insulin resistance based on fasting plasma glucose and insulin such as homeostasis model assessment (HOMA) and the glucose/insulin ratio have been proposed during the last years. These indirect measures were studied among pubertal IVF and control children in our study (Chapter 3). Especially HOMA appeared to be correlated to clamp-derived
insulin sensitivity\textsuperscript{7}. An important advantage of HOMA is that this measure instead of the glucose/insulin ratio behaves qualitatively as expected across a broad spectrum of insulin sensitivity and resistance\textsuperscript{31}. The glucose/insulin ratio only behaves appropriately in subjects with normal fasting glucose, a condition which applied to the children examined in our study.

Today, skinfold thickness measurements are widely utilized to assess body composition due to important advantages such as relative ease of administration, cost effectiveness and high validity and reliability potential. Skinfold measurements (triceps, biceps, subscapular, and supra-iliac) were performed in triplicate on the non-dominant side of the body by means of a Harpenden caliper (Chapter 4). Furthermore, in a pubertal subpopulation body composition was further evaluated by means of DXA (Chapter 4). During the last decades, DXA has emerged as a valuable tool to measure body composition non-invasively using a three-compartment model, including bone mass, fat mass and lean mass\textsuperscript{30}. Skinfold measurements, which measure only subcutaneous body fat, and body fat measured by DXA, which comprises all internal and subcutaneous body fat, appeared to be highly correlated. According to several studies, this could be explained by the fact that during childhood and adolescence most of the total body fat is deposited subcutaneously\textsuperscript{13,40}.

In Chapter 5, clinical signs related to pubertal development according to Tanner, skeletal maturation and sex hormone levels were examined. Tanner criteria are widely used by researchers and clinicians to gain information about the breast and genital developmental stages and pubic hair growth of children. Bone age was assessed from left-hand radiographs using the Greulich and Pyle method by one observer (MC)\textsuperscript{17}. Furthermore, it should be acknowledged that strong variations in hormonal secretory activity during puberty could complicate the investigation of sex hormone levels in children.

Over the last years, growth during infancy and childhood in relation to cardiovascular risk factors in later life has been increasingly investigated. A commonly used measure is weight gain\textsuperscript{12,24}, whereas others prefer measures of body size that include height in addition to weight, such as BMI, which reflect adiposity better than weight alone\textsuperscript{3,42}. Early postnatal growth in relation to blood pressure levels and skinfold thickness was investigated in the present follow-up study by means of weight gain during infancy and early childhood (Chapter 6).

In the Netherlands, a large majority of the children undergo a national test of educational achievement, known as the CITO, just before leaving primary school around the age of 12 to determine high school entrance level. The CITO score of the children was used in the present follow-up study (Chapter 7). CITO has been suggested to be a valuable instrument to assess individual differences in cognitive abilities and the test results have been found to correlate with IQ \((r = 0.63)\)\textsuperscript{4}. Furthermore, educational level, the need for educational assistance and the occurrence of learning and developmental disorders were investigated (Chapter 7).
Although birth weight in relation to gestational age was used in the present thesis as an indication for fetal growth, it is important to realize the limitations of this measure. The ideal assessment of the determinants of fetal growth would start from the expected growth trajectory for each infant through the course of pregnancy and quantify the deviation in actual growth relative to the expected pattern. However, as longitudinal information on fetal growth is rarely available, for most studies, including ours, birth weight is often the only feasible measure. Nevertheless, it is important to realize that environmental stimuli that may affect embryonic and/or fetal growth trajectories can result in altered postnatal physiology without an effect on birth weight. Furthermore, it should be acknowledged that exposure to early prenatal life effects may even induce developmental adaptations in organ development and function that are not accompanied by changes in fetal growth characteristics.

Confounding and statistical analyses

We collected information on various variables (potentially) related to our outcome variables of interest. This provided us with the opportunity to examine differences in these outcome variables between IVF children and controls while adjusting for potentially confounding factors. Examples are birth weight, gestational age, parental educational level, maternal smoking during pregnancy, cause of subfertility and family history of chronic diseases. It is possible that we have underestimated the true association between IVF and certain outcome parameters by adjusting for intermediate factors (e.g. sum of skinfolds in case of evaluated blood pressure). There is currently controversy about interpretation of results when models adjust for current body size when assessing early life predictors of blood pressure. Additionally, the slight attenuation of the blood pressure differences by adjusting for birth weight and gestational age was to be expected. IVF is known to be associated with lower birth weight and shorter gestational age, while these factors themselves have been found to increase blood pressure.

Furthermore, due to the large numbers of outcome variables that were collected in the present study, multiple statistical significance tests were performed. Although various cardiovascular measures were examined in view of the “developmental origins of health and disease” hypothesis, other aspects of growth and development did not follow from a specific hypothesis. Therefore, it is important to bear in mind that chance findings could have occurred.

Pathophysiological considerations

The “developmental origins of health and disease” hypothesis, which proposes that many adult diseases originate in early life, has been extensively examined during the last decades. Adaptations in the structure and metabolic function of organ systems are considered to enhance survival chances of the conceptus in the short-term, but may represent detrimental health
consequences in later life. The health effects caused by prenatal environmental conditions depend on their timing during gestation and body systems developing during that critical time window. Today, numerous studies regarding the influence of early prenatal nutrient restriction on the developing conceptus provided a compelling body of evidence that the periconceptional period represents a susceptible phase of early life. Moreover, the variety of animal and human studies which have been published on growth and development in ART offspring during recent years provided additional insight into the importance of considering environmental exposures that occur very early in conception or even just prior to conception.

The findings presented in this thesis contribute to the current knowledge of periconceptional exposure effects with regard to the development of both short- and long-term consequences in humans. The observed abnormalities in IVF children in terms of blood pressure, glucose metabolism and body composition show striking similarities with the health effects reported in experimental animal studies following exposure to maternal dietary restriction or ART-related techniques during the periconceptional period. For example, reductions in the availability of vitamin B12, folate and the amino acid methionine from maternal diet around the time of conception in sheep appear to be associated with high blood pressure, increased adiposity, insulin resistance, and altered immune function in adult offspring. Intriguingly, embryo culture conditions during the preimplantation period in mice were demonstrated to have detrimental postnatal effects on blood pressure, enzymatic regulators of cardiovascular and metabolic physiology, body mass and adiposity in progeny. The lack of marked differences between IVF and control children in other examined developmental parameters including pubertal development and schoolfunctioning in the current thesis support the idea that similar body systems and functions are affected in offspring conceived after early prenatal nutrient restriction, assisted animal reproduction and IVF treatment in humans.

Although the effect of underlying parental fertility problems on the growth and development of offspring in general is unclear, the observed differences between IVF and control children presented in this thesis might (partly) originate from adaptations of the developing conceptus to the IVF procedure. Important events occur during periconception, including the transition of maternal to embryonic control and epigenetic reprogramming of the genome, when the developing conceptus is extremely sensitive to environmental influences. Developmental plasticity following exposure to early prenatal insults appears to be mediated, at least in part, by epigenetic mechanisms, which control the establishment and maintenance of gene expression patterns in the placenta and conceptus. ART-related techniques used in animal studies have repeatedly been associated with aberrant methylation and expression of important genes related to fetal growth and development with long-term consequences. Interestingly, similar epigenetic changes in one of those genes were recently found in adults who were conceived in famine during the Dutch Hunger Winter. These data are the first to contribute empirical support for the hypothesis that early-life environmental conditions can cause epigenetic changes in humans that persist throughout life. Additionally, the associations between
ART, periconceptional induced imprinting defects and postnatal cardiometabolic dysfunction in animal studies and the increased frequency of rare epigenetic disturbances observed in IVF children further support the hypothesis that the present findings in IVF children could originate from epigenetic adaptations to artificial aspects of the IVF procedure.

**Current implications and recommendations for future research**

An important question is whether our findings should have clinical implications for daily practice in reproductive medicine. We feel that cautious interpretation is required. First, further monitoring of IVF offspring is warranted to shed more light on the potential effects of the IVF procedure on growth and development during the distinct phases of life. This is the first follow-up study examining various aspects of growth and development in 8- to 18-year-old IVF and control children born from subfertile parents. Essentially, our results regarding early childhood growth and cardiometabolic functioning, body composition, pubertal development and school functioning during late childhood and adolescence need to be reproduced by other sufficiently large and well designed studies. It is important to keep in mind that the multiple statistical significance testing, especially for those findings that did not follow from a specific and detailed hypothesis, could have led to chance findings. The need for additional investigation is underscored by the current rate of children born after IVF. In addition, a growing demand for IVF is expected due to increasing delayed childbearing and the availability of new technologies such as pre-implantation genetic diagnosis to prevent transmission of severe or lethal diseases in offspring. Obviously, in case further research will consistently confirm IVF-induced health problems in conceived offspring, couples considering IVF treatment should be notified in order for them to weigh the benefits and harms of IVF treatment. For now, it is necessary to inform those couples about the current lack of consistent knowledge regarding potential long-term health consequences for IVF offspring.

Not only the present findings in IVF children but also the existing literature regarding the reprogramming of embryos and fetuses in relation to early prenatal stimuli indicate that the periconceptional period represents a particular susceptible phase of early life with long-lasting health consequences. Public health campaigns promoting healthy behaviors among intended parents should pay attention to the potential impact of the environmental exposures prior and shortly after conception. There is a widespread recognition of the importance of periconceptional folic acid supplementation to prevent neural tube defects. Nevertheless, public awareness of the possible relation between periconceptinal exposure to harmful conditions and other longlasting health effects should be reinforced. Advantageously, women appear to be particularly receptive to advice about diet and lifestyle habits before and during pregnancy.

We specifically recommend continuing monitoring of cardiovascular risk factors in IVF offspring, including blood pressure, body fat composition and glucose/insulin metabolism. In addition to
the need for continued monitoring of the cohort examined in the present study, new cohorts of IVF children should be followed-up to reproduce our findings. The apparently small differences in cardiometabolic measures between IVF and control children may have clinical consequences in later life. It cannot be excluded that raised blood pressure in IVF children may be amplified throughout life. The meta-analysis performed by Chen et al. recently reinforces the concept that blood pressure tracks from childhood to adulthood and that elevated blood pressure in childhood is likely to help predict adult hypertension. Additionally, although it is currently uncertain whether and to what extent these relations apply to IVF offspring, slightly elevated adult blood pressure increases future risks for cardiovascular disease considerably. Risk of stroke or coronary heart disease mortality has been demonstrated to double with each increment of 20 mm Hg systolic blood pressure or 10 mm Hg diastolic blood pressure in 40- to 69-year-old adults. Furthermore, the clinical relevance and implications of the differences in body composition and fasting glucose between IVF and control children with respect to cardiovascular risk in adult life should be established. An appreciation of the potential for greater susceptibility for health problems among IVF offspring could offer further opportunities for targeted interventions in the near future.

Furthermore, it could be useful to take several practical issues into account during the planning of future research. The identification of eligible children and the subsequent recruitment of participants appeared challenging and time consuming tasks in the current study. A lot of effort has been put into these important tasks, especially because they constitute prerequisites for the recruitment of a representative study sample of sufficient size. Extensive recruitment techniques were used to enhance participation rates such as the noncommittal and personal approach and information providing on repeated occasions as well as the flexible attitude towards the families regarding the planning of hospital visits. Similarly, the data collection involving various growth and developmental aspects in almost 500 children was a demanding period of approximately three years. Therefore, considerable time and manpower should be budgeted for future follow-up research projects concerning large numbers of participants and/or extensive measurements.

An important key area for future research is the further exploration and disentanglement of the interactions between parental characteristics, periconceptional conditions and later life risk factors. Especially the potential influences of parental subfertility history, the hormonal stimulation and in vitro culture conditions on postnatal parameters in IVF offspring should be ascertained in an attempt to unravel the origins of the adverse postnatal IVF outcome. Clues can be obtained from observational studies investigating postnatal outcome after IVF according to type/severity of subfertility problems, type of ovarian stimulation and type of in vitro culture. In order to identify involved biological mechanisms, additional research is necessary to investigate whether similar environmentally induced epigenetic modifications during the periconceptional period precede the developmental differences in IVF children as has been found in animal assisted reproduction. Lastly, it would be worthwhile to pay attention to specific subgroups of children, including twins and children born after ICSI (intracytoplasmatic sperm injection) treatment and embryo
cryopreservation in future research. In addition to the importance of postnatal health investigation in IVF twins, twin research offers the opportunity to distinguish between environmental and genetic aspects that contributed to the induction of health effects after IVF treatment. ICSI is the most manipulative form of assisted reproduction currently used in humans, by injecting a single sperm directly into an oocyte. Therefore, postnatal developmental and health aspects of ICSI children should be monitored to assess the long-term safety of this procedure.

**Final conclusions**

This thesis addressed distinct aspects of postnatal growth and development in 8- to 18-year-old IVF children and matched controls born to subfertile couples. Whereas results with respect to cognitive abilities and school functioning of IVF children were reassuring, a tendency towards a less favorable cardiovascular risk profile was observed in IVF children compared with controls. Although results need to be confirmed by others and underlying mechanisms remain to be identified, our observations suggest that the periconceptional period represents a sensitive time window in humans during which environmental stimuli can clearly perturb developmental potential. The findings presented in this thesis provide several starting points for future research. In addition, further monitoring of IVF offspring is needed to gain more insight into postnatal development and potential health risks in later life.
References


Samenvatting
In vitro fertilisatie (IVF) is in de loop van de jaren wereldwijd uitgegroeid tot een routine procedure binnen de voortplantingsgeneeskunde voor koppels met vruchtbaarheidsproblemen. Sinds de eerste IVF geboorte in 1978 zijn over de gehele wereld naar schatting 3 miljoen kinderen geboren na IVF of een gerelateerde vorm van geassisteerde voortplanting. Ongeveer 2,3% van de Nederlandse baby's geboren in 2005 is verwekt met behulp van geassisteerde voortplantingstechnologieën. Tegenwoordig wordt in toenemende mate erkend dat de IVF procedure mogelijk invloed kan hebben op kwetsbare processen die plaatsvinden tijdens de conception en de vroege embryonale ontwikkeling. Uit wetenschappelijk onderzoek is gebleken dat IVF kinderen een verhoogd risico lopen op een ongunstige perinatale uitkomst, waaronder een laag geboortegewicht, vroeggeboorte en perinatale sterfte. Bovendien hebben verschillende studies een verhoogde incidentie van aangeboren afwijkingen en zeldzame epigenetische aandoeningen bij IVF kinderen beschreven. Als gevolg van het ontbreken van systematische follow-up van deze groep kinderen is nog grotendeels onduidelijk of IVF substantiële consequenties heeft voor de ontwikkeling in latere levensstadia van IVF nakomelingen. Daarom behandelt dit proefschrift verschillende aspecten van postnatale groei en ontwikkeling van 8-18 jaar oude IVF eenlingen en spontaan verwekte controle kinderen geboren van subfertiele ouders. In Hoofdstuk 1 zijn de doelstellingen van dit proefschrift uiteen gezet.

Hoofdstuk 2 presenteert een uitgebreid overzicht van actuele literatuur omtrent de groei en ontwikkeling van kinderen geboren na een IVF behandeling. Perinatale uitkomst van IVF zwangerschappen, het voorkomen van aangeboren afwijkingen, verstoorde genomic imprinting en maligniteiten, en postnatale groei karakteristieken in IVF nakomelingen worden uitvoerig besproken. Verscheidene meta-analyses en overige methodologisch sterke studies hebben substantieel bewijs geleverd dat IVF kinderen een verhoogd risico lopen op een ongunstige perinatale uitkomst, aangeboren afwijkingen en zeldzame epigenetische afwijkingen. Tot op heden is er geen consensus of de geobserveerde gezondheidsproblemen gerelateerd zijn aan de IVF procedure zelf en/of aan de onderliggende vruchtbaarheidsproblemen van de ouders. Studies die postnatale groei, ontwikkeling en morbiditeit onderzoeken zijn schaars en tonen conflicterende resultaten. Lange termijn onderzoek bij IVF kinderen gericht op andere aandachtsgebieden staat nog in de kinderschoenen.

Hoofdstuk 3 is gericht op onderzoek naar verschillende cardiometabolische parameters bij 8-18 jarige IVF eenlingen en spontaan verwekte kinderen van subfertiele ouders. Voorgaande studies hebben aangetoond dat nadelige omstandigheden tijdens het vroege prenatale leven geassocieerd zijn met cardiometabolisch dysfunctioneren in het postnatale leven. De bloeddruk van 225 IVF kinderen en 225, op leeftijd en geslacht gematchte, spontaan verwekte kinderen is onderzocht. Verschillende indicatoren van insuline resistentie zijn bestudeerd in een puberale sub-populatie. Systolische en diastolische bloeddruk was hoger bij IVF kinderen dan bij de controle kinderen (respectievelijk 109 ± 11 vs. 105 ± 10 mm Hg, P < 0.001 en 61 ± 7 vs. 59 ± 7 mm Hg, P < 0.001). IVF kinderen bleken ~2 maal meer kans te hebben om zich in de hoogste systolische en diastolische
bloeddruk kwartieren te bevinden. Bovendien werden significant hogere nuchter glucosewaarden gevonden in puberale IVF kinderen (5.0 ± 0.4 vs. 4.8 ± 0.4 mmol/l bij controle kinderen, P = 0.005). Verschillen in bloeddruk en nuchter glucose konden niet worden verklaard door huidige lichaamsomvang, geboortegewicht of andere factoren in de vroege levensfase, noch door parentale karakteristieken inclusief subfertilité. Derhalve veronderstellen wij dat de IVF procedure mogelijk bijdraagt aan het programmeren van de cardiometaabolische fysiologie en het cardiometaabolische functioneren in IVF nakomelingen. De bevindingen beschreven in dit hoofdstuk onderstrepen het belang de cardiometaabolische monitoring van IVF kinderen voort te zetten om de klinische consequenties in het verdere leven te evalueren.

In Hoofdstuk 4 is de lichaamssamenstelling van 233 IVF eenlingen en 233 spontaan verwekte controle kinderen van subfertiele ouders onderzocht door middel van antropometrisch onderzoek. Tevens is de lichaamssamenstelling bepaald in een puberale subpopulatie met behulp van dual energy X-ray absorptiometrie (DXA). Andere studies hebben een link aangetoond tussen nadelige stimuli tijdens de periconceptie in relatie tot een verstoorde vetweefselontwikkeling en obesitas in het postnatale leven. Kinderen geboren na IVF hadden een significant lagere subcapulaire triceps huidplooi ratio en een significant hogere perifeer lichaamsmassa, en een hoger percentage perifeer lichaamsvet in vergelijking met de controle kinderen. Alhoewel statistische significantie niet werd bereikt, suggereren zowel DXA als huidplooi metingen dat het totale lichaamsvet bij IVF kinderen verhoogd is. De verschillen in perifeer vet onderzocht met behulp van antropometrie tussen IVF kinderen en controle kinderen kon niet worden verklaard door huidige en vroege risico factoren, noch door parentale eigenschappen zoals oorzaak van subfertilité. In bot mineraal samenstelling werden geen verschillen aangetroffen tussen IVF kinderen en controle kinderen. Aangezien onze observaties suggereren dat de lichaamsvet samenstelling bij IVF kinderen verstoorde is, is het van groot belang dat monitoring van IVF kinderen plaatsvindt – van adolescente tot en met volwassenheid – om zicht te krijgen op lichaamsvet patronen en potentiële gerelateerde gezondheidsproblemen.


Diverse studies hebben een relatie aangetoond tussen vroege postnatale groei en bloeddruk en
lichaamsvet bij kinderen en adolescenten. Aangezien weinig bekend is over postnatale groei bij IVF nakomelingen en het effect op bloeddruk en lichaamsvet compositie tijdens de kinderjaren en adolescentie, zijn deze aspecten bestudeerd in Hoofdstuk 6. Gegevens uit groeiboekjes tot aan de leeftijd van 4 jaar zijn gebruikt om vroege postnatale groei van 193 IVF en 199 controle kinderen van subfertiele ouders te bestuderen. IVF kinderen hadden significant lagere gewicht, lengte en BMI standaard deviatie scores (SDS) bij 3 maanden leeftijd en gewicht SDS bij 6 maanden leeftijd in vergelijking met controle kinderen. Ook vertoonden IVF kinderen een grotere toename in gewicht SDS, lengte SDS en BMI SDS gedurende het eerste levensjaar. Gewichtstoename tijdens de vroege kinderjaren (1-3 jaar) was gerelateerd aan bloeddruk bij IVF kinderen maar niet bij controle kinderen. Groei tijdens het eerste levensjaar was niet gerelateerd aan huidplooi dikte bij IVF kinderen – in tegenstelling tot de controle kinderen. Groei tijdens de vroege kinderjaren was zowel bij IVF kinderen als controle kinderen geassocieerd met huidplooi dikte. Geconcludeerd werd dat groei bij IVF nakomelingen tijdens de vroege kinderjaren gerelateerd is aan verschillende cardiovasculaire risico factoren tijdens de late kinderjaren en adolescentie.


Tenslotte zijn in Hoofdstuk 8 methodologische aspecten, pathofysiologische consideraties, potentiële implicaties van onze bevindingen en aanbevelingen voor toekomstig onderzoek behandeld.

Concluderend, in dit proefschrift zijn verschillende aspecten van postnatale groei en ontwikkeling onderzocht bij 8–18 jarige IVF kinderen en gematchde controle kinderen van ouders met vruchtbaarheidsproblemen. Naast geruststellende resultaten op gebied van cognitief vermogen en schoolprestaties zijn ook aanwijzingen gevonden voor een minder gunstig cardiovasculair risico profiel bij IVF kinderen in vergelijking met controle kinderen. Alhoewel deze resultaten door anderen bevestigd dienen te worden en onderliggende mechanismen opgehelderd zouden moeten worden, suggereren onze bevindingen dat periconceptie bij de mens een kwetsbare periode betreft waarin omgevingsfactoren mogelijk het ontwikkelingspotentieel kunnen beïnvloeden. De bevindingen gepresenteerd in dit proefschrift leveren diverse aanknopingspunten voor toekomstig onderzoek. Een continuering van follow-up onderzoek bij IVF nakomelingen is nodig om meer inzicht te verwerven in postnatale ontwikkeling en eventuele gezondheidsrisico’s later in het leven.
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About the author
Manon Ceelen was born on September 20, 1977 in Amsterdam, the Netherlands. After graduating from secondary school in 1995 (KSH, Hoofddorp), she started her study Medical Biology at the VU University in Amsterdam. As part of this study, she participated in a research project regarding cell cycle control in cancer patients at the Department of Otolaryngology/Head and Neck Surgery (section Tumor Biology) at the VU University medical center, Amsterdam. At the Subdivision of Reproductive Endocrinology and Fertility/IVF Center of the VU University medical center, she performed her second research project focusing on biochemical biomarkers of embryo quality. After receiving her MSc degree in 1999, she contributed to the Hoorn Screening Study during an internship at the EMGO Institute for Health and Care Research in Amsterdam. In September 2001, after working as a research fellow for one year, she started as a PhD student at the Department of Pediatrics of the VU University medical center. Her PhD-project regarding postnatal growth and development of children born after IVF treatment was performed in collaboration with the Department of Obstetrics and Gynaecology of the VU University medical center and the Department of Epidemiology of the Netherlands Cancer Institute. From 2001 to 2003, she was a member of the Scientific Research Committee of the VU University medical center. Since April 2007, she works as an epidemiological researcher in the field of forensic medicine at the Department of Epidemiology, Documentation and Health Promotion of the Public Health Service of Amsterdam.