Health of offspring of subfertile couples

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Introduction
Nowadays, couples who do not achieve a pregnancy despite trying for over 12 months after the start of regular unprotected intercourse, meet the criteria for subfertility. This turns subfertility into an uncommon diagnosis as it concerns couples instead of individuals. The underlying mechanism(s) causing the couple’s subfertility may find their origin in female, in male, or in both. The treatment possibilities for unwanted childlessness following infertility have increased in the last decades.

In 1978 a major event in the world of Reproductive Medicine occurred; Louise Brown, the first in vitro conceived baby, was born. The English scientists, Robert Edwards and Patrick Steptoe, managed to successfully fertilize an oocyte outside the human body and transfer the resulting embryo to the womb where it implanted and developed naturally. The fact that mankind was able to do this is still considered one of the most spectacular medical discoveries of the 20th century. In 1983 the birth of the first in vitro fertilization (IVF) baby who was cryopreserved as an embryo was accomplished. This was an important achievement as during the IVF procedure the goal is to obtain multiple embryos, while - at least in Nordic European countries - nowadays usually only one or a maximum of two embryos are transferred into the womb. The knowledge on preservation of an embryo by freezing allowed the use of the embryos that were not used in the initial IVF treatment at a later time. In 1992 the method of intracytoplasmic sperm injection (ICSI) was added to the array of assisted reproductive techniques (ART). In ICSI a ‘handpicked’ single sperm cell is selected and then directly injected across the zona pellucida into the oocyte cytoplasm. With this discovery most couples with a severe subfertile male with very low sperm counts could also be offered an effective treatment. In 1995, the first live births after IVF with preimplantation genetic screening (PGS) were reported. In IVF with PGS an attempt is made to select only euploid embryos for embryo transfer. In theory transferring only euploid embryos to the womb was supposed to improve pregnancy and live birth rates after IVF.

After 25 years of IVF/ICSI the first million IVF/ICSI babies were born. Surprisingly, it only took two years until the second million IVF/ICSI babies were delivered. This well illustrates the rapid increase in the use of IVF and ICSI.

With the increased use of IVF and ICSI the concerns about the health consequences for the offspring, which arose directly after the first use of IVF (e.g. risks for the future child were unknown and that therefore IVF was unethical), arose as well. The concerns deepened especially after David Barker had published the ‘Fetal Origins of adult disease’ (also known as the Barker Hypothesis) in 1990. In the early nineties, David Barker and colleagues investigated the rate of cardiovascular mortality in men of whom accurate information on birthweight and growth during infancy was available. It turned out that children who were small at birth and were small at one year of age had a higher risk of dying from ischaemic heart disease later in life. Barker hypothesized that intrauterine growth restriction might have predisposed the offspring to heart disease...
in later life. This developmental concept formed the foundation for the Developmental Origins of Health and Disease (DOHaD) theory. The DOHaD theory describes how parental and environmental factors may influence fetal development in early life so that it may result in an increased vulnerability for health related problems in later life. This implies that the in vitro culture procedure, ovarian hyperstimulation and parental subfertility all could affect the health of the IVF offspring.

So while the first studies on the outcome of IVF focused on pregnancy and/or live-birth rates, consecutive studies also focussed on the health of IVF offspring. Initially the studies focussed on the increase in the rate of multiple births and on the consequences of the phenomenon of the so-called vanishing twin in IVF pregnancies. Studies showed that twin and singleton survivors of a vanishing twin had a lower birthweight compared to singletons conceived with IVF. Later studies dealt with other short-term outcomes, like congenital abnormalities and preterm birth. Adverse outcomes were more common in IVF offspring (more details follow on page 16). Next, studies started to address long-term outcomes of IVF offspring.

The aim of this thesis is to analyse the possible effect of different IVF components, i.e., controlled ovarian hyperstimulation (COH), the in vitro culture procedure, PGS, and the underlying subfertility on long-term child health and development. First I will briefly summarize the DOHaD theory. In the next sections IVF and its components and subfertility will be discussed. This is followed by a brief review of the literature about short and long-term health outcomes of IVF offspring. Finally, the aim and the outline of this thesis will be addressed.

Developmental Origins of Health and Disease Theory (DOHaD)

In 1986 David Barker published a study in which he demonstrated an association between geographical location in England, infant mortality in 1921-25 and ischaemic heart disease in 1968-78. Barker hypothesised that an association exists between infant mortality and ischaemic heart disease in a population. Later Barker and colleagues published two more articles regarding fetal development; development in the first year of a child and health conditions in later adult life. The first one describes how children born between 1911-1930 with a weight at birth below 3.4 kg and with a weight below 8.2 kg at one year of age had the highest death rates from ischaemic heart disease in later life. The second one reports how undernutrition during different periods of gestation lead to different phenotypes: undernutrition in early pregnancy may result in symmetrically small, low birthweight babies; undernutrition in mid-pregnancy may result in no noticeable retardation of fetal growth; undernutrition in late pregnancy is associated with thin foetuses with a wasted appearance. These three articles laid the foundation for the ‘Fetal Origins of adult disease’ hypothesis, also known as the Barker Hypothesis. This theory stimulated interest in the fetal origins of adult disorders. In order to broaden the scope of this hypothesis, its name was later changed into the Developmental Origins of Health and Disease (DOHaD) theory. Since then evidence has been accumulating on how environmental exposures during pregnancy may have an influence on the offspring’s health in later life. A good example is the study by Roseboom et al. regarding the long-term effects of the Dutch famine during World War II, now 75 years ago on the offspring. Roseboom and colleagues found indications that undernutrition during gestation may affect health of the offspring in later life even without affecting size at birth. Furthermore, the authors showed that the timing of prenatal exposure to undernutrition plays an important role: (1) prenatal exposure to the Dutch famine in the first trimester is among others associated with lower LDL cholesterol levels in adult life, obesity and cardiovascular disease; (2) in the second trimester e.g. with obstructive airway disease in the offspring and microalbuminuria; (3) and in the third trimester with higher plasma glucose and insulin levels in adult life. Together with the information on how undernutrition during different trimesters of gestation may lead to different phenotypes by epigenetic reprogramming it became clear that the human embryo and/or foetus can differentially adapt to timing, type and degree of environmental exposure.

With this in mind, it is conceivable that subfertility, COH, the in vitro culture procedure and/or PGS all could have their influences on the natural development of the embryo and may affect the offspring’s health in later life.
Subfertility

Fecundity is the so-called ability of women to have offspring. The monthly chance of a woman to achieve pregnancy resulting in a live birth during a single menstrual cycle with adequate exposure to sperm and no contraception use is called fecundability. The fecundability of a women decreases rapidly after the age of 30 years. This may lead to subfertility - or infertility - which is defined as the inability to conceive after 12 months of frequent pregnancy initiated intercourse. The time between pregnancy initiated intercourse and pregnancy is called time to pregnancy (TTP). TTP is regarded as a proxy for the severity of subfertility. Subfertility has a heterogeneous origin: causes of subfertility vary from ovulation disorders, endometriosis and acquired tuba malformations as a result of pelvic inflammatory disease, to low sperm counts, in male partner and in one third of subfertility cases no cause is found, so-called idiopathic subfertility. These different causes of subfertility call for different types of infertility treatment.

Assisted Reproductive Techniques (ART)

ART is the umbrella term given to describe a number of different fertility treatments, in which fertilization takes place in culture in vitro, that can be performed to help couples to achieve pregnancy. In this thesis we use the term ART to describe the conventional form of IVF/ICSI, IVF/ICSI within a modified natural cycle (MNC) and PGS. This means that this thesis zooms in on fertility treatments in which an oocyte is obtained by means of COH or minimal ovarian stimulation of the ovary, oocyte retrieval is performed and the retrieved oocyte(s) are inseminated outside the human body with preselected sperm and the embryo resulting from the fertilization is cultured for several days (in vitro) before embryo transfer to the uterus.

Controlled ovarian hyperstimulation (COH)

To a large degree the conventional form of IVF is performed with COH. In the early years of IVF human menopausal gonadotropins were used, but for safety reasons (impurity of urinary products) COH by means of recombinant FSH (rec-FSH) became more common. In COH follicle-stimulating hormone (FSH) is given in combination with a gonadotropin-releasing hormone (GnRH) agonist or a GnRH antagonist. FSH stimulates the growth of follicles, whereas the GnRH-agonist or antagonist inhibits the gonadotropin production of the pituitary gland. This prevents spontaneous ovulation of the follicles due to suppression of luteinising hormone (LH) release from the pituitary. These two hormones are given until several follicles (5-15) have reached a diameter of 18-20 millimetre. In the meantime the growth and development of the follicle(s) is monitored with the help of ultrasonography. When follicles have reached a diameter of 18-20 millimetre human chorionic hormone (hCG) or rec-hCG or a GnRH-agonist is administrated to mimic the LH surge. The function of hCG is to mimic the LH surge so that the first meiotic division and morphological changes of oocyte occur. After 34-36 hours of hCG administration oocyte aspiration is performed, just before ovulation takes place. Next, due to downregulation of the pituitary gland and the supra-physiologic oestrogen levels in the follicular phase in IVF cycles, which results in an insufficient luteal phase, progesterone is vaginal administrated for multiple days to prepare the endometrium for implantation after embryo transfer. The oocytes are transferred to the lab for the in vitro procedure after aspiration. The live birth rates per COH-IVF cycle are in general around 20-25% (heavily depending on the age of the female partner).

Modified Natural Cycle (MNC)

The MNC differs from COH by the fact that only in the late follicular phase of the menstrual cycle, when the one naturally dominant follicle has reached a diameter of ≥ 14 mm, a GnRH antagonist and FSH are administrated. The total dosages of these hormones per started cycle are minimal compared to COH and are solely used to compensate for the decrease in FSH levels as a result of the use of an GnRH antagonist. Besides the dose of hormonal stimulation there are other differences between COH and MNC: (1) in an MNC cycle usually only one dominant follicle will grow and be obtained whereas in COH the goal is to obtain multiple oocytes; (2) due to a lower total dose of hormonal stimulation in a shorter timespan a MNC cycle has fewer side effects of the hormonal treatment and an negligible risk of ovarian hyperstimulation syndrome; (3) COH results in an altered endocrine environment with higher oestrogen and progesterone levels in early pregnancy compared to MNC; (4) live birth rates per MNC-IVF cycle are 9-16%.

The In vitro Fertilization laboratory procedures

To fertilize an oocyte sperm is needed. Sperm ‘work-up’ takes place in order to select high quality sperm, as sperm quality is a significant factor in predicting fertilization and pregnancy rates in IVF. In a culture droplet of medium the sperm is used to fertilize one or more oocyte(s): IVF. In order to prevent low fertilization rates in case of severe male factor infertility ICSI may offer a solution. In ICSI one ‘handpicked’ sperm cell (based on, among other things, shape and progressive motility) is injected directly into the cytoplasm of the oocyte, in
Preimplantation Genetic Screening (PGS)

Pregnancy rates after IVF are lower in couples of which the female partner is of advanced age (women of 35 years) than in couples of which the female partner is under the age of 35 years. For instance, the European IVF-Monitoring Consortium reported that the cumulative pregnancy rate was 34.7% in women younger than 35 years and 27.5% in women aged 35-39. The presence of an abnormal number of chromosomes in the ageing oocytes, i.e. aneuploidy, causes the poorer pregnancy rates in women of advanced age. Aneuploidy is a known risk factor for miscarriage and more common in women of advance age, woman with recurrent miscarriage and in woman with repeated implantation failure.

To improve the pregnancy rates after IVF and ICSI for these populations PGS has been developed. The theoretical advantage of embryo selection with the use of PGS was to transfer only euploid embryos into the womb and thereby decrease miscarriage and implantation failure rates.

The aspired blastomeres of the embryo were screened for aneuploidies in 5- to 9 chromosomes with fluorescence in situ hybridization. In the early days of PGS, this aneuploidy screening was performed using a day-3 cleavage stage embryo biopsy. Only the euploid embryos were selected for transfer. Unfortunately multiple randomized controlled trials (RCT) showed that day-3 cleavage stage PGS did not increase, but unexpectedly reduced pregnancy and live birth rates after IVF in women of advanced age. There is evidence that cleavage stage human embryos resulting from IVF are mosaic: indicating that the exact chromosomal constitution of the human embryo may not be revealed by the analysis of a day-3 embryo biopsy.

Due to the fact that day-3 cleavage stage does not increase live birth rates new technological developments have taken place. Nowadays, comprehensive chromosome screening allows screening for aneuploidy of all 24 chromosomes. In addition, laboratory experiments suggested that day-5 blastocyst biopsy might be less harmful for the embryo than a biopsy at the day-3 stage. Nevertheless, up until now sufficiently powered RCT has been carried out to prove that PGS using day-5 blastocyst biopsy increases pregnancy and live birth rates in women of advanced age. Meanwhile, day-3 cleavage stage PGS continues to be carried out in many countries and clinics for three reasons: (1) not all embryos reach blastocyst stage; (2) fertility clinics are not competent with day-5 blastocyst biopsy; and (3) private clinics make a lot of money by adding PGS to IVF.

Another form of embryo diagnostics is preimplantation genetic diagnosis (PGD). PGD was developed for couples with a known risk of transmitting a genetic disorder to their offspring. The biopsied cells are screened for genetic pathology and only unaffected embryos are transferred. Although PGD has another indication than PGS, the biopsy procedure is the same. The application of embryo biopsy inherent to PGS and PGD has induced questions on its safety with regard to child development as in PGD and PGS the embryo is manipulated to a higher extent than in IVF.

Health Outcome of IVF Offspring

Short-term outcomes of ART

Although the short-term health outcomes of IVF offspring have been studied comprehensively, there are still no well-defined core outcome sets. The systematic review by Helmerhorst et al. of controlled studies published between 1985-2002 and the meta-analysis of Jackson et al. including studies from 1978-2002 indicated that singletons conceived with the help of IVF had a 2.0-fold higher risk of being born preterm, having a low birthweight and being small for gestational age compared with singletons conceived with the help of ICSI transfer. But over time the ART procedures further developed and changed. For example blastocyst transfer was introduced and other culture media were used as it became clear that in vitro culture could affect the offspring’s birthweight. As some offspring outcome studies carried out after 1998 did not demonstrate differences in birthweight between ART and natural conceived singletons, the call for a new meta-analysis became urgent. In 2013 Pinborg and colleagues conducted a meta-analysis which demonstrated a tendency of a lower perinatal risk in IVF/ICSI singletons for preterm birth, low birthweight and being small for gestational age compared to the previous meta-analysis: for example a decrease from a 2.0-fold higher risk to a 1.5 higher risk in preterm birth. In addition, this latter meta-analysis showed that subfertility is a risk factor for preterm birth in singletons conceived after ART. As preterm birth is a known risk factor for health problems in later life (e.g., 35% of preterm born children at school-age are in need of special educational services due to neurodevelopmental deficits at school; preterm born children tend to have a higher systolic blood pressure [SBP] of 3.8 mmHg (95% CI: 2.6-5.0 mm Hg) compared to term born children; and preterm birth is associated with a lifelong impaired lung function), it is of importance to investigate whether ART offspring is at risk for an less optimal health at later age.
Long-term outcomes of ART

Cardiovascular and metabolic health
Cardiovascular disease is the number one cause of death worldwide. In 2012 an estimated 31% of all global deaths were due to cardiovascular diseases. Coronary heart disease, causing a heart attack, and cerebrovascular disease, leading to stroke, contribute most to this mortality rate. Risk factors for the development of cardiovascular disease are high blood pressure (BP), high body mass index (BMI) and higher skinfold thickness. The Dutch famine study showed that adults who were prenatally exposed to undernutrition as early embryo’s in the first trimester are at increased risk of the development of coronary heart disease compared to non-exposed adults: (9% versus 3%; adjusted odds ratio for sex [95% CI]: 3.0 [1.1, 8.1]). Those exposed during the second or third trimester did not have an increased risk for the development of coronary heart disease. As mentioned before it was also demonstrated that in vitro culture could affect the birthweight of the offspring. As it is known that the early environment of an embryo may affect its physiology and thereby may lead to diseases in later life, it is conceivable that ovarian stimulation, the in vitro culture procedure and PGS affect the health of the offspring. Ovarian hyperstimulation induces epigenetic changes in the oocyte, induces the growth of multiple follicles bypassing the natural selection of the development of one dominant follicle, and leads to higher progesterone and oestrogen levels preceding the laboratory in vitro culture procedure and PGS oocytes, sperm and embryos are cultured in vitro, possibly affecting their developmental phenotype by epigenetic reprogramming. In addition, the aetiology of subfertility may contribute to an increased vulnerability for health related problems in later life in the offspring, even though the etiological pathways are not clear. In the next paragraphs I discuss BP, cardiovascular function, anthropometrics and glucose metabolism in IVF offspring.

Blood pressure
The recent meta-analysis of Guo et al. on BP in ART offspring included ten studies, which altogether recruited 872 children conceived with the help of IVF/ICSI and 3,034 children (aged 4-21 years) who were conceived naturally to fertile couples. The authors reported a higher SBP (mean difference 1.88 mmHg; 95% CI: 0.27, 3.49) and a higher diastolic blood pressure (DBP) (mean difference 1.52 mmHg; 95% CI: 0.34, 2.70) in the IVF/ICSI group compared to the natural conceived group. However, they could not determine if the higher BP levels in the IVF/ICSI group were due to the IVF procedures and/or the underlying parental subfertility, because the authors did not have information on the diagnosis or on the duration of subfertility.

The study of Pontesilli et al. showed that 5-to-6 year-old naturally conceived children born to subfertile couples (n= 220; average TTP 28 months) had a higher DBP (adjusted linear regression coefficient β 1.4 mmHg [95% CI: 0.70, 2.30]) than naturally conceived children born to fertile couple (n= 2,444; average TTP 3 months). This suggests an effect of parental subfertility on the BP of the offspring.

The study of Ceelen et al. reported increased levels of SBP and DBP in Dutch ART children aged 8-18 years (n= 225) compared to age and sex matched subfertile controls (n= 225). The SBP and DBP of IVF children were significantly higher than in controls (109 +/- 11 mmHg versus 105 +/- 10 mmHg and 61 +/- 7 mmHg versus 59 +/- 7 mmHg, respectively). This suggests an effect of the IVF procedures on BP.

The prospective follow-up study of Seggers et al. analysed the specific role of COH in IVF and revealed that at the age of 4 years the SBP percentiles (mean 68, SD 22) of children born after COH-IVF (n= 63) were higher than those of peers (mean 59, SD 24) who were born after MNC-IVF (n= 52). Adjustment for birthweight, TTP or current weight did not alter these results. Although TTP was 1.9 years longer in the COH-IVF group [median [range] 4.0 [0.1, 13.3]] than in the naturally conceived group [median [range] 2.1 [0.1, 11.3]] no differences in BP percentiles were observed between COH-IVF offspring and naturally conceived children to subfertile couples.

Another study compared 8-year-old Belgian children born after ICSI (n= 137) to spontaneously conceived children in fertile couples (n= 143). They reported higher SBP levels in the ICSI group (median [range]: SBP 100 mmHg [80-125] versus 95 mmHg [70-120] and higher DBP levels in the ICSI group than in the control group (median [range] DBP 60 mmHg [45-75] versus 55 mmHg [35-80]). Note, in the analyses only offspring’s height and sex were used as confounders and not the severity of subfertility. At the age of 14 years the authors replicated the study in 217 ICSI conceived children and 223 spontaneously conceived children to fertile couples, but the investigators could not replicate their findings. The authors hypothesized that due to the pubertal growth phase the tracking of BP from childhood into adulthood is perturbed. When the children had reached the age of 18 years the authors replicated the study for a second time in 126 ICSI conceived adults and 133 naturally conceived adults to fertile couples. No differences in BP were observed after adjustment for age, physical activity (hours sport/week), alcohol consumption, current maternal BMI and current paternal BMI. However, this study had a low participation rate (54%), which may have introduced participation bias. As childhood BP is known to track into adulthood it is of importance that future studies further investigate if a part of the IVF procedure and/or subfertility is associated with a higher BP.
Cardiovascular function

Some studies investigated if IVF has an impact on the offspring’s cardiovascular morphology. In a mouse model cardiovascular morphology showed endothelial dysfunction and an increased arterial stiffness in mice conceived with IVF. No systematic analysis has been performed as up until now too few studies addressed this issue.

The prospective cohort study of Valenzuela-Alcaraz et al. assessed the cardiovascular function of foetuses and infants (at 6 months of age). They included 100 foetuses conceived by IVF/ICSI and 100 control pregnancies that were conceived naturally and matched for maternal age. Ultrasonographic examination at 28-30 weeks of gestation showed that IVF/ICSI conceived foetuses had an increased atrial size, thicker myocardial walls, tricuspid ring displacement and impaired cardiac relaxation. In addition, IVF/ICSI conceived foetuses had a lower left ejection fraction (63% [range 57-68%] versus 69% [range 63-73%]). The cardiac output did not differ between the groups. At the age of six months IVF/ICSI offspring showed increased right atrial size (median [range]: 2.70 cm [range 63-73%]). The cardiac output did not differ between the groups. At the age of six months IVF/ICSI offspring showed increased right atrial size (median [range]: 2.70 cm² [2.6-3.0] versus 2.50 cm² [2.2-2.9]), thicker right ventricular walls (median [range]: 3.2 mm [2.9-3.3] versus 2.8 [2.6-3.2]), thicker aortic intima-media thickness (median [range]: 0.64 mm [0.62-0.67 mm] versus 0.52 mm [0.45-0.56 mm]). The differences remained significant after adjustment for gestational age, birthweight percentile and preeclampsia.

A second study assessed the vascular function of 11-year-old children (n= 65) born after fresh and frozen IVF/ICSI and 12-year-old naturally conceived controls (n= 57). The systolic pulmonary artery pressure at high altitude (at the high-altitude research station at the Jungfraujoch [Switzerland, 3450 m]) was higher in children conceived by IVF/ICSI than controls (39 ± 11 versus 30 ± 9 mmHg). Carotid intima-media thickness was significantly thicker in IVF/ICSI children (410±30 versus 370±20 μm). Others from the same research group found differences in cardiovascular function between IVF offspring and naturally conceived children at high altitude, but not at low altitude.

A fourth research group compared the hospitalization rates in 2,603 children conceived with IVF and 237,863 naturally conceived children up to the age of 18 years for valvular disorders, hypertension, arrhythmias, cardiomyopathy, ischemic heart disease, and heart failure. Hospitalization rates were comparable between the two groups and were not increased compared to the general population. Note that this study compared two groups of children up to the age of 18. Cardiovascular disease has, in general, a later onset than 18 years. Overall, these results highlight the importance of studies on the vascular function of IVF offspring later in adult life.

Anthropometrics

The recent meta-analysis of Guo et al. on BMI included 14 studies, which altogether recruited 1,914 IVF/ICSI offspring and 3,881 naturally conceived participants to fertile couples aged 2 to 22 years. The authors reported no statistically significant difference in BMI: the mean difference was -0.04 kg/m² (95% CI: -0.28, 0.20). They did not report other anthropometric outcomes.

In line with this meta-analysis, Ceelen et al. found no differences in BMI between 233 IVF children aged 8-18 years and 233 age and sex matched naturally conceived controls who were born to subfertile couples. However, they did find indications that the body fat composition in IVF children was different from that of naturally conceived controls. The 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 than the controls. This suggests that the IVF procedures may alter the body fat distribution of the offspring. As the peripheral body fat of IVF offspring was higher than that of natural conceived offspring to subfertile couples this might indicate a disadvantageous cardiovascular profile.

Belva et al. analysed the body fat distribution of 217 ICSI singletons (116 boys, 101 girls) and 223 naturally conceived singletons to fertile couples (115 boys, 108 girls) at the age of 14. The study did find differences in anthropometrics - a difference that was restricted to the subgroup of girls. Compared to naturally conceived girls, the ICSI girls had a significantly higher sum of peripheral and central sum of skinfolds (difference of 2.8 mm), had a higher waist circumference (difference of 2.1 cm), a higher BMI (difference of 1.1 kg/m²) and a higher percentage of body fat mass expressed by waist-to-hip ratio (difference of 2%) after adjustment for multiple confounders including gestational age, sports frequency and maternal BMI. It should be noted that a number lifestyle confounders were excluded (such as diet or type of sport). In addition, the study compared a subfertile ICSI group with a fertile control group. This means that the unfavourable anthropometrics could also be attributed to subfertility.

When the offspring had reached the age of 19 years the authors replicated their study on body fat distribution in 126 ICSI conceived singletons (56 men, 70 women) and 223 naturally conceived singletons (53 men, 80 women). ICSI conceived men had a higher peripheral fat deposition than naturally conceived peers (difference of 4.6 mm after adjustment for physical activity). Body fat distribution and serum levels of leptin did not differ between ICSI offspring and naturally conceived offspring. Still this study could not determine whether the difference in peripheral fat deposition could be attributed to subfertility or the IVF procedures.

A recent study showed that a part of the IVF procedure (culture medium) has an influence on body composition of offspring. The prospective cohort study of Zandstra et al. compared two different embryo culture media used during IVF/ICSI treatment and showed that the nature of the in vitro culture medium for human embryos affects anthropometric outcomes at the age of 9 years. For example after correction for confounders (such as sex, IVF/ICSI, amount of physical exercise), the adjusted difference
attributable to culture medium in weight was 1.58 kg (95% CI: 0.01, 3.14) and the adjusted difference in BMI attributable to culture medium was 0.84 kg/m² (95% CI: 0.02, 1.67). The authors underline however, that the results did not indicate which one of the two in vitro culture groups could be considered as the healthiest or which group would be more vulnerable for the development of disease in later life. This study demonstrates how media may affect long-term child anthropometrics. This warrants a close follow-up of IVF offspring.

Glucose and lipid metabolism

Mouse models of IVF showed that IVF could result in alterations in glucose metabolism: mice conceived with IVF had higher levels of peak glucose and peak insulin than naturally conceived mice.41,42 The meta-analysis of Guo et al. included seven studies investigating the glucose profiles of 477 IVF/ICSI offspring and 1,852 naturally conceived offspring.44 Fasting insulin levels of IVF/ICSI offspring was higher (0.38 mIU/L [95% CI: 0.08, 0.68]) than that of the naturally conceived controls. However, fasting glucose in both groups was comparable (-0.03 mM [95% CI: -0.13, 0.06]). Furthermore, the lipid profiles of 332 IVF/ICSI conceived children and 1,701 naturally conceived children were in general comparable.44 IVF/ICSI offspring only had significant lower levels of LDL cholesterol. No differences in HDL cholesterol, total cholesterol and triglycerides were observed, but a trend for lower total cholesterol, lower triglycerides, and higher HDL cholesterol was seen. These findings indicate a favourable lipid profile in IVF offspring.

Belva et al. assessed the glucose and lipid metabolism of 18-year-old men and women conceived by ICSI (n= 56 and n=70, respectively) and naturally conceived control men (n= 53) and women (n= 80).43 ICSI conceived men had lower mean HDL cholesterol concentrations in comparison to controls. However, the HDL cholesterol concentrations of ICSI conceived men were still well within the normal range. No differences in total cholesterol, LDL cholesterol, triglycerides, glucose, and insulin were observed.

In summary, multiple studies described a cardiovascular risk profile in ART offspring compared to naturally conceived children. These poorer results could often not be attributed to confounders such as preterm birth or a low birthweight, but it is not clear if they could be attributed to a specific component of the IVF procedure (the hyperstimulation of the ovaries as well as the culture media and/or parental subfertility).

Asthma

Asthma is a reversible pulmonary condition in which the airways narrow causing wheezing and shortness of breath.84 Due to the high incidence among children and the impact of asthma on child health, asthma has been studied widely in the general population. This has led to the identification of many risk factors for the development of asthma. As IVF is associated with risk factors for the development of asthma (i.e. preterm birth and caesarean section) an association between IVF and asthma is plausible.85 Others suggested that IVF per se has been as a causative factor for asthma in the offspring.86

One of the first studies addressing the possible association between asthma and IVF is the study of Carson et al.86 Within the Millennium Cohort study (n= 13,041) the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used to assess the asthma prevalence and the use of asthma medication in 5 and 7-year-old IVF conceived children (n= 104) and naturally conceived children of fertile (n= 6575) and subfertile couples (n= 505). Children born after IVF had an increased prevalence of asthma (adjusted odds ratio: 2.65 [95% CI: 1.48, 4.76]) compared to children born to fertile couples.

The second study is the one of Källén et al. who used five or more prescriptions for asthma drugs as an indicator of asthma in 2-to-25-year-olds participating in the Swedish birth register cohort study (n= 2,628,728). They found an association between IVF and asthma in children, which was due to parental subfertility.87 However, it should be noted that drugs used in asthma treatment are also prescribed for other indications than asthma, i.e. viral infections.88

Cetinkaya et al. used a telephone adaption of the ISAAC questionnaire in a case control study to investigate the prevalence of asthma in 4.6-year-old children born after IVF (n=158) and children of fertile couples (n=102). They found no statistically significant difference in asthma prevalence, allergic rhinitis and atopic dermatitis between the two groups. However, they did not adjust their comparisons for potentially confounding factors, such as parental smoking or birthweight, and prevalences of asthma and allergic rhinitis were not provided.89

In summary, the above results suggest that there could be a link between IVF and asthma, but it is not clear if this is due to the IVF procedures, parental subfertility or a combination of both.

Pubertal development

The primary sexual characteristics are present at birth, while the secondary sexual characteristics emerge and develop during puberty. Puberty is initiated by hormonal signals from the brain to the gonads and result (among other things) in changes in the breast development, growth of sexual hair, height, growth of reproductive organs and increase in body weight.

A limited amount of studies evaluated the pubertal development of ART offspring.90 Most studies focussed on the semen quality and pubertal development of ICSI boys. The growth during pubertal development in IVF/ICSI boys and girls was investigated by multiple studies with overall reassuring findings.91-93
Belva et al. investigated the development of secondary sexual characteristics and menarche in 14-year-old IVF offspring (n = 101) and 14-year-old naturally conceived controls (n = 108) using parental questionnaires and by physical examination. Age at menarche did not differ between ICSI and naturally conceived girls: 13.1 ± 1.2 versus 13.1 ± 1.4 years. Furthermore, differences regarding breast development at the age of 14 years were observed. Breast development was less advanced in ICSI girls. Adjustment for multiple confounders (age, BMI, maternal age at menarche, maternal educational level, birthweight, gestational age and maternal parity) did not alter this result: adjusted odds ratio: 0.5 (95% CI: 0.3, 0.8). The authors stated that this was an unexpected finding as breast development and menarche follow a fixed pattern and no difference in menarche was observed. Another study could not replicate this finding regarding breast development.

Belva et al. investigated the development of secondary sexual characteristics and menarche in 14-year-old IVF offspring (n = 71) aged 18 to 22 years and naturally conceived controls (n = 81). No abnormalities were noted in either reproductive hormone profiles or in ovarian morphology. While these are overall reassuring findings, it should be noted that this study was limited by single blood measurements, had a relatively small sample size, had limited information on the type hormonal contraceptive (77% of the study population used hormonal contraceptives) and had limited information on the menstrual cycle of the participants.

Males

Determined by parental questionnaires and physical examination one study showed that Tanner staging was comparable in 14-year-old ICSI conceived boys (n = 116) and naturally conceived peers (n = 115). The salivary testosterone levels of 58/116 ICSI boys and of 62/115 naturally conceived boys did not differ: 113 ± 42 pg/ml and 123 ± 56 pg/ml, respectively. The salivary testosterone levels of ICSI conceived boys of fathers diagnosed with and without oligozoospermia did not differ. At the age of 18-22 years 54 males conceived with ICSI had more often inhibin B levels below the 10th percentile and FSH levels above the 90th percentile than 57 naturally conceived males without and with adjustment for confounders (such as age, BMI and season). In men lower levels of inhibin B and higher levels of FSH are associated with low total sperm counts. ICSI conceived men had more often sperm concentrations below 15 million/mL (adjusted odds ratio: 2.7 [95% CI: 1.1, 6.7]) and a total sperm count below 39 million (adjusted odds ratio: 4.3 [95% CI: 1.7, 11.3]) than naturally conceived peers after correction for confounders including abstinence period and time from ejaculation to analysis. Men with sperm concentrations below 15 million/mL and/or a total sperm count below 39 million are considered to have a low sperm count. The data indicate that future studies addressing the reproductive abilities of IVF and ICSI offspring are certainly needed.

Neurological development

In the last few decades the development of the human brain and nervous system of ART offspring received substantial attention. The systematic review of Middelburg et al. conducted in 2008, which included nine large registry based studies, suggested that IVF/ICSI per se does not increase the risk for severe neuromotor handicaps such as cerebral palsy (CP). The previously reported association between IVF/ICSI and CP could be attributed to perterm birth and low birthweight and disappeared after adjustment. This may imply that the neurological sequelae observed in IVF offspring is due to the adverse obstetric outcomes in IVF pregnancies.

However, there are several studies indicating that parental subfertility could have an adverse effect on the neurodevelopment of the offspring. The study group of Zhu et al. described that offspring of subfertile couples (n = 4,351) at 18 months had a pattern of psychomotor development that did not differ from the offspring fertile couples (n = 37,897). However, an increasing TTP correlated with a modest delay in the achievement of motor milestones at 18 months. Adjustment for gestational age did not alter this result.

Siegers et al. and Schendelaar et al. found that an increased TTP is associated with a suboptimal neurological condition in 2-year-old (n = 209) and 4-year-old singletons (n = 195) born to subfertile couples, respectively. The neurological condition was assessed using the detailed and age-specific neurological examination according to Hempel (see box 1).

In 7-year-old children Zhu et al. found that children conceived naturally to subfertile couples (n = 1,614) had a slightly higher risk of developmental coordination disorder (DCD [see box 1]) compared to children conceived naturally to fertile couples (n = 14,928). Additional adjustment for preterm birth did not alter this result. The study did not have any further information about duration of TTP longer than 12 months and therefore could not address the question if an increased TTP was associated with a higher risk of DCD.

On the other hand, several studies did not demonstrate a difference in the neurological development of ART offspring and naturally conceived children. For example the study of Knoester et al. reported no significant difference after adjustment for parity in neuromotor development, between 5-to-8-year old ICSI (n = 81) and natural conceived children (n = 85), assessed with an age-specific assessment for minor neurological dysfunction (MND, see box 1). The study of Levi-Shiff et al. evaluated the neurological development at the age of 9-10 years of 51 children conceived with IVF and 51 naturally conceived children. A general paediatric examination showed no pathological differences between the two groups. Note, that the studies of Levi-Shiff et al. and Knoester et al. did not adjust for...
infertility, but still did not find a difference regarding neurological condition between IVF/ICSI children and naturally conceived children. In other words no negative effect of subfertility on the neurological condition was seen.

Well-designed, well-powered, follow-up studies that start before and end after the neurodevelopmental transition that takes place between 7 and 9 years are needed to investigate if parental subfertility and/or IVF procedures influence neurological development of ART offspring.  

**Box 1**

**The Hempel assessment for MND**

The Hempel neurological assessment is an age-specific detailed neurological examination for preschool age children (1.5-4 years). It pays special attention to the presence of minor neurological dysfunction (MND). Dysfunction is assessed in five domains of function, that can be classified as typical or deviant: (1) Fine motor function; (2) Gross motor function; (3) Posture and muscle tone; (4) Reflexes; (5) Visual motor function.

Children are classified as neurologically normal, simple MND or complex MND. Neurologically normal implies that no domains are deviant or only the domain of reflexes. Simple MND means the presence of one deviant domain (except reflexes) and it indicates a suboptimal, yet normal form of brain function. Complex MND implies the presence of multiple deviant domains; it represents the clinically relevant form of MND. Children with this form of MND have an increased risk for ASD, ADHD, and DCD.

The Hempel examination may also be used to calculate the neurologic optimality score and a fluency score by determining the number of items scored in a predefined optimality range. The neurologic optimality score (range: 0-58) evaluates subtle differences in neurological outcome, since the range for optimal behaviour is narrower than that for normal behaviour. The fluency score (range: 0-13), a sub-score of the NOS, deals with the fluency of motor behaviour.

**Developmental coordination disorder (DCD) questionnaire**

DCD occurs when a delay in the development of motor skills or coordinating movements results in a child being unable to perform common everyday tasks. The DCD questionnaire is a parental questionnaire consisting of 15 items and was designed to screen for these coordination disorders in children, aged 5 to 15 years. Parents are asked to compare their child’s motor performance with peers using a 5-point Likert scale. A total score (15-75 points) consists of the sum of each item (1-5 points). A high score suggests no DCD. In the study of Zhu et al. DCD is defined as a total score of 46 or below (as recommended by the developers of the DCD questionnaire).

**Behavioural and Cognitive development**

Investigation of behavioural development of children is difficult. Typically, it is already difficult to control for parental attitudes towards their children, parental expectations of their children and the environment in which children grow up. Moreover, families in which parents conceived with the help of ART may be different in a variety of ways (parental age, family size and socioeconomic status) from that of families in which parents conceived naturally. In addition, it is possible that ART parents are more aware of having a child as they spent much time and effort to conceive and therefore may appreciate the value of their offspring and of parenthood more intensely. This all could influence a child’s behavioural development and the parents’ perception of this development.

Overall, studies showed reassuring results: no differences were reported in the incidence of behavioural problems in IVF/ICSI conceived children compared to naturally conceived peers. Some studies hypothesized that due to perinatal adversities related to IVF (e.g. preterm birth), IVF offspring is at increased risk for attention deficit hyperactive disorder (ADHD) and autism spectrum disorders (ASD). The pilot study of Zachor et al. conducted in an ASD centre (n= 507; 11% was conceived with ART), identified ART as a risk factor for ASD. However, a large case-control study, which included 4,053 ASD cases and 16,143 control cases, did not find an association between IVF and ASD. Regarding ADHD, Källen et al. performed a data linkage study of prescriptions for ADHD drugs, but did not find an association between the use of ADHD prescriptions and IVF. Beydoun et al. tried to unravel if IVF was associated with the prevalence of ADHD, but their study suffered from a low response rate (31%) on a self-administered questionnaire. The authors did not find an association between IVF and ADHD.

The cognitive development of ART offspring has been studied in a variety of ways. Overall, findings were reassuring: the IVF procedures do not seem to have a negative influence on the cognitive function of the offspring. Regarding subfertility, the study of Zhu et al. found that a longer TTP was associated with a delay in achieving certain cognitive and language developmental milestones at the age of 1.6 years (n= 3,309). Researchers who investigated whether TTP influenced the cognitive development of children participating in the Millennium Cohort Study at the age of 3 years (n= 11,790) and at the age of 5 years (n= 12,136) did not find evidence that subfertility per se has an effect on cognitive ability scores.

**Ocular health**

The formation of the eye in the human embryo starts at week three and ends in the tenth week of embryonic development. During this period the eye expresses estradiol and progesterone receptors in multiple ocular structures. After COH-IVF serum progesterone and estradiol concentration are higher in early pregnancy (three and two
times higher, respectively), lasting up until week 6, compared to natural pregnancies.\textsuperscript{126} Therefore it is possible that the hormones used in COH-IVF may indirectly affect the eyes of offspring.\textsuperscript{127} In addition, it is well known that preterm born children have an increased risk of adverse ophthalmic conditions, such as refractory errors and amblyopia.\textsuperscript{128} This would imply that IVF offspring is at increased risk of ophthalmic conditions.

One study (n = 24,628) found an increased risk for visual impairment among children born after IVF, which may be secondary to subfertility.\textsuperscript{129} Three smaller studies also addressed visual acuity of IVF offspring. However, the conclusions of these studies were inconsistent, probably due to the relative small sample sizes of the studied groups.\textsuperscript{130-132} The systematic review by Toro et al. concluded that the available data are inadequate to draw a firm conclusion regarding the possible influence of ART on ocular function of the offspring.\textsuperscript{127}

**Twins**

Since 2000 the number of IVF twin pregnancies has been gradually decreasing in Europe due to (elective) single embryo transfer.\textsuperscript{133} Nevertheless, a large cross-county variation in IVF twin birth is seen, with in 2012 small numbers in the Nordic European countries (multiple delivery rate per cycle: 5.2 in Iceland; 11.4 Norway; 7.4 in the Netherlands) and larger numbers in East European countries (multiple delivery rate per cycle: 31.7 Montenegro; 34.1 Serbia; 22.8 Ukraine).\textsuperscript{133} In general twin pregnancies are associated with an increase in maternal pregnancy complications (such as pregnancy induced hypertension and gestational diabetes) and with worse perinatal outcomes (preterm birth and low birthweight) compared to singletons pregnancies.\textsuperscript{134-136} The same holds true when comparing IVF twin pregnancies with IVF singleton pregnancies.\textsuperscript{137} In addition, neonatal and maternal outcomes were significantly better for women undergoing two IVF singleton pregnancies compared with one IVF twin pregnancy (after dual embryo transfer).\textsuperscript{138} Some studies even suggested that the perinatal outcome of IVF twin pregnancies might be worse than that of naturally conceived twin pregnancies; they showed an increased risk of obstetrical complications, preterm birth and perinatal mortality. However others did not demonstrate differences between IVF twin pregnancies and naturally conceived twin pregnancies.\textsuperscript{139,140}

Although an increased risk of perinatal adversities was observed in twin pregnancies conceived after IVF compared to singleton pregnancies conceived after IVF, only a few studies addressed the long-term developmental outcomes after the perinatal period in IVF conceived twins and in IVF conceived singletons. In the next paragraph I discuss the neurodevelopment health, cognitive development, BP and anthropometrics of twins conceived with IVF and singletons conceived with IVF.

The large controlled, national register based Danish cohort study analysed the prevalence of CP, mental retardation, severe mental developmental disturbances, and retarded psychomotor development in 3,393 twins and 5,130 singletons conceived by using ART and 10,236 naturally conceived twins.\textsuperscript{141} Physicians made the neurological diagnoses when the children were 2-to-7 years old. The study did not show a difference regarding the neurological outcomes (e.g. prevalence of CP and psychomotor retardation) between twins born after IVF/ICSI, singletons born after IVI/ICSI and naturally conceived twins. Adjustment for zyosity, low birthweight or sex did not alter the results. No differences were found in neurological outcomes between twins conceived after IVF and ICSI. In addition, a population-based retrospective Swedish cohort study included all twins born between 1982-1995 and compared the prevalence of CP in 2,060 twins born after IVF and 4,120 naturally conceived control twins.\textsuperscript{142} With respect to risk of neurological sequelae the study concluded that twins born after IVF did not differ from control twins: prevalence rates of CP were 7.4 and 6.9 per 1000 children in IVF conceived twins and spontaneously conceived twins, respectively.

Whereas the neurological development of IVF conceived twins and IVF conceived singletons did not differ, two studies suggested that the cognitive development of IVF conceived twins and IVF conceived singletons do differ. The study of Bonduelle et al. reported that the cognitive development at 2 years, as measured by the Bayley Scales of Infant Development, of twins born after IVF/ICSI (n = 61) was significantly worse than that of singletons born after IVF/ICSI (n = 69).\textsuperscript{143} The study of Pinborg et al. demonstrated that 3-4-year-old IVF/ICSI conceived twins (n = 454) were more likely to receive speech therapy than IVF/ICSI conceived singletons (n = 656).\textsuperscript{144} The difference in speech therapy remained statistically significant after adjustment for birthweight.

Studies comparing the anthropometrics and cardiovascular health of twins born with the help of IVF and IVF conceived singletons are rare. The study by Saunders et al. from 1996 compared the growth and physical development at the age of 2 years of 196 singletons and 47 sets of twins born after IVF.\textsuperscript{145} Weight percentiles did not differ between the twins and singletons when age was corrected for prematurity. No other comparison in this study was made between twins conceived after IVF and singletons conceived after IVF.

All in all, there is little information on the health and development of IVF twins and no information beyond the age of seven years.

**Health after PGS**

The invasiveness of the embryo biopsy inherent to PGS has induced questions on its safety. The study of Mastenbroek et al. demonstrated a decrease of live birth rates after IVF/ICSI with PGS performed with day-3 embryo biopsy and the study of Twisk et al. found no evidence of an increase in live birth rates after IVF/ICSI with PGS performed with
day-5 biopsy embryo biopsy. Still PGS is increasingly used and as PGS includes more extensive embryo manipulation than IVF, PGS has raised multiple questions with regard to child development. But despite the extensive use of PGS only a few studies have addressed the long-term outcomes of PGD/PGS and only one systematic review has been carried out.

Multiple studies have investigated whether neurodevelopment of PGD/PGS offspring differs from that of IVF/ICSI offspring without PGD/PGS. At the age of 2 years two studies evaluated the psychomotor and neurological outcomes of PGD and PGS offspring. One study used a prospective, case-controlled design and compared two-year-old singletons born after PGD/PGS (n = 70) with singletons conceived after ICSI (n = 70) and naturally conceived singletons (n = 70). No effects of PGD/PGS with an unknown day of biopsy on psychomotor development and neurological functioning were found. The prospective follow-up study of a RCT on PGS with day-3 embryo biopsy (carried out by our research group), reported that 2-year-old PGS offspring (n= 54) had a lower neurological optimality score (see box 1, page 30) than 2-year-old IVF offspring (n = 77). This follow-up study reported that at the age of 4 years this difference persisted on the fluency score (see box 1, page 30) in twins, but had disappeared in singletons. At the age of 5-6 years two studies have investigated the safety of day-3 and day-5 embryo biopsy for psychomotor development by comparing three groups of singletons: (1) PGD offspring; (2) IVF/ICSI offspring; (3) naturally conceived offspring. The studies found no effect of PGD on psychomotor development.

Three study groups investigated the behavioural development of PGS/PGD offspring. Two study groups did not find a difference between day-3 or day-5 PGD offspring and IVF/ICSI offspring and naturally conceived children at the age of 5-6 years. The other study group described a similar behavioural development of day-3 PGS and IVF/ICSI offspring at the age of 2 years (54 PGS children and 77 controls) and 4 years (49 PGS children and 64 controls).

Three study groups addressed the cognitive development of PGD/PGS offspring. Two studies investigated the safety of day-3 and day-5 embryo biopsy (PGD) on the cognitive development at the age of 5-6 years. They reported no differences between the PGD, IVF/ICSI conceived and naturally conceived singletons. The third study found that day-3 PGS does not affect the behavioural development of 4-year-old singletons and twins.

Two study groups have evaluated the anthropometrics (including BMI, height, weight and skinfolds) and BP of children born after IVF with and without PGD/PGS. Both groups did not demonstrate an effect of PGD/PGS on BP and anthropometrics at the age of 2. However, it was not recorded if the group evaluating PGD used day-3 or day-5 embryo biopsy. At the age of 5-6 years the authors replicated the study in 87 children born after PGD with day-3 embryo biopsy and 87 ICSI conceived peers. The study found no differences in height, weight, body fat distribution or BP between the two groups. The other study group found no effect of day-3 PGS on BP and anthropometrics at the age of 4 years.

Although PGS with day-3 and day-5 embryo biopsy is increasingly used, the knowledge on long-term consequences of embryo biopsy is limited. Detailed follow-up studies are needed to study if there is an effect of PGS on the offspring.

**Aim of the Thesis**

The aim of this thesis is to determine whether ovarian hyperstimulation, the *in vitro* culture procedure and PGS are associated with one or more of the following health parameters of children born to subfertile couples: anthropometrics, asthma, BP, neurodevelopment and visual acuity. If the studies of this thesis will provide evidence for adverse effects of components of the ART procedure, effort should be made to improve the ART technique in order to reduce long-term health effects of the offspring. In order to investigate the possible associations we used the ‘Groningen ART cohort study’ and the ‘PGS-trial’ conducted by Mastenbroek et al.

**The Groningen ART cohort study**

The Groningen ART prospective cohort study is unique in its design. This longitudinal assessor-blinded follow-up study makes it possible to separately evaluate the effects of IVF and its components on the offspring’s health. Couples who successfully conceived after receiving ART or conceived naturally while on the waiting list for infertility treatment at the Department for Reproductive Medicine at the University Medical Center Groningen (UMCG) and had a term date during 1 March 2005 and 31 December 2006 were approached during the third trimester of pregnancy to participate in the Groningen ART cohort study. The born singletons were divided into the following groups of singletons:

1. singletons born after COH-IVF;
2. singletons born after MNC-IVF;
3. singletons who were conceived naturally (Subfertile-naturally conceived; Sub-NC).

The composition of the study groups allowed us to make three comparisons, depicted in Figure 2. Placement in the COH-IVF group depended on the presence of ovarian hyperstimulation. Within the Groningen ART cohort study the overall doses of FSH used during COH is on average (median [range]) 1500 (650-6400) IU. Criteria for inclusion in the MNC-IVF group were female age between 18 and 36 years, no ovarian hyperstimulation, no previous unsuccessful COH-IVF treatment or first IVF treatment after pregnancy, regular ovulatory menstrual cycle of 26-35 days and a BMI of 18-28 kg/m². In the MNC-IVF group when a lead follicle with a mean diameter of at least 14 mm was observed a daily dose of 150 IU FSH, up to the day of ovulation triggering was given, which was by average
3 days (average total dose median [range]: 450 [150-1800] IU). Couples who conceived naturally after 1 year after the start of unprotected intercourse and were waiting for fertility treatment at the department of Reproductive Medicine formed the Sub-NC group. The presence of the Sub-NC group prevents the overestimation of the possible effect of IVF. Only pregnancies resulting from fresh embryo transfer were included. Children born after oocyte or embryo donation were excluded. The twins resulting from the pregnancies after COH-IVF, MNC-IVF and Sub-NC pregnancies and with a term date in the same period as the singletons of the ART cohort study were followed with the same assessment procedures as the singletons of the ART cohort study.

The children of the Groningen ART cohort were neurodevelopmentally assessed at several ages: 2 weeks, 3 months, 4 months, 10 months, 1.5 years, 2 years, 4 years and 9 years. At the age of 4 a questionnaire on general health outcomes, including asthma, the measurement of BP and anthropometrics was added to the neurodevelopmental follow-up. Up to and including 4 years of age neurological, cognitive and behavioural development of the COH-IVF, MNC-IVF and Sub-NC group was similar. Yet, subfertility was associated with less favourable outcome on these parameters. This means that the ovarian stimulation used in IVF and the in vitro culture procedure did not affect the neurodevelopmental outcomes up until 4 years. On the other hand, the follow-up at 4 years revealed that the SBP levels in the COH-IVF group were higher than those in the MNC-IVF group. In addition, we found an increased subcapular skinfold thickness in children conceived with COH-IVF in comparison to the Sub-NC group. This suggests that the conventional ovarian stimulation in IVF may have a minor negative effect on the child’s cardiovascular profile. At the age of 9 we reassessed these outcome parameters.

Figure 3 shows the flow chart of the singletons participating in the Groningen ART cohort up until the age of 9 years. Attrition in the three groups of the Groningen Cohort study after 9 years of follow-up was 21%, which is acceptable.

The PGS-Trial

The PGS-trial was a multicenter trial (Academic Medical Center in Amsterdam and UMCG) on PGS. The trial initially started as a double blinded RCT evaluating the efficacy of day-3 cleavage stage PGS on ongoing pregnancy rates. The trial consisted of two groups...
receiving a reproductive technique (IVF or ICSI), one with PGS (PGS group) and one without PGS (control group) (see Figure 4). Inclusion criteria were: (1) female age of 35 through 41 years; (2) no previous failed IVF cycles; (3) no objection to a possible double-embryo transfer. Women who fulfilled these criteria and provided written informed consent were randomly assigned to IVF with or without PGS between May 2003 and November 2005 by a computer program with a minimization procedure for age (35 through 37 years and 38 through 41 years), IVF/ICSI and with stratification according to study center, before the first follicular aspiration. The PGS-trial showed that PGS significantly reduced the rates of ongoing pregnancies and live births after IVF in women of 35 through 41 years. After having evaluated the ongoing pregnancy rates, the focus of the PGS-trial shifted towards the health and development of PGS offspring. At the age of 2 years children born after IVF with PGS showed lower neurologic optimality scores than the control children. At the age of 4 years this difference in neurologic optimality scores persisted in twins, but had disappeared in the singletons. Furthermore, at the age of 4 years BP, anthropometrics, behavioral and cognitive development did not differ between the two groups.

Chapter 8 of this thesis addresses the offspring’s outcome at the age of 9 years. This chapter also includes the flow-chart of the PGS-trial.

Figure 4. Figure 4 shows an overview of the two groups. Comparing the two groups reveals the effect of PGS.
Outline of this thesis

The studies in this thesis concern the anthropometric, behavioural, cardiovascular, cognitive, neurologic, ocular and pulmonary outcomes of children born to subfertile couples following IVF with or without PGS. The thesis is divided into four parts;

Part I: IVF twins

Chapter 2 focuses on IVF twins. It is well known that in general more pregnancy complications and poorer obstetric outcomes are seen in twins versus singleton pregnancies. This chapter addresses whether IVF contributes to adverse health outcomes in IVF twins at the age of 4 years.

Part II: The Groningen ART cohort study

In this prospective cohort study we separately evaluate the effect of ovarian hyperstimulation and the in vitro culture procedure on the prevalence of asthma, visual acuity and cardiovascular outcomes by evaluating these outcomes in children born following COH-IVF, MNC-IVF and naturally conceived children born to subfertile couples.

Chapter 3 describes the prevalence of asthma and use of asthma medication in 4-year-old offspring of the three groups of the Groningen ART cohort study.

Chapter 4 uses the ISAAC questionnaire to assess the prevalence of asthma and use of asthma medication in 9-year-old offspring of the three groups of the Groningen ART cohort study.

Chapter 5 focusses on the possible effect of ovarian hyperstimulation and the in vitro culture procedure on IVF offspring’s visual acuity at the age of 9.

Chapter 6 reports on the possible effect of ovarian hyperstimulation and the in vitro culture procedure on the cardiovascular health of the 9-year-old children born to subfertile couples.

Chapter 7 presents the cardiovascular outcomes of the Groningen ART cohort study as one subfertile group and compares the outcomes to a fertile reference group at the age of 9. In this way we could study the effects of the presence of subfertility on cardiovascular health at 9 years.

Part III: The PGS-Trial

Chapter 8 focuses on PGS. Because of the relatively low pregnancy rates after IVF, new technologies, like PGS have been developed to improve the efficiency of IVF. In PGS an embryo is biopsied and screened for aneuploidies. Yet, very little is known on the long-term consequences of this type of embryo manipulation. This chapter describes the consequences of day-3 cleavage-stage PGS on the neurodevelopment and cardiovascular health of 9-year-old IVF offspring.

Part IV: General discussion, future perspectives and summary

Chapter 9 Contains the general discussion and future perspectives.

Chapter 10,11 Summarizes the content of the studies in English and Dutch
References


