

# Fertility treatment: long-term growth and mental development of the children

*Bjørn Bay*

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Tutors: Ulrik Schiøler Kesmodel & Erik Lykke Mortensen

Official opponents: Tine Brink Henriksen (chairman), Anja Pinborg & Bengt Källén

Correspondence: Department of Obstetrics and Gynecology, Aarhus University Hospital, Brendstrupgaardsvej 100, 8200 Aarhus N. Denmark.

E-mail: bjornbay@me.com

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## List of scientific papers

- I. Assisted reproduction and child neurodevelopmental outcomes: a systematic review.  
Bay B, Mortensen EL, Kesmodel US.  
Fertil Steril. 2013 Sep;100(3):844-53
- II. Fertility treatment and risk of childhood and adolescent mental disorders: register based cohort study.  
Bay B, Mortensen EL, Hvidtjørn D, Kesmodel US.  
BMJ. 2013 Jul 5;347:f3978
- III. Fertility treatment and child intelligence, attention, and executive functions in 5-year-old singletons: a cohort study  
Bay B, Mortensen EL, Kesmodel US.  
BJOG. 2014 Jun 9. Epub ahead of print.
- IV. Is subfertility or fertility treatment associated with long-term growth in the offspring: a cohort study  
Bay B, Mortensen EL, Kesmodel US.  
Fertil Steril. 2014 Aug 11. Epub ahead of print.

## INTRODUCTION

Fertility treatment as we know it today, is a rather young discipline in medicine. Yet, infertility and fertility treatment has been recognized for thousands of years. Long before fertilization was described as the union of egg and spermatozoa, Hippocrates (460 BC) proposed numerous treatments for the problem. In 1562,

Bartolommeo recommended that husbands should put their finger in the vagina after intercourse to encourage conception. Later, in 1785 John Hunter, a Scottish surgeon from London, advised a man with hypospadias to collect his semen and have his wife inject it into her vagina. Almost 100 years later in the US, professor William Pancoast inseminated a woman using the semen of the best looking student. Since insemination to a great extent resembles spontaneous conception, a major breakthrough was made in 1978 when Louise Brown was born as the first live born baby after fertilization of an oocyte in vitro.

Today, more than 5 million children have been born after in vitro fertilization alone. However, the indication for treatment is inevitably changing along with continuing advancement of the techniques. With pre-implantation genetic diagnosis, biopsy of the 8-cell-stage embryo is carried out in order to perform investigations for heritable diseases present in the parents. And with the technique of vitrification (rapid freezing of oocytes) the possibility of "social egg freezing" arises. Here, a woman undergoes hormonal treatment and oocyte retrieval in order to freeze a number of unfertilized eggs merely to delay pregnancy for a variety of social reasons.

Along with the increasing use and advancement follows a responsibility to continuously monitor the safety of these procedures applied to millions of current and future lives. We conducted a range of epidemiological studies investigating the long-term mental health and growth of children born to parents conceiving after fertility treatment compared with that of spontaneously conceived controls. In this thesis, the background, methods, and results are presented along with a discussion relating the findings to previous studies and addressing specific study limitations. Finally, more general points regarding the potential association between fertility treatment and child development are discussed.

## BACKGROUND

### FERTILITY AND INFERTILITY

Despite the planning of a pregnancy, conceiving is not a matter of course. The chance of achieving a spontaneous pregnancy within a given period of relevant trying is defined as the fecundability. Traditionally, the natural fecundability has been determined as the waiting time to a pregnancy after onset of marriage in populations, which do not allow contraceptives or abortions such as the Amish. Pregnancy rates per month in such populations are approximately 25%.<sup>[1]</sup> This may be biased by high coitus rate commonly seen at the onset of marriage. However, similar preg-

nancy rates have been confirmed in prospective studies,[2] although lower and higher first month clinical pregnancy rates have been reported depending on the study methodology such as inclusion criteria (e.g. couples with or without previous pregnancies).[3, 4] The cumulative pregnancy rate after 6 months is 60-80%[2-5] and approximately 90% after 12 months.[4] After 12 months of unsuccessfully trying, only a small portion of the couples will conceive spontaneously. An estimated 5% will suffer from complete infertility defined as the inability to achieve a spontaneous pregnancy.[4] However, the chance of and time to spontaneous conception are strongly dependent on the woman's age, with considerably lower chance at older age.[6] Infertility has been defined as a failure to conceive within 12 months[7] although some couples subsequently will conceive spontaneously or after treatment. Thus, a better term may be subfertility.[8] Failure to achieve a spontaneous pregnancy may be caused by a large variety of causes originating in the male and/or female reproductive system. Thus, for all couples seeking fertility treatment, the cause may be originating in failure of the ovulation, fallopian tubes, sperm quality, a mix of these, or other aetiologies such as endometriosis or sexual dysfunction (Figure 1).[9] Even so, for a large proportion of couples or single women seeking fertility treatment, there is no obvious explanation for their subfertility.

#### FERTILITY TREATMENT

Various definitions exist for the treatments of infertility. Fertility treatment is a broad term and may be defined as any procedure or method to enhance the chance of a pregnancy. More specifically defined, medically assisted reproduction covers reproduction brought about through in vitro procedures, ovulation induction, controlled ovarian stimulation, ovulation triggering, and intrauterine, intracervical, and intravaginal insemination with sperm of husband/partner or donor.[7] Finally, the term assisted reproductive technology (ART) is limited to all treatments or procedures that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of establishing a pregnancy.[7]

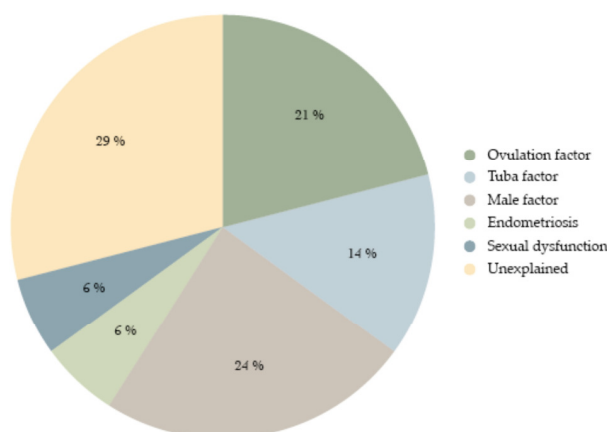


Figure 1. Causes of infertility.  
Hull et al, BMJ 1985

#### Hormonal treatment

The overall aim of the hormonal treatment is to enhance the growth and development of one or more oocytes and subsequent

ovulation from the ovary in order for the oocyte(s) to be available for fertilization. The drugs interact with the natural hormones in the female reproductive endocrine system mainly constituted of the hypothalamus, pituitary gland, and the ovaries. Hormones may be used to induce ovulation in women who do not ovulate on their own and typically, the goal is to produce a single, healthy egg. This treatment is termed ovulation induction (OI), and fertilization is achieved by coitus. Further, hormonal treatment may be used in women who naturally ovulate to increase the number and growth of oocytes to increase the chance of one or more to be fertilized. This treatment is termed ovarian stimulation. When the ovaries have been stimulated to produce one or more mature follicles the release of the oocyte from the follicle may be triggered, and subsequently the sperm can be made available to the oocyte by means of coitus, insemination or in vitro fertilization (IVF).

Clomid or clomiphene citrate is a commonly prescribed drug used to stimulate ovulation in women who have infrequent or absent ovulation. It works by causing the pituitary gland to secrete more follicle stimulating hormone. The higher level of FSH stimulates the development of ovarian follicles that contain oocytes.

Follicle stimulating hormone (FSH) is a recombinant or natural extracted gonadotrophin that stimulates the ovary to produce one or more mature follicles and an appropriate estrogen level. It is often prescribed for injection in anovulatory women who have tried clomiphene without success or to women whose pituitary glands produce inadequate amounts of FSH. Additionally, FSH is used prior to IVF to induce development of multiple follicles containing oocytes to retrieve and fertilize.

Human Chorionic Gonadotrophin (hCG) is a hormone used to trigger the process of ovulation. It is similar in chemical structure and function to the natural gonadotrophin luteinizing hormone (LH). As such, an injection mimics the natural LH surge causing the release of the oocyte from the dominant follicle in the ovary. Thus, in a cycle with controlled ovarian stimulation (e.g. with Clomid or FSH), the growth and development of the follicles may be monitored with ultrasound and when mature, injection of hCG will trigger ovulation after approximately 36 hours.

Gonadotrophin releasing hormone (GnRH) agonists or antagonists are chemically modified synthetics of the natural GnRH, which is a hormone released from the hypothalamus stimulating the secretion of gonadotrophins (FSH and LH) from the pituitary gland. The purpose of GnRH analogs is a block-replacement strategy to control the levels of the gonadotrophins and to prevent premature ovulation due to an endogenous LH peak prior to IVF.

#### Ovulation induction and insemination

The simplest form of treatment is insemination. With this treatment, the sperm from the husband or a donor is placed in the vagina, or the uterine cavity through a catheter (intrauterine insemination (IUI)). With the exception of the insemination of single or lesbian women without reproductive disorders, insemination is often preceded by OI.

#### In vitro procedures

IVF may be indicated if the ovarian stimulation followed by coitus or insemination did not result in a pregnancy, if the fallopian tubes are damaged, in women with endometriosis, or in the case of severe male infertility. Additionally, IVF may be performed as part of treatment with pre-implantation genetic diagnosis or in women who volunteer as oocyte donors. When the ovaries have

been stimulated to produce one or more mature follicles containing an oocyte, these are retrieved by an ultrasound-guided transvaginal aspiration. Subsequently, the oocytes are fertilized in vitro with the husband's or donor sperm and develop 2-5 days in a culture medium while situated in an incubator. Eventually, one, two, or in some countries even more embryos are transferred to the uterine cavity in order to implant and achieve a viable pregnancy.

Microinsemination may be indicated if the sperm count is low, has a low motility, a high percentage of morphologically abnormal sperm, the ejaculation is obstructed, or if IVF has failed repeatedly. With intracytoplasmic sperm injection (ICSI), a single spermatozoon is injected directly into the oocyte using a microscope and a micropipette.

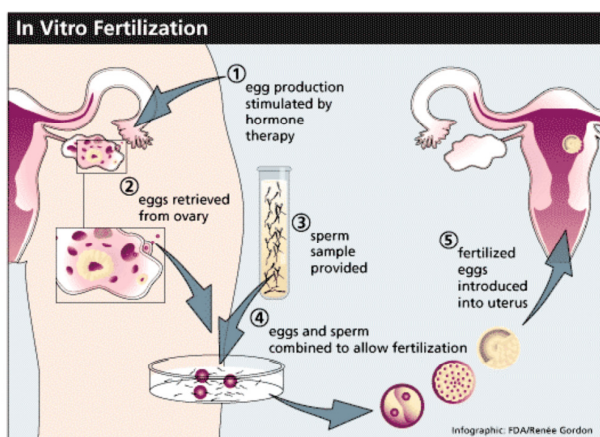


Figure 2. The process of *in vitro* fertilization, US Food and Drug Administration

### Increasingly used and useful treatments

Whether the fecundability in the civilized world is declining remains a question unanswered.[10] Although several studies report of declining sperm quality and fecundability as well as innumerable theories about potential chemical causes, no consistent evidence has been published.[11] Nevertheless, the number of involuntarily childless couples and single women seeking medical treatment is increasing.[12, 13] Further, an increasing number of infertile couples are seeking help after a shorter time of unsuccessfully trying to conceive.[12] [14]

Moreover, fertility rates are well below replacement level in several developed countries.[15] Thus, fertility treatment may be considered a desirable option for couples struggling with involuntary childlessness at the personal as well as at the society level. Worldwide more than 1.5 million IVF cycles are performed each year, producing more than 350,000 children.[16] Denmark has the highest number of treatments per inhabitant (2726 cycles/million) and correspondingly the largest proportion of newborn conceived after fertility treatment.[16] In 2012, a total of 5.4% of new Danes were conceived after IVF/ICSI, and an additional 3% were born by parents conceiving after insemination.[17] The chance of a pregnancy in a single cycle depends on the type of treatment. After insemination with sperm from the partner or donor sperm the pregnancy rate is averaging approximately 8% and 13%, respectively.[18] With the use of IVF and ICSI the pregnancy rate amounts to approximately 29% per aspiration.[18] The cumulative live-birth rate for six cycles is 50-70%, although age dependent.[19]

### SAFETY OF THE TREATMENTS

Fortunately, fertility treatment only rarely leads to medical or surgical complications. The medical ovarian stimulation may occasionally result in ovarian hyperstimulation syndrome (OHSS). Although this complication occurs in milder and more common forms, severe OHSS is a potentially life threatening condition. It occurs only sporadically after ovulation induction alone, but in 0.1-2 % after IVF/ICSI.[20] OHSS may lead to swollen ovaries, vascular permeability, intracellular fluid depletion, and accumulation of fluid in the abdominal cavity and lungs, and clotting of the blood. Due to changes in treatment protocols during the last decades,[14] the incidence is given to decline and recently, the overall incidence in Europe was reported to be 0.4%.[18] The surgical procedure of transvaginal aspiration of oocytes may cause bleeding or infection. However, these complications are uncommon with very low incidences of 0.1% and 0.01% per ART cycle, respectively.[18]

By far, the highest risk of complications after fertility treatment is related to the risk of multiplicity. Compared to spontaneous conception, where typically one oocyte is developed, matured, and released, the medical ovarian stimulation leads to development and maturation of several oocytes. Additionally, transfer of more than one oocyte has been standard practice in IVF until recently, where the practice of single embryo transfer started to increase.[21] Regardless of conception mode, multiples carry a higher risk of obstetrical and perinatal complications, although it may be slightly higher for multiples born after assisted reproduction compared to spontaneously conceived multiples.[22, 23] Nevertheless, the increased risk associated with multiplicity is important to acknowledge in studies investigating obstetric, perinatal, or childhood outcomes of children born after fertility treatments.

### Obstetrical complications

In a recent review and meta-analysis, the risk of obstetrical complications was increased for singleton pregnancies after conception by IVF/ICSI compared to spontaneously conceived children. Assisted conception was found to be associated with a higher risk of ante-partum haemorrhage, hypertensive disorders of pregnancy, preterm rupture of membranes, and caesarean section.[24] Further, an increased risk of venous thrombosis has been suggested in pregnancies after IVF.[25]

### Short-term offspring consequences

The perinatal risk after fertility treatment and parental subfertility was recently summarized.[26] In a meta-analysis, singletons conceived after IVF/ICSI were shown to have an increased risk of preterm birth and further, the risk of low birth weight was increased compared to spontaneously conceived controls. When compared with children born to subfertile parents either directly or through a sibling design with one child spontaneously conceived and one conceived after IVF/ICSI, the risk difference was diminished although still significant. Similarly, singletons born after OI and/or IUI had an increased risk of preterm birth and low birth weight compared to spontaneously conceived singletons.[26]

The risk of malformations in children conceived after IVF has consistently been found to be increased, although most likely associated with parental subfertility rather than the fertility treatment.[27, 28]

## **Long-term offspring consequences**

### *Somatic health*

Significant, albeit weak associations have been found for a range of specific childhood somatic health outcomes such as cardio-metabolic and endocrine disorders.[29] Recently, a Swedish study suggested an association between IVF conception and development of asthma in childhood. Although some of the association may have been explained by gestational age or maternal health, parental subfertility seemed to be the most important predictor.[30] Further, a recent meta-analysis concluded that the risk of all cancers, haematological cancers, central nervous system/neural cancers and other solid cancers was increased in childhood and/or adolescence.[31] The absolute risk was small and the risk associated with fertility treatment could not be separated from the potential risk associated with the underlying parental subfertility.

### *Mental health*

While several studies have been published, no consistent deficits have been revealed to be related to either ART or OI, regarding specific neurodevelopment outcomes, or for any age groups of the children.

Cognitive development such as intelligence and language development seem comparable to that of spontaneously conceived children regardless of age. The association between conception mode and cognitive development has not been investigated among infants. Among toddlers, studies showed that ICSI children scored lower on tests of mental development, although the association was only significant for boys in the one study[32] and reflected lower chronological age in study participants in the other.[33] A majority of studies on toddlers showed no associations with cognitive[34-44] or language development.[33, 42-47] In a Dutch study, ICSI children scored significantly lower on tests of intelligence compared with spontaneously conceived children.[48] However, as for toddlers, most studies on preschoolers and children in mid-childhood showed no associations between conception mode and cognitive or language development.[49-54] Although only sparsely investigated among teens (13+ years), the few studies with follow-up on this age group generally report of comparable development.[55-58]

With regard to behavioural and socio-emotional development, McMahon and Gibson found that 4-month-old infants born after IVF were rated more difficult on aspects of temperament and displayed more negative behaviour on the Still Face procedure.[59] At a later follow-up, they found more behavioural difficulties as reported by mothers among toddlers conceived after IVF. In contrast, other studies showed no differences in behavioural or socio-emotional development between toddlers conceived after assisted reproduction and spontaneous conceived controls.[33, 34, 37, 43, 44, 60] Studies investigating behaviour or socio-emotional development among preschoolers or in mid-childhood are also reassuring. Although more problems on social development have been reported in a small sample of ART twins with very low birth weight compared to spontaneously conceived twins,[61] and another study showed lower scores on socio-emotional- and behavioural development and higher levels of depression, aggression and anxiety compared to spontaneously conceived children,[51] most studies showed no associations.[49, 62-69]

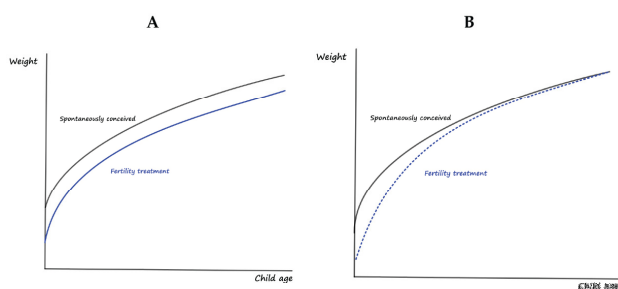
The risk of mental disorders has primarily been assessed in Danish and Swedish health register studies. Two large register-

based studies from Sweden including children from infancy to early adulthood showed increased risks of attention deficit/hyperactivity disorder[70] and behavioural problems[71] in children born after IVF compared with spontaneously conceived children. However, statistical significance was lost when adjusting for length of involuntary childlessness and restriction to term-infants, respectively. While Wennerholm et al[72] found comparable risk of developmental delays in 18 months old toddlers after extraction of records from Swedish Habilitation Centres, another Swedish study showed increased risks of developmental delays in terms of higher risk of disabilities, neurological impairments or handicaps in children up to 14 years old.[73] However, these findings were largely explained by multiplicity, low birth weight and gestational age. Pinborg and colleagues found no risk of mental retardation, cerebral palsy or imprinting disorders in singletons up to 13 years[74] or in twins up to 7 years[75] born after IVF/ICSI. Nor did they find increased risk of autism, Asperger Syndrome or retarded psychomotor development in IVF/ICSI twins[75] compared to spontaneously conceived peers. The risk of autism has also been evaluated in two Danish and one Israeli study with inconsistent results: While one of the Danish studies[76] reported lower risk of infantile autism in a case-control study of 33 ART children and 913 spontaneously conceived children, another study showed no overall increased risk of autism spectrum disorders depending on conception mode among 588,967 children born after IVF, OI or spontaneous conception. However, they showed a slightly increased risk in girls born after OI after stratification for gender.[77] In contrast, a case-control study from Israel showed that significantly more children with Autism Spectrum Disorders (ASD) were conceived after ART compared to a large unmatched reference population of spontaneously conceived children, but the number of cases in the ART group was low.[78] The risk of 54 imprinting disorders were extensively studied based on information on 442,349 singletons from the Danish national health registers yielding comparable risks among ART children and spontaneously conceived children up to 7 years, except for sleeping disturbances and cerebral palsy,[79] where IVF/ICSI children carried significantly higher risks in unadjusted analyses.

Thus, neurodevelopmental outcomes in children conceived after fertility treatment have mostly been investigated in younger children and only sparsely beyond the age of preschoolers. More reliable measures of neurodevelopmental outcomes such as intelligence and more specific cognitive functions (e.g. executive function) may not be obtained until older ages. Further, cognitive deficits may change as the child grows older: Early cognitive deficits may not reflect long-term influences on cognitive development, while deficits in more complex cognitive functions may only be detectable in later childhood or adolescence. Indeed, many mental disorders are diagnosed at school age or later.[80]

### *Long-term growth*

Singletons born after fertility treatment have been shown to have lower birth weight compared to spontaneously conceived children.[26] Impaired fetal growth and rapid post-natal catch-up



**Figure 3**  
Hypotheses on postnatal growth in children conceived after fertility treatment compared to spontaneously conceived children. Panel A: Difference in birth weight persist throughout childhood indicating different general metabolism. Panel B: Postnatal catch-up growth as a compensatory mechanism for intrauterine growth restraints.

may be related to increased risk of cardiovascular disease, obesity, and type 2 diabetes in adult life.[81-84] Still, it remains unclear whether smaller anthropometric size at birth in children conceived after fertility treatment persists later in childhood. Despite differences in birth weight, several studies have reported comparable weight and height later in childhood or adolescence for singletons born by parents conceiving after fertility treatment compared with spontaneously conceived children.[85-88] In addition, a long-term follow-up of Dutch IVF children and controls showed that the anthropometric differences observed at birth and at 3 months of age were no longer present after 6 months.[89] These results indicate that the IVF children may have increased postnatal growth velocity (Figure 1, panel B). For IVF children, a rapid weight gain during early childhood has been related to higher blood pressure levels independently of birth weight, gestational age and body size at follow-up in 8-18 year-old children.[89] In contrast, other studies have concluded that differences in birth weight persist into childhood,[90, 91] which may indicate general differences in the metabolism (Figure 3, panel A).

**BIOLOGICAL PLAUSIBILITY FOR NEURODEVELOPMENTAL DEFICITS**  
According to the developmental origin of health and disease hypothesis, environmental factors acting during the early embryonic development interact with the genotype and change the capacity of the organism to cope with its environment in later life.[92] Thus, events in early life may affect the later susceptibility to certain diseases. Especially the identification of the association between low birth weight and ischemic heart disease in adult life (the Barker hypothesis)[93] has been ground breaking in the understanding of the importance of fetal development for later health outcomes.

There are several biological reasons for increased vigilance on the mental development of children conceived after ART or OI. The procedures involve medical hyperstimulation, handling, and culture of gametes and early embryos at a particularly vulnerable period of development.[94, 95] Studies imply that ART may affect the epigenetic control in early embryogenesis,[96, 97] and ART has been associated with an increased risk of imprinting disorders in both experimental animal studies and epidemiological human studies.[98, 99] Medical ovarian hyperstimulation may impact fetal neurodevelopment,[98] and the use of different culture media used for IVF may affect the phenotype of the offspring by significantly altering physical and biochemical parameters such as birth weight[100], blood pressure, fasting glucose, pubertal gonadotrophin levels[101], growth factors and blood lipids[102].

The altered selection of the fertilizing spermatozoa may be of importance - especially in the case of ICSI where a single spermatozoon is introduced into the oocyte by micro insemination. However, possible neurodevelopmental deficits in children born after ART or OI may origin from several non-procedural related factors rather than the treatments. The procedures are major contributors to multiple gestations, which are at risk of preterm delivery, low birth weight and small for gestational age;[103] three risk factors for neurodevelopmental deficits.[104-106] But even when restricted to singletons, children born after ART and OI have a higher rate of preterm delivery and reduced fetal growth.[26, 107] Further, differences in neurodevelopment could be due to the underlying subfertility[108] or conditions in the parents, e.g. men with low sperm quality are more likely to have chromosomal abnormalities that they may pass on to the offspring.[109] Finally, predictors of neurodevelopment (e.g. age, parity, and intelligence) may differ between infertile couples and couples with no problems conceiving spontaneously.[49, 110-112]

### AIM OF THE THESIS

The overall aim of the thesis was to explore the long-term mental development of children born to subfertile parents or parents conceiving after fertility treatment. We aimed to cover major outcomes such as the risk of mental disorders as well as minor outcomes such as level of cognitive functioning in healthy children. Further, we aimed to investigate the long-term growth in these children.

### STUDY I

The study aimed to summarize the existing literature on neurodevelopmental outcomes in children born to parents conceiving after fertility treatment compared to spontaneously conceived children. In particular, the study aimed to address methodological limitations in previous studies exploring this association.

### STUDY II

The study aimed to assess the risk of mental disorders in children and adolescents born to parents conceiving after fertility treatment compared to spontaneously conceived children.

### STUDY III

The study aimed to assess the intelligence, attention and executive functions in 5-year-old singletons born to subfertile parents or parents conceiving after fertility treatment compared to the cognitive development of spontaneous conceived children. Further, the study aimed to explore the importance of accounting for maternal intelligence level as the first study ever in this field.

### STUDY IV

The study aimed to assess anthropometric measures at birth and at the age of 5 in singletons born to subfertile parents or parents conceiving after fertility treatment compared to the growth of spontaneously conceived children.

### METHODS

The studies included in this thesis are based on information obtained from the Danish national health registers, the Danish National Birth Cohort,[113] and the Lifestyle During Pregnancy Study.[114] Although the Danish health registers are primarily maintained for administrative reasons, they offer unique possibilities to conduct epidemiological research.[115] Every contact,

treatment, and diagnosis is registered at the individual level with the use of the unique personal identification number assigned to all live-born children in Denmark. Thus, accurate individual-level linkage between all national registers can be assured, which is essential in register-based epidemiology.

Below is a description of each data source of information used to conduct the studies presented in this thesis. This is followed by a specification of each study with regard to exposure, outcome, covariates, and statistical analyses.

## DATA SOURCES

### ***The Medical Birth Register***

The Medical Birth Register was established in 1968 and contains complete information from 1973 about all births of newborn as well as stillborn babies at Danish hospitals or at home.[116] This information includes a wide range of data covering date of birth, gender, multiplicity, Apgar scores (1 and 5 min). Further it has a wide range of information about procedures and complications related to labour as well as information about the parents.

### ***The IVF register***

We assessed exposure to IVF and ICSI through the Danish IVF register,[117] which registers information from all public and private fertility clinics. The register contains information on each woman's personal identification number, underlying aetiology of infertility, type of treatment (IVF, ICSI, fresh/frozen embryo, egg or semen donation), and type of medication used, as well as information on pregnancy outcomes and the personal identification number of the resulting children since the start of the register in 1994. It is mandatory by law to report all initiated treatments to the register. The register is updated yearly.

### ***The Danish National Prescription Registry***

The Danish National Prescription Register (DNPR) contains individual level data of all redeemed prescription medications sold at outpatients pharmacies including date of dispensing, drug name and dose units. The register is maintained by Statistics Denmark and is a sub-register to The Register of Medicinal Products Statistics held by the Danish Medicines Agency. DNPR has complete data since 1995 and incomplete data from 1994.[118] We identified women who redeemed prescriptions on all types of medications used for OI and IUI. As these medications can be prescribed for up to three months at a time, we included women who redeemed the prescription at a date within 12 weeks before and four weeks after the last menstrual period. The same medications may also be used for IVF/ICSI and thus, we excluded any women who occurred in the IVF register with the same date of last menstrual period.

### ***The Danish Psychiatric Central Research Register***

The Danish Psychiatric Central Research Register (DPCRR) contains individual-level data on all admissions and outpatient contacts on public psychiatric hospitals, including diagnoses and dates.[119] Since 1994 diagnoses have been registered according to the International Classification of Diseases 10th revision (ICD-10). Based on Classification of Mental and Behavioural Disorders chapter 7-9 a selection of outcome variables of mental disorders in childhood or adolescence was identified and included for analysis.

### ***Statistics Denmark***

Statistics Denmark is a state institution under the Ministry of

Economic Affairs and the Interior, collecting, compiling and publishing statistics on the Danish society. For the purpose of this study we used The Populations Education Register at Statistics Denmark. This register contains information on the highest completed level of education at Danish teaching institutions for each individual in the Danish population. Data for the register has been systematically collected since 1974. For educations completed before 1974 and for immigrants without Danish schooling records, the information comes from the Population and Housing Census in 1970 and the Immigrant Census in 1999.[120, 121] The register has been reported to be of high validity and to have a high degree of completeness. With 0-3% misclassification and non-missing for 97%, the register represents a valid source of information.[120] Further, we assessed information on migration and death for each individual in the study period in order to assure that a given child only contributed with time at risk, while alive and living in Denmark.

### ***The Danish National Birth Cohort***

The Danish National Birth Cohort (DNBC) is a large follow-up study including more than 100,000 pregnant women and their children.[113] The enrolments took place during the period 1997–2003. The pregnant women were recruited at their first antenatal visit by their general practitioner, who routinely is the first healthcare professional to see a pregnant woman in Denmark. The only exclusion criterion was the inability to speak Danish. Exposure information was collected by computer-assisted telephone interviews with the women twice during pregnancy (week 12 and 30) and when their children were six and 18 months old. The database includes 101,042 pregnancies, which corresponds to approximately 60% of the women invited and approximately 30% of all pregnant women in Denmark during the enrolment period. Besides the possibility to link the data in the DNBC to information in the Danish health registers, the children have been followed up at age 7, and a sample of the children at age 5. Currently, the 11-year follow-up is being conducted.

### ***The Lifestyle During Pregnancy Study***

The Lifestyle During Pregnancy Study (LDPS) study is a prospective follow-up on a sample of participants from the DNBC. The overall aim of the LDPS was to examine the relation between maternal lifestyle during pregnancy and offspring neurodevelopment at the age of 5.[114] Exclusion criteria in the LDPS were impaired hearing or vision loss inhibiting the performance on neuropsychological tests, or if a child was affected by a congenital disorder associated with mental retardation.

Mothers were invited to participate in the study by letter approximately 3-10 (mean=5.8, standard deviation (SD)=0.7) weeks before their child's fifth birthday. Mothers were offered further information by telephone, if needed. Those agreeing to participate were contacted by telephone and provided more detailed project information, and an appointment for testing the child was made. Child outcome measures were obtained during a 3-hour assessment when the child was between 5.0 and 5.3 years old. To minimize the travel distance for all mothers and children, the assessment took place in the four largest cities in Denmark. Prior to this, the parents had been mailed a self-explanatory questionnaire regarding the child's general postnatal health and development, as well as maternal and paternal postnatal lifestyle and socio-demographic characteristics. If permission was obtained from the parents, questionnaires were subsequently mailed to the child's day-care centre. At the follow-up, the children were



examined with an eyesight test, audiometric evaluation, anthropometric measurements, and a comprehensive neuropsychological test battery.

A sample of 3,478 singletons from the DNBC was invited to the LDPS and 1782 (51.2%) participated in the assessments. There were no substantial differences between the participants and non-participants in the LDPS with regard to maternal age, parity, body mass index (BMI), prenatal smoking or alcohol consumption, marital status, child gender, birth weight or gestational age at birth.[122]

The objective neurodevelopmental assessment was performed by 10 psychologists with a Master of Science in Psychology (MSc psych). To avoid bias due to inter-rater variability they regularly and blindly rescored subtest administered by other psychologist. The ongoing blind rescoring of test protocols was followed by discussions among the psychologists to obtain consensus on scoring criteria. Typically, there was 97–97.5% agreement before discussion.

### STUDY I – REVIEW OF PREVIOUS STUDIES

This study was designed as a systematic review and adhered to the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).[123] With the use of the bibliographic databases Medline, Embase, Web of Science and Scopus, a computerized literature search was conducted in corporation with a medical librarian. Keyword searches were used to identify all potentially relevant studies. The search was extended with free text terms to include non-indexed new literature. The electronic search was supplemented with a review of the bibliographies of included studies to identify all papers relevant to this review. Titles and abstracts of all identified studies were screened for study design, type of exposure and outcome according to the a priori determined criteria described below. Articles deemed relevant or potentially relevant, were obtained and critical read in full text. Cohort or case-control studies were included and studies were excluded if they were case studies or case series. The searches were not imposed by restrictions in study language or year of publication.

**Table 1.**  
Overview of studies included in the thesis

	Study I	Study II	Study III	Study IV
Topic of interest	Review of previous studies	Risk of mental disorders	Neurodevelopment	Growth
Study design	Systematic review	Cohort study	Cohort study	Cohort study
N	31 - 2,446,044	588,967	1782	1773
Child ages	0-26 years	0-17 years	5 years	5 years
Data sources	Medline, Embase, Web of Science and Scopus	MBR, IVF-register, DNPR, DPCRR, Statistics Denmark	DNBC, LDPS	DNBC, LDPS
Exposure	ART or OI	IVF/ICSI or OI/IUI	IVF/ICSI/OI/OS	IVF/ICSI/OI/OS
Outcome	Mental disorders, and cognitive, behavioural, and psychomotor development	Developmental disorders, mental retardation, and behavioural and emotional disorders	Intelligence, attention, and executive function	Height, weight, BMI, head circumference
Statistical analysis	---	Cox proportional hazard regression	Linear regression	Linear regression

MBR: medical birth register; DNPR: Danish national prescription register; DPCRR: Danish psychiatric central research register; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection.

### Exposure

The included studies had to encompass an exposure group with children born after OI or ART as defined by the WHO.[7] Each study could additionally include children born after gamete dona-

tion, adoption, or surrogacy, but the study was excluded if the exposure group was limited to the latter groups. Further, studies were only included if the neurodevelopmental outcomes were compared with a reference group of spontaneously conceived children.

### Outcomes

Based on each specific outcome investigated in the articles constituting the existing scientific literature, each outcome was categorized into one of four domains: 1. Cognitive development including educational achievement and language development; 2. Behavioural and socio-emotional development including coping and temperament; 3. Psychomotor development; and 4. Mental disorders. Based on this categorization, the results were sorted based on age group into infants (first year of life), toddlers (1-2 years), preschoolers (3-5 years), middle childhood (5<age<12 years) and teens (≥12).

### Statistical analysis and strategy

No statistical analyses were performed. We did not combine the results of the different studies in order to obtain a quantified synthesis. In particular, the assessments of outcomes in the included studies differed considerably. Thus, a meta-analysis was not considered suitable.

### STUDY II – RISK OF MENTAL DISORDERS

This study was designed as a historical cohort study with prospective follow-up of all children born in Denmark from January 1, 1995, to December 31 2003. Based on the unique personal identification number, we established the cohort based on data from the Danish Medical Birth Register.[124] Information about exposure, outcome and covariates were obtained from the IVF register,[117] the Danish National Prescription Registry,[118] the Danish Psychiatric Central Research Register,[119] and Statistics Denmark[120] (Figure 3)

### Exposure

The main exposure was IVF and ICSI or OI and ovarian stimulation with or without intrauterine insemination. The children born after conceptions by IVF or ICSI were grouped into one exposure group (IVF/ICSI), OI/IUI represented an additional exposure group, and the reference group was children born to parents conceiving spontaneously.

In secondary analyses, other exposures of interest were specific type of ART (IVF; ICSI), type of hormonal medication (Follicle Stimulating Hormone; Clomiphene Citrate; Humane Chorionic Gonadotrophin; Gonadotrophin Releasing Hormone), aetiology of infertility (male factor; tuba factor; ovulation factor; mixed aetiology), and type of gamete (fresh embryo; thawed embryo; donor sperm; donor oocyte). Still, the reference group was children born to parents conceiving spontaneously.

### Outcomes

Based on information from the Danish Psychiatric Central Research Register, the following variables were dichotomized for each individual in the cohort: Any mental disorder (F70.0-F99.9); any mental retardation and degrees of mental retardation (F70.0-F79.9); developmental disorders, all (F80.0-F89.9); developmental disorders of speech and language (F80x); scholastic skills (reading, spelling or calculating disorders) (F81x) or motor function (F82x); developmental disorders, mixed (F83x), pervasive developmental disorders (autism spectrum disorders) (F84x); developmental

disorders, other (F88.0-F89.9); behavioral and emotional disorders, all (F90.0-F98.8); hyperkinetic disorder (F90x); conduct, emotional or social disorder (F91.0-F94.9); Tic disorders (F950-F95.9); Behavioral and emotional disorders, other (F98.8) and mental disorders, other (F99x). Since very few were diagnosed with profound mental retardation we collapsed this category with severe mental retardation. Both primary and supplementary diagnoses were included.

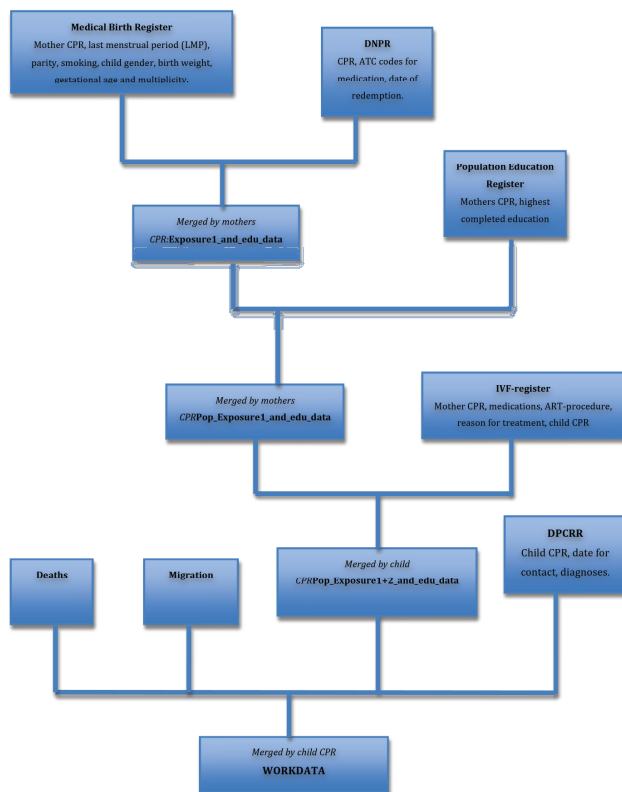


Figure 4. Flowchart of register-based information for study II: Bay et al, *BMJ* 2013;347:f3978

### Statistical analysis and strategy

Each of the children in the population contributed with time at risk beginning at the day of birth and ending on one of the following dates, whichever came first: Date of diagnosis, death, emigration or end of follow-up on February 16 2012. The date of diagnosis was considered the first date a child received the first diagnosis (any mental disorder) or the date a child received the specific diagnosis relevant for the outcome in question. The risk of mental disorders associated with conception mode was estimated using standard Cox proportional hazards regression analyses. In order to account for correlations between siblings, all analyses were made using robust standard errors taking into account the non-independence of subsequent pregnancies in the same woman. Further, estimation of the association of subtypes of procedures, hormonal treatment, embryo types and cause of infertility with the risk of mental disorders was assessed. In all analyses adjustments were made for a priori determined potential confounding variables. In the main analyses, these included maternal age, parity, maternal smoking in pregnancy, maternal

educational level, maternal psychiatric history, multiplicity, child gender, and year of birth. Birth weight and gestational age were considered potential intermediate factors and were not included in the regression model. Furthermore, additional analyses were performed stratifying for multiplicity and child gender as well as restricting analyses to infant survivors (surviving >12 months). The proportional hazards assumption was evaluated by graphical assessment, and the assumption was met. In reporting the results the risks are referred to as hazard ratios with 95% confidence intervals (CI). P-values less than 0.05 were considered statistically significant.

As all women receiving fertility treatment were more than 20 years old, only children of mothers above this age were included in the study.

**STUDY III AND IV –NEURODEVELOPMENT AND GROWTH AT AGE 5**  
These follow-up studies are based on information from the DNBC[113] and the LDPS.[114] By combining exposure information obtained in the DNBC with the outcome measures from the LDPS, we were able to investigate whether fertility treatment is associated with the child's neurodevelopment and growth at age 5.

### Exposure

Information on conception method was obtained in the first of two prenatal interviews in the DNBC at a median of 17 weeks of gestation. The women were asked about any treatment for childlessness prior to the index pregnancy including type of treatment as well as waiting time to pregnancy (TTP). Due to relatively low numbers of exposed, all children born to parents conceiving after fertility treatment were grouped. Fertility treatment was defined as IVF, ICSI, and ovarian stimulation or ovulation induction with or without intrauterine insemination. Further, we included an additional exposure group of children born to subfertile parents conceiving spontaneously, but after waiting 12 months or longer before conceiving (TTP>12). The reference group remained the children born to parents successfully conceiving spontaneously within 12 months.

Information on self-reported fertility treatments has previously been validated in the DNBC and found to have high positive predictive value.[125]

### Outcomes

In study III we investigated the children's intelligence, attention and executive functions.

The child's intelligence quotient (IQ) was measured with the Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R).[126] The test is a widely used measure of intelligence among 3- to 7-year-old children and comprises five verbal and five performance (non-verbal) subtests. In this study only three verbal and three performances subtests were used in order to reduce the total testing time. Due to lack of Danish WPPSI-R norms the derived scaled scores and IQs were based on Swedish norms, which is unlikely to affect the internal comparison within the sample or the external validity since Sweden is a neighbouring country and in general considered very similar to Denmark. The child's attention was measured with the Test of Everyday Attention at Five (TEACh-5).[127] This recently developed test minimises demands on task comprehension, memory, reasoning, language and motor skills in order to obtain pure measures of attention. Briefly, the test comprises 5 subtests, testing selective, sustained, and overall attention, using visual and auditory stimuli



in the form of a storybook. Selective attention is assessed by two subtests: 1) the child counts balloons with and without visual distracters (The Great Balloon Hunt) and 2) identifies whether dog barking is present in a series of 14 animal sounds in 10 seconds (Hide and Seek II). Each of the two test scores is standardized (to a mean of 0 and a SD of 1) and the sum of these is then re-standardized to a composite selective attention score. Sustained attention is assessed as the child is counting dog barks with varying numbers and intervals (Barking) and tracing a line with a pen as slowly as possible without stopping (Draw a Line). These two test scores are standardized (to a mean of 0 and a SD of 1) and the sum of these is then re-standardized to a composite sustained attention score. Further, a composite overall mean attention score is calculated as the sum of the four standardized scores (The Great Balloon Hunt, Hide and Seek II Barking, and Draw a Line), which is re-standardized for use in the analyses. Executive function was assessed with the Behaviour Rating Inventory of Executive Functions (BRIEF) consisting of a parent and a teacher form each containing 86 different statements about the child's daily life measuring eight different aspects of executive function (inhibit, shift, emotional control, initiate, working memory, plan/organise, organization of materials, and monitor).[128] No Danish BRIEF norms were available at the time of the study, and based on the whole sample a normalizing T-score transformation for the observed BRIEF scores was computed, with higher scores indicating more executive function difficulties. The transformed scores were further combined in two broader indexes for the parent and teacher form, respectively: the behavioural regulation index (BRI) and meta-cognition index (MI), which are summed to get the Global Executive Composite (GEC).

In study IV we investigated the long-term growth of children born to subfertile parents or parents conceiving after fertility treatment compared to the growth of spontaneously conceived children. Based on the physical examination in the 5-year follow-up in the LDPS, we included the following outcome measures: Height at 5-years, weight at 5-years, BMI at 5-years, and head circumference at 5-years.

### **Covariates**

Information on important covariates was obtained from the DNBC prenatal interview, the 5-year follow-up, and the Danish national health registers. From the DNBC prenatal interview, information was obtained on the mother's parity at the time of the DNBC index pregnancy, prenatal maternal smoking, maternal alcohol consumption during pregnancy, and maternal BMI. From the 5-year follow-up in the LDPS, information was recorded on parental educational level, marital status, maternal anthropometrics (height, weight, and head circumference), family/home index (dichotomised as normal or suboptimal if two or more of the following conditions was present: living with only one biological parent, changes in primary care givers, day care more than 8 hours per day before the age of 3 years, separation from the parents for 14 days or more, irregular breakfast, maternal depression, parental alcohol intake above the recommendations from the Danish National Board of Health at the time of follow-up), and number of full –or half siblings. Maternal age was obtained directly from the unique Danish personal identification number, as was gender of the child and age of the child at testing. Birth weight and gestational age were obtained from the Danish Medical Birth Register. Maternal intelligence was assessed during the follow-up session. Standardised scores on two verbal subtests of

the Wechsler Adult Intelligence Scale (information and vocabulary)[129] and on the non-verbal intelligence provided by Raven's Standard Progressive Matrices[130] were weighted equally and standardised to a combined IQ with a mean of 100 and SD of 15.

### **Statistical analysis and strategy**

The main exposure of interest in the LDPS was maternal alcohol consumption during pregnancy. The participants were sampled from the DNBC on the basis on their self-reported average alcohol intake and pattern of binge drinking during or prior to pregnancy. Sampling fractions were calculated in order to weight the analyses with sampling weights defined as the inverse probability of being sampled. Sampling fractions were calculated by dividing the number sampled by the actual number identified in the DNBC in the given category. For the participants sampled based on their alcohol intake prior to pregnancy, the probability of being sampled took into account the probability that the individual was not sampled on the basis on the alcohol intake during pregnancy. The sampling fractions were included in the multiple regression analyses.

In study III, the intelligence, attention and executive functions in children born to subfertile parent or parents conceiving after fertility treatment were compared with that of spontaneously conceived children. The analyses were conducted using multiple linear regression analyses with robust variance estimates to allow for any departures from normality in the test scores. We included a set of a priori determined covariates in the regression analyses. In the main analyses this set was comprised of maternal age, maternal intelligence score, parental educational level, maternal BMI, maternal smoking in pregnancy, maternal alcohol consumption in pregnancy, parity, child gender, child age at test, and examiner. In order to evaluate the importance of adjusting for maternal intelligence and educational level we subsequently conducted analyses where these variables were excluded from the regression. Further, based on the a priori determined analysis plan analyses of all outcomes were subsequently performed in a model including the potentially mediating factors birth weight and gestational age. For all continuous covariates we assessed and found no evidence of a non-linear association with the outcomes. Complete information on all covariates was provided by 1731 out of 1782 participants (97%), whereas 51 (3%) had missing values for a maximum of one covariate. A total of 1771 (99%) completed all three intelligence test and 1511 (85%) had full information on all scores in the attention test. For the questionnaire (BRIEF), complete information was provided by 1778 (99.8%) of the parents, but only 1544 (86.6%) of the teachers. There were no significant differences in the proportion of missing information between the groups for either covariates or outcomes. The results are based on complete case analyses.

In study IV, the long-term growth was compared between children born to subfertile parents or parents conceiving after fertility treatment with spontaneously conceived children. Adjusted mean differences for all outcome measures at 5 years between the exposure groups and the reference group were estimated using multiple linear regression with robust variance estimation. In the main analyses, we included a set of a priori determined covariates including maternal age, parity, smoking in pregnancy, alcohol consumption in pregnancy, postnatal parental smoking, home-index, health-index, child gender, and age at testing. When the outcome was body weight or BMI at age 5 years the model additionally included maternal pre-pregnancy

BMI, whereas maternal height and head circumference were included when the outcomes were child height and head circumference, respectively. To evaluate the importance of the potential mediating factors, birth weight and gestational age, we conducted secondary analyses including these variables in the regression analyses. Since couples conceiving after fertility treatment or long waiting time to pregnancy might also differ from the reference group with respect to socioeconomic variables we conducted additional analyses further adjusting for parental educational level, maternal IQ, and marital status as well as for the child's participation in organized sport at age 5. Complete information for all covariates included in the main analyses was available for 1722 children (97.1%), while 44 children (2.5%) had missing values for one covariate, and 7 children (0.4%) had missing values for two covariates. Information on all covariates included in secondary models was complete for 1577 children (89%), while 1754 children (99%) had missing information for a maximum of two covariates. A total of 1761 children (99%) had complete information on all outcomes, whereas 11 children (0.6%) had a missing value for one of the outcomes, and one child (0.06%) had missing values on two of the outcomes. The results are based on complete case analyses

## MAIN RESULTS

### A SYSTEMATIC REVIEW (STUDY I)

The comprehensive searches yielded a total of 750 articles, of which 617 investigated irrelevant outcomes or did not meet the inclusion criteria. The remaining 133 publications were obtained and critically appraised in full text, and of these 79 were included in the review (Appendix I). For infants, studies on psychomotor development showed no deficits, but few investigated cognitive or behavioural development. Studies on toddlers (1-2 years) and preschoolers (3-5 years) generally report of normal cognitive, behavioural, socio-emotional and psychomotor development. For children in mid-childhood (5<age<12 years), development seems comparable in children born after assisted reproduction and controls, although fewer studies have been conducted with follow-up until this age. Very few studies have assessed neurodevelopmental outcomes among teens (≥12years), and the results are inconclusive. Studies investigating the risk of diagnoses of mental disorders are generally large, with long follow-up, but the results are inconsistent.

### RISK OF MENTAL DISORDERS (STUDY II)

A total of 588,967 live-born children were included in the study (Appendix II). Of these 555,828 were born after spontaneous conception and 33,139 were conceived after any type of fertility treatment (14,991 born after IVF/ICSI and 18,148 after OI/IIUI). The total number of children diagnosed with one or more of the included mental disorders was 23,278 (4%). The absolute risk (AR) was 3.9% among children born after spontaneous conception, 3.5% in children conceived after IVF/ICSI, and 4.1% in children conceived after OI/IIUI. The proportion of children diagnosed with any mental disorder was significantly higher among boys (5.8%) than among girls (2.1%)( $P<0.001$ ). The mean age at the time of any diagnosis was 9.3 years (SD 3.4 years, range 8 days to 17 years).

### Hazards associated with conception methods

Compared with children born after spontaneous conception, children born after IVF or ICSI had a higher hazard ratio of tic disorders (Table 2). This difference remained significant after

adjustment for potential confounding variables (HR 1.41 (1.05-1.87), absolute risk (AR) 0.3%) and after restricting analyses to infant survivors (data not shown). When analyses were stratified for multiplicity or gender of the child, the hazard of tic disorders was not significantly increased. There were no other significant crude or adjusted hazard ratios of mental disorder in children or adolescents born after IVF/ICSI and spontaneously conceived controls. Children conceived after OI/IIUI had significantly increased hazards of any mental disorder in both crude and adjusted analyses (HR 1.20 (1.11-1.31), AR 4.1%)(Table 2). The increased hazards were significant for both singletons, multiples, boys, girls and infant survivors (data not shown). When considering categories of mental disorders, OI/IIUI were not associated with the hazard of mental retardation or unspecified mental disorders, but systematically related to disorders of psychological development (HR 1.17 (1.05-1.31), AR 2.2%)) as well as behavioural and emotional disorders (HR 1.22 (1.11-1.35), AR 2.8%). Within these categories the increased hazards was primarily confined to ASD, hyperkinetic disorder, tic disorders and conduct, emotional or social disorders. After stratification, we found that the increased hazards of mental disorders within these categories were more consistent in boys than in girls, where it fell short of significance for hyperkinetic disorder, tic disorders and conduct, emotional or social disorders (data not shown). Stratification for child gender did not reveal any significant differences in estimates between boys and girls, and thus no effect modification was present.

**Table 2.** Mental disorders<sup>1</sup> in 0-17 years old children born after IVF/ICSI (n=14,991) or OI/IIUI (n=18,148). Reference: Spontaneously conceived children (SC, n=558,828)

	SC N (Absolute risk, %)	Fertility treatment	
		Adjusted <sup>2</sup> hazard ratio (95% CI)	
<b>Any Mental Disorder</b>	<b>22,009 (3.9)</b>	<b>1.04 (0.94-1.15)</b>	<b>1.20 (1.11-1.31)</b>
<b>Mental Retardation</b>	<b>3,217 (0.6)</b>	<b>0.84 (0.63-1.12)</b>	<b>1.02 (0.81-1.28)</b>
Mild	1,910 (0.3)	0.76 (0.51-1.13)	0.94 (0.69-1.27)
Moderate	610 (0.1)	1.29 (0.80-2.10)	1.11 (0.68-1.83)
Severe to profound	145 (0.03)	0.52 (0.14-1.94)	0.46 (0.12-1.85)
Other or Unspecified	552 (0.1)	0.73 (0.31-1.70)	1.38 (0.84-2.25)
<b>Disorders of Psychological Development</b>	<b>11,099 (2.0)</b>	<b>1.02 (0.89-1.17)</b>	<b>1.17 (1.05-1.31)</b>
Speech and Language	1,568 (0.3)	0.78 (0.49-1.24)	1.14 (0.84-1.55)
Scholastic skills	709 (0.1)	0.95 (0.54-1.68)	0.94 (0.58-1.51)
Motor function	587 (0.1)	1.01 (0.57-1.81)	0.52 (0.27-1.02)
Mixed	1,989 (0.4)	1.08 (0.77-1.51)	1.25 (0.97-1.62)
Autism Spectrum Disorders	7,060 (1.3)	1.02 (0.87-1.20)	1.20 (1.05-1.37)
Other or Unspecified	940 (0.2)	1.04 (0.68-1.65)	1.11 (0.77-1.60)
<b>Behavioural and Emotional Disorders</b>	<b>15,563 (2.8)</b>	<b>1.08 (0.95-1.22)</b>	<b>1.22 (1.11-1.35)</b>
Hyperkinetic disorder (ADHD)	8,252 (1.7)	1.01 (0.86-1.19)	1.23 (1.08-1.40)
Conduct, Emotional or Social Disorders	4,836 (0.9)	1.09 (0.87-1.36)	1.21 (1.02-1.45)
Tic Disorders	1,424 (0.3)	1.40 (1.01-1.95)	1.51 (1.16-1.96)
Other	3,424 (0.6)	1.00 (0.77-1.29)	1.03 (0.83-1.28)
<b>Unspecified Mental Disorder</b>	<b>647 (0.1)</b>	<b>0.93 (0.48-1.77)</b>	<b>1.16 (0.72-1.86)</b>

<sup>1</sup>As defined by International Classification of Diseases, 10<sup>th</sup> revision

<sup>2</sup>Adjusted for maternal age, parity, educational level, smoking in pregnancy, maternal psychiatric history, birth year, child gender and multiplicity.

### Type of ART and cause of infertility

Beside conception after OI/IIUI there were no systematic association with type of treatment, nor when looking at children conceived after IVF and ICSI as separate groups. Similarly, there were no associations with the reported aetiology of infertility (only available for IVF/ICSI group).

### Type of specific hormones and type of gamete or embryo

We found no association between treatment with Clomiphene Citrate, hCG or GnRH medications and the hazard of any mental disorders, but an increased hazard of any mental disorder after treatment with FSH, although this was not significant within any category of mental disorders. Information on type of embryo

(fresh, frozen or donor egg, donor sperm) used for the conception was available for IVF/ICSI treatments. We found an increased hazard of any mental disorder after IVF-conception with donor sperm and increased hazard of behavioural and emotional disorders after conception with donor oocyte, but found no significantly increased hazard associated with fresh or cryopreserved embryos.

#### INTELLIGENCE, ATTENTION AND EXECUTIVE FUNCTION (STUDY III)

Of the 1782 participants, 1577 children were born after spontaneous conception with no problems conceiving (TTP<12 months)(fertile parents group). A total of 134 children were born after spontaneous conception but to parents who had experienced difficulties obtaining a planned pregnancy (TTP>12 months) (subfertile group). The number of children born to mothers who were treated with fertility treatment was 71 (fertility treated parents group). Significant group differences were found for maternal age, parity and birth weight, but otherwise the groups were homogeneous with respect to health, lifestyle and socioeconomic characteristics.

The mean score on the three IQ-scales of children born to fertile parents were 105.6 (SD 12.9), 104.8 (SD 10.8), and 105.1 (16.2) for the full-scale, verbal and performance IQs respectively. Children born to parents conceiving after fertility treatment scored lower on all three IQ scales, but the differences did not reach statistical significance (Table 3). There were no significant differences in either overall, selective or sustained attention scores on the Teach-5 except for a slightly increased selective attention score in children conceived by subfertile parents. There were no significant differences in either parent -or teacher-rated executive function between children born to subfertile parents or parents conceiving after fertility treatment and the children born to fertile parents (Table 3).

**Table 3**  
Adjusted<sup>a</sup> Mean Differences in Intelligence, Attention and Executive Functions Between 5-Year-Old Singletons Born by Subfertile Parents or Parents Conceiving After Fertility Treatment and Children Born by Fertile Parents (Reference Group). Denmark, 2003-2008 (N=1782).

	Fertile parents group <sup>b</sup> (n=1577)		Subfertile parents group <sup>c</sup> (n=134)		Fertility treated parents group <sup>d</sup> (n=71)
	Mean (SD)		Mean difference	(95% CI)	Mean difference (95% CI)
<b>Intelligence (WPPSI-R)</b>					
Full scale IQ score	105.6 (12.9)		0.6	-2.2, 3.4)	-2.8 (-7.8, 2.2)
Verbal IQ score	104.8 (10.8)		0.2	-2.1, 2.5)	-1.9 (-5.8, 2.5)
Performance IQ score	105.1 (16.2)		0.9	-3.4, 5.3)	-3.1 (-9.1, 2.9)
<b>Attention (Teach-5)</b>					
Overall attention score	0.0 (1.0)		0.1	-0.2, 0.4)	-0.1 (-0.6, 0.3)
Sustained attention score	0.0 (1.0)		-0.1	-0.4, 0.2)	-0.2 (-0.5, 0.1)
Selective attention score	0.0 (1.0)		0.3	0.1, 0.5)	0.0 (-0.4, 0.5)
<b>Executive functions (BRIEF)</b>					
<i>Parent version</i>					
General Executive Composite	50.0 (10.0)		1.0	-1.8, 3.7)	-0.1 (-3.0, 2.9)
Behavioural Regulation Index	50.0 (9.9)		0.2	-2.5, 2.9)	-0.3 (-3.7, 3.1)
Metacognition index	50.0 (9.9)		1.5	-1.3, 4.3)	-0.5 (-3.4, 2.4)
<i>Teacher version</i>					
General Executive Composite	50.0 (9.9)		1.5	-1.5, 4.5)	1.8 (-0.9, 4.6)
Behavioural Regulation Index	50.0 (9.9)		1.5	-1.4, 4.3)	1.6 (-1.5, 4.7)
Metacognition index	50.0 (9.9)		1.4	-1.6, 4.4)	1.6 (-1.1, 4.4)

<sup>a</sup>Adjusted for maternal age, intelligence, parental educational level, parity, smoking in pregnancy, average alcohol consumption in pregnancy, maternal body mass index, child gender, age at testing, and testing psychologist.

<sup>b</sup>Spontaneous conception with a waiting time to pregnancy of less than 12 months. Reference group.

<sup>c</sup>Spontaneous conception with a waiting time to pregnancy of more than 12 months.

<sup>d</sup>Comprised of *in vitro* fertilization, intracytoplasmic sperm injection, and ovarian stimulation or ovulation induction with or without intrauterine insemination.

When including the potential intermediate factors, birth weight and gestational age, the conclusions were essentially unchanged and the differences remained insignificant (data not shown). To evaluate the importance of including maternal intelligence score and parental educational level in the assessment of an association between fertility treatment and child neurodevelopment, analy-

ses were conducted without these two variables. These analyses showed systematically lower performance in the children in the fertility treated parents and subfertile parents groups compared to the fertile group with respect to intelligence and executive function but not attention. The difference in intelligence scores reached a clinically relevant level with the children in the fertility treated parents group scoring 5.1 points (95% CI (-10.6; 0.4)) lower on full IQ and 5.6 IQ points (12.0; 0.7) lower on performance IQ compared to the spontaneously conceived children. Nonetheless, the differences remained just short of statistical significance.

#### GROWTH (STUDY IV)

In total, 1773 singleton children were included in the study. Of these, 1572 children were born after spontaneous conception, 132 children were born to subfertile parents, and 69 children were born to parents conceiving after fertility treatment. The families were generally homogeneous with respect to health, lifestyle and socioeconomic characteristics, although significant group differences were found for maternal age and parity, the proportions of women who had at least one episode of alcohol binge drinking in early pregnancy, and the proportions of women giving birth by caesarean section. There were no significant differences in child vision, hearing or somatic health status between the three groups.

#### Fetal growth

There were significant differences in birth weight ( $p<0.01$ ), gestational age ( $p=0.04$ ), and fetal length at birth ( $p<0.01$ ) between the three groups. Compared to spontaneously conceived children born to fertile parents, the children born after fertility treatment weighted 243 grams less at birth (95% CI (-368; -118)) and were 0.8 cm shorter (-1.4; -0.3). After exclusion of children born pre-term (gestational age less than 37 weeks,  $n=53$ ), the results were generally comparable. The difference in birth weight and length for term infants were -239 grams (-360; -118) and -0.8 cm (-1.3; -0.2), respectively. There were no significant differences in the gestational age in the pair wise comparison between children born after fertility treatment and the reference group. After adjustment for maternal age, parity, smoking in pregnancy, average alcohol consumption in pregnancy, parental educational level and maternal BMI, the mean difference in birth weight diminished to -194 grams (-318; -70) but fell just short of statistical significance in the model adjusting for sampling weights (mean difference: -162 grams (-359; 35). However, the length at birth remained significantly shorter for children born by parents conceiving after fertility treatment compared with spontaneously conceived children.

For children born to subfertile parents, there were no significant differences in birth weight, length at birth or gestational age compared to spontaneously conceived children.

#### Childhood growth

At the age of 5 years, the mean body weight of children born after spontaneous conception by fertile parents was 20.4 Kg (SD 2.6), whereas the children born by subfertile parents and parents conceiving after fertility treatment had a mean body weight of 20.2 (2.5) and 19.9 kg (3.0), respectively (Table 4). No systematic differences were observed for body weight, height, BMI or head circumference at age 5 in either crude or adjusted analyses. When the statistical adjustment included the potential intermediate variables birth weight and gestational age, the results were

essentially the same (data not shown). Further extensions of the statistical model with additional adjustment for the socioeconomic factors maternal marital status, maternal IQ and parental education, child physical activity or duration of breastfeeding did not change the conclusions either (data not shown).

**Table 4**  
Adjusted mean differences in long-term growth between singletons born by subfertile parents or parents conceiving after fertility treatment and children born by fertile parents (reference group). N=1773.

	Fertile <sup>1</sup> n=1572	Subfertile <sup>2</sup> n=132	Fertility treatment <sup>3</sup> n=69
	Mean $\pm$ SD	Mean difference (95% CI)	Mean difference (95% CI)
<b>Childhood growth</b>			
Weight at 5 years (Kg) <sup>4</sup>	20.4 $\pm$ 2.6	0.1 (-0.6; 0.8)	-0.1 (-1.2; 0.9)
Height at 5 years (cm) <sup>5</sup>	113.7 $\pm$ 4.4	0.4 (-0.6; 1.5)	0.1 (-1.4; 1.5)
BMI at 5 years (Kg / m <sup>2</sup> ) <sup>6</sup>	15.8 $\pm$ 1.3	0.0 (-0.3; 0.4)	-0.1 (-0.6; 0.5)
Head circumference at 5 years (cm) <sup>7</sup>	51.8 $\pm$ 1.5	0.0 (-0.2; 0.3)	-0.2 (-0.7; 0.3)

<sup>1</sup>Spontaneous conception with a waiting time to pregnancy of less than 12 months. Reference group.

<sup>2</sup>Spontaneous conception with a waiting time to pregnancy of more than 12 months.

<sup>3</sup>Treated with in vitro fertilization, intracytoplasmic sperm injection, or ovarian stimulation or ovulation induction with or without intramembrane insemination.

<sup>4</sup>Adjusted for maternal age, parity, smoking in pregnancy, alcohol consumption in pregnancy, postnatal parental smoking, home-index, health-index, child gender, age at test, and maternal pre-pregnancy BMI.

<sup>5</sup>Adjusted for maternal age, parity, smoking in pregnancy, alcohol consumption in pregnancy, postnatal parental smoking, home-index, health-index, child gender, age at test, and maternal height.

<sup>6</sup>Adjusted for maternal age, parity, smoking in pregnancy, alcohol consumption in pregnancy, postnatal parental smoking, home-index, health-index, child gender, age at test, and maternal head circumference.

## DISCUSSION

### MAIN FINDINGS IN RELATION TO PREVIOUS STUDIES

Essentially, we did not find any evidence of impaired neurodevelopment or growth in children born to parents conceiving after IVF/ICSI. We reported an increased risk of mental disorders in children born to parents conceiving after ovulation induction or ovarian stimulation. Based on secondary analyses we excluded a systematic causal association between the hormonal medications and the risk of mental disorders, except for an increased risk of the composite of any mental disorders in children born to mothers conceiving after treatment with FSH. Thus, the result are generally reassuring with regard to the IVF procedure, whereas the results for children conceived after hormonal stimulation without IVF call for a more thorough discussion of a potential causal or confounded association.

### Risk of mental disorders

In line with our results on the risk of mental disorders (study II), other Danish register-based cohort studies showed no association between conception after IVF/ICSI and development of mental disorders.[74, 79] Further, a long-term Swedish register-based follow-up showed no excess risk of autism or mental retardation in IVF singletons compared to spontaneously conceived controls.[131] The study subsequently investigated the risk associated with various types of ICSI treatments. For singletons, ICSI was not associated with mental retardation or autism except for a small increased risk of mental retardation in ICSI singletons conceived with ejaculated sperm used in fresh and frozen cycles. When using surgically extracted sperm, ICSI posed no apparent risk in singletons. When including multiples the authors found that ICSI treatment where the sperm had been surgically extracted and used in a fresh embryo transfer was associated with an increased risk of autism and mental retardation compared to children conceived after IVF or spontaneous conception. The association was found for both preterm and term infants, which indicates no mediation by gestational age. These results may suggest a biological association between ICSI and mental disorders. However, a parental aetiology may also explain the association, e.g. if an unmeasured confounder is associated with low sperm count and the risk of mental disorders. Since ICSI may also be used in the case of unsuccessful IVF treatments, the increased

risk may also be due to underlying parental subfertility. But actually, the results remained unchanged after accounting for years of involuntary childlessness. TTP may however, not be the optimal indicator of subfertility in the case of severe male factor aetiology, since these couples may seek treatment earlier. Further, information on time to pregnancy was collected at the first antenatal visit, and hence differential misclassification may have occurred. Couples conceiving spontaneously may underestimate the waiting time or simply recall less precisely compared to couples undergoing treatment. Finally, the analyses were unadjusted for parental education or socioeconomic variables, which may have confounded the association.

In the same Swedish study, secondary analyses dismissed an association between mental disorders and hormonal treatment without subsequent IVF. This is in contrast to the results presented in study II of this thesis. In the Swedish study, they did not show any risk associated with hormonal treatment alone. However, the collection of this information seems based on self-reported information and may be considered less valid compared to the register-based information based on redeemed prescriptions included in our study. Further, the authors did not state which hormones this statement included.

In contrast to our results, other large register-based studies from Sweden showed increased risks of attention deficit/hyperactivity disorder (ADHD)[70] and behavioural problems[71] in children conceived after IVF, although statistical significance was lost when adjusting for length of involuntary childlessness and restriction to term-infants respectively. Nevertheless, the studies highlight important aspects of a potential association between assisted reproduction and mental disorders - especially ADHD. Couples conceiving after fertility treatment are considerably different with regard to socioeconomic determinants, some of which are related to lower risk of mental disorders in the offspring. Failure to account for such associations may thus hide a true association. In a large Swedish study the risk of ADHD in children conceived after IVF compared to spontaneously conceived children was only present after accounting for cohabitation status.[70] Almost all women conceiving after IVF are cohabitating. In contrast, parents with traits or even diagnoses of ADHD may be more likely to become pregnant outside a stable relationship, which would affect the risk of similar disorders in the children for genetic reasons.

Very few studies report long-term neurodevelopmental follow-up of children born after OI. In a previous shorter follow-up investigating the risk of autism in our cohort, a significantly increased risk was found in girls born after OI.[77] In another Danish study[132] the risk of developmental difficulties fell short of significance for all treatments, although OI carried the highest risk for behavioural problems.

Contradictory to our results, a population-based sample from Finland showed increased risk of psychological, developmental and emotional disorders after IVF/ICSI[133] but no association between these disorders and conception after OI, even though OI children showed poorer perinatal health and more episodes of long hospitalization than the control children.[134] These studies included ICD-10 diagnoses from a hospital discharge register and child disability allowance before the age of 2 years, but did not include outpatient contacts. Since many psychological, developmental and emotional disorders are diagnosed later in childhood and often managed in outpatient clinics, this may explain the

different findings. Further, results from the UK Millennium Cohort Study showed no effect of either IVF/ICSI or OI on cognitive development of children up to 5 years, but the number of exposed children was small.[47]

### **Intelligence**

The results of the present study confirm the findings of most previous studies of the association between fertility treatment and offspring intellectual development. One study showed that 6-year-old Dutch children born after ICSI scored on average 7.1 IQ points lower than children born after spontaneous conception.[48] In contrast, a Belgian study showed that 8-year-old children born after ICSI achieved higher IQ scores compared to spontaneously conceived children.[111]\_ENREF\_134\_ENREF\_134 However, in line with our results the most consistent finding is that child intelligence is not associated with fertility treatment.[42, 50, 51, 54, 135-137] Further, several other studies have investigated the association between type of conception and performance on broader cognitive developmental indices in children at preschool age or older. Similar to the studies comparing scores on intelligence tests, these studies show no differences in intellectual development.[49, 52, 53, 68] Only very few studies have investigated the intellectual development in children born after fertility treatment at later ages. The findings are similarly reassuring showing no association between conception mode and educational achievement[58] or school functioning.[57] In fact, a study on 8-17 year-old American children showed that children born after IVF scored higher on tests of academic achievement compared to the national mean and higher than a group of spontaneously conceived controls for the 3-9 graders.[56] However, the comparison did not account for differences in parental educational level or other key demographical parental covariates that are likely to significantly affect child school performance.

### **Attention**

Investigations on neurodevelopment in the domain of attention among children conceived after fertility treatment are very limited. No association was found between mode of conception and sustained and selective attention in objective tests measuring speed and accuracy of attention as well as the ability to distinguish between objects and subsequently make the correct reaction.[58] As mentioned above, a large register-based study from Sweden showed slightly increased risk of ADHD in children born after IVF[70] compared with spontaneously conceived children. The statistical significance was lost when adjusting for length of involuntary childlessness.

### **Executive function**

No previous studies have evaluated executive functions in children born after fertility treatment compared to spontaneously conceived children. However, behavioural and socio-emotional development seems comparable in children born after fertility treatment or spontaneous conception, although only few long-term studies have been conducted.[49, 63, 64]

### **Growth**

Despite differences in birth weight, several studies have reported comparable weight and height later in childhood or adolescence for singletons conceived after fertility treatment compared with spontaneously conceived children [85-88]. In addition, a long-term follow-up of Dutch IVF children and controls showed that the anthropometric differences observed at birth and at 3 months

of age were no longer present after 6 months [89]. These results indicate that the IVF children may have increased postnatal growth velocity. Such catch-up growth may be compensatory in order to account for intrauterine growth restraint [89]. It has been suggested that both impaired fetal growth and a subsequent early postnatal catch-up may lead to an increased risk of cardiovascular diseases and type-2-diabetes in later life.[81-84] For IVF children, a rapid weight gain during early childhood has been shown to relate to higher blood pressure levels independently of birth weight, gestational age and body size at follow-up in 8-18 year-old children.[89]

In contrast, other studies have concluded that differences in birth weight persist into childhood, which may indicate general differences in the metabolism. The results are, however, inconsistent with regard to the trend of anthropometric progress. A population-based cohort study from Finland showed that the weight but not height was lower in IVF singletons compared to controls at 1, 2, and 3 years of age.[90] Additionally, in a cohort study from New Zealand, pre-puberty singletons born by mothers conceiving after hormonal ovarian stimulation had a shorter stature compared to children born by subfertile or fertile parents. [91] Still, the opposite results have been shown in other studies. Green and colleagues found that 6-year-old singletons born at term conceived after fresh IVF cycles were taller compared to children born after thawed IVF cycles and spontaneously conceived controls.[138] However, all children were within the normal range. These results were confirmed in a similar cohort from the same fertility provider, which also reported higher IGF-I and IGF-II levels in the IVF children. Yet, others studies have shown no significant differences in anthropometric measures at birth and at follow-up in children at ages from 3 months to 12 years.[42, 62, 139-142]

### **Causal association: Parental or procedural aetiology?**

In general, morbidity (if any) in a child conceived after fertility treatment may be caused by one of two basic aetiologies: i) the treatment or ii) underlying genetic, socioeconomic, or health factors in the couples or women seeking fertility treatment. As outlined in Background of this thesis, mechanisms related to the treatment may involve altered epigenetic regulation in the fetus due to hormonal stimulation or the microenvironment in the culture medium.[98, 100] Since placenta-associated obstetric disorders and birth weight seems to be related to fertility treatments, the general development of the fetus, and the function of the placenta may also be affected by the treatments. Further, the ex vivo handling of gametes may play a part. The natural selection of the gametes is not yet fully understood and hence, neither is the consequence of circumvention such as in ICSI, where a single spermatozoon is introduced into the oocyte by micro insemination. Transfer of more than one embryo is associated with an independent iatrogenic risk whatever caused by a pregnancy with multiples or vanishing twin syndrome.[26, 143]

In contrast, the procedures may be complete safe, and any adverse effect related to underlying parental factors. Indeed, couples seeking fertility treatment are more likely to have chromosomal abnormalities, which they may pass on to the offspring, namely in the case of men with low sperm count.[144, 145] Diseases and health-related factors related to infertility may pose an individual risk for the offspring – either through inheritance or influences in utero. Thus, maternal depression and anxiety, epilepsy, asthma, anaemia and metabolic disorders such as diabetes may have an impact on growth and fetal development.[146]



Further, couples or women seeking fertility treatment may differ from couples conceiving spontaneously with respect to age, socioeconomic position, and parenting behaviour with treated couples being older, having higher socioeconomic position, and investing more in their parenting.[49, 112] These factors may reduce the risk of neurodevelopmental deficits. Thus, parental aetiology may be based on genetic factors, hormonal and metabolic factors, socioeconomic, or health related factors.

### *Parental subfertility*

Recently, studies have shown that the underlying parental subfertility itself seems to affect the risk of adverse perinatal outcomes in singletons such as low birth weight, preterm birth,[26] and malformations.[147] Yet, studies attempting to investigate the impact of parental subfertility on child neurodevelopment rather than the procedures of fertility treatment are still few.

Depending on the research question, the choice of exposure and reference groups is important. Especially if the aim is to assess the risk associated with fertility treatment independently of parental subfertility, the study design is essential. In study III and IV we included a separate exposure group of couples conceiving spontaneously but after a period of involuntary childlessness of 12 months. Not only does this minimize the risk of misclassification, it also raises the possibility of distinguishing causation by the treatment from that of the underlying subfertility.

As mentioned above, the increased risk of ADHD in Swedish children born after IVF of non-single women became insignificant only after adjustment for waiting time to pregnancy.[70] Similarly, longer waiting time to pregnancy has been shown to be significantly associated with delays in cognitive and language development[148] and with developmental coordination disorder.[149] In contrast, Zhu and colleagues showed that children born after a time to pregnancy of more than 12 months but no infertility treatment had a behavioural pattern similar to that of children born to fertile parents.[132] Similarly, the studies in this thesis did not show any association between subfertility and intelligence, attention, executive function, and anthropometrics at age 5. Thus although subfertility seems to pose a risk itself in other studies, it may not be the only contributor to potentially adverse outcomes in singletons born after fertility treatment. With regard to long-term growth, very few other studies [86, 89, 91] have explored the impact of parental subfertility and only one study [91] included a reference group of children born by parents with no problems conceiving spontaneously.

### *Hormones*

In study II, we assessed the risk of mental disorders associated with the individual medications used in the treatments in a cohort design. Although not yet investigated in a long-term perspective, similar research question may be answered by comparing children born to parents conceiving after standard IVF preceded by hormonal stimulation with children conceived after natural cycle IVF. ENREF\_148 A Dutch study using this design showed that blood pressure was lower in children conceived after natural cycle IVF compared with standard IVF using ovarian stimulation.[150] While such a study design may separate the effects of hormonal treatment and the IVF procedure individually, a risk of confounding by indication remains.

Further, several studies have compared pregnancy outcomes after transfer of fresh and frozen embryos. Since the medical stimulation often results in several oocytes, embryos may either

be transferred directly after the hormonal stimulation and IVF or be frozen (e.g. embryos in excess). Later on, the embryos may be thawed and transferred either if pregnancy was not achieved in the stimulated cycle or in order to obtain another pregnancy. Studies suggest that the treatment with frozen embryos leads to a significantly lower risk of perinatal complications such as low birth weight compared to children born after fresh embryo transfer.[26, 151] This indicates that the superphysiological hormone due to the hormonal stimulation in the “fresh embryo IVF” may have an impact on the developing fetus. However, many women receiving frozen embryos may be multiparous, which alone would reduce the risk of perinatal complications. Further, the better prognosis of frozen embryos may be due to selection where the weaker embryos are discharged and the remaining represent higher quality embryos associated with better growth and development. Although mainly investigated for short-term outcomes, a large register-based study from Sweden did not show any association with long-term risk of mental disorders.[131]

### *Culture and handling of embryos*

Several studies have compared children conceived after ICSI with children conceived after IVF. Although an increased risk of male genital malformations may exist, the groups are generally comparable with regard to perinatal and long-term outcomes.[140, 152, 153]

Other studies have compared risks associated with type or length of embryo culture. No long-term studies exist, but parallels may be drawn to short-term outcomes. Results indicate that type of culture media affects the birth weight of the offspring,[100] which indeed suggests that the treatment, in addition to parental subfertility, may contribute to adverse effects in the offspring. Whether longer period of embryo culture is associated with increased risk of preterm birth is still controversial. Longer embryo culture allows for selection of the best embryos, but an early meta-analysis showed very low grade of evidence that culture time affects the risk of preterm birth.[26] In contrast, a more recent meta-analysis including slightly more studies showed that transfer of embryos at the cleavage-stage (day 2-3 after egg collection) was associated with an increased risk of preterm birth compared to transfer at the blastocyst stage (day 5-6).[154] A long-term follow-up on the risk of mental disorders revealed no significant differences between blastocyst and cleavage-stage transfer.[131] Study design where siblings act as controls may present pivotal results to the discussion on the association between fertility treatment and child development.[108, 155] With this design, parental health, socioeconomic, and genetic factors are eliminated. Although studies using this design are still few and so far with inconsistent results, lower birth weight in children conceived after IVF compared to a spontaneously conceived sibling might indicate that the treatment plays a role in adverse neonatal outcome.[155]

### **STRENGTHS OF THE STUDIES IN THE THESIS**

Each study was based on an a priori determined analysis plan. Thus, exposure, outcome and confounders were all selected and pre-specified with regard to the types and categorizations of variables. Similarly, all analyses were conducted according to the analysis plan and only when requested by reviewers after submission for peer review, supplementary analyses were done exploratively.

In study I, the literature was systematically reviewed. In particular, several methodological limitations were identified in order

to overcome such limitations in our own subsequent studies included in this thesis. Overall, these limitations consisted of limited sample sizes, short length of follow-up, lack of sufficient confounder control, or lack of blind assessment.

Most studies investigating the neurodevelopment of children born after fertility treatment were small with less than 500 participants and with short term follow-up until the age of preschool or less (5 years).[156] These limitations are essential, since many neurodevelopmental deficits or mental disorders are diagnosed later in childhood.[80] In this thesis, the study II investigating the risk of mental disorders is one of the largest studies to date with long-term follow-up into adolescence.

Further, several previous studies were not blinded[157] for the exposure which may bias the results, most likely in the direction of overestimating the association between exposure to ART and neurodevelopmental deficits in the offspring. In the studies included in this thesis, the assessment of IQ, attention, teacher-rated executive function, and anthropometric measures were performed unaware of conception mode (study III+IV). However, blinding was not possible when assessing parent-rated executive function (study III) or in the study assessing the risk of mental disorders since it relied on register-based diagnoses (study II). Several of the included studies in the review (study I) were unmatched or unadjusted for potentially confounding variables.[157] Couples seeking fertility treatment may differ from couples conceiving spontaneously with respect to important factors likely to affect neurodevelopmental outcomes in the offspring such as parental age, parity and socioeconomic status.[51, 56, 158, 159] Failure to adjust for such variables is a major limitation. Although cognitive abilities in parents and offspring are strongly associated [160, 161] several studies with cognitive outcomes were unadjusted for parental education or socioeconomic status.[38, 41, 52, 53, 61, 135, 162, 163] In particular, no studies on child intelligence accounted for parental intelligence level.[157] All results reported in this thesis were based on analyses adjusted for a range of important covariates. In study III, we assessed the association between fertility treatment or subfertility and child intelligence level while accounting for maternal intelligence level. Such analyses have not been conducted in previous studies.

## LIMITATIONS

Study II investigating the risk of mental disorders was large and included numerous outcomes. Although all analyses conducted were based on an apriori specified analysis plan, the numerous subgroup analyses in this study might lead to a risk of finding isolated significant associations because of chance alone (Type I error). However, the significant associations in the main analyses were systematically distributed with regard to both the exposure and the outcomes. The few isolated significant associations in the subanalyses were, however, more likely to have been caused by chance. In contrast, study III and IV investigating the neurodevelopment and growth at age 5 might have been underpowered. However, based on power calculations the studies could have detected differences that may be considered clinically significant.

## Selection bias

In study I, the existing literature was systematically reviewed and the conclusions thus rely on the papers published. For all studies, there is a risk of publication bias where studies with positive associations tend to be more likely to be submitted and published than studies with negative findings, leading to an overall interpre-

tation that ART or OI may be more harmful than it is. However, this bias may work in both directions, since several studies have shown better performances in children born after fertility treatments.[39, 45, 53, 56, 57, 76, 135, 159, 164-168]

In study II, the risk of mental disorders was assessed using Danish national health registers. The population was generated using the medical birth register. The entry is based on birth notifications, completed by midwives at birth (all births in Denmark, home births included, are attended by midwives).[169] Generally, register-based studies carry no risk of selection bias due to non-responders if reporting to the register is fairly complete.[170] Since the data can be anonymously obtained with the appropriate permissions from Danish data authorities, the participation is unrelated to patient demography or will to participate. Further, loss to follow-up is similarly negligible. Follow-up ended at diagnosis, emigration, death, or end of study period. Thus, the only risk of loss to follow-up was disappearance, which may be considered very uncommon in Denmark.

In study III and IV, the neurodevelopment and growth at age 5 were assessed. The invited mother-child pairs were sampled from the DNBC. Although the participants in the DNBC are likely to be strongly selected, this causes only little if any bias in within DNBC analyses.[171] Further, there were no substantial differences between the participants and non-participants in the LDPS with regard to maternal age, parity, BMI, prenatal smoking or alcohol consumption, marital status, child gender, birth weight or gestational age at birth.[122]

The participation rate was 51% in the LDPS. The mothers' choice to participate in the 5-year-follow-up may have been associated with neurodevelopmental or growth deficits in their offspring as well as fertility treatment or a long waiting time to pregnancy. Although the nonparticipants were generally comparable to participants with respect to key demographic covariates, this may potentially have underestimated an association.

## Information bias and misclassification

In study II, information on exposure, outcome and covariates were obtained from Danish health registers. Exposure to IVF or ICSI was assessed from the IVF register, and although the coverage is believed to be close to 100% for the treatment reports it may be less for the pregnancy outcome during the first years of the register.[117] Thus, a small portion of the IVF/ICSI children could be misclassified as spontaneously conceived, which would bias the estimates towards the null. But when restricting our cohort to the 520,610 children born from 1996-2003, and thus removing the first years of the IVF-register's activity, there were still no associations between IVF/ICSI and mental disorders (data not shown). Similarly, exposure to OI/IUI was assessed from the Danish National Prescription Register which contains complete information on all dispatches,[118] but no information as to whether the woman actually took the prescribed medicine. Thus, a small number of spontaneously conceived children could be misclassified as OI/IUI children, which would lead to underestimated associations. But since the women are highly motivated for the treatment we believe the risk of bias is very small. Since exposure to both IVF/ICSI and OI/IUI are based on Danish health registers, we only included children born after fertility treatment in Danish clinics. However, Denmark has a long tradition for cost-free fertility treatment for infertile couples and fertility tourism out of Denmark is primarily practiced by a small number of cou-

ples seeking egg donation (low availability in Denmark), simultaneous sperm and egg donation, or surrogacy (illegal in Denmark). The Danish Psychiatric Central Research Register includes information on all persons diagnosed with a given disorder, but only cases with admissions or outpatient contacts are included. Previous studies have shown that children born after IVF or OI are more frequent users of hospital care compared to spontaneously conceived children.[71, 134] Whether this is driven by poorer health outcomes related to the fertility treatment or the underlying subfertility, or by general concerns about the health of these children is still debatable.[71, 134] However, it may lead to risk of detection bias and thus, overestimation of any association between fertility treatment and hospital diagnoses. Previous studies have not investigated differences in the use of the health care system between IVF/ICSI and OI/IUI children. Since we found increased hazard estimates after OI/IUI but not after IVF/ICSI, we do not consider such differences a plausible explanation of our findings.

In study III and IV, the information on exposure, outcome, and covariates were obtained from the DNBC and the LDPS. The information on fertility treatment and TTP was self-reported, although based on a structured prenatal interview in the DNBC. Information bias, in particular misclassification where women underestimate their TTP may have biased the results towards the null. Similarly, misclassification of fertility treatment may have caused an underestimation of any association. However, the information on self-reported fertility treatment has been validated in the DNBC and found to have a positive predictive value of 88% and a sensitivity of 83%.[172]

### Confounding

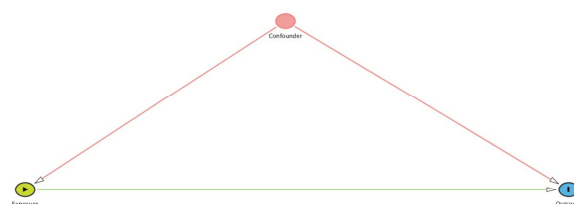
When a variable is associated with the exposure and are an extraneous risk factor for the outcome, but not lies on the causal pathway, it is defined as a confounder (Figure 5). Thus, a confounder is a factor that explains all or part of the difference between the measure of exposure and the outcome.[173] In general, control for confounding factors is a crucial issue in epidemiological research. It represents an important statistical tool, and when performed adequately, it may ensure that the groups only differ with regard to the exposure. While the outcome and exposure are typically determined by the potential association under investigation, the identification of confounders is not obvious.[174] Several strategies exist for this identification of potential confounding factors. A number of widely used approaches such as stepwise regression or change-of-estimate rely solely on statistical associations in the dataset at hand. However such strategies may lead to bias. Hence, the identification of confounders should mainly rely on subject-matter knowledge about the potential causal association in question.[174] Subsequently, some of the selected variables may be excluded from the analyses, for example based on too little variation, too many missing data, or too much measurement error.[175]

If a given variable is considered a potential confounder, the only reason for omitting the variable from the analysis is a trade-off between a reduction in variance vs. a reduction in bias. Since a statistically significant, but biased result may be misleading, the confounder-selection methods used in the literature will often focus on bias reduction rather than this trade-off. In other words, since the number of variables included in the statistical model affects the precision of the estimates, the number of variables must be determined according to the amount of data. For this

reason, the maximal complexity of the models was determined by the sample size for continuous or the number of events for categorical outcomes, respectively.[176] Eventually, the number of parameters to include in the model was allocated based on a prioritized list of variables.

In study II, the analyses were adjusted for a range of important covariates. Yet, residual confounding may persist. It is possible that women with ADHD or other neuropsychiatric illnesses are more likely to become unplanned pregnant outside a stable relationship which would affect the probability for the child to develop similar pathology for genetic reasons. As previous described, this was the case in a Swedish study where an association with ADHD among children born after IVF/ICSI did not become statistically significant until the analysis was restricted to cohabiting women[70]. Unfortunately we did not have access to data regarding cohabitation status at the time of conception. This potential source of bias would have attenuated the estimates, but we believe that this risk is very small in this study. Among other things, the analyses were adjusted for maternal history of psychiatric disorders as well as for maternal education and smoking in pregnancy.

The analyses were unadjusted for paternal factors (e.g. age and education). Further, we did not adjust for place of living or ethnicity of the family. There may be regional or cultural differences in the frequency of seeking medical advice for both fertility treatment and mental problems, which may have affected our results. However, the analyses were adjusted for maternal education, which is likely to be associated with these variables, and in addition the number of immigrants in Denmark is relatively low. Most importantly, if any of the above-mentioned potential sources of confounding should explain the results, we would expect the associations to be similar for both the IVF/ICSI and OI/IUI groups. Since we consistently found increased hazards for children born after OI/IUI but not after IVF/ICSI, we believe that these potential sources of residual confounding most likely did not cause systematic bias in this study. Still, we cannot eliminate that other sources of residual confounding might partly explain our results.



**Figure 5.**  
A confounder is statistically associated with the exposure, an extraneous risk factor for the outcome and do not lie on the causal pathway between exposure and outcome.

Information on maternal psychiatric history was based on information from the Danish Psychiatric Central Research Register and only disorders that required contact to the psychiatric health care system are registered here. Thus, we do not have information on less severe cases of mental disorders in the mothers. Since it is well known that mental disorders run in families[177], this could explain our findings if children conceived after OI/IUI were born by parents with a higher prevalence of mental disorder.

ders. This would be true if the conditions leading to infertility in the OI/IUI group were biologically associated with mental disorders or if couples with mental disorders had a higher risk of infertility. The recommended first line medical treatment for women with polycystic ovary syndrome is ovulation induction[178], and these women have increased risk of mental disorders[179]. In the secondary analyses, we investigated the association between the aetiology of infertility in couples treated with IVF/ICSI and the risk of mental disorders. As the only positive finding, ovulation factor infertility (e.g. anovulation) was associated with a 4-fold increased of the category of unspecified mental disorders. Further, women with mental disorders may take longer to conceive[180], which may lead to low-tech fertility treatment such as OI, even though these couples do not have any history or aetiology of infertility. In any case, the increased risk could be due to underlying parental factors and not the fertility treatment.

In study III, neurodevelopment performance including intelligence was assessed while accounting for a wide range of important covariates. In particular, this was the first study to assess maternal intelligence and include this factor in the analyses. To investigate the importance of this adjustment, additional analyses excluding maternal intelligence and parental education were conducted. In these analyses, intelligence scores were systematically lower in the fertility treated parents group and subfertility parent group compared to the spontaneously conceived children. Especially, for the group of children born after fertility treatment, the differences were of a magnitude that may affect every day life, although just short of statistical significance. Still, these results emphasise the importance of including key covariates in studies investigating the association between fertility treatment and child neurodevelopment. To evaluate the model fit, we assessed the coefficient of determination ( $R^2$ ) for both models. In fact, for the Full-scale IQ score the  $R^2$  was 0.21, indicating that 21% of the variation in IQ could be explained by the explanatory variables. This corresponds to the explained variance in offspring intelligence previously reported for the LDPS sample.[181] For the model not including maternal intelligence and parental educational level, the adjusted  $R^2$  was 0.05, indicating that only five percent of the variation was explained by the remaining explanatory variables. This suggests that maternal IQ and parental education explain most of the variance, and that the other factors included in this study explained relatively little additional variance.[181] Again, these results emphasize that one of the methodological strengths of the present study III is the ability to adjust for these important variables, which has not been done before in studies investigating long-term neurodevelopment in children born to parents conceiving after fertility treatment.

### Mediation

An intermediate variable is a variable that lies on the causal pathway between the exposure and the outcome. It is important to identify potential intermediates, since adjustment for these variables may introduce bias, typically towards the null. Indeed, this may be a problem when estimating the association between fertility treatment or parental subfertility and childhood health outcomes. There is high quality evidence that IVF singletons have higher risk of preterm birth and low birth weight. Furthermore, these perinatal factors are important predictors of childhood morbidity. As a consequence, birth weight may be considered an intermediate variable, and controlling for birth weight may produce biased estimates, whether performed by restriction, stratification, or regression adjustment. However, adjusting for birth

weight may be appropriate in order to evaluate a direct causal pathway if one such exists (Figure 6, panel A). Yet, the risk of introducing bias persists if birth weight is a collider for one or more unknown confounders (Figure 6, panel B).[182] Thus, the direct causal pathway may only be estimated in the absence of unknown confounders associated with birth weight and childhood growth, for example genetic factors.

Adjusting for birth weight and/or gestational age was done in a majority of studies in the existing literature investigating the association between fertility treatment and long-term growth in the children. This may partly explain the inconsistency in the results. Several studies restricted the participants to term infants,[42, 87, 88, 138, 183] a few studies matched the IVF children with controls on birth weight,[35, 184]\_ENREF\_183 and yet other studies have included birth weight as a covariate in their analyses of long-term growth.[89, 140, 141, 183] According to the above, adjusting for intermediate variables such as birth weight may introduce bias - typically towards the null.[185] Interestingly, of all studies that report a significant difference in anthropometric measures,[90, 91, 138, 183, 184] only one study adjusted for birth weight.[183] Correspondingly, all studies but one,[183] which conducted birth weight adjusted analyses in the assessment of an association between fertility treatment and childhood growth reported of no long-term association. Since adjusting for birth weight may be appropriate in order to evaluate a direct causal pathway, this may suggest that no direct causal pathway exist. In the present study we evaluated the importance of the potential intermediates by including them in additional analyses. This did, however, not substantially change the association, which supports the hypothesis of no direct causal pathway independent of birth weight between fertility treatment and childhood anthropometric measures.



Figure 6. Birth weight is an intermediate variable and adjustment may be appropriate to estimate a direct effect between fertility treatment and child neurodevelopment (Panel A). However, if birth weight is a collider for one or more unknown confounders there is a risk of introducing bias (Panel B)

### CONCLUSIONS

Overall, the results of this thesis provide reassurance with regard to the long-term mental development and growth of children conceived after fertility treatment. Children born after IVF or ICSI had a low risk of mental disorders, and compared to spontaneous conceived children the risk was generally not increased. In contrast, children born after ovulation induction with or without insemination had a low, but significantly increased risk of mental disorders. There was no risk sys-

tematically related to any specific type of hormonal medication, which may indicate that other factors than the treatment are responsible for this apparent adverse effect.

Further, there were no significant association between fertility treatment or parental subfertility and offspring intelligence, attention, or executive functions.

Finally, no systematic differences were observed for body weight, height, BMI or head circumference at age 5 in children conceived after fertility treatment, by subfertile parents compared to spontaneous conceived children.

## PERSPECTIVES

Fertility treatment represents an essential option for infertile couples. Regardless of treatment procedure, the desirable endpoint is the birth and upbringing of a healthy child. Thus, information on the fetal and childhood development after conception by fertility treatment is highly requested.

The long-term development seems overall comparable, albeit final conclusions are not yet guaranteed. However, for couples with a desired and unmet child wish, it is reassuring that conception by the means of medical assistance holds no obvious large risk of long-term adverse effects related to the mental health. For some techniques a small risk may exist and additionally, the subfertility per se may contribute to difference in fetal or childhood development. Nevertheless, the size of such risk is unlikely to cause couples to opt out of treatment since the risk estimates are small in comparison to the result of the treatment.

Increasingly invasive techniques may be related to more severe adverse outcomes. A moral obligation exist to continuously survey potential impacts on offspring development. Thus, investigations of short and long-term development after treatment with novel procedures are warranted.

As concluded in the present studies included in this thesis, any differences in the growth or mental health of the children conceived after fertility treatment may reflect unknown differences between couples conceiving easy and couples with difficulties in obtaining a spontaneous pregnancy. In example, unknown genetically differences may be present and a future research strategy could include investigations of such potential differences. With regard to fetal and long-term growth in children conceived after fertility treatment, a more thorough characterization of the timing and cause of impaired fetal growth is desired. This may be achieved by several consecutive measurements during the total length of gestation.

## SUMMARY

Fertility treatment has been associated with obstetrical and perinatal complications. It is, however, uncertain whether fertility treatment or parental subfertility is associated with long-term development of the children. We aimed to assess the growth and mental health of children and adolescents conceived after fertility treatment compared to spontaneously conceived controls.

First, we evaluated all previous studies comparing neurodevelopmental outcomes between children conceived after fertility treatment and spontaneous conceived controls. The systematic review clarified methodological limitations in the existing literature on the long-term development of children conceived after medical assisted reproduction. Although several studies had been published, large, well-controlled studies with long-term follow-up

and thorough statistical adjustments were still few.

Second, we evaluated the children's mental health by assessing the risk of mental disorders. We studied a population of 555,828 children conceived after spontaneous conception and 33,139 children conceived after fertility treatment with follow-up in 2012 when the children were 8-17 years old. The absolute risk and hazard ratio of overall and specific mental disorders were estimated while adjusting for potential confounding variables. Further, we estimated the association between subtypes of procedures, hormonal treatment, gamete types and cause of infertility on the one hand and the risk of mental disorders on the other. Children conceived after ovulation induction had a low, but significantly increased risks of autism spectrum disorders, hyperkinetic disorders, conduct, emotional, or social disorders, and tic disorders. Children conceived after IVF or ICSI showed no increased risk, except for a small risk of tic disorders. There was no risk systematically related to any specific type of hormone drug treatment. Thus, the increased risks may rely on residual confounding such as unknown parental factors associated with infertility in the ovulation induction group.

Third, we assessed the intelligence, attention and executive functions in 1782 5-year-old singletons. Compared with children conceived after spontaneous conception, there were no differences in test scores in children conceived by subfertile parents waiting more than 12 months before conceiving naturally or children born by parents conceiving after fertility treatment.

Finally, we evaluated the growth of children born after fertility treatment or to subfertile parents. Compared to children conceived spontaneously, children born after fertility treatment or by subfertile parents had a significantly lower birth weight -an important predictor of mental development. In contrast, no differences on height, weight, or head circumference were found at the age of 5 years.

In conclusion, we found no differences on long-term growth and neurodevelopment of children conceived after fertility treatment or by subfertile parents compared with spontaneously conceived children. Children born after ovulation induction had a low, but increased risk of mental disorders in childhood or adolescence, although this risk may rely on unknown parental factors associated with infertility.

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