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Development and Health after ICSI

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Chapter 1

General Introduction

1.

Ever since the introduction of intracytoplasmic sperm injection (ICSI), the most recent major development in artificial reproduction techniques (ART), concerns have been voiced because the ICSI-procedure involves more invasive technical manipulation than established ART-procedures. Therefore, follow-up studies have been carried out after birth and in the first years of life about the health and development of children who were born after ICSI. The present thesis describes a follow-up study of ICSI-singletons at 5 to 8 years of age.

Background

In 2002, 1.3-4.2% of all child births in Europe resulted from ART,[1] of which in vitro fertilisation (IVF) and ICSI were the main contributing procedures. IVF was successfully introduced in 1978[2] with the birth of the first 'test-tube' baby, Louise Brown. In the Netherlands, IVF is the first-choice therapy for tubal factor and idiopathic subfertility. ICSI was introduced in 1992[3] and has been particularly successful in the treatment of male factor infertility and when fertilisation does not succeed with IVF. We will first describe the technical procedures, to point out the differences between IVF and ICSI.

The IVF-procedure consists of the following phases. In the mother, multiple follicle development is achieved by the administration of gonadotrophins (FSH). To avoid a spontaneous LH surge and thus ovulation, the woman's endogenous gonadotrophin production is down-regulated with gonadotrophin-releasing-hormone (GnRH) agonists. The number and size of mature follicles in the ovaries is monitored ultrasonically and/or by measuring the oestradiol level. These parameters determine when the ovulation process can be ignited by human Chorionic Gonadotrophin (hCG). About 36 hours thereafter, oocytes can be retrieved by an ultrasound guided transvaginal ovarian punction and subsequently cultured. Selected spermatozoa are added to an oocyte in vitro and fertilisation takes place. This is confirmed by the presence of two pronuclei after 18 to 20 hours. The zygote remains in culture for another two days. Several cell cleavages take place and the zygote is now called an embryo. The morphologically superior embryos are selected to be transvaginally placed in the uterus. To support the endometrium, progesterone is administered and this treatment is continued until the seventh week if pregnancy occurs.[4]

The ICSI-procedure is similar to the IVF-procedure up to the stage of oocyte retrieval. With ICSI, the next step is to strip the oocyte of its cumulus cells and corona radiata, in order to check whether the first polar body has been extruded (oocyte is in metaphase II). Subsequently, one 'good looking', motile spermatozoon is selected for fertilisation by the embryologist and aspirated tail-first into a microinjection pipette. Finally, the spermatozoon is injected across the zona pellucida and oocyte membrane into the oocyte's cytoplasm (Figure 1). After fertilisation, the procedure is again similar to IVF.

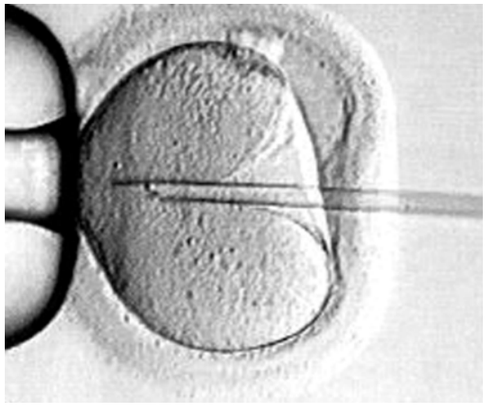

Figure 1. Injection of the spermatozoon into the oocyte

The potential drawbacks of ICSI follow naturally from the procedure. First, natural selection barriers are circumvented, which may result in fertilisation with sperm of minor quality (e.g. with genetic abnormalities). This argument is very relevant as ICSI is the main treatment for male factor infertility and therefore relies on spermatozoa that would not have achieved fertilisation and pregnancy in a natural way. Second, damage may be done to structures and processes in the oocyte. The microinjection pipette may damage the ooplasm and organelles, or disturb the meiotic spindle, which may lead to aneuploidy. Damage to the oocyte may also be caused by agents that are injected along with the spermatozoon (e.g. polyvinylpyrrolidone, a sperm slowing chemical). Third, in vitro culture may disturb the methylation process that is a part of epigenetic programming (imprinting).[5-8]

ICSI follow-up

Based on these potential drawbacks, concerns related to the health and development of ICSI-children have been voiced from the moment the procedure first came into use. The first follow-up studies focused mainly on early adverse events: chromosomal abnormalities and congenital malformations,[9-16] and suggested an increased prevalence of de-novo (mainly sex-chromosomal) abnormalities and inherited chromosomal aberrations.[11, 13-15] This increment persisted in later studies; the total prevalence of chromosomal aberrations in ICSI-offspring was found to be 1.5-3.5% versus <1% in the reference population.[8, 17-19]

No increase in congenital malformations was found in ICSI-children in these early studies. However, such reassuring results were later adjusted to a relative risk of 1.3-1.4 for singletons born following ART (ICSI as well as IVF) as compared to children born following natural conception.[20-25] Furthermore, genetic imprinting disorders (e.g. Angelman syndrome, Prader-Willi syndrome, and Beckwith-Wiedemann syndrome) have been suggested to be associated with ART,[26-35] but the results are not conclusive.[36-38]

In the second stage of ICSI follow-up, when the number of children born after ICSI had increased and the first cohort had reached two years of age, perinatal

outcome[11,39-44] and psychomotor development generated interest.[11,42,45-49] A tendency towards high rates of prematurity and low birth weight was noted after ICSI, which could not be explained solely by an increase in multiple births. IVF-singletons are known to suffer more adverse perinatal outcomes than naturally conceived singletons[50,51] and in more recent studies increased rates of prematurity and low birth weight after ICSI as compared to natural conception have been confirmed.[20,22,49,52] Several studies in which ICSI and IVF-singletons were mutually compared showed no differences[18,40,53]; others[52,54] found an increase in prematurity and low birth weight after IVF.

Psychomotor testing placed greater emphasis on cognitive development than neuromotor development at age two. When neuromotor development was investigated, the examinations were limited to gross and fine motor skills and did not involve a full neurological examination. In 1998, Bonduelle et al.[45] reported normal mental development in two-year-old ICSI-children, although information on parental educational background was limited. Simultaneously, Bowen et al.[47] showed that ICSI-children scored significantly lower than IVF and naturally conceived children on the mental scale of a developmental test, at age one. Although the study was criticised for methodological flaws[55,56] and subsequent results were reassuring,[42,46,49] the findings of Bowen et al. carried a serious warning.

At age five, cognitive and neuromotor development could be assessed more accurately and general health and growth have been used as additional outcome measures. The results up to age five have been reassuring: cognitive as well as neuromotor development,[57-62] general health,[20,57,63] and growth[20,57,63] of ICSI-singletons appeared mostly normal. Nevertheless, according to the findings of Bonduelle et al.,[20] ICSI-children required more hospital admissions, surgery and remedial therapy than naturally conceived children. Recent results of Belva et al.[57] disagree with these findings, showing no significant differences between children born after ICSI and natural conception on hospitalisation, surgery, or remedial therapy.

Aim

In this thesis, on a wide scope of outcome measures we evaluate the potential negative effects in singleton children of ICSI as opposed to IVF and natural conception, at a next step of development: 5-8 years of age. The following are assessed:

(1) Neuromotor development, expressed in minor neurological dysfunctions (MND);
(2) Cognitive development, expressed in IQ;
(3) Health: perinatal outcome, congenital malformations and dysmorphic features, general health, medical care utilisation, and growth;
(4) Psychosocial well-being: behaviour and quality of life of the child as well as parental stress.

By comparing ICSI and IVF-children, we strive to evaluate the difference that is due to the ICSI-procedure itself, given the background of an infertile couple, maternal hormonal stimulation and fertilisation in vitro. With the control group of naturally conceived children, we investigate the overall difference between ICSI and natural conception, as this represents the pivotal question of future ICSI-parents: '(How) will the health and development of my child differ if it is born after ICSI rather than after natural conception – given our parental characteristics up to the time of conception?'. Second, with a more biological approach, we attempt to assess the net effect of ICSI as compared to natural conception, by controlling for known intermediate factors such as prematurity.[50,51,64-66]

Methods

Design

The design of this study can be described as a controlled or matched follow-up. The entire cohort of ICSI-children born between June 1996 and December 1999 after treatment in the Leiden University Medical Center and who were alive in September 2003, were asked to participate (n=110). June 1996 represents the first ICSI-birth at this centre; the limit was set to December 1999 to achieve a reasonable group size. Two matching control groups were constituted, drawn from either an IVF-population or a population of naturally conceived births.

Selection

Inclusion criteria for ICSI and IVF-children were: live birth between June 1st 1996 and December 31st 1999 after treatment in the Leiden University Medical Center, singleton birth, and alive in September 2003. Exclusion criteria were: oocyte or sperm donation, cryopreservation of the embryo, and selective embryo reduction with medical indication. Naturally conceived control children were enrolled via regular pre-schools and primary schools (i.e. not providing special education) in the region of the university hospital. Inclusion criteria were: born between June 1st 1996 and December 31st 1999 or 5-8 years of age, singleton child, and born after natural conception.

Although multiple pregnancies are the most harmful complication of ICSI-treatment,[67,68] selection was restricted to singleton children. First, including multiples would introduce confounding as the prevalence of multiplicity is increased after ART and multiplicity is a risk factor for adverse health and developmental outcomes.[67] Second, if one compares ICSI-multiples to control-multiples, the negative effect of multiplicity might conceal the actual effect of the ICSI-procedure.[50,68,69]

We decided to use regular pre-schools and primary schools as a source for naturally conceived control children. Alternative options would have been relatives (e.g. cousins) and classmates or friends of those in the ICSI-group. As will be discussed in more detail in the next paragraph, we intended to match the group of naturally conceived children to the group of ICSI-children for various (demographic) characteristics. Choosing children from randomly selected pre-schools and primary schools at least results in matching for age. With relatives, matching might cover both socio-economic status and genetic factors; with classmates or friends socio-economic status and age would be captured. An additional advantage of these two alternatives would be the control for unmeasurable confounders, but both choices would also have limitations. First, if ICSI-parents decided on which relative, classmate or friend to enrol, they might be subjective in their choosing. They might anticipate that their own

child would come out better if they were to bring a control child with less optimal development. Second, relatives (e.g. cousins) of the same age are rare, particularly in ART-families as ART-parents are generally older when they have their first child. Another disadvantage of classmates and friends would be that ICSI-parents might be less likely to volunteer, because when they have to bring a classmate or friend this would entail informing other parents concerning the aim of this study and their own history of infertility.

In conclusion, we chose to invite children from regular pre-schools and primary schools as naturally conceived controls. These schools, in the region of the university hospital, were selected if their estimated social class distribution based on their zip codes corresponded to the social class distribution of the ICSI-cohort. In this way, we achieved (group-) matching on socio-economic status in addition to matching on age. We acknowledge the disadvantage that the children who attend these regular schools are inherently healthy and have developed to such a degree that they can follow mainstream education.

Matching

Matching was applied to ensure comparability between the groups for all but the outcome variables. Because matching increases research efficiency, the number of controls can be reduced. This was a desirable consequence considering that the IVF-population was not infinitely large and we lacked the capacity to have a natural conception control group that could be several times as large as the ICSI-group.

ICSI and IVF-children were individually matched for gender, socio-economic status, gestational age [preterm/term], maternal age at the time of pregnancy [± 3 years] and birth date [closest]. Socio-economic status was based on the zip code/socio-economic status indicator of Statistics Netherlands, which combines home price and income to determine low, medium, or high social status.[70]

ICSI and naturally conceived children were group-matched for age, gender, and socio-economic status. Matching on age was established by inviting control children from schools within the age range of the ICSI-cohort. During the period of enrolment, the distribution of gender in the natural conception control-group was monitored. If the boy/girl ratio had deviated from the ratio in the ICSI-group, measures would have been taken to achieve comparability. As mentioned in the previous paragraph, matching on socio-economic status was established by selecting schools based on zip code and hence socio-economic status of the area from which the children were drawn.

Individual matching of the natural conception control group would have been complex as no information on the children was available until the parental consent to enrol had been given. We did not match this group of controls for maternal age, because mothers conceiving naturally at older age (i.e. ages comparable to ICSI-mothers) are rare. Instead, we have adjusted for maternal age in the statistical analysis. The reason for not matching the natural conception group for gestational age is different: as described in the 'aim' section, our intention was to measure both the overall and, in special cases, the net effect of ICSI versus natural conception. The overall effect is the potential direct effect of ICSI in addition to the effect of increased prematurity and low birth weight rates. The net effect is the potential direct ICSI-effect given term birth or after stratification for term/preterm birth. Matching for gestational age would have made the measurement of the overall effect impossible.

Examinations

After six months of preparation, the examination period ran from March 2004 to May 2005. To narrow the age difference within the groups and to make sure that all children were at least 5 years old, we assessed the children by age ranking. The older children were assessed first, followed by the younger, so all children were between 5 and 8 years old at the time of follow-up.

The instruments that were used to assess the various outcomes are listed in Table 1 (see also the Appendix). The parents received two questionnaires by mail that were to be completed at home (questionnaire 'child health' and the Child Behaviour Checklist). The children visited the hospital once to undergo intelligence testing, as well as a physical examination assessing neuromotor development, congenital malformations and dysmorphic features, and growth. In addition, the children themselves completed a questionnaire (Dux25 Child). These assessments took an average of 2-2.5 hours, during which the parents filled out the remaining four questionnaires. During intelligence testing, to avoid interference and promote the reliability of the test, parents were asked to wait elsewhere.

A single trained investigator carried out the neurological examinations. To guarantee the quality of the examinations, a specialist in neuromotor developmental assessment reviewed a random sample of 32 children on videotape. Both observers were blinded to the mode of conception of the children. Nine trained examiners who were also blinded to the children's conception modes performed the intelligence tests. In the General Discussion we discuss the issue of multiple observers in more detail.

Table 1. Instruments used to measure the outcome variables

Outcome variable	Instrument
Neuromotor development	Touwen examination
Cognitive development	Revised Amsterdam Child Intelligence Test
Health	
Perinatal outcome	Questionnaire 'pregnancy and birth'
Congenital malformations	Physical examination and questionnaire 'pregnancy and birth'
General health	Questionnaire 'child health'
Medical care utilisation	Questionnaire 'child health'
Growth	Physical examination
Psychosocial well-being	
Behaviour	Child Behaviour Checklist and questionnaire 'child health'
Parenting stress	Parenting Stress Index (NOSI)
Quality of life	Dux25 Parent en Dux25 Child
Health-related quality of life	TACQOL

Outline of this thesis

Chapter 2 describes the study in which we compare neuromotor development between children born following ICSI and children born following IVF and natural conception. We measured neuromotor development with the Touwen examination,[71] which focuses on minor neurological dysfunctions (MND).[72,73] In Chapter 3, we compare the cognitive development of ICSI-children to IVF and natural conception. Children were tested with the short version of the Revised Amsterdam Child Intelligence Test.[74] Chapter 4 addresses the medical history and general health of ICSI-children, including pregnancy and perinatal outcome, congenital malformations and dysmorphic features, medical care utilisation, general health, and growth. In Chapter 5 we assess the psychosocial well-being of ICSI-children and their parents, as expressed by child behaviour, parenting stress and child quality of life. Chapter 6 contains the General Discussion on the results of this project.

References

(1) Andersen AN, Gianaroli L, Felberbaum R, De Mouzon J, Nygren KG. Assisted reproductive technology in Europe, 2002. Results generated from European registers by ESHRE. *Hum Reprod.* 2006; 21(7):1680-1697.

(2) Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet.* 1978; 2(8085):366.

(3) Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet.* 1992; 340(8810):17-18.

(4) Van der Westerlaken LAJ. Technology assessment of assisted reproduction. Leiden: Leiden University, 2005.

(5) Schultz RM, Williams CJ. The science of ART. *Science.* 2002; 296(5576):2188-2190.

(6) Sutcliffe AG. Intracytoplasmic sperm injection and other aspects of new reproductive technologies. *Arch Dis Child.* 2000; 83(2):98-101.

(7) Tournaye H. ICSI: a technique too far? *Int J Androl.* 2003; 26(2):63-69.

(8) Bonduelle M, Van Assche E, Joris H, Keymolen K, Devroey P, Van Steirteghem A et al. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod.* 2002; 17(10):2600-2614.

(9) Bonduelle M, Desmyttere S, Buysse A, Van Assche E, Schietecatte J, Devroey P et al. Prospective follow-up study of 55 children born after subzonal insemination and intracytoplasmic sperm injection. *Hum Reprod.* 1994; 9(9):1765-1769.

(10) Bonduelle M, Legein J, Derde MP, Buysse A, Schietecatte J, Wisanto A et al. Comparative follow-up study of 130 children born after intracytoplasmic sperm injection and 130 children born after in-vitro fertilization. *Hum Reprod.* 1995; 10(12):3327-3331.

(11) Bonduelle M, Wilikens A, Buysse A, Van Assche E, Wisanto A, Devroey P et al. Prospective follow-up study of 877 children born after intracytoplasmic sperm injection (ICSI), with ejaculated epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI. *Hum Reprod.* 1996; 11 Suppl 4:131-155.

(12) Bonduelle M, Legein J, Buysse A, Van Assche E, Wisanto A, Devroey P et al. Prospective follow-up study of 423 children born after intracytoplasmic sperm injection. *Hum Reprod.* 1996; 11(7):1558-1564.

(13) Govaerts I, Englert Y, Vamos E, Rodesch F. Sex chromosome abnormalities after intracytoplasmic sperm injection. *Lancet.* 1995; 346(8982):1095-1096.

(14) In 't Veld P, Brandenburg H, Verhoeff A, Dhont M, Los F. Sex chromosomal abnormalities and intracytoplasmic sperm injection. *Lancet.* 1995; 346(8977):773.

(15) Liebaers I, Bonduelle M, Van Assche E, Devroey P, Van Steirteghem A. Sex chromosome abnormalities after intracytoplasmic sperm injection. *Lancet.* 1995; 346(8982):1095.

(16) Palermo GD, Colombero LT, Schattman GL, Davis OK, Rosenwaks Z. Evolution of pregnancies and initial follow-up of newborns delivered after intracytoplasmic sperm injection. *JAMA.* 1996; 276(23):1893-1897.

(17) Aboulghar H, Aboulghar M, Mansour R, Serour G, Amin Y, Al Inany H. A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. *Fertil Steril.* 2001; 76(2):249-253.

(18) Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P et al. Neonatal data on a cohort of 2889 infants born after ICSI (1991-1999) and of 2995 infants born after IVF (1983-1999). *Hum Reprod.* 2002; 17(3):671-694.

(19) Jozwiak EA, Ulug U, Mesut A, Erden HF, Bahceci M. Prenatal karyotypes of fetuses conceived by intracytoplasmic sperm injection. *Fertil Steril.* 2004; 82(3):628-633.

(20) Bonduelle M, Wennerholm UB, Loft A, Tarlatzis BC, Peters C, Henriet S et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Hum Reprod.* 2005; 20(2):413-419.

(21) Hansen M, Bower C, Milne E, de Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects--a systematic review. *Hum Reprod.* 2005; 20(2):328-338.

(22) Katalinic A, Rosch C, Ludwig M. Pregnancy course and outcome after intracytoplasmic sperm injection: a controlled, prospective cohort study. *Fertil Steril.* 2004; 81(6):1604-1616.

(23) Klemetti R, Gissler M, Sevon T, Koivurova S, Ritvanen A, Hemminki E. Children born after assisted fertilization have an increased rate of major congenital anomalies. *Fertil Steril.* 2005; 84(5):1300-1307.

(24) Olson CK, Keppler-Noreuil KM, Romitti PA, Budelier WT, Ryan G, Sparks AE et al. In vitro fertilization is associated with an increase in major birth defects. *Fertil Steril.* 2005; 84(5):1308-1315.

(25) Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. *J Assist Reprod Genet.* 2004; 21(12):437-443.

(26) Cox GF, Burger J, Lip V, Mau UA, Sperling K, Wu BL et al. Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet.* 2002; 71(1):162-164.

(27) DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet.* 2003; 72(1):156-160.

(28) Gicquel C, Gaston V, Mandelbaum J, Siffroi JP, Flahault A, Le Bouc Y. In vitro fertilization may increase the risk of Beckwith-Wiedemann syndrome related to the abnormal imprinting of the KCN1OT gene. *Am J Hum Genet.* 2003; 72(5):1338-1341.

(29) Halliday J, Oke K, Breheny S, Algar E, Amor J. Beckwith-Wiedemann syndrome and IVF: a case-control study. *Am J Hum Genet.* 2004; 75(3):526-528.

(30) Maher ER, Brueton LA, Bowdin SC, Luharia A, Cooper W, Cole TR et al. Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). *J Med Genet.* 2003; 40(1):62-64.

(31) Moll AC, Imhof SM, Cruysberg JR, Schouten-van Meeteren AY, Boers M, Van Leeuwen FE. Incidence of retinoblastoma in children born after in-vitro fertilisation. *Lancet.* 2003; 361(9354):309-310.

(32) Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: a call for investigation. *Am J Hum Genet.* 2004; 74(4):599-609.

(33) Orstavik KH, Eiklid K, Van der Hagen CB, Spetalen S, Kierulf K, Skjeldal O et al. Another case of imprinting defect in a girl with Angelman syndrome who was conceived by intracytoplasmic semen injection. *Am J Hum Genet.* 2003; 72(1):218-219.

(34) Sutcliffe AG, Peters CJ, Bowdin S, Temple K, Reardon W, Wilson L et al. Assisted reproductive therapies and imprinting disorders--a preliminary British survey. *Hum Reprod.* 2006; 21(4):1009-1011.

(35) Tesarik J, Mendoza C. Genomic imprinting abnormalities: a new potential risk of assisted reproduction. *Mol Hum Reprod.* 1996; 2(5):295-298.

(36) Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. *Birth Defects Res A Clin Mol Teratol.* 2005; 73(3): 162-169.

(37) Lidegaard O, Pinborg A, Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. *Hum Reprod.* 2005; 20(4):950-954.

(38) Manning M, Lissens W, Bonduelle M, Camus M, De Rijcke M, Liebaers I et al. Study of DNA-methylation patterns at chromosome 15q11-q13 in children born after ICSI reveals no imprinting defects. *Mol Hum Reprod.* 2000; 6(11):1049-1053.

(39) Bonduelle M, Camus M, De Vos A, Staessen C, Tournaye H, Van Assche E et al. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum Reprod*. 1999; 14 Suppl 1:243-264.
(40) Govaerts I, Devreker F, Koenig I, Place I, Van den BM, Englert Y. Comparison of pregnancy outcome after intracytoplasmic sperm injection and in-vitro fertilization. *Hum Reprod.* 1998; 13(6):1514-1518.
(41) Loft A, Petersen K, Erb K, Mikkelsen AL, Grinsted J, Hald F et al. A Danish national cohort of 730 infants born after intracytoplasmic sperm injection (ICSI) 1994-1997. *Hum Reprod*. 1999; 14(8):2143-2148.
(42) Sutcliffe AG, Taylor B, Li J, Thornton S, Grudzinskas JG, Lieberman BA. Children born after intracytoplasmic sperm injection: population control study. *BMJ*. 1999; 318(7185):704-705.
(43) Wennerholm UB, Bergh C, Hamberger L, Nilsson L, Reismer E, Wennergren M et al. Obstetric and perinatal outcome of pregnancies following intracytoplasmic sperm injection. *Hum Reprod*. 1996; 11(5):1113-1119.
(44) Wisanto A, Bonduelle M, Camus M, Tournaye H, Magnus M, Liebaers I et al. Obstetric outcome of 904 pregnancies after intracytoplasmic sperm injection. *Hum Reprod*. 1996; 11 Suppl 4:121-129.
(45) Bonduelle M, Joris H, Hofmans K, Liebaers I, Van Steirteghem A. Mental development of 201 ICSI children at 2 years of age. *Lancet*. 1998; 351(9115):1553.
(46) Bonduelle M, Ponjaert I, Steirteghem AV, Derde MP, Devroey P, Liebaers I. Developmental outcome at 2 years of age for children born after ICSI compared with children born after IVF. *Hum Reprod*. 2003; 18(2):342-350.
(47) Bowen JR, Gibson FL, Leslie GI, Saunders DM. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. *Lancet*. 1998; 351(9115):1529-1534.
(48) Place I, Englert Y. A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilization. *Fertil Steril*. 2003; 80(6): 1388-1397.
(49) Sutcliffe AG, Taylor B, Saunders K, Thornton S, Lieberman BA, Grudzinskas JG. Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study. *Lancet.* 2001; 357(9274):2080-2084.
(50) Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ*. 2004; 328(7434):261-264.
(51) Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol.* 2004; 103(3):551-563.
(52) Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. *Obstet Gynecol*. 2004; 103(6):1144-1153.
(53) Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. *Fertil Steril.* 2005; 84(3):611-617.
(54) Ombelet W, Cadron I, Gerris J, De Sutter P, Bosmans E, Martens G et al. Obstetric and perinatal outcome of 1655 ICSI and 3974 IVF singleton and 1102 ICSI and 2901 IVF twin births: a comparative analysis. *Reprod Biomed Online.* 2005; 11(1):76-85.
(55) Sutcliffe AG, Taylor B, Grudzinskas G, Thornton S, Lieberman B. Children conceived by intracytoplasmic sperm injection. *Lancet.* 1998; 352(9127):578-579.
(56) Te Velde ER, Van Baar AL, Van Kooij RJ. Concerns about assisted reproduction. *Lancet*. 1998; 351(9115):1524-1525.

(57) Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born >=32 weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod*. 2007; 22(2):506-515.
(58) Leslie GI, Gibson FL, McMahon C, Cohen J, Saunders DM, Tennant C. Children conceived using ICSI do not have an increased risk of delayed mental development at 5 years of age. *Hum Reprod*. 2003; 18(10):2067-2072.
(59) Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. Cognitive and motor development of 8-year-old children born after ICSI compared to spontaneously conceived children. *Hum Reprod.* 2006; 21(11):2922-2929.
(60) Pinborg A, Loft A, Schmidt L, Greisen G, Rasmussen S, Andersen AN. Neurological sequelae in twins born after assisted conception: controlled national cohort study. *BMJ*. 2004; 329(7461):311.
(61) Ponjaert-Kristoffersen I, Tjus T, Nekkebroeck J, Squires J, Verte D, Heimann M et al. Psychological follow-up study of 5-year-old ICSI children. *Hum Reprod*. 2004; 19(12):2791-2797.
(62) Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, Nekkebroeck J, Loft A, Wennerholm UB et al. International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. *Pediatrics.* 2005; 115(3):e283-e289.
(63) Bonduelle M, Bergh C, Niklasson A, Palermo GD, Wennerholm UB. Medical follow-up study of 5-year-old ICSI children. *Reprod Biomed Online*. 2004; 9(1):91-101.
(64) Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *JAMA*. 2002; 288(6):728-737.
(65) Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med.* 2005; 352(1):9-19.
(66) Veen S, Ens-Dokkum MH, Schreuder AM, Verloove-Vanhorick SP, Brand R, Ruys JH. Impairments, disabilities, and handicaps of very preterm and very-low-birthweight infants at five years of age. The Collaborative Project on Preterm and Small for Gestational Age Infants (POPS) in The Netherlands. *Lancet*. 1991; 338(8758):33-36.
(67) Multiple gestation pregnancy. The ESHRE Capri Workshop Group. *Hum Reprod*. 2000; 15(8): 1856-1864.
(68) Pinborg A. IVF/ICSI twin pregnancies: risks and prevention. *Hum Reprod Update*. 2005.
(69) Stromberg B, Dahlquist G, Ericson A, Finnstrom O, Koster M, Stjernqvist K. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet*. 2002; 359(9305):461-465.
(70) Van Duijn C, Keij I. Sociaal-economische status indicator op postcodeniveau. *Maandstatistiek van de bevolking*. 2002; 2:32-35.
(71) Touwen BCL. *Examination of the Child with Minor Neurological Dysfunction.* 2 ed. London; Philadelphia: Spastics International Medical Publications: William Heinemann Medical Books; J. B. Lippincott Co.; 1979.
(72) Hadders-Algra M. Two distinct forms of minor neurological dysfunction: perspectives emerging from a review of data of the Groningen Perinatal Project. *Dev Med Child Neurol.* 2002; 44(8):561-571.
(73) Hadders-Algra M. Developmental coordination disorder: is clumsy motor behavior caused by a lesion of the brain at early age? *Neural Plast.* 2003; 10(1-2):39-50.
(74) Bleichrodt N, Resing WCM, Drenth PJD, Zaal JN. *Intelligentie-meting bij kinderen.* Lisse: Swets & Zeitlinger BV; 1987.

Chapter 2

Matched follow-up study of 5 - 8-year-old ICSI-singletons: comparison of their neuromotor development to IVF and naturally conceived singletons

2.

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Abstract

Background: Intracytoplasmic sperm injection (ICSI) is an invasive technique of artificial reproduction. We investigated the effect of ICSI on neuromotor development in 5 - 8-year-old singletons.

Methods: Follow-up of ICSI-singletons born between 1996 and 1999 after treatment in the Leiden University Medical Center and comparison with matched controls born after in vitro fertilisation (IVF) and natural conception (NC). Children underwent a thorough neurological examination that focuses on minor neurological dysfunction (MND).

Results: No differences in outcome between ICSI (n=81) and IVF-children (n=81), all born at term: MND prevalence 66.3% versus 61.3%, prevalence ratio (PR) 1.08 [0.83; 1.29]. MND prevalence among all ICSI-children (n=87) was higher than among NC-controls (n=85) (66.3% vs. 50.6%, PR 1.31 [1.02; 1.55]). After adjustment for maternal age and parity the PR remained elevated but was no longer statistically significant (Adj. PR 1.22 [0.86; 1.52]). When comparing only term ICSI and NC-children (n=81; n=85) the PR adjusted for maternal age and parity was 1.20 [0.83; 1.51].

Conclusions: Neuromotor outcome of 5 - 8-year-old singletons born at term after ICSI or IVF was similar; ICSI-children (both the total group and term children only) deviated slightly from NC-controls. Part of this effect was explained by a difference in parity, but not prematurity.

Introduction

Intracytoplasmic sperm injection (ICSI) is a technique of artificial procreation, in which a single spermatozoon is injected into the oocyte and once fertilised the zygote is transferred to the prestimulated uterus.[1] Due to the invasive character of the procedure, e.g. the in vitro manipulation of the gametes and the bypassing of natural selection barriers,[2-5] long-term follow-up of ICSI-children is warranted.

In the present study, we investigated the effect of ICSI on neuromotor development at the age of 5-8 years. Children born through artificial reproductive techniques are known to be at risk for prematurity and low birth weight,[6,7] both risk factors for disturbed neuromotor development.[8,9] By comparing ICSI-children with carefully matched IVF-controls we assessed the excess risk of the ICSI-procedure on neuromotor development, given the common characteristics of underlying infertility, hormonal stimulation of the mother, in vitro manipulation of the gametes and an increased risk of prematurity and low birth weight. In a comparison with naturally conceived (NC) control children, we studied both the overall effect of ICSI on neuromotor outcome, including the increased risk due to prematurity, and the net effect of ICSI in children born at term.

Previous studies on neurological and psychomotor development of ICSI-singletons painted a reassuring general picture,[10-16] but only one study has reached beyond the child age of 5 years old.[10,11] The test-instruments used in former studies were except for one[10] limited to gross and fine motor assessment or diagnosed neurological sequelae. In the present study, neuromotor development was recorded with well-defined outcome measures based on the assessment of posture, muscle tone, reflexes, gross and fine motor function, associated and involuntary movements, sensory deficits, and cranial nerve dysfunctions.

Patients and methods

All ICSI singleton children born between June 1996 and December 1999 after fertility treatment in the Leiden University Medical Center were invited. Exclusion criteria were: oocyte or sperm donation, cryopreservation of the embryo and selective embryo reduction with medical indication. Similar inclusion criteria were applied in the selection of IVF-children, who were matched person-to-person to ICSI-participants for gender, socio-economic status (SES), gestational age [preterm/term], maternal age at the time of pregnancy [±3 years] and birth date [closest]. SES-level low, medium or high was ascribed according to the zip code/socio-economic status indicator of Statistics Netherlands,[17] based on home price and income. If no match was available within the maternal age range of ±3 years, larger deviations were permitted.

Regular pre-schools and primary schools (i.e. schools not providing special education) with zip codes that indicated social class distributions similar to the ICSI-cohort were approached for the sampling of naturally conceived singletons. Teachers distributed letters among singletons within the defined age range 5 to 8 years old or born between June 1996 and December 1999) without further selection. In this way, we applied group matching for socio-economic status, birth date, and additionally gender.

Paternal educational level was indexed according to the SOI-register of Statistics Netherlands.[18] Demographical information on ICSI and IVF non-participants was obtained from the Leiden University Medical Center database to evaluate selection bias.

The study design was approved by the Ethics Committee of the Leiden University Medical Center and written informed consent was obtained from at least one parent.

Examination and outcome measures

All children underwent a standardised neurological examination developed by Touwen,[19] which focuses on minor neurological dysfunction (MND) and is applicable between 4 and 18 years old. Outcome measures consist of total neuromotor outcome and clusters of dysfunction separately (Table 1): posture and tone, reflexes, involuntary movements, gross motor development, fine motor development, associated movements, sensory deficits and cranial nerve dysfunctions.[20]

Simple MND (1 or 2 clusters of dysfunction) reflects the presence of a normal, but non-optimally functioning brain, and forms the lower tail of the distribution of the quality of brain function, which is seen as non-pathological.[21] Complex

MND (more than 2 clusters of dysfunction) can be considered as a distinct form of perinatally acquired brain dysfunction that is likely to be associated with a structural deficit of the brain.[21] Children with dysfunctional patterns in two or more clusters (complex MND) who meet the criteria of cerebral palsy (CP) are classified as CP. CP is defined as: movement and posture deviations due to a defect or lesion of the immature brain that manifest early in life and are permanent and non-progressive.[22] Clinically, a child with coordination problems, fine motor dysfunction, and excessive associated movements would be reported as complex MND; CP would be diagnosed in the case of e.g. hemiplegia.

One trained investigator who was blinded for the mode of conception did all neurological examinations. Blinding was achieved by scheduling and assessing the children in order of birth date. No information on mode of conception was available in this procedure. During the examination, we instructed the parents not to reveal the family name or the conception mode of the child. The assessments were videotaped and a sample of 32 children was reviewed by a specialist in neurological developmental assessment, who was also blinded for mode of conception. The sample included ten children haphazardly chosen with score 'normal' (10%), 15 children with 'simple MND' (10%), all six children with 'complex MND', and the one child with CP (excluded from main analysis due to prematurity).

General characteristics and additional information on the study groups were obtained through questionnaires.

Statistical analysis

We performed statistical analysis using the SPSS 11.0 for Windows package (SPSS Inc., Chicago, IL). The original power calculation was based on an intelligence test that was carried out in parallel (RAKIT, mean 100, SD 15; minimal detectable difference 7.5, power 0.80, $n \geq 63$). Additionally, a post-hoc power calculation on MND showed that a sample size larger than 59 was required to detect an increase in MND prevalence from a baseline of 25% in the NC-group (anticipated from Hadders-Algra et al.)[21] to 50% in the ICSI-group, with a power of 0.80. Cross tabulations and logistic regression analyses provided odds ratios (OR) and the corresponding 95% confidence intervals (95%CI). If the prevalence of outcome values exceeded 10%, the OR did not sufficiently approximate the relative risk any longer and therefore, all odds ratios and 95%CIs were translated to prevalence ratios (PR) (relative risks) using the method of Zhang[23]: $PR = OR/((1 - Po) + (Po \times OR))$, with Po= the prevalence of outcome of interest in non-exposed.

We used the Pearson chi-square test to assess the distribution of outcome values between groups if outcome consisted of more than two categories. Multiple logistic regression analysis was done to adjust for possible confounders.

Table 1. Clusters of minor neurological dysfunction (MND) based on the neurological examination of Touwen (1979)[20]

Cluster of dysfunction	Based on	Criteria for dysfunctional cluster
Dysfunctional muscle tone regulation	Muscle tone Posture during sitting, crawling, standing and walking	One or more of the following: - consistent mild deviations in muscle tone - consistent mild deviations in posture
Reflex abnormalities	Abnormal intensity and/or threshold or asymmetry in: - biceps reflex - knee jerk - ankle jerk Foot sole response: uni- or bilateral Babinski sign	Presence of at least two signs
Choreiform dyskinesia	Spontaneous motor behaviour Test with extended arms Movements of face, eyes, tongue	Presence of at least one of the following: - marked choreiform movements of distal and facial muscles - slight or marked choreiform movements of proximal muscles, eyes or tongue
Coordination problems	Finger-nose test Fingertip-touching test Diadochokinesis Kicking Knee-heel test Reaction to push (sitting, standing) Romberg Tandem gait Standing on one leg	Presence of age-inadequate performance of at least two tests
Fine manipulative disability	Finger-opposition test: - smoothness - transition Follow-a-finger test Circle test	Presence of age-inadequate performance of at least two tests
Rarely occurring miscellaneous disorders	Motor behaviour of face, eyes, pharynx, tongue Associated movements during diadochokinesis, finger-opposition test, walking on toes or heels	Evidence of at least one of the following: - mild cranial nerve palsy - excessive amount of associated movements for age

Age specific criteria for simple and complex MND[20]

Age	Simple MND	Complex MND
4yr to onset of puberty	1-2 MND clusters of dysfunction	>2 MND clusters of dysfunction

We performed both ICSI-IVF and ICSI-NC analyses in an unpaired design. ICSI-IVF analyses were suitable for paired testing, as we had matched the children person to person. The advantage of unpaired testing was the possibility of presenting the results as crude data instead of differences only. A possible disadvantage was the slight widening of the 95%CI.

The ICSI-NC comparison was carried out in two ways: First, to assess the overall difference in neuromotor development between ICSI and NC-children; the clinical question that parents are interested in. For this purpose the data were analysed without controlling for intermediate factors that are associated with both ART and neuromotor outcome, such as prematurity. Second, to assess the net difference between ICSI and NC-children provided term birth. For this purpose, preterm born children were excluded from the analyses.

Results

Selection

Overall response in the ICSI-group was 97/110 (88%), of which 87 children enrolled (90% of responders, 79% of all children invited) and 10 refused for various reasons. Participating and non-participating children were comparable for gender, SES, maternal age, and gestational age (data not shown). Higher participation rates were seen in the higher SES groups (participation percentage: high SES: 91%, medium SES: 71%, low SES: 59%).

In the IVF group, 257 children met the inclusion criteria. To find a match for each ICSI-child, 126 IVF-children were invited. Overall response was 100/126 (79%), of whom 92 participated (92% of responders, 73% of all invited) and 8 refused. Because no matches within the range of [-3, +3 years] for maternal age were available, larger deviations were permitted in 11 cases. Reasons for refusal were similar as for ICSI-families. The 92 participants differed from the 34 non-participants in gender-distribution (male gender in participants 49% vs. 71% in non-participants), but were comparable for maternal age, gestational age and birth weight (data not shown). The participation rates according to SES approximated those of the ICSI-group (high: 81%, medium: 73%, low: 50%). In five cases, two IVF-matches were available for one ICSI-child. By selecting the best match we restricted n=92 to n=87.

Of the 87 ICSI-children, 6 were born preterm. For 4 out of 6 cases we failed to include an IVF-match. As two children and their matches could not represent the preterm born children in the ICSI and IVF cohorts, we decided to exclude them from

Table 2. Demographic characteristics of parents and children: ICSI versus IVF and ICSI versus NC

	ICSI n=81	**IVF** n=81	**ICSI** n=87	**NC** n=85
Gender: male, n(%)	40 (49)	40 (49)	44 (51)	47 (55)
Age at examination, mean (range)	6.1 (5.3-7.7)	6.2 (5.3-8.3)‡	**6.1 (5.3-7.7)**	**6.3 (5.1-8.0)**
Parity: first-born, n(%)	61 (75)	59 (73)	**65 (75)**	**31 (37)**
Birth parameters				
gestational age, mean (range)	40.1 (37-43)	39.8 (37-42)	39.9 (35-43)	39.8 (37-43)
birth weight, mean (range)	3447 (2300-4750)	3379 (1835-4730)	**3370 (1485-4750)**	**3555 (2300-4800)**
prematurity (gest. age <37 wks)	0 (0)	0 (0)	**6 (7)**	**0 (0)**
birth weight <2500g, n(%)	3 (4)	3 (4)	**7 (8)**	**1 (1)**
small for gestational age †, n(%)	4 (5)	2 (3)	6 (7)	1 (1)
if Apgar score available, n(%):	57 (70)	58 (72)	60 (69)	62 (73)
Apgar 1min<5 or 5min<7, n(%)	2 (4)	2 (3)	2 (3)	1 (2)
Caesarian section, n(%)	11 (14)	9 (11)	12 (14)	6 (7)
Vanishing twin	6 (7)	7 (9)	9 (10)	-
timing unknown	1	4	3	-
<9 wks	4	1	4	-
9-21 wks	0	2	1	-
>21 wks	1	0	1	-
Parental age at pregnancy, mean (range)				
mother	32.8 (22-41)	33.4 (24-42)	**32.8 (22-41)**	**30.6 (20-41)**
father	36.9 (23-65)	37.3 (27-60)	**36.9 (23-65)**	**32.6 (20-49)**
Diagnosed infertility factor, n(%)				
mother	**13 (16)**	**37 (46)**	**15 (17)**	**0 (0)**
father	**64 (79)**	**11 (14)**	**70 (80)**	**0 (0)**
Pregnancy complications, n(%)	17 (21)	27 (33)	23 (26)	17 (20)
Medication during pregnancy, n(%)	10 (13)*	8 (10)	10 (12)*	14 (17)§
Smoking during pregnancy, n(%)				
mother	*		*	
no	70 (88)	70 (86)	76 (88)	75 (88)
yes, <10 per day	9 (11)	10 (12)	9 (11)	8 (9)
yes, >10 per day	1 (1)	1 (1)	1 (1)	2 (2)
father		‡		*
no	57 (70)	61 (77)	61 (70)	62 (74)
yes, <10 per day	**7 (9)**	**11 (14)**	**9 (10)**	**15 (18)**
yes, >10 per day	**17 (21)**	**7 (9)**	**17 (20)**	**7 (8)**
Ethnicity II, n(%)				
mother: non-Caucasian	7 (9)	9 (11)	9 (10)	8 (9)
father: non-Caucasian	8 (10)	8 (10)	10 (12)	11 (13)

Socio-economic status, n(%)				
low	8 (10)	8 (10)	10 (12)	7 (8)
medium	26 (32)	26 (32)	27 (31)	18 (21)
high	47 (58)	47 (58)	50 (58)	60 (71)
Level of education, n(%)				
mother		*		
no education	0 (0)	1 (1)	**0 (0)**	**0 (0)**
low	25 (31)	25 (31)	**27 (31)**	**11 (13)**
medium	28 (35)	27 (34)	**29 (33)**	**37 (44)**
high	28 (35)	27 (34)	**31 (36)**	**37 (44)**
father	*		*	
no education	0 (0)	2 (3)	0 (0)	1 (1)
low	28 (35)	26 (32)	31 (36)	22 (26)
medium	26 (33)	16 (20)	26 (30)	26 (31)
high	26 (33)	37 (46)	29 (34)	36 (42)
child, special education	1 (1)	2 (2)	1 (1)	0 (0)

* 1 missing value

† birth weight for gestational age< -2SDS[24]

‡ 2 missing values

§ 3 missing values

|| Turkey classified under non-Caucasian

bold p<0.05

further analyses in the ICSI/IVF-comparison (n=81). This decision was in line with our aim to investigate the effect of ICSI compared to IVF other than via low birth weight and prematurity.

From sixteen schools 87 children enrolled, of which one was excluded for being a twin and one for being conceived with intrauterine insemination (n=85). Forty-three children refused for various reasons. Response rate for all children invited and selection were hard to estimate in the NC-group, not knowing the exact size of the target group neither the characteristics of non-responders. However, of those who responded, 67% participated. Within the schools, the response was higher among NC-children of higher SES.

The ICSI-NC comparison was initially not restricted to term children because we aimed to assess the overall effect of ICSI on the outcome measures (ICSI n=87; NC n=85). However, in parallel we assessed the net effect of ICSI on neuromotor development by excluding preterm children from the analysis (ICSI n=81; NC n=85).

Characteristics

Parental and child characteristics are listed in Table 2. The ICSI and IVF-groups were comparable except for diagnosed infertility factors, incidence of pregnancy complications, paternal smoking behaviour, and paternal educational level.

Table 3. Crude and adjusted outcomes of neuromotor development: ICSI versus IVF and ICSI versus NC

Score	**ICSI** n=81*	**IVF** n=81*		
	n (%)	n (%)		
Normal	27 (34)	31 (39)		p=0.802
Simple MND†	50 (63)	46 (58)		
Complex MND	3 (4)	3 (4)		
Cerebral palsy	0 (0)	0 (0)		

Score	**ICSI** n=87*	**NC** n=85		
	n (%)	n (%)		
Normal	29 (34)	42 (49)		p=0.087
Simple MND	54 (63)	42 (49)		
Complex MND	3 (3)	1 (1)		
Cerebral palsy	0 (0)	0 (0)		

ICSI versus IVF				
Score	**ICSI** n=81*	**IVF** n=81*	**PR [95%CI] §**	**Adj. PR [95%CI] ||**
	n (%)	n (%)		
Normal	27 (34)	31 (39)	1.08 [0.83; 1.29]	1.09 [0.83; 1.30]
MND‡	53 (66)	49 (61)		

ICSI versus NC, total groups				
Score	**ICSI** n=87*	**NC** n=85	**PR [95%CI]**	**Adj. PR [95%CI]****
	n (%)	n (%)		
Normal	29 (34)	42 (49)	1.31 [1.02; 1.55]	1.22 [0.86; 1.52]
MND	57 (66)	43 (51)		

ICSI versus NC, children born at term				
Score	**ICSI** n=81*	**NC** n=85	**PR [95%CI]**	**Adj. PR [95%CI]****
	n (%)	n (%)		
Normal	27 (34)	42 (49)	1.31 [1.01; 1.55]	1.20 [0.83; 1.51]
MND	53 (66)	43 (51)		

* 1 missing value

† MND = minor neurological dysfunction

‡ simple MND and complex MND combined

§ Prevalence Ratio with 95% Confidence Interval

|| adjustment for maternal age, parity, and low birth weight

** adjustment for maternal age and parity

Despite the matching, the ICSI and NC-groups varied in age at the time of examination, parity, parental age, diagnosed infertility factors, paternal smoking behaviour, SES and maternal level of education. Mean birth weight was lower after ICSI and a higher frequency of prematurity, low birth weight, small for gestational age, and caesarean sections was found for ICSI-children compared to NC-controls.

Of the participating ICSI-children, all but one attended regular pre-schools and primary schools. This justified the retrieval of NC-controls via regular education.

Neuromotor Development

The investigator (MK) and the reviewing specialist (SV) agreed in 30 out of 32 cases that were reassessed (rate of agreement 0.94). The two cases with disagreement were analysed according to the score of the investigator. One ICSI-boy did not complete the examination and questionnaires were incompletely returned in three ICSI-cases. Two children in the IVF-group did not undergo the physical examination because of (i) severe developmental delay of the child (estimated total score of complex MND was assigned, based on parents' interview), and (ii) many previous hospital visits due to a congenital malformation (no score assigned).

Total neuromotor outcome in the ICSI and IVF-groups was similar (Table 3). The outcomes of simple and complex MND were combined to outcome MND in the second part of Table 3. The crude Prevalence Ratio (PR) of ICSI versus IVF considering neuromotor development normal versus MND was 1.08 (95%CI [0.83; 1.29]). To further investigate the effect of ICSI, we performed logistic regression analysis with the following covariates: maternal age, parity, and low birth weight. After adjustment, the ICSI-procedure still was not a predictor for neuromotor development (Adj. PR= 1.09 95%CI [0.83; 1.30]). Adjustment for differences in patient characteristics between the two groups (pregnancy complications, paternal smoking, and paternal education) did not result in a material change of this prevalence ratio.

Neither the occurrence of the specific clusters of dysfunction, nor movement-quantity and quality was different between ICSI and IVF (Table 4). We found a doubled frequency of children who had ever required physical therapy in the IVF-group and a third fewer IVF-children received speech therapy as compared to ICSI-children. The increase in physical therapy after IVF was mainly due to a higher frequency of gross or fine motor delay (ICSI n=3, IVF n=8). The decrease in speech therapy after IVF disappeared if the comparison was limited to speech therapy due to articulation problems and deviating mouth behaviour, the most relevant causes in this study on neuromotor development.

Comparing ICSI versus NC showed that ICSI-children were more often classified as simple MND than NC-controls (63% vs. 49%) (Table 3). The crude prevalence ratio of ICSI versus NC considering neuromotor development normal versus MND, was 1.31 95%CI [1.02; 1.55]. Logistic regression analysis adjusting for maternal age and parity showed an increase in risk of 22%, which was not statistically significant (Adj. PR= 1.22 95%CI [0.86; 1.52]) (Table 3). Parity seemed to account for a part of the crude ICSI-effect, as first-born children performed worse than children born with higher parity and ICSI-children were more often first born

Table 4. Clusters of dysfunction ICSI versus IVF and ICSI versus NC

	ICSI n=81		**IVF** n=81		**PR***	**[95%CI PR]**
	n (%)		n (%)			or p-value
Posture and tonus †	9 (11)		4 (5)	§	2.19	[0.70; 5.86]
Reflexes	20 (25)		21 (27)	§	0.93	[0.52; 1.50]
Involuntary movements	5 (6)		3 (4)	§	1.63	[0.39; 5.86]
Coordination	43 (54)	‡	35 (44)	§	1.21	[0.87; 1.55]
Fine manipulative disability	1 (1)		3 (4)	§	0.33	[0.03; 2.88]
Associated movements	0 (0)		0 (0)	§	-	-
Sensory deficits	0 (0)		0 (0)	§	-	-
Cranial nerve dysfunction	0 (0)		1 (1)	§	-	p=0.310
Quantity of movement	0 (0)		0 (0)	§	-	-
Quality of movement						
normal, fluent	76 (94)		72 (91)	§	-	p=0.747
moderate	2 (2)		2 (3)			
abnormal	3 (4)		5 (6)			
Physical therapy	8 (10)	§	16 (20)		0.51	[0.22; 1.10]
Speech therapy	16 (20)	‡	10 (12)		1.62	[0.78; 3.01]

	ICSI n=87		**NC** n=85	**PR***	**[95%CI PR]**
	n (%)		n (%)		or p-value
Posture and tonus ||	9 (10)		3 (4)	2.93	[0.83; 8.69]
Reflexes	21 (24)		14 (16)	1.46	[0.79; 2.45]
Involuntary movements	5 (6)		1 (1)	4.89	[0.59; 29.6]
Coordination	47 (55)	‡	34 (40)	1.37	[0.99; 1.72]
Fine manipulative disability	1 (1)		2 (2)	0.49	[0.04; 4.91]
Associated movements	0 (0)		2 (2)	-	p=0.150
Sensory deficits	0 (0)		0 (0)	-	-
Cranial nerve dysfunction	0 (0)		0 (0)	-	-
Quantity of movement**	1 (1)		3 (4)	0.33	[0.03; 2.90]
Quality of movement					
normal, fluent	81 (93)		79 (93)	-	p=0.800
moderate	2 (2)		1 (1)		
abnormal	4 (5)		5 (6)		
Physical therapy	8 (9)	§	10 (12)	0.79	[0.31; 1.83]
Speech therapy	18 (21)	‡	17 (20)	1.05	[0.56; 1.79]

* PR = Prevalence Ratio
† ICSI: 1 hypertonic, 8 hypotonic; IVF: 1 hypertonic, 1 hypotonic, 2 changing hypo-/hypertonic
‡ 1 missing value
§ 2 missing values
|| all hypotonic and hyperlax, except for 1 ICSI-child
** all hyperkinetic

(PR parity= 1.33 95%CI [0.96; 1.64]). Furthermore, ICSI and NC-groups varied in age at the time of examination, paternal age, paternal smoking behaviour, SES, and maternal educational level, but none of these factors influenced the adjusted prevalence ratio.

The occurrence of the specific clusters of dysfunction, the frequencies of supporting physical and speech therapy, and the occurrence of abnormalities in movement-quantity and quality (Table 4) were not significantly different between ICSI-children and NC-controls. However, a dysfunction on the cluster Coordination (gross motor skills) occurred in 55% of ICSI-children vs. 40% of NC-controls (PR=1.37 95%CI [0.99; 1.72]). Stratification for parity revealed that this difference was only present in first-born children. When reasons for physical therapy were compared, gross and/or fine motor dysfunction was equally frequent in the ICSI and NC-group (ICSI n=3 and NC n=4). Frequencies of speech therapy due to articulation problems or deviating mouth behaviour were comparable (articulation ICSI n=9 (10.3%) vs. NC n=10 (11.8%); mouth behaviour ICSI n=2 (2.3%) vs. NC n=5 (5.9%)).

When considering only term ICSI and NC-children (n=81; n=85), the crude and adjusted prevalence ratios for MND ICSI versus NC were 1.31 [1.01; 1.55] and 1.20 [0.83; 1.51] (Table 3). A non-significant elevation of 34% was found on the Coordination cluster (ICSI versus NC PR=1.34 [0.96; 1.71], data not shown); similarly as in the comparison of the total ICSI and NC-groups this elevation was limited to first-born children.

Discussion

From this detailed neurological investigation of 5 - 8-year-old singleton children conceived by ICSI, in comparison to children conceived by IVF and naturally conceived children, two conclusions can be drawn. First, there was no effect of the ICSI-procedure in itself on neuromotor development in comparison to the more common IVF-procedure, neither on total MND score, nor on subscores. Second, in the comparison of ICSI-children with naturally conceived control children, the crude data showed a higher prevalence of simple and overall MND among ICSI-children in both the total group and term children only. This difference largely disappeared upon controlling for mother's parity.

The comparison between ICSI and IVF was limited to children born at term. The number of premature ICSI-children was small, and we had difficulty in finding matching prematurely born IVF-children. Prematurity is more frequent after ART[6,7] and is associated with neuromotor delay.[8,9] A comparison of term singletons from ICSI to those from IVF, allowed us to assess if there was any extra effect of ICSI over IVF in addition to the risks following prematurity.

In the comparison between ICSI and naturally conceived children at first the complete groups were compared. Premature ICSI-children were left in, as we wanted to assess what the future parents of an ICSI child might expect on neuromotor development in comparison to natural conception (assuming similar parental characteristics up to the time of conception). The same argument was considered in the adjustment for confounding factors. Secondly, we compared ICSI and NC-children who were born at term to assess the net effect of ICSI.

We found that the overall difference in MND between ICSI and NC-children largely disappeared when controlling for parity. In our study, primiparous children performed worse on neuromotor development than multiparous children. As ICSI-children were more often first-born, this influenced our crude results. The way in which parity affects neuromotor development is unclear. Although adjustment for parity lowered the prevalence ratio to non-significance, it did not completely explain the findings between the ICSI and NC groups. As we found similar outcomes for ICSI and IVF-children, a shared factor of ICSI and IVF that was not present in NC-children may explain the difference. In a post-hoc analysis, we found that low birth weight, and being small for gestational age had only minimal effects in our data. Residual factors that may play a role are infertility status, hormonal stimulation of the mother, and in vitro manipulation of the oocyte.

The clinical significance of the difference in MND-outcome between ICSI and NC after correction for parity is moderate. However, a slight shift of ICSI-outcomes to poorer neuromotor development is (i) a topic of interest in the scope of science and (ii) may not only result in children shifting from normal to simple MND, but also from simple MND to complex MND.

The increase in rate of physical therapy and decrease in rate of speech therapy in IVF-children seem contradictory. The doubled rate of physical therapy for IVF versus ICSI remained when we limited the children to those who ever needed therapy for fine or gross motor movement. Regarding speech therapy, the most relevant categories in the scope of neuromotor development are articulation and mouth behaviour. The one-third decrease in speech therapy for IVF versus ICSI-children was not present if only these categories were considered. A hypothetical reason why the doubled rate of physical therapy was not reflected in the MND outcomes may be that IVF-children in origin had poorer neuromotor development than ICSI-children, which was captured at a young age and was compensated for with physical therapy up to age 5-8, the age at which we examined the children.

Strengths and weaknesses of the study

What our study adds to those previously carried out is the assessment at a higher age and with a more specific test-instrument with well-defined outcome measures. As children should reach more milestones with aging, assessment at a higher age allows for a more precise distinction in neuromotor development. Further, the strength of our study lies in the matched controlled design, the blinded examination of each individual child by one trained investigator in a single centre, and the blinded review of video-recordings.

A limitation of the study is that in the ICSI-IVF comparison we can only draw conclusions on term children. At the same time, this allowed us to focus on the potential effect of the procedure itself, irrespective of any difference in prematurity rate.

Composing the NC-group of children from regular pre-schools and primary schools had the disadvantage that control children were inherently neurologically developed to a degree that they could attend regular education. As only one participating ICSI-child relied on special education, we were confident that the ICSI and NC-children had similar educational backgrounds.

By the matching process, we intended to increase the validity of the comparisons. The benefit of this matching procedure was that we needed less control for confounding in the analyses, but the downside was that our sample sizes decreased. Although we reached the number of children to achieve a power of 80%, the difference in MND-prevalence that was found between the two groups was smaller than the difference used for the power calculation. This led to broader confidence intervals than aimed for, but the results remain interpretable.

With response rates of 79% and 73%, respectively, we assumed to have retrieved representative samples of ICSI and IVF-children. Part of the non-responders may never have been reached due to expired home addresses and of the 97 ICSI-responders (88% of those invited) 87 participated (90%). Of the 126 invited IVF-children 100 had responded (79%), of which 92 (92%) enrolled. The increased participation rate in higher SES families compared to lower will not have influenced our outcomes as we matched for SES and the rates were comparable between ICSI and IVF. The higher rate of male gender in IVF non-participants as compared to participants was unexpected. Hypothetically, the non-participating boys may have had more neuromotor problems and their parents may have been less keen to volunteer them for the study. In that case, the prevalence of neuromotor problems in IVF-children would be an underestimation and finding no differences between ICSI and IVF would reflect a higher than average neuromotor development in ICSI-children.

The NC-group should represent that part of the general population that matches the ICSI-group, but the prevalence of MND in the NC-group was higher than in the general population (51% versus 21% for Dutch children aged 9)[21,25]. Reassuringly, this increase involved mainly simple MND, which represents non-optimality. However, combined with the apparently high prevalences of physical therapy and speech therapy among NC-children, the impression may rise that the controls were 'too pathological': NC-parents may have been keener to volunteer when they worried about their child's health or development. If such selection happened, this would change our results and conclusions: the true difference in MND-prevalence between ICSI and NC-children might be larger, and might remain after adjustment for confounding factors.

The high rates of physical and speech therapy do not necessarily point to such selection. Statistics Netherlands reports that 4.6% of the children aged 0-11 years visited the physical therapist at least once in the year 2005.[26] Our data cover the complete history of the children. When excluding children with indications that could not have occurred in 2005 (e.g. hyperextension at infant age), we found that 7.1% of the NC-controls had visited a physical therapist. As this percentage covers several years, we consider it comparable to the 4.6% of the Dutch population that relates to a single year. Speech therapy (including language therapy) is also common in the Netherlands. This is mainly the result of screening at age 5 in schools. In a sample of 20.000-30.000 children covered by about 15 health services (GGD-NL), 7-33% of the children were referred to a speech therapist.[27]

Selection bias based on the child's health or development was not seen in outcomes that were measured in parallel to the current study either. The mean IQ of NC-children was 110; 35% of the NC-children did not visit a general practitioner once in the past year; NC-parents considered their children healthier than other children in 31%, equally healthy in 67%, and less healthy in 2% (data not shown). These findings are not in line with the potential selection of children from worried parents. Another argument against selection bias is that a large part of the children that scored simple MND would have never been recognised by the parents as such, as the deviations are minor. Finally, parents had been asked for their reasons to volunteer; in the NC-group answers were mainly 'to support medical sciences/help other people' and 'being keen to follow my child's development'. 'Worries' were mentioned in four cases, of which only one involved neuromotor development.

Alternative explanations for the increased MND rate in our findings as compared to the reference population could be: (i) a stricter method of examination, (ii) a hypothetical increase in MND in children over the past decades (norm population was born in the '70s), and (iii) other differences between the NC-control group and the norm population (e.g. parity).

We did not take into consideration the possibility of hereditary minor neurological dysfunctions. Diagnosing MND among the parents would have required their full assessment. An alternative would have been to acquire information on their needs for physical and speech therapy due to motor delay in childhood.

The death of a co-twin in utero (vanishing twin) may cause neurological sequelae in the surviving 'singleton'.[28-30] In our study, the number of vanishing twins was comparable for ICSI and IVF. No information was available on the incidence after natural conception, but this was probably lower than after ICSI or IVF, as the incidence of twinning is also lower after natural conception. However, as being the survivor of a vanishing co-twin did not influence our adjusted prevalence ratio in regression analysis, bias due to vanishing twins was excluded.

Related studies

Our findings of no significant differences in neuromotor development between ICSI and IVF, neither between ICSI an NC, at least after adjustment for parity, are in line with the literature.[12,14;10,11,13-16] Ponjaert-Kristoffersen et al.[13] noted significantly lower scores on the Peabody Developmental Motor Scales for Gross Motor and Fine Motor abilities comparing ICSI-children to NC-controls at age 5. The authors explained that after stratification for site, the difference in Gross Motor Quotient was only present in one (New York) of the two centres (New York and Brussels) studied. This finding could be in line with the effect of adjustment for parity in our study: in Brussels only primiparous women had been included, while in New York a higher number of primiparous women in the ICSI-group compared to the NC-group might have led to worse motor outcomes for the ICSI-group.

From the present study, in which ICSI-children underwent a complete and detailed neuromotor examination at the more advanced age of 5-8 years, we can conclude that: neuromotor outcome of 5 - 8-year-old singleton children born at term after the ICSI or IVF-procedure was similar, but ICSI-children (both the total group and term children only) deviated slightly from NC-controls. Part of the latter effect might be explained by a difference in the mother's parity. Thus, the overall conclusion of this study on neuromotor development is in line with the literature, and is reassuring for future parents of ICSI-children.

References

(1) Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet.* 1992; 340(8810):17-18.

(2) Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: a call for investigation. *Am J Hum Genet.* 2004; 74(4):599-609.

(3) Schultz RM, Williams CJ. The science of ART. *Science.* 2002; 296(5576):2188-2190.

(4) Sutcliffe AG. Intracytoplasmic sperm injection and other aspects of new reproductive technologies. *Arch Dis Child.* 2000; 83(2):98-101.

(5) Tournaye H. ICSI: a technique too far? *Int J Androl.* 2003; 26(2):63-69.

(6) Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ.* 2004; 328(7434):261-264.

(7) Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol.* 2004; 103(3):551-563.

(8) Marlow N, Wolke D, Bracewell MA, Samara M. Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med.* 2005; 352(1):9-19.

(9) Veen S, Ens-Dokkum MH, Schreuder AM, Verloove-Vanhorick SP, Brand R, Ruys JH. Impairments, disabilities, and handicaps of very preterm and very-low-birthweight infants at five years of age. The Collaborative Project on Preterm and Small for Gestational Age Infants (POPS) in The Netherlands. *Lancet.* 1991; 338(8758):33-36.

(10) Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born >=32 weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod.* 2007; 22(2):506-515.

(11) Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. Cognitive and motor development of 8-year-old children born after ICSI compared to spontaneously conceived children. *Hum Reprod.* 2006; 21(11):2922-2929.

(12) Pinborg A, Loft A, Schmidt L, Greisen G, Rasmussen S, Andersen AN. Neurological sequelae in twins born after assisted conception: controlled national cohort study. *BMJ.* 2004; 329(7461):311.

(13) Ponjaert-Kristoffersen I, Tjus T, Nekkebroeck J, Squires J, Verte D, Heimann M et al. Psychological follow-up study of 5-year-old ICSI children. *Hum Reprod.* 2004; 19(12):2791-2797.

(14) Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, Nekkebroeck J, Loft A, Wennerholm UB et al. International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. *Pediatrics.* 2005; 115(3):e283-e289.

(15) Sutcliffe AG, Taylor B, Li J, Thornton S, Grudzinskas JG, Lieberman BA. Children born after intracytoplasmic sperm injection: population control study. *BMJ.* 1999; 318(7185):704-705.

(16) Sutcliffe AG, Taylor B, Saunders K, Thornton S, Lieberman BA, Grudzinskas JG. Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study. *Lancet.* 2001; 357(9274):2080-2084.

(17) Van Duijn C, Keij I. Sociaal-economische status indicator op postcodeniveau. *Maandstatistiek van de bevolking.* 2002; 2:32-35.

(18) Statistics Netherlands (CBS). Standaard Onderwijs Indeling (SOI). *StatLine, Central Bureau of Statistics.* 1998; (Accessed January 9, 2006, at http://www.cbs.nl).

(19) Touwen BCL. *Examination of the Child with Minor Neurological Dysfunction.* 2 ed. London; Philadelphia: Spastics International Medical Publications: William Heinemann Medical Books; J. B. Lippincott Co.; 1979.

(20) Hadders-Algra M. Developmental coordination disorder: is clumsy motor behavior caused by a lesion of the brain at early age? *Neural Plast.* 2003; 10(1-2):39-50.

(21) Hadders-Algra M. Two distinct forms of minor neurological dysfunction: perspectives emerging from a review of data of the Groningen Perinatal Project. *Dev Med Child Neurol.* 2002; 44(8):561-571.

(22) Bax M, Goldstein M, Rosenbaum P, Leviton A, Paneth N, Dan B et al. Proposed definition and classification of cerebral palsy, April 2005. *Dev Med Child Neurol.* 2005; 47(8):571-576.

(23) Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA.* 1998; 280(19):1690-1691.

(24) Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand.* 1991; 80(8-9):756-762.

(25) Hadders-Algra M, Huisjes HJ, Touwen BC. Perinatal correlates of major and minor neurological dysfunction at school age: a multivariate analysis. *Dev Med Child Neurol.* 1988; 30(4):472-481.

(26) Statistics Netherlands (CBS). Geboorte; kerncijfers. *StatLine, Central Bureau of Statistics.* 2005; (Accessed December 12, 2006, at http://www.cbs.nl).

(27) Sluijmers J, Ter Horst H. Reactie van vakgroep logopedie van GGD-NL op het TNO-rapport "Signalering van spraaktaal-ontwikkelingsstoornissen bij kleuters" (Van der Ploeg e.a., 2005). pag1-9. 17-5-2005.

(28) Melnick M. Brain damage in survivor after in-utero death of monozygous co-twin. *Lancet.* 1977; 2(8051):1287.

(29) Pharoah PO, Adi Y. Consequences of in-utero death in a twin pregnancy. *Lancet.* 2000; 355(9215):1597-1602.

(30) Pinborg A, Lidegaard O, La Cour FN, Nyboe AA. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod.* 2005; 20(10):2821-2829.

Chapter 3

Cognitive development of singletons born after ICSI in comparison to IVF and natural conception

3.

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Fertility and Sterility, in press

Abstract

Objective: To investigate cognitive development of ICSI-singletons at 5-8 years of age.

Design: Follow-up study.

Setting: University medical centre, assessments between March 2004 and May 2005.

Patients: Singletons born between June 1996 and December 1999 following ICSI at the Leiden University Medical Center were compared with matched singletons born following IVF and natural conception (NC).

Intervention: Mode of conception.

Main outcome measure: IQ was measured with the Revised Amsterdam Child Intelligence Test (RAKIT, short form). The investigators were blinded to conception mode.

Results: ICSI-singletons (n=83) achieved lower IQ-scores than IVF-singletons (n=83) (adjusted mean difference IQ: 3.6, 95%CI [-0.8; 8.0]). After categorising IQ-outcomes into <85; 85-115; >115 no significant difference in the distribution of IQ was found (p=0.268). ICSI-singletons (n=86) achieved lower IQ-scores than NC-singletons (n=85); the adjusted mean difference varied between 5 and 7 points (5.6, 95%CI [0.9; 10.3]; 7.1, 95%CI [1.7 to 12.5]) depending on the covariates included in the model. Adjustment for prematurity did not change the results. Percentages in IQ-categories <85; 85-115; >115 were 12%; 64%; 24% for ICSI and 6%; 54%; 40% for NC (p=0.019).

Conclusion: In the relatively limited sample investigated, cognitive development among ICSI-singletons was lower than among IVF and NC-singletons. Infertility factors or unmeasured confounders may play a role.

Introduction

Artificial reproductive techniques (ART) such as in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) currently account for between 0.2-3.9% of childbirths in Europe.[1] Since the introduction of ICSI in 1992,[2] the health status and development of ICSI-children have been a matter of concern, as the technique is rather invasive.[3-6] In ICSI, because the spermatozoon is selected by the laboratory technician and injected into the oocyte with a microinjection pipette, several natural selection barriers are bypassed. Fertilisation with spermatozoa of uncertain quality, and the possible damage caused by the in vitro manipulation of the oocyte warrant the study of possible long-term effects on ICSI-children.

In this study we compared the cognitive development of 5 - 8-year-old singletons that were born after an ICSI-procedure with two control groups: children born after standard in vitro fertilisation (IVF) and children born after natural conception (NC). ICSI and IVF-procedures are similar in maternal hormonal stimulation and in fertilisation taking place in vitro, but differ in sperm selection and oocyte penetration, which are not manipulated during IVF. By comparing ICSI-children and IVF-children, we intended to investigate potential differences, given a similar background of an infertile couple, maternal hormonal stimulation and fertilisation in vitro. The second control group, consisting of children conceived naturally, was used to assess the cognitive outcome of ICSI-children compared to children born after natural conception in two ways: first we investigated the overall difference in cognitive development between ICSI and NC, as this represents the main clinical question of future ICSI-parents. Second, with a more biological approach, we investigated whether a net effect of ICSI existed on cognitive development as compared to NC, by controlling for known intermediate factors such as prematurity.[7-9]

All previous follow-up studies on the cognitive development of ICSI-children have concerned children up to the age of 5, except one.[10] All studies but one[11] found no differences in cognitive development.[10, 12-18]

Our study assessed children beyond the age of 5. By strict selection, careful matching and adjustment for demographic variables, and by blinded assessment in a single centre, we aimed to enhance the validity of the comparisons between the different modes of conception.

Materials and methods

Institutional Review Board approval was obtained and at least one parent of each child signed for informed consent. The authors have no conflicts of interest to disclose. The assessments were carried out between March 2004 and May 2005.

Participants

Live birth ICSI-singletons born between June 1996 and December 1999 after fertility treatment in the Leiden University Medical Center laboratory were invited to participate. Exclusion criteria were: oocyte or sperm donation, cryopreservation of the embryo, and selective embryo reduction with medical indication. Similar criteria were used for the inclusion of IVF-children, who were selected to match person-to-person to the ICSI-participants on gender, socio-economic status, gestational age [preterm/term], maternal age at the time of pregnancy [±3 years] and date of birth [closest]. Socio-economic status (low, medium, or high) was ascribed using the zip-code/socio-economic status indicator of Statistics Netherlands,[19] which is based on home price and income. If no match was available within the maternal age range of ±3 years, larger deviations were permitted.

Pre-schools and primary schools with zip-codes that indicated social class distributions similar to the ICSI-cohort assisted in the sampling of naturally conceived singletons. We applied group matching on gender, socio-economic status, and date of birth. As the NC-children all had a cognitive development sufficient to attend regular education until the age of assessment, we excluded ICSI-children attending special education from the ICSI/NC comparison. A similar restriction was not required in the ICSI/IVF comparison, as IVF-children had been recruited without prior information on education.

Demographical information on ICSI and IVF non-participants was obtained from the Leiden University Medical Center database to evaluate selection bias.

Response

One hundred and ten ICSI-children met the inclusion criteria. Overall response was 97/110 (88%), 87 children joined in (90% of responders, 79% of all children invited) and 10 refused for various reasons. Participants and non-participants were comparable for gender, maternal age, and gestational age (data not shown). The rate of participation was higher in the upper socio-economic groups (91% among high socio-economic status, 71% among medium status, and 59% among the low status group).

In the IVF group 257 children met the inclusion criteria, and 126 were invited to participate. Overall response was 100/126 (79%); 92 participated (92% of responders, 73% of all invited IVF-children) and 8 refused. A deviation in maternal age of more than +/- three years was permitted in 11 cases. Reasons for refusal to participate were similar to those for ICSI-families. The 92 IVF participants differed from the 34 non-participants by gender (among participants 49% were male vs. 71% among non-participants), but the groups were comparable for maternal age, gestational age and birth weight. The participation rates according to socio-economic status approximated those of the ICSI-group (high socio-economic status: 81%, medium: 73%, low: 50%). In five cases, two IVF-matches were available for an ICSI-child. The best match was selected and n= 92 was restricted to n= 87. Of the original 110 ICSI-children, eight had been born prematurely. Six enrolled on the study, but in four cases no premature IVF-match could be found, reducing the n to 83.

Sixteen schools participated in the recruitment of NC-children and 85 of the 87 children that applied met the inclusion criteria (one twin boy and one child born after intrauterine insemination were excluded). Forty-three children refused for various reasons. A response rate could not be estimated for the NC-group, as we did not know the exact size of the target group. However, of those who responded, 67% participated. The response was higher among NC-children of higher socio-economic status: of the 16 schools that participated 9 were approached specifically to obtain the group of 7 low socio-economic status children. One ICSI-boy (1%) attended special education and was therefore excluded from the ICSI/NC comparison (n=86).

Assessment and outcome measures

When the study began, the Dutch norms for the WISC III had not yet been approved and we chose to use the Revised Amsterdam Child Intelligence Test (RAKIT),[20] the short version. This test is applicable for children aged 4-11 years. The six subtests measure the subscales: perceptual reasoning (Exclusion, Discs, Hidden Figures), verbal learning (Verbal Meaning, Learning Names), spatial orientation and speed (Discs), and verbal fluency (Idea Production).[20] The test correlates 0.93 with the IQ of the complete version,[20] which was not applied because of time limitation. The sum score of the subtest scores (mean 15, SD 5) is translated into a short version RAKIT-IQ (mean 100, SD 15), allowing for child age. Nine trained investigators administered the tests. The observers were scheduled independently of child characteristics and were blinded to the mode of conception.

General characteristics and additional information on the study groups were obtained through questionnaires.

Statistical analysis

For statistical analyses we used the SPSS 11.0 for Windows package (SPSS Inc., Chicago, IL). The principal investigator performed the data-analysis. To reach a power of 0.80 with a standard deviation of 15 and a minimal detectable difference of 7.5 IQ-points[21] (half a standard deviation) ≥ 63 children had to be included per group. We compared continuous data using the Student's t-test with a significance level of 0.05 and linear regression analysis was applied to adjust for potential confounders. As ICSI and IVF-children had been matched person to person, paired testing was appropriate: paired t-tests and linear mixed model analysis with couple number as a random factor. Through ordinal regression analysis we analysed and adjusted categorical data. We performed a one-way ANOVA to assess potential differences in scoring between the investigators.

The ICSI-NC comparison was carried out to assess both the overall difference in cognitive development between ICSI and NC-children (clinical question), as well as the net difference (biological question). In answering the former question, the data were analysed without controlling for intermediate factors that are associated with both ART and cognitive outcome, such as prematurity.[7-9] In answering the latter question, we indeed adjusted for these factors, assessing a potential net effect of ICSI on cognitive development.

Results

Characteristics

Table 1 compares the characteristics of the parents and children for the three groups. Maternal subfertility was more frequent among IVF-couples than among ICSI-couples, which was the inverse for paternal subfertility. ICSI-mothers had a lower frequency of pregnancy complications than IVF-mothers. Paternal educational level was lower in the ICSI-group (indexed according to the register of Statistics Netherlands)[23]. Primary language spoken at home other than Dutch was 1% for ICSI-children and 4% for IVF-children. ICSI-fathers smoked more heavily than IVF-fathers. Furthermore the groups were comparable in drug use and excessive-drinking habits of the parents (data not shown).

When comparing the ICSI and NC-group, 74% of the ICSI-children were first-born versus 37% of the NC-children. The mean birth weight of ICSI-children was lower. ICSI-children showed a higher incidence of premature birth, low birth weight, small for gestational age characteristics, and caesarean sections than NC-controls. Parental mean ages were higher for ICSI-children. NC-controls were of higher socio-economic status and maternal educational level than ICSI-children.

Table 1. Characteristics of parents and children: ICSI versus IVF and ICSI versus NC

	ICSI n=83	**IVF** n=83	**ICSI** n=86	**NC** n=85
Gender: male, n(%)	41 (49)	41 (49)	43 (50)	47 (55)
Age at time of examination, mean	6.1 (5.3-7.7)	6.2 (5.3-8.3)§	6.1 (5.3-7.7)	6.3 (5.1-8.0)
Parity: first born, n(%)	63 (76)	61 (74)	**64 (74)**	**31 (36)**
Birth parameters				
gestational age, mean	40.0 (35-43)	39.7 (36-42)	39.9 (35-43)	39.8 (37-43)
birth weight, mean	3409 (1485-4750)	3349 (1725-4730)	**3361 (1485-4750)**	**3555 (2300-4800)**
prematurity (gest. age < 37 wks), n(%)	2 (2)	2 (2)	**6 (7)**	**0 (0)**
birth weight < 2500g, n(%)	5 (6)	4 (5)	**7 (8)**	**1 (1)**
small for gestational age †, n(%)	5 (6)	3 (4)	**6 (7)**	**1 (1)**
if Apgar score available, n(%)	59 (71)	58 (70)	59 (69)	62 (73)
Apgar 1min<5 or 5min<7, n(%)	2 (3)	2 (3)	2 (3)	1 (2)
Caesarean section, n(%)	12 (15)	10 (12)	**12 (14)**	**6 (7)**
Parental age at pregnancy, mean				
mother	32.8 (22-41)	33.4 (24-42)	**33.0 (22-41)**	**30.6 (20-41)**
father	36.9 (23-65)	37.2 (27-60)	**37.0 (23-65)**	**32.6 (20-49)**
Diagnosed infertility factor, n(%)				
mother	**13 (16)**	**38 (46)**	**15 (17)**	**0 (0)**
father	**66 (80)**	**11 (13)**	**69 (80)**	**0 (0)**
Pregnancy complications, n(%)	**19 (23)**	**29 (35)**	23 (27)	17 (20)
Medication during pregnancy, n(%)	10 (12)*	8 (10)	9 (11)*	14 (17)§
Smoking during pregnancy, n(%)				
mother	*		*	
no	72 (88)	72 (87)	76 (89)	75 (88)
yes, <10 per day	9 (11)	10 (12)	9 (11)	8 (9)
yes, >10 per day	1 (1)	1 (1)	0 (0)	2 (2)
father		‡		*
no	**58 (70)**	**63 (78)**	**61 (71)**	**62 (74)**
yes, <10 per day	**8 (10)**	**11 (14)**	**9 (11)**	**15 (18)**
yes, >10 per day	**17 (21)**	**7 (9)**	**16 (19)**	**7 (8)**
Ethnicity II, n(%)				
mother: non-Caucasian	7 (8)	9 (11)	9 (10)	8 (9)
father: non-Caucasian	8 (10)	8 (10)	10 (12)	11 (13)
Socio-economic status, n(%)				
low	8 (10)	8 (10)	**10 (12)**	**7 (8)**
medium	26 (31)	26 (31)	**26 (30)**	**18 (21)**
high	49 (59)	49 (59)	**50 (58)**	**60 (71)**
Level of education, n(%)				
mother		*		
no education	0 (0)	1 (1)	**0 (0)**	**0 (0)**
low	25 (30)	25 (31)	**27 (31)**	**11 (13)**
medium	28 (34)	29 (35)	**28 (33)**	**37 (44)**
high	30 (36)	27 (33)	**31 (36)**	**37 (44)**
father	*		*	
no education	**0 (0)**	**2 (2)**	0	1 (1)
low	**28 (34)**	**27 (33)**	31 (36)	22 (26)
medium	**26 (32)**	**16 (19)**	25 (29)	26 (31)
high	**28 (34)**	**38 (46)**	29 (34)	36 (42)
child	*		*	
regular pre-/primary school	72 (88)	69 (83)	76 (89)	79 (93)
regular school, repeat class	7 (9)	8 (10)	7 (8)	4 (5)
regular school, remedial teaching	2 (2)	2 (2)	2 (2)	2 (2)
special education	1 (1)	4 (5)	0	0

* 1 missing value

† Sweden, Niklasson[22]: birth weight for gestational age< -2SDS

‡ 2 missing values

§ 3 missing values

|| Turkey classified under non-Caucasian

bold: differences considered as of potential confounding effect

Primary language other than Dutch spoken at home was 1% among ICSI-children and 5% among the NC control group. ICSI-fathers tended to smoke more heavily than the NC-fathers who smoked. Furthermore the groups were comparable in drug use and excessive-drinking habits of the parents (data not shown).

Cognitive Development

The outcomes of cognitive developmental testing are listed in Table 2 and 3 and Figure 1. No difference was found by analysis of variance (ANOVA) among the mean IQ-scores for the nine investigators (p=0.843). Three IVF-children did not undergo the RAKIT because of (i) developmental delay of the child (n=2, an estimated total IQ-score of 84 was assigned) and (ii) many previous hospital visits due to a congenital malformation (n=1, regular education, no score assigned). The latter child was thus excluded from the analyses of cognitive development, but as congenital malformations were studied in parallel, the child was not replaced by another.

Table 2. Mean RAKIT-IQ and mean subtest scores

	ICSI	IVF	Crude difference
	n=83	n=82	[95% Confidence Interval]
Mean RAKIT-IQ	103	107	3.9 [-0.7; 8.4]
Mean subtest scores:	n=83	n=80	
Exclusion	15.4	16.8	1.3 [-0.2; 2.9]
Verbal Meaning	16.1	17.3	1.1 [-0.6; 2.8]
Discs	15.3	15.7	0.4 [-1.2; 2.0]
Learning Names	15.5	16.8	1.2 [-0.2; 2.7]
Hidden Figures	16.4	16.8	0.3 [-1.4; 2.1]
Idea Production	15.2	16.3	1.0 [-0.6; 2.6]

	ICSI	NC	Crude difference
	n=86	n=85	[95% Confidence Interval]
Mean RAKIT-IQ	103	110	**6.8 [2.0; 11.6]**
Mean subtest scores:			
Recognise Figures*	-	13.0	-
Exclusion	15.4	16.3	0.9 [-0.6; 2.3]
Verbal Meaning	16.1	17.9	**1.8 [0.3; 3.4]**
Discs	15.3	16.0	0.7 [-0.8; 2.1]
Learning Names*	15.5	17.5	**1.9 [0.4; 3.5]**
Hidden Figures*	16.3	18.4	**2.1 [0.5; 3.6]**
Idea Production	15.3	16.6	1.3 [-0.3; 2.8]

* if child age <5.2 years: Learning Names and Hidden Figures are replaced by Recognise Figures; NC: Recognise Figures n=1, Learning Names n=84 and Hidden Figures n=84.
bold p<0.05

The mean RAKIT-IQ for ICSI-children was 3.9 points lower than for IVF-children (103 vs. 107; 95%CI [-0.7; 8.4]) (Table 2). Mean subtest scores were all lower in the ICSI-group, with mean differences ranging from 0.3 to 1.3 points. The largest differences were found for the subtests Exclusion and Learning Names. The results were consistent in age categories <6; 6-7; 7-8; >8. The difference among boys was greater than among girls (mean difference boys 5.4, 95%CI [-2.6; 13.4]; girls 2.4, 95%CI [-4.0; 8.8]). When the continuous RAKIT-IQ was divided into three categories based on the standard deviation, the percentages in each group were as follows: IQ <85 ICSI 11% vs. IVF 9%; IQ 85-115 ICSI 65% vs. IVF 60%; IQ >115 ICSI 24% vs. IVF 32% (ordinal regression analysis p=0.268) (Figure 1).

Adjustment of the crude mean difference of 3.9 for the characteristics in which ICSI and IVF had differed (i.e. paternal education and pregnancy complications (Table 1)) resulted in a decrease of the difference to 3.6 95%CI [-0.8; 8.0] (Table 3). The minimal change was due to the opposite influence of pregnancy complications and paternal education. Correction for paternal smoking had no further effect. The adjusted p-value for the difference in distribution over the three IQ-categories was 0.303.

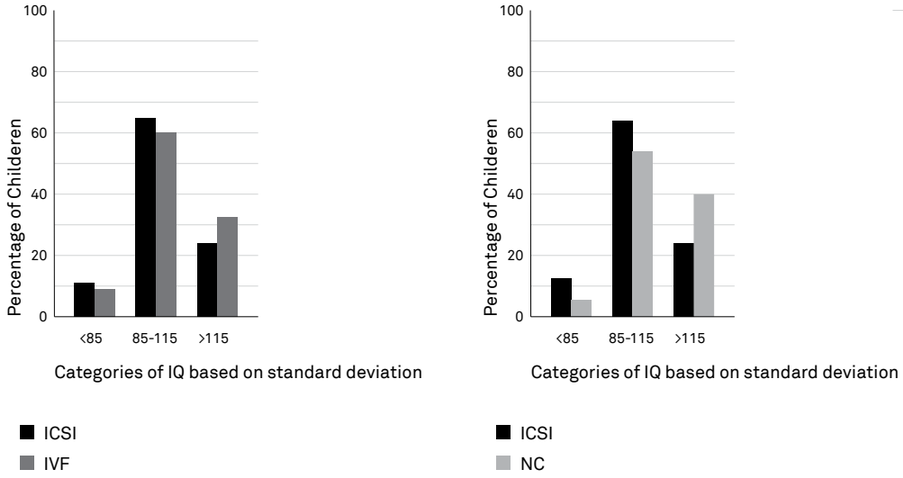

Figure 1. Percentage of children with IQ-scores <85, 85-115, and >115: ICSI versus IVF (p=0.268) and ICSI versus NC (p=0.019)

When comparing ICSI-children with NC-controls, both groups attending regular education, we found a difference in mean RAKIT-IQ of nearly 7 points in favour of NC-controls (ICSI 103, NC 110, mean difference 6.8, 95%CI [2.0; 11.6]) (Table 2). ICSI-children performed worse on all subtests with differences in mean scores ranging from 0.7 to 2.1. Significance was reached for the subtests Verbal Meaning, Learning Names and Hidden Figures. The results were consistent in age categories <6; 6-7; 7-8; >8 and for gender. In the three IQ-categories based on the standard deviation, the percentages were: IQ <85 ICSI 12% vs. NC 6%; IQ 85-115 ICSI 64% vs. NC 54%; IQ >115 ICSI 24% vs. NC 40% (ordinal regression analysis p=0.019) (Figure 1).

Regarding the clinical question, about the overall difference in cognitive development between ICSI and NC-children, the adjusted difference varied between 5 and 7 (Table 3), with the confidence intervals excluding zero. The two models included the main variables that are generally considered important in determining a child's IQ and in which the groups differed; with exception of variables that are assumed to be in the causal pathway from artificial conception procedure to intelligence outcome. The difference in paternal smoking habits did not account for the difference in IQ. The adjusted p-values for the difference in distribution over the three IQ-categories were 0.045 and 0.064. Regarding the biological question, about the net difference, we additionally adjusted for: caesarean section, premature birth, birth weight, low birth weight and small for gestational age (Table 3). Due to an effect of low birth weight, the adjusted mean difference decreased to 5.0, 95%CI [0.2; 9.8]. The p-value for the difference in distribution over the three IQ-categories was 0.067 after correction for maternal education, parity, socio-economic status, prematurity, and low birth weight.

Table 3. Linear regression analysis on the effect of conception mode on IQ-score

ICSI and IVF	Mean difference*	95%CI
Conception crude	3.9	[-0.7; 8.4]
Adjustment for:		
paternal education, pregnancy complications	3.6	[-0.8; 8.0]

ICSI and NC	Mean difference †	95%CI
Conception crude	6.8	[2.0; 11.6]
Clinical question, adjustment for:		
maternal education, parity, SES ‡	5.6	[0.9; 10.3]
maternal education, parity, SES, maternal age, paternal age	7.1	[1.7; 12.5]
Biological question, adjustment for:		
maternal education, parity, SES, caesarian section	5.6	[0.9; 10.3]
maternal education, parity, SES, prematurity	5.4	[0.5; 10.2]
maternal education, parity, SES, birth weight	5.7	[0.9; 10.4]
maternal education, parity, SES, low birth weight	5.0	[0.2; 9.8]
maternal education, parity, SES, small for gestational age	5.5	[0.7; 10.2]
maternal education, parity, SES, prematurity, low birth weight	5.1	[0.3; 9.9]

* in favour of IVF

† in favour of NC

‡ SES = socio-economic status

The number of children attending special education was 1 out of 83 (1.2%) among ICSI-children and 4 of 83 (4.8%) among IVF-children; of the latter four, two had been born preterm. In the total group of ICSI-children, including prematurely born children, 1 of 87 (1.1%) followed special education. In the Dutch population 1.0% of children aged 5-7 years attended special schools in 2004/2005.[24]

Discussion

This study of the cognitive development of 5 - 8-year-old ICSI-children has found a lower adjusted mean IQ (not statistically significant) among ICSI-children in comparison with IVF-children and a lower adjusted mean IQ (statistically significant) among ICSI-children relative to naturally conceived controls. As compared to NC-children, ICSI-children had statistically significant lower scores on three subtests (verbal learning and perceptual reasoning scales) and the IQ-distribution in total shifted to lower IQ-scores. The percentage of ICSI-children attending special education was similar to the reference population.[24]

The difference in IQ between ICSI and IVF-children was 3.6 points and was greater in boys than in girls. Based on this study, the IQ of children conceived through ICSI may be expected to be between 5 and 7 points lower than of those conceived naturally, among parents with similar characteristics up to the time of conception. The net difference in IQ between ICSI and NC-children, i.e. the difference after additional adjustment for prematurity, (low) birth weight, small for gestational age status, and caesarean section, was 5 points.

ICSI and IVF-children were invited independently of school performance and could be analysed without limitations. Because all NC-controls were recruited from regular pre- and primary schools, the ICSI-NC comparison was restricted to children attending regular education.

Assigning an estimated score of 84 to the IVF-girls with developmental delay might be an overestimation of their skills. Assigning a score of 70 would have resulted in an adjusted mean IQ-difference between ICSI and IVF of 3.2 [-1.2; 7.7].

The use of multiple observers did not influence our results as they were blinded and haphazardly distributed over the children. Besides, the analysis of variance showed no differences in IQ-scores between the investigators.

The clinical significance of the differences in IQ between ICSI-children and both IVF and NC-controls is debatable. On the one hand, the mean IQ of ICSI-children was within the normal range and the mean differences of 3-7 points were less than half a standard deviation (population mean of 100, standard deviation 15; Dutch children attending regular education, 1987)[20]. On the other hand, a shift of the total ICSI-population to lower IQs may result in children crossing borders at the lower edge of the normal range. Indeed, ICSI-children more often scored <85 than NC-children.

Strengths and weaknesses of the study

The strength of this study lies in the assessment within a single centre/laboratory, the careful selection criteria, the matched and controlled design, and the blinded assessment of each individual child. We have compared ICSI-children with both IVF-children and children born after natural conception. Additionally, we have assessed the children at a later age, which increases the predictive value of the test outcomes.[25]

Our sample size is not large, but this is less important in a study with positive (difference found) than with negative results (no difference found). Larger sample sizes permit controlling for multiple confounders. By strict matching we have decreased the number of confounders to control for and as a consequence the precision of our results is fairly high despite the smaller sample size.

With response rates of 79% and 73% we assume that the samples are representative of the population of ICSI and IVF-children at this centre. Selection bias could have occurred if parents decided to enrol their child based on the child's (low or high) developmental status and if this selection differed between the ICSI and IVF-group. However, with the common background of infertility we have assumed that ICSI and IVF-parents had comparable motives to participate and that selection bias will not have influenced our results. The higher rate of participation among

upper socio-economic status families will not have influenced our outcomes as we matched for socio-economic status and the rates were comparable between ICSI and IVF. The higher rate of male gender in IVF non-participants (n=34) was unexpected and could not be explained.

A limitation of our study was that 4 of the 6 preterm ICSI-children were excluded as we had difficulty finding a matching preterm IVF-child. Our conclusions therefore mainly apply to full-term ICSI and IVF-children.

The representativeness of the natural conception control group might be a point of discussion. This group may have been subject to selection as we cannot examine potential differences between responders and non-responders. The low socio-economic status group might have been at highest risk for selection bias, as of the 16 schools that participated, 9 were schools with low socio-economic status, while eventually only seven control children with low socio-economic status applied. In the ICSI-group, 59% of low socio-economic status children participated. Excluding the children of low socio-economic status from the ICSI versus NC analysis indeed resulted in a decrease of the adjusted difference from 5.6 to 4.5 [-0.4; 9.4]. An argument against selection bias might be found in the fact that the direction of the difference in IQ was similar to the difference when ICSI and IVF-children were compared.

When comparing ICSI and IVF-children, the effect of the procedure can never be detached from the type of underlying infertility, since ICSI will be the treatment of choice in couples with male infertility, while in couples with female infertility IVF will generally be offered first. A comparable drawback in the comparison of ICSI and NC was that known important differences between the ICSI and NC-group were adjusted for, but we could not assure that we allowed for all appropriate factors (residual confounding). Obtaining parental IQ-scores would have been a valuable extension.

Related studies

With one exception,[11] previous studies comparing the cognitive development of ICSI and IVF-children found no differences.[12-14, 16] Bowen et al.[11] showed that ICSI-children had a lower mean developmental score than IVF-children, a difference that was larger among boys than girls – as discussed by Te Velde et al.[26] Our findings are in line with those of Bowen et al., but their study has been criticised for using an unstandardised testing system, insufficient adjustment for demographic differences between groups, and inclusion of cryo and multiple pregnancies.[5, 13, 26, 27] However, the majority of studies had one or more of these or other limitations (e.g. low response rates, young age of the study group, unblinded observers).[12-14, 16] In the present study we accounted for these important points of critique.

No indication of delayed cognitive development in ICSI versus NC-children has been found[10, 14-18] apart from the report of Bowen et al.[11] Leunens et al.,[10] reported higher IQ-levels in 151, 8-year-old ICSI-singletons as compared to 153 NC-controls, although this effect might have been due to a difference in maternal educational level. In their study, the higher prevalence of prematurity in the NC-group combined with the lower IQ-scores of premature NC-children as compared to term NC-children may have also lowered the mean IQ-scores of the NC-group. The study by Ponjaert-Kristoffersen et al.[16] was potentially the most reassuring, finding no differences in cognitive development between ICSI and NC-children, including 511 ICSI and 488 NC-children at age 4.5-5.5 years and allowing for appropriate matching and correction. Why our findings in children aged 5-8 years differ substantially is unclear, although of course we examined a different population of children.

In conclusion, in the relatively limited sample investigated, the cognitive development (IQ) of 5 - 8-year-old ICSI-singletons was slightly lower than of matched IVF and NC-children. We tried to safeguard the validity of our results by using blinded observers and by careful matching of singleton children between the ICSI and the IVF-group. Although selection bias and unmeasured confounders may still play a role in the origin of these differences, an effect of ICSI per se cannot be excluded.

References

(1) Andersen AN, Gianaroli L, Felberbaum R, De Mouzon J, Nygren KG. Assisted reproductive technology in Europe, 2001. Results generated from European registers by ESHRE. *Hum Reprod.* 2005; 20(5):1158-1176.

(2) Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet.* 1992; 340(8810):17-18.

(3) Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: a call for investigation. *Am J Hum Genet.* 2004; 74(4):599-609.

(4) Schultz RM, Williams CJ. The science of ART. *Science*. 2002; 296(5576):2188-2190.

(5) Sutcliffe AG. Intracytoplasmic sperm injection and other aspects of new reproductive technologies. *Arch Dis Child.* 2000; 83(2):98-101.

(6) Tournaye H. ICSI: a technique too far? *Int J Androl*. 2003; 26(2):63-69.

(7) Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *JAMA*. 2002; 288(6):728-737.

(8) Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ*. 2004; 328(7434):261-264.

(9) Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol.* 2004; 103(3):551-563.

(10) Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. Cognitive and motor development of 8-year-old children born after ICSI compared to spontaneously conceived children. *Hum Reprod.* 2006; 21(11):2922-2929.

(11) Bowen JR, Gibson FL, Leslie GI, Saunders DM. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. *Lancet.* 1998; 351(9115):1529-1534.

(12) Bonduelle M, Joris H, Hofmans K, Liebaers I, Van Steirteghem A. Mental development of 201 ICSI children at 2 years of age. *Lancet.* 1998; 351(9115):1553.

(13) Bonduelle M, Ponjaert I, Steirteghem AV, Derde MP, Devroey P, Liebaers I. Developmental outcome at 2 years of age for children born after ICSI compared with children born after IVF. *Hum Reprod.* 2003; 18(2):342-350.

(14) Leslie GI, Gibson FL, McMahon C, Cohen J, Saunders DM, Tennant C. Children conceived using ICSI do not have an increased risk of delayed mental development at 5 years of age. *Hum Reprod.* 2003; 18(10):2067-2072.

(15) Ponjaert-Kristoffersen I, Tjus T, Nekkebroeck J, Squires J, Verte D, Heimann M et al. Psychological follow-up study of 5-year-old ICSI children. *Hum Reprod.* 2004; 19(12):2791-2797.

(16) Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, Nekkebrocck J, Loft A, Wennerholm UB et al. International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. *Pediatrics*. 2005; 115(3):e283-e289.

(17) Sutcliffe AG, Taylor B, Li J, Thornton S, Grudzinskas JG, Lieberman BA. Children born after intracytoplasmic sperm injection: population control study. *BMJ.* 1999; 318(7185):704-705.

(18) Sutcliffe AG, Taylor B, Saunders K, Thornton S, Lieberman BA, Grudzinskas JG. Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study. *Lancet*. 2001; 357(9274):2080-2084.

(19) Van Duijn C, Keij I. Sociaal-economische status indicator op postcodeniveau. *Maandstatistiek van de bevolking*. 2002; 2:32-35.

(20) Bleichrodt N, Resing WCM, Drenth PJD, Zaal JN. *Intelligentie-meting bij kinderen.* Lisse: Swets & Zeitlinger BV; 1987.

(21) Leslie GI. Mental development of children conceived using intracytoplasmic sperm injection. The current evidence. *Minerva Ginecol.* 2004; 56(3):247-257.

(22) Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand*. 1991; 80(8-9):756-762.

(23) Statistics Netherlands (CBS). Standaard Onderwijs Indeling (SOI). *StatLine, Central Bureau of Statistics*. 1998; (Accessed January 9, 2006, at http://www.cbs.nl).

(24) Statistics Netherlands (CBS). Basisonderwijs; leerlingen. *StatLine, Central Bureau of Statistics.* 2005; (Accessed January 9, 2006, at http://www.cbs.nl).

(25) Sternberg RJ, Grigorenko EL, Bundy DA. The predictive value of IQ. *Merril-Palmer Quarterly.* 2001; 47(1):1-41.

(26) Te Velde ER, Van Baar AL, Van Kooij RJ. Concerns about assisted reproduction. *Lancet.* 1998; 351(9115):1524-1525.

(27) Sutcliffe AG, Taylor B, Grudzinskas G, Thornton S, Lieberman B. Children conceived by intracytoplasmic sperm injection. *Lancet.* 1998; 352(9127):578-579.

Chapter 4

Perinatal outcome, health, growth, and medical care utilisation of 5 - 8-year-old ICSI-singletons

4.

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Fertility and Sterility, in press

Abstract

Objective: To evaluate short and long-term health in ICSI-singletons.

Design: Follow-up study.

Setting: University medical centre, assessments between March 2004 and May 2005.

Patients: Singletons born between June 1996 and December 1999 after ICSI in the Leiden University Medical Center laboratory were compared with matched singletons born after IVF and natural conception (NC).

Intervention: Mode of conception.

Main outcome measures: An examiner blinded to the conception mode of the child assessed congenital malformations and growth. Information on pregnancy, perinatal period, birth defects, general health, and medical consumption was obtained through questionnaires.

Results: Outcomes of ICSI and IVF-children (n = 81/81, preterm infants excluded) were comparable or even more positive for ICSI. Perinatal outcomes were poorer after ICSI than NC: prematurity: p = 0.014; low birth weight: OR = 7.4, 95%CI [0.9; 62.5]; mean birth weight: Δ = 186g, 95%CI [21; 351]. ICSI-mothers had more pregnancy complications (n = 33 vs. 18) and in-hospital deliveries (PR = 1.36, 95%CI [1.17; 1.48]). No further differences were found between ICSI and NC-children on congenital malformations, health, growth, and medical consumption (n = 87/85, preterm infants included).

Conclusions: No adverse health outcomes were identified in ICSI-singletons up to age 5-8 as compared to IVF and NC-singletons, besides poorer perinatal outcomes after ICSI versus NC.

Introduction

Intracytoplasmic sperm injection (ICSI) is an invasive method of artificial reproduction. Besides the mechanical damage that may occur due to the injection, fertilisation may take place with oocytes and spermatozoa of lesser quality because natural selective barriers are circumvented.[1-4] To evaluate the potential of negative consequences of the ICSI-procedure, ICSI-offspring is closely monitored on a wide range of outcome measures: e.g. chromosomal aberrations,[5] birth defects,[6] perinatal outcome,[7,8] and development.[9] Although the technique was introduced in 1992,[10] follow-up studies have not reached beyond the age of 5 years except for one recent project.[11,12]

In the present study we focus on pregnancy and perinatal outcome, congenital malformations and dysmorphic features, general health, growth, and medical care utilisation up to age 5-8. By assessing this wide scope of outcomes in one defined group of children (born after ICSI, IVF or natural conception (NC)) we aim to minimise selection and information bias. Besides, in reviewing the literature, general health, growth, and medical care utilisation of ICSI-children appear not to have been studied widely beyond the perinatal period into school-age.[11,13,14]

We differentiate two research questions. First, we assess the potential negative effect of ICSI superimposed to IVF on the various outcome measures. Both ICSI and IVF-children have a background of parental subfertility, maternal hormonal stimulation, fertilisation in vitro, and an increased risk of prematurity and low birth weight; the procedure of fertilisation differs. Second, we investigate the overall effect of ICSI as compared to NC to answer the future parents' question: will the health outcome of my child differ if it is born after ICSI instead of natural conception, given similar parental characteristics up to the time of conception?

Materials and methods

The Institutional Review Board approved the study design. The authors have no conflicts of interest to declare. At least one of the parents signed for informed consent. Data collection was carried out between March 2004 and May 2005.

Selection and matching

ICSI-singletons born between June 1996 and December 1999 after fertility treatment in the Leiden University Medical Center were invited. Exclusion criteria were: oocyte or sperm donation, cryopreservation of the embryo and selective embryo reduction with medical indication. Identical inclusion criteria were used in the selection of IVF-children, who were matched person-to-person to ICSI-participants for gender, socio-economic status, gestational age [preterm/term], maternal age at the time of pregnancy [±3 years] and birth date [closest]. Socio-economic status low, medium or high was ascribed using the zip-code/socio-economic status indicator of Statistics Netherlands,[15] based on home price and income. If no match was available within the maternal age range of ±3 years, larger deviations were permitted.

Regular pre-schools and primary schools with zip-codes that indicated social class distributions similar to the ICSI-cohort assisted in the recruitment of naturally conceived singletons. We applied group matching on socio-economic status, gender and birth date. The composition of the NC-control group from regular schools was reasonable as only one ICSI-child attended special education.

Data collection

Three detailed questionnaires (see Appendix) were filled out by the parents: 1. General information, 2. Pregnancy and birth, and 3. Health of the child. Parental educational level was indexed according to the SOI-register (standard education classification) of Statistics Netherlands.[16] The parents were requested to bring the 'baby book' (given to all mothers at the infant welfare centre, where growth and other parameters are measured) or the obstetric data form to guide them through questions on birth parameters. Information on the incidence of vanishing twins in ICSI and IVF-pregnancies and on the time of vanishing was retrieved from obstetric records. As a part of the questionnaire on child's health we used the WHO/Region survey[17-19] to assess airway symptoms. Outcome consisted of prevalence and severity of the following symptom clusters and diagnoses: shortness of breath, wheeze, asthma,

cough and phlegm, cough, runny/congested nose, and pneumonia. The questions covered the full history of the child as well as the past 12 months only.

In a physical examination, congenital malformations and dysmorphic features were reported by an investigator who was blinded for mode of conception. A clinical geneticist, also blinded for conception mode, categorised the malformations in major malformations and minor malformations/dysmorphic features.

We examined 3D-vision with a stereo test, using a Polaroid 3D Vectograph. Growth of the child was assessed by measuring height, weight on a calibrated balance, and head circumference using a non-stretching measuring tape.

The use of three detailed questionnaires resulted in missing values. The number of missing values was referred to in the tables by various symbols as explained in the legends.

Definitions

International definitions were followed for preterm (gestational age< 37 weeks), very preterm (gestational age< 32 weeks), low birth weight (< 2500g), very low birth weight (< 1500g), and small for gestational age (birth weight for gestational age< -2SDS).[20]

Other definitions include: gestational hypertension: hypertension without proteinuria developing in the latter part of pregnancy in a previously normotensive woman. Pre-eclampsia: onset of hypertension and proteinuria after 20 weeks of gestation or proteinuria superimposed upon chronic hypertension. Gestational diabetes: glucose intolerance of variable degree with onset or first recognition during pregnancy. Ovarian hyperstimulation syndrome: combination of ovarian enlargement due to multiple ovarian cysts and an acute fluid shift out of the intravascular space.

Congenital malformations and dysmorphic features were studied on the basis of the Q-codes (Q0-99) of the ICD10 and on the textbook by Aase.[21] Major malformations were defined to cause functional impairment and/or to require surgical correction. Complexity of the malformation and rarity of occurrence were also considered.

Statistics

Statistical analysis was performed with the SPSS 11.0 for Windows package (SPSS Inc., Chicago, IL). Continuous data were analysed with an independent t-test if a normal distribution was likely and with a Mann-Whitney test if the distribution was skewed. Categorical data were analysed using Pearson's Chi-square test. We performed linear and logistic regression analysis to adjust for confounders. Statistical significance was reached if $p<0.05$. Differences in continuous data were presented as a mean difference and 95% confidence interval (95%CI). Differences in categorical data (2x2) were expressed in terms of Odds Ratios (OR) and 95%CI if the prevalence of the outcome was <10% in at least one group. If the prevalence exceeded 10% in both groups the Prevalence Ratio (PR) and 95%CI was given, as the OR would over- or underestimate the relative risk in that situation.[22] P-values were provided for categorical data with more than two categories. Multi-testing correction was not performed; instead data were interpreted with caution and in the light of previous literature.

Results

Selection

One hundred and ten ICSI-children met the inclusion criteria, 87 of whom enrolled on the study (79%). Of the 257 eligible IVF-children 126 potential matches were invited and 92 (73%) participated. Two IVF-controls were available for five ICSI-children, and the best match was selected (n=87). Extension of the range of maternal age beyond ±3 years was required in 11 cases. Among the total cohort of ICSI-children, eight had been born prematurely, six of whom entered the study. Proper IVF-matches could only be found for 2 of the 6 preterm children. We decided to restrict the ICSI-IVF comparison to children born at term, because the two preterm ICSI/IVF-couples could not represent all preterm ICSI and IVF-children and confounding would be introduced if preterm ICSI-children were matched with term IVF-children (n=81/81).

Eighty-seven children from sixteen grade schools were enrolled, of whom 2 were excluded; one for being a twin and one for being conceived with intrauterine insemination (n=85). The ICSI-NC comparison was not restricted to term children because we wanted to assess the overall effect of ICSI on the outcome measures. This included the potentially negative effect of prematurity.

Demographic characteristics

Table 1 shows the demographic characteristics. Naturally conceived children were slightly older than ICSI-children at the time of the assessment (mean 0.2 years, 95%CI [0.02; 0.4]). In the ICSI-group parity was lower than in the NC-control group (PR first-born 2.0 95%CI [1.7; 2.3]). The level of education of NC-mothers was higher than of ICSI-mothers ($p=0.016$). At the time of pregnancy, the age of ICSI-parents was higher than of NC-parents (mean difference maternal age 2.3 [1.1; 3.5], paternal age 4.3 [2.7; 5.9]). The differences in prevalence and type of infertility factors were inherent to differences in modes of conception, as the choice for ICSI or IVF largely depends on type of infertility. NC-mothers were less likely than ICSI-mothers to use folic acid for the full period of -4 to +8 weeks around conception, with 15 ICSI-mothers (17%) and 35 NC-mothers (42%) not taking folic acid at all ($p<0.001$). ICSI-fathers smoked more heavily than smoking IVF and NC-fathers (PR ICSI vs. IVF for smoking >10 cigarettes per day per smoker: 1.8 [1.0; 2.3], PR ICSI vs. NC: 2.1 [1.1; 2.7]).

When differences in main outcome measures were detected between ICSI and IVF or ICSI and NC, we investigated the association of the abovementioned variables with the particular outcome measure to explore the possibility of confounding. If an association was present and reasonable, the variable was entered as a covariate in a regression model or used for stratification.

Table 1. Demographic characteristics of parents and children: ICSI versus IVF and ICSI versus NC

	ICSI	IVF	ICSI	NC
	n=81	n=81	n=87	n=85
Gender: male, n(%)	40 (49)	40 (49)	44 (51)	47 (55)
Age at examination, mean	6.1 (5.3-7.7)	6.2 (5.3-8.3)†	**6.1 (5.3-7.7)**	**6.3 (5.1-8.0)**
Parity: first-born, n(%)	61 (75)	59 (73)	**65 (75)**	**31 (37)**
Socio-economic status, n(%)				
low	8 (10)	8 (10)	10 (12)	7 (8)
medium	26 (32)	26 (32)	27 (31)	18 (21)
high	47 (58)	47 (58)	50 (58)	60 (71)
Level of education, n(%)				
mother		*		
no education	0 (0)	1 (1)	**0 (0)**	**0 (0)**
low	25 (31)	25 (31)	**27 (31)**	**11 (13)**
medium	28 (35)	27 (34)	**29 (33)**	**37 (44)**
high	28 (35)	27 (34)	**31 (36)**	**37 (44)**
father	*		*	
no education	0 (0)	2 (3)	0 (0)	1 (1)
low	28 (35)	26 (32)	31 (36)	22 (26)
medium	26 (33)	16 (20)	26 (30)	26 (31)
high	26 (33)	37 (46)	29 (34)	36 (42)
Ethnicity §, n(%)				
mother: non-Caucasian	7 (9)	9 (11)	9 (10)	8 (9)
father: non-Caucasian	8 (10)	8 (10)	10 (12)	11 (13)
Primary language, n(%)				
Dutch	74 (91)	73 (90)	78 (90)	76 (89)
other	1 (1)	3 (4)	1 (1)	4 (5)
bilingual	6 (7)	5 (6)	8 (9)	5 (6)
Parental age at pregnancy, mean				
mother	32.8 (22-41)	33.4 (24-42)	**32.8 (22-41)**	**30.6 (20-41)**
father	36.9 (23-65)	37.3 (27-60)	**36.9 (23-65)**	**32.6 (20-49)**
Diagnosed infertility factor, n(%)				
mother	**13 (16)**	**37 (46)**	**15 (17)**	**0**
uterus pathology	0	2	0	0
hormonal	5	10	7	0
tuba pathology	7	23	7	0
endometriosis	1	2	1	0
father II	**64 (79)**	**11 (14)**	**70 (80)**	**0**
ATZ e causa ignota	54	11	59	0
ATZ chemotherapy	5	0	6	0
ATZ vasectomy	4	0	4	0
ATZ chromosomal	1	0	1	0
Medicine during pregnancy, n(%)	10 (13)*	8 (10)	10 (12)*	14 (17)‡
Folic acid use: -4 to +8 weeks, n(%)	59 (73)	57 (70)	**62 (71)**	**32 (39)†**
Smoking during pregnancy, n(%)				
mother	*		*	
no	70 (88)	70 (86)	76 (88)	75 (88)
yes, <10 per day	9 (11)	10 (12)	9 (11)	8 (9)
yes, >10 per day	1 (1)	1 (1)	1 (1)	2 (2)
father		†		*
no	57 (70)	61 (77)	61 (70)	62 (74)
yes, <10 per day	7 (9)	11 (14)	9 (10)	15 (18)
yes, >10 per day	17 (21)	7 (9)	17 (20)	7 (8)
Family situation, n(%)				
parents living together	71 (88)	77 (95)	77 (89)	75 (88)

* 1 missing value
† 2 missing values
‡ 3 missing values
§ Turkey classified under non-Caucasian
II AsthenoTeratooligoZoospermia
bold p<0.05

Pregnancy and perinatal period

Table 2 summarises the pregnancy and perinatal parameters. In the comparison between ICSI and IVF, we found a non-significant increase in the number of mothers with a pregnancy complication in the IVF-group (PR 1.6 [0.9; 2.4]), with a similar distribution of pregnancy complications in both groups except for gestational diabetes (p=0.04).

The ICSI and NC groups were different in total number of pregnancy complications, which was mainly due to an increase in prematurity (ICSI n=6, NC n=0, p=0.01; all ≥32 wks and <37 wks) and the occurrence of vanishing twins. No information on vanishing twins was available for NC-controls, but the expected incidence was low due to a low general incidence of twinning after natural conception. Stratification by parity and maternal education mainly showed that the difference between ICSI and NC in the occurrence of vanishing twins was highest in low parity women.

The frequency of hospital admissions of the mother in the period around labour due to complications of mother or child (e.g. hypertension, instrumental delivery, small for gestational age) was higher after IVF than after ICSI (PR 1.6 [1.1; 2.1]), but comparable after ICSI versus NC. Except an increased frequency of labour induction, the increase in admissions of IVF-mothers was not due to one or more specific complications.

Birth parameters for ICSI and IVF-children were similar. ICSI-children had lower birth weights than NC-controls (mean difference 186g [21; 351]; low birth weight OR 7.4 [0.9; 62.5]). After correction for parity, maternal education, and parental age the difference in mean birth weight was no longer statistically

Table 2. Pregnancy, birth, and perinatal period: ICSI versus IVF and ICSI versus NC

	ICSI n=81	IVF n=81	ICSI n=87	NC n=85
Pregnancy complications, n				
hypertension	4	5	5	3
pre-eclampsia	2	3	2	1
gestational diabetes	**0**	**4**	0	2
ovarian hyperstimulation syndrome	3	1	3	0
extra-uterine gravidity	0	1	0	0
vanishing twin	6	7	9	.
term unknown	1	4	3	.
<9 wks	4	1	4	.
9-21 wks	0	2	1	.
>21 wks	1	0	1	.
prematurity (gest. age <37 wks)	0	0	**6**	**0**
other	5	7	8	12
total no. of pregnancy compl.	20	28	33	18
mothers with pregn compl., n(%)	17 (21)	27 (33)	23 (26)	17 (20)
Hospital admission mother, n(%)				
during pregnancy	6 (7)	7 (9)	7 (8)	8 (10)†
peri-labour due to complication	**24 (30)**	**38 (48)***	28 (32)	30 (35)
duration (days), median	3 (1-49)*	2 (1-20)*	3 (1-49)*	2 (1-42)
Caesarian section, n(%)	11 (14)	9 (11)	12 (14)	6 (7)
Birth parameters				
gestational age, mean	40.1 (37-43)	39.8 (37-42)	39.9 (35-43)	39.8 (37-43)
birth weight, mean	3447 (2300-4750)	3379 (1835-4730)	**3370 (1485-4750)**	**3555 (2300-4800)**
birth weight <2500g, n(%)	3 (4)	3 (4)	**7 (8)**	**1 (1)**
small for gestational age §, n(%)	4 (5)	2 (3)	6 (7)	1 (1)
if Apgar score available, n(%):	57 (70)	58 (72)	60 (69)	62 (73)
Apgar 1min<5 or 5min<7, n(%)	2 (4)	2 (3)	2 (3)	1 (2)
Hospital admission child				
admission at birth, n(%)	17 (21)	24 (30)	23 (26)	23 (27)
duration (days), median	4 (1-10)*	3 (1-42)‡	4.5 (1-19)*	2.5 (1-45)*
Feeding after birth				
breastfeeding, n(%)	64 (79)	58 (73)*	70 (81)	67 (80)*
duration (months), median	4 (0-36)*	4 (0-21)	4 (0-36)*	5 (0-40)
problems with feeding, n(%)	14 (17)	20 (25)	16 (18)	17 (20)*

* 1 missing value

† 2 missing values

‡ 3 missing values

§ Sweden, Niklasson,[20] birth weight for gestational age< -2SDS

bold p<0.05

significant. The OR for low birth weight decreased to 4.7 [0.5; 40.8] after correction for maternal education and maternal age. ICSI-children tended to be small for gestational age more often than NC-children (OR 6.2 [0.7; 52.8], adjusted OR 4.4 [0.5; 39.4]). Of the 6 preterm children 4 were low birth weight children (including 1 very low birth weight) and 2 were small for gestational age.

Congenital malformations

Estimation of major malformation risk (Table 3) was not possible, due to the small size of the study cohorts. ICSI-children did not differ in the prevalence of minor malformations and dysmorphic features from IVF-children (40% vs. 43%, PR 0.9 [0.6; 1.3]) or NC-children (39% vs. 32%, PR 1.2 [0.8; 1.7]). The numbers of minor malformations per child were also similar (p=0.256 and p=0.134).

Table 3. Congenital malformations: ICSI versus IVF and ICSI versus NC

	ICSI n=81	IVF n=81	ICSI n=87	NC n=85
Major malformation, n(%)*	5 (6.2)	3 (3.8)	6 (6.9)	5 (5.9)
iris coloboma	x		x	
aniridia	x		x	
triple ventricular septal defect				x
atrium septum defect				x
pulmonary artery stenosis				x
submucous cleft palate	x		x	
duodenum atresia		x		
anus atresia		x		
undescended testes †	x		x	x
hypospadia	x		x	
additional muscle in arm				x
primary lymph edema			x	
alopecia				x
tuberous sclerosis		x		
Minor malformation/dysmorphic feature, n(%)*	32 (40)	34 (43)	34 (39)	27 (32)
1 minor malformation	23 (72)	26 (77)	25 (74)	24 (89)
2 minor malformations	9 (28)	6 (18)	9 (27)	3 (11)
3 or more minor malformations	0	2 (6)	0	0

* number of children (percentage); major malformations not mutually exclusive

† undescended testes needing surgery

Table 4. General health up to age 5-8 years: ICSI versus IVF and ICSI versus NC

	ICSI	IVF	ICSI	NC
	n=81	n=81	n=87	n=85
Pulmonary symptoms, n(%)				
shortness of breath	12 (15)†	10 (13)*	12 (14)†	9 (11)
wheezing	24 (31)‡	29 (36)*	26 (31)‡	25 (29)
asthma	7 (9)*	5 (6)	8 (9)*	10 (12)
coughing of sputum	10 (13)*	6 (8)*	10 (12)*	12 (14)
cough	28 (35)*	29 (36)	29 (34)*	20 (24)
runny nose	39 (49)*	31 (38)	40 (47)*	39 (46)
pneumonia	11 (14)*	9 (11)*	12 (14)*	7 (8)
Severity if symptoms, mean				
shortness of breath	2.5 (1-5)	2.8 (1-5)	2.5 (1-5)	2.9 (1-6)
wheezing	2.9 (1-8)	3.0 (1-12)	2.8 (1-8)	2.5 (1-7)
asthma	3.0 (1-9)	3.0 (1-5)	2.8 (1-9)	1.9 (1-7)
coughing of sputum	1.5 (1-4)	1.3 (1-3)	1.5 (1-4)	1.7 (1-2)
coughing	1.9 (1-5)	1.8 (1-5)	1.8 (1-5)	1.8 (1-4)
runny nose	2.1 (1-11)	1.8 (1-4)	2.1 (1-11)	2.0 (1-4)
pneumonia	1.3 (1-2)	1.4 (1-3)	1.3 (1-2)	1.1 (1-2)
Adenoidectomy	19 (23)	11 (14)	22 (25)	16 (19)
Cystic fibrosis	0	0	0	0
Bladder infection				
never	70 (86)	72 (89)	76 (87)	76 (89)
once	7 (9)	4 (5)	7 (8)	6 (7)
twice or more	4 (5)	5 (6)	4 (5)	3 (4)
Testis down-migration at birth	n=40†	n=40	n=44†	n=47
two-sided	35 (92)	35 (88)	39 (93)	41 (87)
one-sided	1 (3)	4 (10)	1 (2)	2 (4)
undescended testes	2 (5)	1 (3)	2 (5)	4 (9)
Term of descent	n=3	n=5	n=3	n=6§
<2 years old	2	4	2	2
>2 years old	1	1	1	0
Surgical descent	1	0*	1	1
Inguinal hernia	2 (3)	3 (4)	2 (2)	3 (4)
Umbilical hernia	2 (3)	2 (3)	2 (2)	0 (0)
Convulsion				
with fever	7 (9)*	4 (5)	7 (8)*	2 (2)
without fever	1 (1)II	2 (3)	1 (1)II	0 (0)*
epilepsy	0 (0)*	2 (3)	0 (0)*	0 (0)
Any cancer	0 (0)	1 (1)	0 (0)	0 (0)
Ear and hearing				
deviation	5 (6)	5 (6)	5 (6)	8 (9)
ear tubes	11 (14)*	6 (7)	14 (16)*	6 (7)
hearing aid	0*	0	0*	0
Eye and vision				
vision				
good	72 (92)‡	76 (95)*	78 (93)‡	79 (93)
moderate, needs glasses	5 (6)	4 (5)	5 (6)	5 (6)
severely impaired	0 (0)	0 (0)	0 (0)	1 (1)
blind one eye	1 (1)	0 (0)	1 (1)	0 (0)
blind two eyes	0 (0)	0 (0)	0 (0)	0 (0)
colour blindness	0 (0)*	3 (4)	0 (0)†	1 (1)
strabismus	4 (5)	2 (3)	4 (5)	1 (1)
treated	3	2	3	1
surgery	1	1	1	1
3D-vision		†		*
optimal	74 (91)	78 (99)	80 (92)	82 (98)
semi-optimal	5 (6)	0 (0)	5 (6)	1 (1)
bad	2 (3)	1 (1)	2 (2)	1 (1)
General problems past 6 months				
sleeping	9 (11)	3 (4)*	9 (10)	7 (8)
eczema	15 (19)	17 (21)	15 (17)	19 (22)
eating	10 (12)	6 (7)	12 (14)	5 (6)
crying	6 (7)	6 (7)	6 (7)	5 (6)
restless	10 (13)*	14 (17)	12 (14)*	11 (13)
headache	21 (26)	16 (20)	21 (24)	20 (24)
stomachache	5 (6)	5 (6)	5 (6)	3 (4)
bellyache	33 (41)	34 (42)	34 (39)	36 (42)
nauseous	13 (16)	22 (27)	13 (15)	21 (25)
vomiting	**18 (22)**	**33 (41)**	18 (21)	24 (28)
Allergy	13 (16)	15 (19)*	14 (16)	10 (12)
Parental perception child health		†		
healthier than peers	13 (16)	21 (27)	14 (16)	26 (31)
as healthy as peers	66 (82)	56 (71)	71 (82)	57 (67)
less healthy than peers	2 (3)	2 (3)	2 (2)	2 (2)

* 1 missing value
† 2 missing values
‡ 3 missing values
§ 4 missing values
II 5 missing values
bold p< 0.05

General health up to age 5-8 years

General health up to the examination at 5-8 years of age (Table 4) was very similar when ICSI-children were compared to IVF or NC-control children. ICSI-children were not at a higher risk to develop urogenital complications. Two IVF-children had been diagnosed with epilepsy.

Hearing deviations were noted by the parents in 5 ICSI (6%) and 5 IVF-children (6%), and reflected conductive hearing loss, hypersensitive hearing, and hemifacial microsomia. 11/80 children born after ICSI and 6/81 children born after IVF had ventilation tubes (OR 2.0 [0.7; 5.7]). Comparing ICSI and NC-children, ICSI-parents reported hearing deviations in 6% and NC-parents in 9%. All reports reflected conductive hearing loss. In the ICSI-group ventilation tubes tended to be more frequent (OR 2.6 [0.9; 7.0]).

Regarding eyes and vision, ICSI-children were comparable to IVF and NC-controls, except for a trend towards more colour blindness among IVF-children (p=0.082). Two ICSI-children had a congenital malformation of the eye. Astigmatism was present in one IVF-child and two NC-controls, and one NC-girl had had an asymmetrical growth rate of the irises.

The only significant difference that was found on general symptoms as noticed by the parents in the past 6 months, was an increased frequency of vomiting by IVF as compared to ICSI-children (PR IVF/ICSI = 1.8 [1.2; 2.6]).

We found a tendency of ICSI-parents showing a more reserved attitude in the perception of their child's health as compared to IVF and NC-parents: ICSI-parents were less inclined to perceive their child healthier than his or her peers (ICSI vs. IVF p=0.262; ICSI vs. NC p=0.078).

Table 5. Growth parameters at birth and at age 5-8 years: ICSI versus IVF and ICSI versus NC

	n	ICSI	IVF	*n*	ICSI	NC
Birth						
height (cm) (range)	*47/42*	50.5 (41-57)	50.5 (45-55)	*51/58*	50.3 (41-57)	50.9 (46-59)
weight (g)	*81/81*	3447 (2300-4750)	3379 (1835-4730)	*87/85*	**3370 (1485-4750)**	**3555 (2300-4800)**
head circumference (cm)	*13/13*	35.5 (33-37)	36.1 (33-39)	*17/24*	34.7 (28-37)	35.6 (33-40)
Age 5-8 years						
height (cm)	*81/79*	120.8 (107-142)	121.1 (108-142)	*87/85*	120.6 (107-142)	121.4 (108-141)
weight (kg)	*81/78*	23.2 (17-41)	23.2 (16-38)	*87/85*	23.1 (17-41)	23.8 (15-38)
head circumference (cm)	*81/79*	51.7 (49-58)	52.1 (48-56)	*87/84*	51.7 (49-58)	52.0 (49-55)
BMI* (kg/m^2)	*81/78*	15.8 (13-23)	15.7 (12-23)	*87/85*	15.8 (13-23)	16.0 (13-22)

bold p<0.05

* BMI = body mass index, weight/height^2

Growth

Growth parameters (Table 5) of ICSI and IVF-children were similar both at birth and at the time of examination. ICSI and NC-children had comparable heights and head circumferences, but differed in weight at birth (mean difference 186 g [21; 351]). At age 5-8 mean weights were comparable. If prematurely born children were excluded from the analyses, the mean birth weight of ICSI-children was 3447g (mean difference ICSI versus NC 108g [-48; 264]). This implies that the difference in birth weight was partially due to a higher incidence of prematurity in the ICSI-group.

Medical care utilisation

Table 6 shows the parameters of medical care utilisation. The number of hospital deliveries was similar for ICSI and IVF, but significantly lower for NC-control children (PR 0.7 [0.5; 0.9]). The adjusted prevalence ratio after correction for parity and maternal education was 0.9 [0.7; 1.0], p=0.121. IVF-children were more likely to have ever received physical therapy (OR 2.6 [1.0; 6.6]), largely due to more coordination problems (ICSI n=3, IVF n=8).

No further differences in medical care utilisation were found between ICSI and IVF-children, or between ICSI and NC-children.

Table 6. Medical care utilisation of the child: ICSI versus IVF and ICSI versus NC

	ICSI	**IVF**	**ICSI**	**NC**
	n=81	n=81	n=87	n=85
Hospital labour, n(%)	68 (84)	71 (89)*	**74 (85)**	**53 (62)**
Hospital admission child				
at birth, n(%)	17 (21)	24 (30)	23 (26)	23 (27)
duration (days), median	4 (1-10)*	3 (1-42)†	4.5 (1-19)*	2.5 (1-45)*
later in life, n(%)	24 (30)	31 (38)	27 (31)	28 (33)
no. of admissions =1	14 (58)	25 (81)	16 (59)	17 (61)
no. of admissions =2	6 (25)	0 (0)	6 (22)	7 (25)
no. of admissions ≥3	4 (17)	6 (19)	5 (19)	4 (14)
General practitioner visits, n(%)				
0 times per year	17 (21)	17 (21)	21 (24)	30 (35)
1-2 times per year	45 (56)	45 (56)	46 (53)	38 (45)
3-5 times per year	15 (19)	15 (19)	16 (18)	13 (15)
up to 10 times per year	2 (3)	3 (4)	2 (2)	2 (2)
>10 times per year	2 (3)	1 (1)	2 (2)	2 (2)
Treatment by medical specialist, n(%)	49 (61)	58 (72)	55 (63)	53 (62)
No. of specialists visited per child, n(%)				
1 specialist	25 (51)	35 (60)	27 (49)	26 (49)
2 specialists	16 (33)	17 (29)	19 (35)	21 (40)
≥ 3 specialists	8 (16)	6 (10)	9 (16)	6 (11)

Type of specialism, n(%)				
pediatrician	22 (27)	21 (26)	27 (31)	27 (32)
otolaryngologist	24 (30)	18 (22)	29 (33)	23 (27)
ophthalmologist	11 (14)	13 (16)	11 (13)	12 (14)
dermatologist	4 (5)	10 (12)	4 (5)	4 (5)
orthopedic surgeon	4 (5)	9 (11)	4 (5)	2 (2)
surgeon	6 (7)	8 (10)	6 (7)	9 (11)
urologist	6 (7)	2 (3)	6 (7)	2 (2)
other	9 (11)	11 (14)	11 (13)	7 (8)
total	86	92	98	86
Logopedics, n(%)	17 (21)*	11 (14)	19 (22)*	17 (20)
Physical therapy, n(%)	**7 (9)***	**16 (20)**	7 (8)*	10 (12)
Prescription medication past use, n(%)				
never	33 (41)	29 (36)	36 (41)	43 (51)
often	15 (19)	12 (15)	16 (18)	10 (12)
Prescription medication present use, n(%)				
never	69 (85)	64 (79)	75 (86)	75 (88)
often	5 (6)	7 (9)	5 (6)	6 (7)
Self-medication past use, n(%)				
never	40 (49)	39 (48)	43 (49)	45 (53)
often	1 (1)	2 (3)	1 (1)	3 (4)
Self-medication present use, n(%)				
never	69 (85)	65 (80)	73 (84)	69 (81)
often	0 (0)	2 (3)	0	3 (4)

* 1 missing value

† 3 missing values

bold p<0.05

Discussion

This study offers a reassuring contribution to the total spectrum of long-term follow-up of ICSI-offspring. ICSI-singletons showed very similar outcomes regarding pregnancy, perinatal period, congenital malformations, general health, growth, and medical care utilisation as IVF and NC-singletons up to the age of 5-8 years. As compared to IVF, ICSI-mothers were at lower risk for hospital admission around labour due to medical complications, and ICSI-children less often needed physical therapy. As compared to NC, ICSI-mothers showed an increased number of pregnancy complications, mainly due to a higher frequency of prematurity and vanishing twins. ICSI-children had lower mean birth weights and increased rates of low birth weight and hospital deliveries, but these differences were no longer significant after correction for differences in parental characteristics.

Strengths and weaknesses

Few studies have assessed health and growth at the age of 5 or older, or investigated medical care utilisation in ICSI-children. All children were prospectively examined for congenital malformations/dysmorphic features and growth by an observer blinded to the mode of conception. The presence of two matched, highly selected control groups contributed to the strength of our study.

A disadvantage of the current study was the small power to detect differences in both very rare and very common diseases. For example, our data on major congenital malformations were insufficiently numerous to draw conclusions. We nevertheless describe these data, as well as other results that demand caution in the interpretation, first because in our opinion it is important to be complete in reporting study results. Second, our findings may serve as indications for future research and may be of use in meta-analyses. However, in the present study, we avoid strong interpretations on weaker results and emphasise the need for further research.

The limitation of the ICSI-IVF comparison to term children did not interfere with our research question, as it did not interfere with the comparability of the two groups. However, it is a drawback that our results are only valid for term-born children. In the ICSI-NC comparison, preterm born children were included as we aimed to assess the overall effect of ICSI, irrespective of whether the causal pathway runs through the ICSI-procedure itself and/or through a potential consequence of the procedure, e.g. prematurity.

Selection bias could hardly ever be ruled out as ICSI and IVF-parents might have had different motives to enrol on the study than NC-parents. When ART-children are compared with NC-children, a potential effect of the underlying infertility in the ART-group should always be kept in mind. In future research, this issue may be solved by using NC-children born from previously subfertile couples as controls.[23] However, when comparing ICSI and IVF-children, the potential effect of the procedure can never be separated from the type of underlying infertility; couples with male infertility usually undergo ICSI, whereas couples with female infertility will generally rely on IVF.

In our study we relied on questionnaires about hearing and vision, while clinical examination would have yielded more accurate results. However, we consider the comparisons reliable, as (i) the three conception groups have been treated similarly and filled out the same questionnaire, (ii) children in the Netherlands are screened for hearing and vision disabilities in well-baby clinics and by school doctors (Primary Health Care 0-19 years), so major impairments were known, and (iii) the questions had been clear-cut. Minor impairments may indeed have been missed.

It is important to realise that follow-up may induce an increase in medical care utilisation by closely monitoring the children. Bias may occur if the intensity of follow-up is not similar among the various study groups.

In perspective of the literature

Demographics

The majority of the differences in demographic characteristics between ICSI, IVF, and NC-children could be explained by treatment indication (maternal/paternal infertility), the period of childlessness due to infertility (parental age, parity), or inadequate matching (maternal educational level).

We found an increased rate of periconceptional folic acid intake (from 4 weeks preconception until 8 weeks postconception) by ICSI-mothers as compared to NC-mothers (71% versus 39%). Ludwig et al. found a comparable - although smaller - difference in their study on antenatal care in singleton pregnancies born after ICSI (folic acid intake ICSI: 38%, general population: 6 - 25%).[24] Explanations for this difference between ICSI and NC-mothers may be that (i) ICSI-pregnancies are always planned, (ii) ICSI-pregnancies are usually achieved after a period of unwanted childlessness, so every effort will be done to give birth to a healthy child, and (iii) ICSI-parents are counselled by the gynaecologist or IVF-specialist.

The higher percentages of folic acid use in our study as compared to Ludwig et al.[24] probably reflect a good implementation of folic acid supplementation in Dutch antenatal care. This mainly involves the periconception use (pre- and postconception), which can be concluded from the comparable frequencies of folic acid use 'at some point in pregnancy' (this study: ICSI 83%, NC 58%; Ludwig et al.: ICSI 75%, general population 50-60%).

Pregnancy and perinatal period

Pregnancy complications increase after ART.[25,26] In the present study we found a trend of IVF-mothers being more prone to pregnancy complications than ICSI-mothers, which went along with a significant increase in hospital admissions of the mothers around labour. A potential increase of pregnancy complications in IVF-mothers versus ICSI-mothers was suggested by Kallen et al.,[26] but not confirmed by Govaerts et al. or Bonduelle et al.[14,27] A hypothesis might be that the higher rate of underlying maternal infertility factors in the IVF-group caused this increase (Table 1). Larger studies will have to be carried out to explore our finding that IVF-mothers have a higher incidence of gestational diabetes as compared to ICSI. An increased risk of gestational diabetes in ART-pregnancies with an odds ratio of ± 2.0 was already noted by Maman et al. and in the meta-analysis of Jackson et al.,[25,28] but no separate analyses were done comparing ICSI and IVF.

When ICSI and NC were compared in previous studies, an increased rate of pregnancy complications (both the number of mothers with a pregnancy complication and the total number) was found for ICSI.[14,26,29] We found the percentage of ICSI-mothers and NC-mothers with pregnancy complications to be comparable (i.e. not significantly elevated). However, the total number of pregnancy complications was higher with ICSI, which indicates a higher number of complications per mother. The lack of information on vanishing twins in the NC-group probably resulted in a slight underestimation of the frequency and number of pregnancy complications among NC-controls, but this will most likely not affect our conclusions.

Helmerhorst et al. and Jackson et al.[25,30] showed a clear-cut increase in adverse perinatal outcomes for ART-conceived children. Conflicting results were found on whether ICSI and IVF-pregnancies contribute equally to such an increase. Ombelet et al. showed higher rates of prematurity and low birth weight after correction for maternal age and parity in IVF versus ICSI-singletons,[31] while perinatal outcome of ICSI and NC-children was comparable.[7] These results may indicate that the effect of ICSI in poor perinatal outcome after ART is small and a primary effect of IVF can be hypothesised. However, other studies found perinatal outcomes of ICSI and IVF-singletons to be comparable,[27,32,33] and showed increased risks of prematurity, (very) low birth weight, and caesarean section for ICSI-singletons as compared to NC-children.[8,14,29,34] The contradiction in findings may be caused by differences in matching and adjustment. In the current study the differences in mean birth weight and frequency of low birth weight between ICSI and NC-children decreased upon correction for maternal age, maternal education, parity, and paternal age.

Congenital malformations

The sample size did not allow an extensive report on congenital malformations. From the literature, we know that children born after artificial reproductive techniques run a slightly higher risk for (major) congenital malformations than children born after natural conception, with an odds ratio most likely ranging between 1.3 and 1.4.[6,14,29,35-37] Increases have been reported clustering to the genitourinary,[35,38,39] cardiovascular,[36,40,41] gastrointestinal,[29,38] and musculoskeletal system[35,36,40] and to neural tube defects.[38] In our study ocular developmental defects seemed increased after ICSI as compared to NC-controls, but the validity of this result is poor. An increase in ocular malformations and retinoblastoma after artificial reproduction has been reported previously.[42,43]

Part of the increased risks for congenital malformations in the literature may be explained by underlying infertility factors. Zhu et al.[44] showed that infertile couples who conceived naturally or received fertility treatment were both at a higher risk of congenital malformations than fertile couples (hazard ratios 1.20 (1.07 to 1.35) and 1.39 (1.23 to 1.57)).

General health

Regarding general health, ICSI-children were reassuringly comparable with IVF-children and children born after natural conception. Follow-up studies on general health of ICSI-children at age 5 or beyond are limited.[11,13,14,45] Common diseases and chronic illnesses were found to occur equally among ICSI and NC- children at age 5 and 8,[11,13] although one report showed an increase in significant childhood illness among ICSI and IVF-children at 5 years of age in comparison with NC-controls.[14] In agreement with the current study, hearing and vision parameters were comparable.[11,13,14]

The numbers are too small to draw conclusions on the frequency of epilepsy. Sun et al. showed that children born after ART had an increased risk of epilepsy, which was partially explained by a history of parental infertility and partially by infertility treatment.[46] One child in the IVF-group had been diagnosed with leukaemia. An association between cancer and ART, which might be a consequence of repeated hormonal exposure or genetic modification, has not yet been confirmed.[47,48]
However, a higher rate of retinoblastomas was found by Moll et al.,[43] with a relative risk between 4.9 and 7.2. The isolated increased rate of vomiting in the IVF-group is an unexplained finding and may be due to chance.

The rates of undescended testes might be overestimated by misclassification. As medical records were not consulted and parents are not supposed to differentiate between true undescended testes and retractile testes, part of the testes classified as undescended will in fact have been retractile. As no difference in the amount of misclassification was expected between the conception groups, we assume that this will not have biased our results.

It is remarkable that ICSI-parents tended to consider their child's health less positive as compared to IVF and NC-parents. The more conservative answers despite reassuring health outcomes might reflect a higher rate of concern, or probably a more sober approach as a consequence of their history of infertility. If ICSI-parents seek more medical care for the reason of concern, the equal health outcomes may indicate that ICSI-children are in fact healthier.

Growth

Growth parameters of ICSI-singletons were very similar to those of IVF and NC-singletons at age 5-8. Our results agree with the few previous reports that monitored weight, height and head circumference of ICSI-children beyond age 1,[11,13,14,45,49] with the current benefit of examination at 5-8 years of age.

The difference in birth weight between ICSI and NC was partially due to a higher incidence of prematurity in the ICSI-group.

Medical care utilisation

Various groups have investigated health resource use for IVF-children versus resource use for NC-children.[50-54] The majority concluded that both in the neonatal period and later in life children born after IVF (with or without ICSI) needed more medical care. Multiple births, prematurity, underlying infertility factors, and higher parental concern and help-seeking were mentioned as probable causes, rather than the IVF-procedure itself. Reports on the use of medical care utilisation after specifically ICSI are scarce.[13,14] Our results, with follow-up to the age of 5-8 years, are reassuring. The only difference between ICSI and IVF-children is the two times higher rate of physical therapy in the IVF-group. Although Bonduelle et al. also found an increased need for physical therapy after both ICSI and IVF,[14] their IVF-children were no more at risk than ICSI-children (ICSI 2%, IVF 1%, NC 0%, p=0.032).

When ICSI and NC-children were compared, only a difference in the frequency of hospital deliveries was found, which decreased after adjustment for parity and maternal education. The residual effect might reflect a closer and more careful monitoring of the precious ART-pregnancy. We could not support the findings of Bonduelle et al.[14] that ICSI-children needed more hospital admissions, surgery and remedial therapy[11] than NC-controls.

Conclusion

We compared ICSI and IVF-singletons at 5-8 years of age and found no significantly increased risks for children born after ICSI considering pregnancy, perinatal period, congenital malformations, general health, growth, and medical care utilisation. Long-term outcome of ICSI and NC-children was very similar, despite the fact that ICSI-children were originally prone to poorer perinatal outcomes.

References

(1) Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: a call for investigation. *Am J Hum Genet*. 2004; 74(4):599-609.

(2) Schultz RM, Williams CJ. The science of ART. *Science*. 2002; 296(5576):2188-2190.

(3) Sutcliffe AG. Intracytoplasmic sperm injection and other aspects of new reproductive technologies. *Arch Dis Child*. 2000; 83(2):98-101.

(4) Tournaye H. ICSI: a technique too far? *Int J Androl*. 2003; 26(2):63-69.

(5) Bonduelle M, Van Assche E, Joris H, Keymolen K, Devroey P, Van Steirteghem A et al. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod*. 2002; 17(10):2600-2614.

(6) Hansen M, Bower C, Milne E, De Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects--a systematic review. *Hum Reprod*. 2005; 20(2):328-338.

(7) Ombelet W, Peeraer K, De Sutter P, Gerris J, Bosmans E, Martens G et al. Perinatal outcome of ICSI pregnancies compared with a matched group of natural conception pregnancies in Flanders (Belgium): a cohort study. *Reprod Biomed Online*. 2005; 11(2):244-253.

(8) Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. *Obstet Gynecol*. 2004; 103(6):1144-1153.

(9) Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, Nekkebroeck J, Loft A, Wennerholm UB et al. International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. *Pediatrics*. 2005; 115(3):e283-e289.

(10) Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992; 340(8810):17-18.

(11) Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born >=32 weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod*. 2007; 22(2):506-515.

(12) Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. Cognitive and motor development of 8-year-old children born after ICSI compared to spontaneously conceived children. *Hum Reprod*. 2006; 21(11):2922-2929.

(13) Bonduelle M, Bergh C, Niklasson A, Palermo GD, Wennerholm UB. Medical follow-up study of 5-year-old ICSI children. *Reprod Biomed Online*. 2004; 9(1):91-101.

(14) Bonduelle M, Wennerholm UB, Loft A, Tarlatzis BC, Peters C, Henriet S et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Hum Reprod*. 2005; 20(2):413-419.

(15) Van Duijn C, Keij I. Sociaal-economische status indicator op postcodeniveau. *Maandstatistiek van de bevolking*. 2002; 2:32-35.

(16) Statistics Netherlands (CBS). Standaard Onderwijs Indeling (SOI). *StatLine, Central Bureau of Statistics*. 1998; (Accessed January 9, 2006, at http://www.cbs.nl).

(17) Brunekreef B, Groot B, Rijcken B, Hoek G, Steenbekkers A, De Boer A. Reproducibility of childhood respiratory symptom questions. *Eur Respir J*. 1992; 5(8):930-935.

(18) De Boer A, Steenbekkers A, Brunekreef B, Hoek G, Rijcken B, Groot B. Een vergelijking van twee vragenlijsten naar luchtwegsymptomen bij kinderen: II De samenhang tussen de vragen. *T Soc Gezondheidsz*. 1990; 68(12):483-488.

(19) Steenbekkers A, De Boer A, Brunekreef B, Hoek G, Rijcken B, Groot B. Een vergelijking van twee vragenlijsten naar luchtwegsymptomen bij kinderen: I Vergelijking van prevalenties. *T Soc Gezondheidsz*. 1990; 68(12):478-482.

(20) Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand*. 1991; 80(8-9):756-762.

(21) Aase JM. *Diagnostic Dysmorphology*. New York, USA: Plenum Publishing Corporation; 1990.

(22) Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA*. 1998; 280(19):1690-1691.

(23) Kapiteijn K, De Bruijn CS, De Boer E, De Craen AJ, Burger CW, Van Leeuwen FE et al. Does subfertility explain the risk of poor perinatal outcome after IVF and ovarian hyperstimulation? *Hum Reprod*. 2006; 21(12):3228-3234.

(24) Ludwig AK, Katalinic A, Steinbicker V, Diedrich K, Ludwig M. Antenatal care in singleton pregnancies after ICSI as compared to spontaneous conception: data from a prospective controlled cohort study in Germany. *Hum Reprod*. 2006; 21(3):713-720.

(25) Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol*. 2004; 103(3):551-563.

(26) Kallen B, Finnstrom O, Nygren KG, Otterblad OP, Wennerholm UB. In vitro fertilisation in Sweden: obstetric characteristics, maternal morbidity and mortality. *BJOG*. 2005; 112(11):1529-1535.

(27) Govaerts I, Devreker F, Koenig I, Place I, Van den BM, Englert Y. Comparison of pregnancy outcome after intracytoplasmic sperm injection and in-vitro fertilization. *Hum Reprod*. 1998; 13(6):1514-1518.

(28) Maman E, Lunenfeld E, Levy A, Vardi H, Potashnik G. Obstetric outcome of singleton pregnancies conceived by in vitro fertilization and ovulation induction compared with those conceived spontaneously. *Fertil Steril*. 1998; 70(2):240-245.

(29) Katalinic A, Rosch C, Ludwig M. Pregnancy course and outcome after intracytoplasmic sperm injection: a controlled, prospective cohort study. *Fertil Steril*. 2004; 81(6):1604-1616.

(30) Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ*. 2004; 328(7434):261-264.

(31) Ombelet W, Cadron I, Gerris J, De Sutter P, Bosmans E, Martens G et al. Obstetric and perinatal outcome of 1655 ICSI and 3974 IVF singleton and 1102 ICSI and 2901 IVF twin births: a comparative analysis. *Reprod Biomed Online*. 2005; 11(1):76-85.

(32) Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P et al. Neonatal data on a cohort of 2889 infants born after ICSI (1991-1999) and of 2995 infants born after IVF (1983-1999). *Hum Reprod*. 2002; 17(3):671-694.

(33) Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. *Fertil Steril*. 2005; 84(3):611-617.

(34) Sutcliffe AG, Taylor B, Saunders K, Thornton S, Lieberman BA, Grudzinskas JG. Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study. *Lancet*. 2001; 357(9274):2080-2084.

(35) Klemetti R, Gissler M, Sevon T, Koivurova S, Ritvanen A, Hemminki E. Children born after assisted fertilization have an increased rate of major congenital anomalies. *Fertil Steril*. 2005; 84(5):1300-1307.

(36) Olson CK, Keppler-Noreuil KM, Romitti PA, Budelier WT, Ryan G, Sparks AE et al. In vitro fertilization is associated with an increase in major birth defects. *Fertil Steril*. 2005; 84(5):1308-1315.

(37) Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. *J Assist Reprod Genet*. 2004; 21(12):437-443.
(38) Ericson A, Kallen B. Congenital malformations in infants born after IVF: a population-based study. *Hum Reprod.* 2001; 16(3):504-509.
(39) Wennerholm UB, Bergh C, Hamberger L, Lundin K, Nilsson L, Wikland M et al. Incidence of congenital malformations in children born after ICSI. *Hum Reprod*. 2000; 15(4):944-948.
(40) Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med.* 2002; 346(10):725-730.
(41) Koivurova S, Hartikainen AL, Sovio U, Gissler M, Hemminki E, Jarvelin MR. Growth, psychomotor development and morbidity up to 3 years of age in children born after IVF. *Hum Reprod.* 2003; 18(11):2328-2336.
(42) Anteby I, Cohen E, Anteby E, BenEzra D. Ocular manifestations in children born after in vitro fertilization. *Arch Ophthalmol*. 2001; 119(10):1525-1529.
(43) Moll AC, Imhof SM, Cruysberg JR, Schouten-van Meeteren AY, Boers M, Van Leeuwen FE. Incidence of retinoblastoma in children born after in-vitro fertilisation. *Lancet.* 2003; 361(9354):309-310.
(44) Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ*. 2006; 333(7570):679.
(45) Place I, Englert Y. A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilization. *Fertil Steril*. 2003; 80(6):1388-1397.
(46) Sun Y, Vestergaard M, Christensen J, Zhu JL, Bech BH, Olsen J. Epilepsy and febrile seizures in children of treated and untreated subfertile couples. *Hum Reprod*. 2007; 22(1):215-220.
(47) Lightfoot T, Bunch K, Ansell P, Murphy M. Ovulation induction, assisted conception and childhood cancer. *Eur J Cancer.* 2005; 41(5):715-724.
(48) Raimondi S, Pedotti P, Taioli E. Meta-analysis of cancer incidence in children born after assisted reproductive technologies. *Br J Cancer.* 2005; 93(9):1053-1056.
(49) Agarwal P, Loh SK, Lim SB, Sriram B, Daniel ML, Yeo SH et al. Two-year neurodevelopmental outcome in children conceived by intracytoplasmic sperm injection: prospective cohort study. *BJOG.* 2005; 112(10):1376-1383.
(50) Ericson A, Nygren KG, Olausson PO, Kallen B. Hospital care utilization of infants born after IVF. *Hum Reprod*. 2002; 17(4):929-932.
(51) Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization in Sweden: child morbidity including cancer risk. *Fertil Steril.* 2005; 84(3):605-610.
(52) Koivurova S, Hartikainen AL, Gissler M, Hemminki E, Klemetti R, Jarvelin MR. Health care costs resulting from IVF: prenatal and neonatal periods. *Hum Reprod*. 2004; 19(12):2798-2805.
(53) Leslie GI, Gibson FL, McMahon C, Tennant C, Saunders DM. Infants conceived using in-vitro fertilization do not over-utilize health care resources after the neonatal period. *Hum Reprod.* 1998; 13(8):2055-2059.
(54) Klemetti R, Sevon T, Gissler M, Hemminki E. Health of children born as a result of in vitro fertilization. *Pediatrics*. 2006; 118(5):1819-1827.

Chapter 5

Matched follow-up study of 5 - 8-year-old ICSI-singletons: behaviour, parenting stress and (health-related) quality of life

5.

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Human Reproduction, in press

Abstract

Background: Psychosocial follow-up of ICSI-children is scarce. We compared behaviour, parenting stress, and quality of life for singletons aged 5-8 years born after ICSI, IVF and natural conception (NC).

Methods: All singletons born between June 1996 and December 1999 after ICSI in the Leiden University Medical Center were invited (n=110). Matched singletons born after IVF and natural conception were recruited. Parents completed the Child Behaviour Checklist (CBCL; measures problem behaviour), the Parenting Stress Index (NOSI), and two quality of life questionnaires (Dux25 and TACQOL). Children completed the Dux25 Child form.

Results: 87 ICSI-children (79%), 83 IVF-children (73%), and 85 NC-children enrolled. Prevalence of behavioural disorders – as reported by the parents – was comparable in the three groups. 3/87 ICSI-children had autism or an Autistic Spectrum Disorder (ASD). Problem behaviour scores were similar for ICSI and NC-children; IVF-children (mainly girls) scored less problem behaviour and their scores were less often in the (borderline) clinical range. Parenting stress was similar for ICSI and IVF, but lower for NC-parents mainly on the child scale. Quality of life scores were similar in the three conception groups.

Conclusions: Prevalence of autism/ASD seemed higher after ICSI, but this unexpected finding should be confirmed by future studies with larger group sizes. ICSI-parents experienced more stress than NC-parents, although selection bias cannot be ruled out. The majority of ICSI-singletons assessed at age 5-8 years showed a normal psychosocial well-being.

Introduction

Since the introduction of intracytoplasmic sperm injection (ICSI) in 1992, follow-up studies on ICSI-children have investigated the potential negative influences of this invasive artificial reproduction technique on health and development. Except for an increased risk of congenital malformations[1] and adverse perinatal outcomes,[2] most studies report that ICSI-children have similar health and development as their naturally conceived peers up to the age of 8 years.[3,4]

Follow-up on psychosocial development of ICSI-offspring and their families is rare. Hypothetically, ICSI-children might be at risk for emotional and behavioural difficulties as a result of the parental history of infertility and its potential negative sequelae. First, parental feelings of incompetence or low self-esteem, high expectations of parenthood and child's achievements, and overprotection of the precious child may reflect negatively on the child's psychosocial development. Second, ICSI-children are more often born preterm and prematurity is a known risk factor for behavioural problems.[5]

The majority of these concerns are shared with the more common in vitro fertilisation (IVF) procedure. As IVF had already been introduced in 1978, follow-up studies are more widely available involving IVF than ICSI families. Psychosocial development of the child, psychological adjustment of the parents, and parent-child interaction have been found to be comparable after IVF and natural conception,[6-15] or even superior after IVF.[6, 10, 11, 15, 16] Two reports are in conflict with these reassuring findings. Levy-Shiff et al.[17] have shown that IVF-children obtained lower scores on socio-emotional adjustment and reported more anxiety, aggression and depression at age 9-10 years, and Kallen et al. have found an increased rate of behavioural problems after IVF in a large register study.[18] The few studies that have addressed the psychosocial well-being of ICSI-children and their parents showed no adverse outcomes of ICSI as compared to IVF or natural conception. If any differences were found, they tended to be in favour of ICSI.[19-21]

Except for the predominant focus on IVF-families, various limitations of previous studies included small sample sizes, inadequate matching or adjustment for potential confounders, and young age of the children.

At the Leiden University Medical Center, the first ICSI-child was born in 1996. We designed a follow-up study with matched controls to assess the health and development of singletons born after ICSI in this centre, at 5 to 8 years of age. An IVF control group served to assess a potential effect of the ICSI-procedure itself, given a background of underlying infertility, hormone treatment, and fertilisation in vitro. With a natural conception control group we investigated the overall effect of ICSI, which represents the clinical question that is likely to be important to future ICSI-parents: what are the potential differences in health and development between children born after ICSI and their naturally conceived peers? In the current report, we focus on the psychosocial well-being of the children and their parents. We chose to assess child behaviour, parenting stress, and child (health-related) quality of life. Our main research questions are: (i) do ICSI-children have more behavioural problems, (ii) do ICSI-parents have more stress due to parenting, and (iii) will ICSI-children rate a lower quality of life? Furthermore, we investigated the association[22] between our previous finding of a slight increase of minor neurological dysfunctions[23,24] in ICSI-singletons[25] and problem behaviour in this group. Finally, we hypothesised that high problem behaviour scores would be associated with high parenting stress scores, because stressful parents may judge their child's behaviour more negatively and children who expose problem behaviour may likely cause more stress.

Materials and methods

The Ethics Committee of the Leiden University Medical Center approved the study design and informed parental consent was obtained. Data sampling was carried out between March 2004 and May 2005.

Participants

ICSI-singletons born between June 1996 and December 1999 after fertility treatment in the Leiden University Medical Center were selected. Exclusion criteria were: oocyte or sperm donation, cryo-preservation of the embryo and selective embryo reduction with medical indication. Similar criteria were used in the inclusion

of IVF-children, who were matched person-to-person to ICSI-participants for gender, socio-economic status, gestational age [preterm/term], maternal age at the time of pregnancy [±3 years] and birth date [closest]. Socio-economic status low, medium or high was ascribed using the zip-code/socio-economic status indicator of Statistics Netherlands,[26] based on home price and income. If no match was available within the maternal age range of ±3 years, larger deviations were permitted.

Regular pre-schools and primary schools (i.e. schools not providing special education) with zip-codes that indicated social class distributions similar to the ICSI-cohort assisted in the sampling of naturally conceived (NC) singletons. We applied group matching on gender, socio-economic status, and birth date. The composition of the NC-control group from regular schools was justifiable as only one ICSI-child attended special education.

The assessment of the psychological well-being of ICSI-children and their parents was part of a larger study in which all children have undergone a neurological examination and intelligence testing in the hospital. As neuromotor outcome (minor neurological dysfunction)[23, 24] and IQ have been associated with behaviour,[22, 27, 28] we entered the prevalence of minor neurological dysfunctions[25] and mean IQ-scores in the current study as potential confounding factors. Mean IQ-scores are not shown in Table 1, as these data will be published separately (Chapter 3). Demographic characteristics of the parents and children were obtained through questionnaires.

Measures

Behaviour

The Child Behaviour Checklist (CBCL/4-18)[29, 30] is a parental measurement of emotional and behavioural problems in 4 - 18-year-old children. The 113 items are categorised under nine syndrome scales: 1. withdrawn, 2. somatic complaints, 3. anxious/depressed, 4. social problems, 5. thought problems, 6. attention problems, 7. rule-breaking behaviour, 8. aggressive behaviour, and 9. other problems. Syndrome scales 1, 2 and 3 comprise internalising behaviour, 7 and 8 externalising behaviour; all items together comprise the total problem score. Problem scores of the CBCL are classified as normal (< 85th percentile), borderline clinical (85th – 90th percentile), and clinical (> 90th percentile), for boys and girls separately.[31]

In the CBCL, parents were asked if their child has a (mental) handicap. With this information, in combination with a more general questionnaire on child's health that contained questions on e.g. consulting a paediatrician/psychiatrist, we have evaluated behavioural disorders 'as reported by the parents'.

The CBCL was completed at home after it had been sent to all children at the same time.

Parental stress

The Nijmeegse Ouderlijke Stress Index (NOSI)[32] is the Dutch version of the Parenting Stress Index (PSI)[33] and measures stress due to parenting on the basis of 123 items in two domains: the parent domain and the child domain. The parent domain includes the subscales: sense of competence, restriction of role, attachment, depression, parent's health, social isolation, and relationship with spouse; the child domain consists of: adaptability, mood, distractibility/hyperactivity, demandingness, 'reinforces parent', and acceptability. A total stress score can be calculated by adding up the parent and child domain scores. The NOSI is the only test with separate norms for mothers and fathers. This parental questionnaire was filled out during the examination in the hospital.

(Health-related) quality of life

To measure quality of life, we used two different questionnaires: the Dutch Children TNO AZL Quality of Life questionnaire (Dux25) and the TNO AZL Child Quality of Life questionnaire (TACQOL).[34-36] The difference between the questionnaires is that the Dux25 measures quality of life based on general questions of 'happiness' and the TACQOL relates these questions to the child's health status. Example question of the Dux25: 'How much do you (does your child) like school?'. Example question of the TACQOL: 'Did your child have headaches in the past weeks?/How did she feel at that time?'. Thus, the TACQOL measures the health status of the child as well as his/her coping strategy.

The Dux25 contains 25 items on 4 domains of life quality: physical, home, emotion, and social. The instrument is child-friendly and was completed by both the child and the parent, in the hospital during the examination. The child form was assisted by one investigator blinded to the conception mode of the child.

The TACQOL covers health-related quality of life (HRQoL) with 56 items in seven domains: physical complaints, motor functioning, autonomy, cognitive functioning, social functioning, positive moods, and negative moods. This questionnaire was completed by the parents in the hospital during the examination of their child.

The Dux25 and TACQOL are designed for children aged 6-16 years. To promote reliability we asked the parents of the children who were still 5 years old at the time of the examination to fill out the Dux25 Parent and TACQOL forms for a second time, when the children had turned 6. The questionnaires were completed at home and returned by mail. To ensure that no bias was introduced we compared the scores of the first completion with the scores of the second. Besides, we compared ICSI with IVF and ICSI with NC using the selection including the first completion and the selection including the second. The Dux25 Child could not be filled out at home when children had reached age 6, as objective instructions by the parents would not be guaranteed. Instead, we performed a subanalysis among children aged 6 years and older.

Statistical analysis

Statistical analysis was performed with the SPSS 12.0 for Windows package (SPSS Inc., Chicago, IL). If values were missing within a questionnaire, we followed the user's manual instructions on missing values of the particular test. Continuous data were analysed with an independent t-test if a normal distribution was likely. Categorical data were analysed using Pearson's Chi-square test. Statistical significance was reached if $p<0.05$. Differences in continuous data were presented as a mean difference and 95% confidence interval (95%CI). Differences in categorical

data (2x3) were expressed in terms of p-values. We performed regression analysis (General Linear Model) to adjust for confounders. Potential confounders were identified by combining three sources of information: 1. baseline differences between the study groups, 2. univariate associations of variables with the outcome scales, and 3. prior knowledge on associations: plausibility and direction. Two types of exceptions were made. First, we did not adjust for differences in type of infertility (paternal/maternal) as the choice for ICSI or IVF is largely determined by type of infertility. Second, in the ICSI-NC comparison we did not adjust for factors that chronologically followed conception and might be in the causal pathway from conception mode to outcome, such as prematurity, low birth weight, IQ, and minor neurological dysfunction. In this way we examined the total difference between ICSI and NC children, an important issue from the perspective of future parents.

It is not unlikely that the perception of psychological well-being in child-rearing differs between mothers and fathers. The NOSI has separate norms for both parents. Regarding the other three questionnaires, we compared the proportions of mothers and fathers who completed the forms between the three conception groups and considered whether this variable was a confounding factor or not. These data are not shown, but if we adjusted for 'parent who completed the form', it is indicated by a footnote in the table.

Although IQ-scores are not reported in Table 1, we adjusted for IQ if this variable met the abovementioned criteria for confounding.

We tested our hypothesis of a correlation between problem behaviour and parenting stress by drawing a scatter plot and calculating the regression coefficient for the various scales and conception modes.

Results

Participants

One hundred and ten ICSI-children met the inclusion criteria, 87 of whom enrolled on the study (79%). Of the 257 eligible IVF-children 126 potential matches were selected and 92 (73%) participated. Two IVF-controls were available for five ICSI-children, and the best match was selected (n=87). Extension of the range of maternal age beyond ±3 years was required in eleven cases. Among the total cohort of ICSI-children, eight had been born prematurely, six of whom entered the study. Proper IVF-matches could only be found for 2/6 preterm children. We decided to restrict the ICSI-IVF comparison to children born at term (n=81/81), because the two preterm ICSI/IVF-couples could not represent all preterm ICSI and IVF-children. Matching preterm ICSI-children with term IVF-children would have introduced confounding.

Eighty-seven children from 16 schools were enrolled, of whom one was excluded for being a twin and one for being conceived with intrauterine insemination (n=85). The ICSI-NC comparison was not restricted to term children because we wanted to assess the overall effect of ICSI on the outcome measures. This included the potentially negative effect of prematurity (n=87/85).

Table 1. Demographic characteristics of parents and children: ICSI versus IVF and ICSI versus NC

	ICSI	**IVF**	**ICSI**	**NC**
	n=81	n=81	n=87	n=85
Gender: male, n(%)	40 (49)	40 (49)	44 (51)	47 (55)
Parity: first-born, n(%)	61 (75)	59 (73)	**65 (75)**	**31 (37)**
Gestational age: < 37 wks, n(%)	0 (0)	0 (0)	**6 (7)**	**0 (0)**
Birth weight: < 2500g, n(%)	3 (4)	3 (4)	**7 (8)**	**1 (1)**
Child education, n(%)	*		*	
regular pre-/ primary school	70 (88)	69 (85)	76 (88)	79 (93)
regular school, repeat class	7 (9)	8 (10)	7 (8)	4 (5)
regular school, remedial teaching	2 (3)	2 (2)	2 (2)	2 (2)
special education	1 (1)	2 (2)	1 (1)	0 (0)
Minor neurological dysfunction, n(%)	53 (66)*	49 (61)*	**57 (66)***	**43 (51)**
Parental age at pregnancy, mean (range)				
mother	32.8 (22-41)	33.4 (24-42)	**32.8 (22-41)**	**30.6 (20-41)**
father	36.9 (23-65)	37.3 (27-60)	**36.9 (23-65)**	**32.6 (20-49)**
Ethnicity †, n(%)				
mother: non-Caucasian	7 (9)	9 (11)	9 (10)	8 (9)
father: non-Caucasian	8 (10)	8 (10)	10 (12)	11 (13)
Level of education, n(%)				
mother		*		
no education	0 (0)	1 (1)	**0 (0)**	**0 (0)**
low	25 (31)	25 (31)	**27 (31)**	**11 (13)**
medium	28 (35)	27 (34)	**29 (33)**	**37 (44)**
high	28 (35)	27 (34)	**31 (36)**	**37 (44)**
father	*		*	
no education	0 (0)	2 (3)	0 (0)	1 (1)
low	28 (35)	26 (32)	31 (36)	22 (26)
medium	26 (33)	16 (20)	26 (30)	26 (31)
high	26 (33)	37 (46)	29 (34)	36 (42)
Diagnosed infertility factor, n(%)				
mother	**13 (16)**	**37 (46)**	**15 (17)**	**0**
father	**64 (79)**	**11 (14)**	**70 (80)**	**0**
Smoking during pregnancy, n(%)				
mother	*		*	
no	70 (88)	70 (86)	76 (88)	75 (88)
yes, <10 per day	9 (11)	10 (12)	9 (11)	8 (9)
yes, >10 per day	1 (1)	1 (1)	1 (1)	2 (2)
father		‡		*
no	57 (70)	61 (77)	61 (70)	62 (74)
yes, <10 per day	**7 (9)**	**11 (14)**	**9 (10)**	**15 (18)**
yes, >10 per day	**17 (21)**	**7 (9)**	**17 (20)**	**7 (8)**

Family situation, n(%)				
parents live together	71 (88)	77 (95)	77 (89)	75 (88)
parents live separated	9 (11)	4 (5)	9 (10)	10(12)
one parent has passed away	1 (1)	0 (0)	1 (1)	0 (0)
Family size, median (range)	2 (1-4)	2 (1-4)	**2 (1-4)**	**3 (1-10)**
Daily care-taking, n(%)	*		*	*
mother and father equally	24 (30)	14 (17)	**26 (30)**	**14 (17)**
mainly mother	51 (64)	63 (78)	**55 (64)**	**69 (82)**
mainly father	5 (6)	4 (5)	**5 (6)**	**1 (1)**
Socio-economic status, n(%)				
low	8 (10)	8 (10)	10 (12)	7 (8)
medium	26 (32)	26 (32)	27 (31)	18 (21)
high	47 (58)	47 (58)	50 (58)	60 (71)

* 1 missing value

† Turkey classified under non-Caucasian

‡ 2 missing values

bold p<0.05

Table 2. Behavioural problems in children born after ICSI, IVF, and NC: as reported by parents

	ICSI	**IVF**	**ICSI**	**NC**
Type of disorder	n=81	n=81	n=87	n=85
Mean age (range)	6.1 (5.3-7.7)	6.2 (5.3-8.3)	6.1 (5.3-7.7)	6.3 (5.1-8.0)
Autism/ Autistic Spectrum Disorder	2*	-	3*	1
Anxiety disorder	1	-	1	-
Fear of failure	1	-	1	-
Aggressive behaviour	1	-	1	-
ADHD/ ADD	1*	1	1*	2
auditory hypersensitivity	-	1	-	-
concentration problems	2	2	2	1
Mild cross-gender role	-	1	-	1
Not specified	-	1	-	1

* 1 child with autism and ADHD

Characteristics

Characteristics of the parents and children are shown in Table 1. The ICSI and IVF-group were comparable except for the prevalence of maternal and paternal infertility factors, and smoking behaviour of the father during pregnancy (ICSI-fathers smoked more heavily). ICSI and NC-children were less similar: differences existed on parity (more first-born children in the ICSI-group), gestational age, birth weight, minor neurological dysfunction (all in favour of NC), parental age (ICSI-parents were older), maternal education (NC-mothers were more highly educated), infertility factors (present with ICSI, absent with NC), paternal smoking (ICSI-fathers smoked more heavily), family size (smaller with ICSI), and daily care for the child (ICSI-parents more often shared daily care).

Behaviour

Table 2 lists behavioural disorders in children born after ICSI, IVF and NC, as reported by the parents. No marked differences in behavioural disorders were found between ICSI and IVF-children or ICSI and NC-children. However, the prevalence of ICSI-children with autism or an Autistic Spectrum Disorder (ASD) was 3/87 (3.4%), which seemed unexpectedly high in comparison with the general population estimate of ± 0.3%.[37]

Table 3 and 4 show the group results on the CBCL, stratified by gender. On the problem scales no differences were found between ICSI and IVF-boys, ICSI and NC-boys, or ICSI and NC-girls. ICSI-girls, however, had higher problem scores than IVF-girls on internalising, externalising and total score, allowing for the potential confounders (Table 3). All the mean scores were within the 85th percentile limit of normal behaviour. Table 4 shows the frequencies of children with scores in the normal, borderline clinical and clinical ranges. ICSI-children more often scored in the (borderline) clinical range than IVF-children; a difference that was larger within girls than boys. Outcomes of ICSI and NC-children were comparable. We found no correlation between minor neurological dysfunctions and problem behaviour (data not shown).

Parenting stress

Crude parenting stress was equally high among ICSI and IVF-parents on the parent, child, and total scales (Table 5). No differences appeared after adjustment for IQ of the children. ICSI-parents reported higher stress rates than NC-parents, with mean differences of 6 [-3; 16] points on the parent total scale, 12 [1; 23] points on the child total scale, and 18 [-1; 37] points on the total stress score. Adjustment for child age, maternal educational level, socio-economic status, and parent who completed the questionnaire resulted in minor changes (Table 5). On the subscale level we found a significant difference on distractibility/hyperactivity (mean difference between ICSI and NC = 4 [1; 6]).

Parenting stress was positively correlated with problem behaviour regardless of problem scale or conception mode. Regression coefficients for parenting stress as a function of problem behaviour were: 2.8 [2.3; 3.3] for total problem score, 7.5 [5.8; 9.2] for internalising, and 5.9 [4.7; 7.1] for externalising; 3.4 [2.5; 4.2] for total problem score in ICSI-children, 2.7 [1.6; 3.8] in IVF-children and 2.6 [1.9; 3.4] in NC-children.

Table 3. Child Behaviour Checklist: syndrome scale scores ICSI versus IVF and ICSI versus NC

Gender	Syndrome scale	Norm p50 (range)*	ICSI n=72	IVF n=70	Mean diff. [95%CI]	Adj. mean diff. [95%CI] †
Mean age (range)			6.7 (5.3-8.8)	6.8 (5.3-8.9)	-0.1 [-0.5; 0.2]	
Boys			n=36	n=37		
	Internalising	3 - 4 (0-62)	5	4	1 [-1; 3]	1 [-1; 3]
	Externalising	7 (0-66)	8	7	0 [-3; 3]	0 [-3; 3]
	Total score	19 (0-236)	22	18	4 [-2; 10]	3 [-4; 10]
Girls			n=36	n=33		
	Internalising	4 (0-62)	**6**	**4**	**2 [0; 4]**	**2 [0; 4]**
	Externalising	4 - 5 (0-66)	**9**	**5**	**4 [1; 6]**	**3 [1; 6]**
	Total score	16 (0-236)	**23**	**14**	**9 [4; 15]**	**8 [3; 14]**

Gender	Syndrome scale	Norm p50 (range)*	ICSI n=78	NC n=75	Mean diff. [95%CI]	Adj. mean diff. [95%CI] ‡
Mean age (range)			6.7 (5.3-8.8)	6.7 (5.2-8.8)	0.0 [-0.3; 0.3]	
Boys			n=40	n=40		
	Internalising	3 - 4 (0-62)	5	5	0 [-1; 2]	0 [-1; 2]
	Externalising	7 (0-66)	8	7	0 [-2; 3]	0 [-3; 2]
	Total score	19 (0-236)	22	20	2 [-5; 8]	1 [-5; 7]
Girls			n=38	n=35		
	Internalising	4 (0-62)	6	6	0 [-2; 2]	-1 [-3; 1]
	Externalising	4 - 5 (0-66)	8	7	1 [-1; 4]	0 [-3; 3]
	Total score	16 (0-236)	22	20	2 [-4; 8]	-1 [-8; 5]

* higher score means more problem behaviour
† adjustment for IQ, paternal smoking during pregnancy, and paternal educational level
‡ adjustment for paternal smoking during pregnancy, paternal educational level, and socio-economic status
bold p<0.05

Table 4. Child Behaviour Checklist: numbers and prevalences of children scoring in the normal, borderline clinical, and clinical range

Gender	Syndrome scale	Range	ICSI n(%)	IVF n(%)	p	ICSI n(%)	NC n(%)	p
All	Internalising		n=72	n=70		n=78	n=75	
		normal (< p85)	**50 (69)**	**63 (90)**		55 (71)	56 (75)	
		borderline clinical (p85-p90)	**14 (19)**	**2 (3)**		15 (19)	10 (13)	
		clinical (> p90)	**8 (11)**	**5 (7)**		8 (10)	9 (12)	
					0.004			0.604
	Externalising							
		normal (< p85)	**51 (71)**	**61 (87)**		56 (72)	59 (79)	
		borderline clinical (p85-p90)	**12 (17)**	**3 (4)**		12 (15)	7 (9)	
		clinical (> p90)	**9 (13)**	**6 (9)**		10 (13)	9 (12)	
					0.032			0.499
	Total score							
		normal (< p85)	**54 (75)**	**63 (90)**		59 (76)	59 (79)	
		borderline clinical (p85-p90)	**6 (8)**	**5 (7)**		6 (8)	8 (11)	
		clinical (> p90)	**12 (17)**	**2 (3)**		13 (17)	8 (11)	
					0.019			0.492
Boys	Total score		n=36	n=37		n=40	n=40	
		normal (< p85)	27 (75)	33 (89)		30 (75)	32 (80)	
		borderline clinical (p85-p90)	4 (11)	2 (5)		4 (10)	5 (13)	
		clinical (> p90)	5 (14)	2 (5)		6 (15)	3 (8)	
					0.281			0.556
Girls	Total score		n=36	n=33		n=38	n=35	
		normal (< p85)	**27 (75)**	**30 (91)**		29 (76)	27 (77)	
		borderline clinical (p85-p90)	**2 (6)**	**3 (9)**		2 (5)	3 (9)	
		clinical (> p90)	**7 (19)**	**0 (0)**		7 (18)	5 (14)	
					0.027			0.786

bold p<0.05

Table 5. Parenting stress outcomes in children born after ICSI, IVF, and natural conception

NOSI	**ICSI**	**IVF**	**Mean diff.**	**Adj. mean diff.**	**ICSI**	**NC**	**Mean diff.**	**Adj.mean diff.**
Scale	n=76	n=76	[95%CI]	[95%CI] §	n=81	n=80	[95%CI]	[95%CI] II
Mean age (range)	6.1 (5.3-7.7)	6.2 (5.3-8.3)	-0.1 [-0.4; 0.1]		6.1 (5.3-7.7)	6.3 (5.3-8.0)	-0.2 [-0.4; 0.0]	
Parent total*	108	107	1 [-10; 12]	3 [-8; 14]	107	101	6 [-3; 16]	8 [-3; 19]
competence	22	21			22	21		
restriction of role	16	16			16	14		
attachment	10	10			10	10		
depression	21	22			21	21		
parent's health	12	12			12	11		
social isolation	11	10			11	10		
relationship with spouse	16	15†			16	14‡		
Child total*	133	131	2 [-11; 14]	1 [-12; 13]	**132**	**121**	**12 [1; 23]**	11 [0; 23]
adaptability	29	29			29	27		
mood	18	19			18	17		
distractibility/ hyperactivity	33	31			**32**	**29**		
demandingness	20	19			20	18		
reinforcement	15	14			15	14		
acceptability	18	19			18	17		
Stress total*	241	238	3 [-19; 25]	3 [-19; 25]	239	221	18 [-1; 37]	19 [-1; 40]

* higher score means more stress; norm values - form completed by mother: parent total (104-130), child total (123-162), stress total (227-292); form completed by father: parent total (94-115), child total (122-157), stress total (215-270)
† 1 missing value due to divorce
‡ 3 missing values due to divorce
§ adjustment for IQ
II adjustment for age child, maternal educational level, socio-economic status, parent who completed the form
bold p<0.05

Table 6. Quality of life outcome for ICSI, IVF, and NC-children

DUX 25 child	**ICSI**	**IVF**	**Mean difference**	**ICSI**	**NC**	**Mean difference**
Subscale score*	n=75	n=77	[95%CI] †	n=80	n=83	[95%CI] ‡
Mean age (range)	6.1 (5.3-7.7)	6.3 (5.3-8.3)	-0.1 [-0.3; 0.1]	6.2 (5.3-7.7)	6.3 (5.3-8.0)	-0.2 [-0.4; 0.0]
Physical	82 (58-100)	80 (33-100)	2 [-3; 6]	83 (58-100)	81 (54-100)	2 [-2; 6]
Home	83 (35-100)	85 (50-100)	-1 [-6; 3]	84 (35-100)	84 (45-100)	0 [-4; 5]
Emotion	77 (43-100)	73 (36-96)	3 [-1; 8]	77 (43-100)	73 (39-100)	4 [-1; 8]
Social	79 (21-100)	79 (50-100)	-1 [-5; 4]	79 (21-100)	77 (32-100)	2 [-2; 7]
DUX 25 parent	**ICSI**	**IVF**	**Mean difference**	**ICSI**	**NC**	**Mean difference**
Subscale score*	n=77 §	n=76	[95%CI] II	n=82 §	n=80	[95%CI] ¶
Mean age (range)	6.6 (5.3-7.7)	6.7 (5.5-8.3)	-0.1 [-0.2; 0.1]	6.6 (5.3-7.7)	6.7 (5.6-8.0)	0.0 [-0.2; 0.1]
Physical	79 (29-100)	82 (46-100)	-3 [-8; 2]	79 (17-100)	79 (50-100)	-1 [-6; 4]
Home	85 (60-100)	88 (60-100)	-3 [-7; 0]	85 (35-100)	85 (45-100)	0 [-4; 4]
Emotion	79 (32-100)	82 (39-100)	-3 [-7; 1]	80 (32-100)	80 (46-100)	0 [-4; 4]
Social	81 (50-100)	82 (46-100)	-1 [-4; 3]	81 (43-100)	80 (46-100)	1 [-3; 5]

* scores are mean percentages of maximum quality of life score
† crude difference, no changes with adjustment for IQ
‡ crude difference, no changes with adjustment for parity and socio-economic status
§ one ICSI-parent completed the 'home' scale only: physical, emotion and social n=76 in ICSI vs. IVF and n=81 in ICSI vs. NC
II crude difference, no changes with adjustment: physical for paternal education; home for paternal education & family situation
¶ crude difference, no changes with adjustment: physical for socio-economic status & paternal education; home for parity & paternal age & socio-economic status & paternal education; emotion for socio-economic status; social for socio-economic status & family size
bold p<0.05

Quality of life

Dux25 Child

Quality of life was rated very similarly by ICSI, IVF and NC-children (Table 6). Adjustment for IQ in the ICSI-IVF comparison and for parity and socio-economic status in the ICSI-NC comparison did not result in substantial differences. In the subanalysis of children aged 6 years and older (ICSI n=37, IVF n=42; ICSI n=41, NC n=56), ICSI-children obtained a higher score than both IVF-children and NC-children on the emotion scale (6% [0; 12] and 6% [0; 12]; adjusted values 6% [0; 12] and 5% [-2; 13]).

We checked the reasons for missing forms in the different conception categories to assess whether missing due to behavioural or concentration problems was similar in the three groups. In the ICSI-IVF comparison 3/6 ICSI-children failed to complete the form due to behaviour or concentration loss versus 2/4 IVF-children. In the ICSI-NC comparison these numbers were 3/7 and 0/2. The other main reason why children did not complete the form was poor understanding.

By asking the parents of 5-year-old children to complete the Dux25 Parent and TACQOL once again at age 6 (ICSI n= 39, IVF n=34; ICSI n=41, NC n= 25), the mean child age at testing rose from 6.1 to 6.6 in the ICSI-group, and from 6.3 to 6.7 in the IVF and NC-group. The number of children younger than 6.0 years of age was reduced to 7 in both the ICSI and IVF-group (9%). The corresponding numbers were 8 (10%) and 3 (4%) in the ICSI-NC comparison. We found slight differences that did not influence our conclusions when comparing the crude scores of the first and second completion or the differences between ICSI, IVF and NC in the two selections.

Dux25 Parent

Parents of ICSI-children rated the quality of life of their children slightly lower than IVF-parents, but the differences did not reach statistical significance (Table 6). Child quality of life scores as assigned by the parents were very similar for the ICSI and NC-group. Adjustment for potential confounders resulted in irrelevant changes.

TACQOL

Table 7 shows the sum scores and the corresponding percentages of the maximum score on health status and HRQoL. No differences in health status or HRQoL were found between ICSI and IVF-children. ICSI and NC-children had comparable scores with a small but significant difference on social functioning (health status 0 [-1; 0], HRQol -1 [-2; 0]). Adjustment for confounding factors (ICSI/IVF: maternal age and IQ, ICSI/NC: maternal age, family size, socio-economic status, and parent who completed the form) did not change the results, except that the difference between ICSI and NC on social functioning was no longer statistically significant.

Table 7. Health-related quality of life and health status, parents' reports: ICSI versus IVF and ICSI versus NC

TACQOL	**ICSI**	n=74	**IVF**	n=77	**Mean diff.**
Scales	**Mean**	**%***	**Mean**	**%***	**[95%CI] §**
Mean age (range)	6.6 (5.3-7.7)		6.7 (5.5-8.3)		0.0 [-0.2; 0.2]
Physical complaints					
health status	13	80	13	83	0 [-1; 0]
HRQoL	27	84	27	86	0 [-2; 1]
Motor functioning					
health status	15	95	15	93	0 [0; 1]
HRQoL	31	96	30	95	0 [0; 1]
Autonomy					
health status	15	93	14	90	0 [0; 1]
HRQoL	31	96	30	95	0 [0; 1]
Cognitive functioning					
health status	14	85	14	86	0 [-1; 1]
HRQoL	29	91	29†	91	0 [-1; 1]
Social functioning					
health status	14	88	14	89	0 [-1; 0]
HRQoL	29	91	29‡	92	0 [-1; 1]
Positive emotions	15	93	15	94	0 [-1; 0]
Negative emotions	11	71	11	69	0 [0; 1]

	ICSI	n=79	**NC**	n=79	**Mean diff.**
Scales	**Mean**	**%***	**Mean**	**%***	**[95%CI] ll**
Mean age (range)	6.6 (5.3-7.7)		6.7 (5.6-8.0)		0.0 [-0.2; 0.1]
Physical complaints					
health status	13	80	13	83	0 [-1; 0]
HRQoL	27	84	28	87	-1 [-2; 0]
Motor functioning					
health status	15	95	15	96	0 [-1; 0]
HRQoL	31	97	31	97	0 [-1; 0]
Autonomy					
health status	15	93	15	95	0 [-1; 0]
HRQoL	31	95	31	96	0 [-1; 0]
Cognitive functioning					
health status	14	85	14	88	-1 [-1; 0]
HRQoL	29	91	30	93	-1 [-2; 0]

Social functioning					
health status	**14**	**87**	**14**	**90**	**0 [-1; 0]**
HRQoL	**29**	**91**	**30**	**93**	**-1 [-2; 0]**
Positive emotions	15	93	15	96	0 [-1; 0]
Negative emotions	11	71	12	74	0 [-1; 0]

* percentages of maximum quality of life score: 16 for health status and emotion scales, 32 for HRQoL scales

† 2 missing values

‡ 1 missing value

§ crude mean difference of sum scores, no changes after adjustment: physical HRQoL for maternal age; motor HRQoL for maternal age; cognitive HRQoL for IQ

II crude mean difference of sum scores, no changes after adjustment: physical HRQoL for maternal age & family size & completing parent; motor HRQoL for maternal age & completing parent; cognitive HRQoL for socio-economic status & completing parent; social HRQoL for socio-economic status & completing parent; positive emotions for completing parent; negative emotions for socio-economic status

bold p<0.05

Discussion

At 5-8 years of age we found no differences in behavioural disorders between children born after ICSI, IVF, and natural conception. The prevalence of autism/ASD in the ICSI-group was higher than expected (3.4% versus the general population ± 0.3%).[37] ICSI-girls had higher problem scores than IVF-girls on the Child Behaviour Checklist (CBCL) and more often scored in the clinical range. However, problem behaviour scores of ICSI and NC-children were comparable. ICSI-parents reported similar stress rates as IVF-parents, but NC-parents reported less stress. We found no differences in (health-related) quality of life as measured with the Dux25 Child, Dux25 Parent, and TACQOL. We found no correlation between minor neurological dysfunctions and problem behaviour; parenting stress was positively correlated with problem behaviour regardless of problem scale or conception mode.

Interpretation of the results

Behaviour

Our sample size was too small to draw firm conclusions on differences in behavioural disorders as reported by the parents of ICSI, IVF, and NC-children. Although we did not have enough statistical power to detect a significant difference between the ICSI and NC-group on autism/ASD, we mention the high prevalence of autism/ASD after ICSI (3.4%) compared with the estimated frequency of 0.3-0.4% in the general population.[37] This is a remarkable but uncertain finding that may deserve attention in future research, especially so as ICSI-children have a background of parental infertility and often advanced paternal age at birth, which factors have been associated with autism.[38-40] Causal pathways are suggested to involve epigenetics.[41,42]

The CBCL measures the parental judgement of children's behaviour. We found similar outcomes for ICSI and NC-children, which is in line with the results of two large follow-up studies conducted among 5-year-old singletons.[19,20] We may safely conclude that ICSI-children do not show more problem behaviour than NC-children.

The finding of lower problem behaviour scores in the IVF as compared to the ICSI-group is in conflict with the results of the main other study comparing problem behaviour of ICSI and IVF-children using the CBCL. Barnes et al.[19] have found ICSI and IVF-children to be comparable with large group sizes (n=345/n=301) at five years of age. As follow-up studies in ICSI-children are rare, we explored whether IVF-children have been reported to have less problem behaviour than NC-children, an indirect conclusion from our study. Cederblad et al.[6] compared 73 IVF-children with a Swedish population group and found no differences in CBCL problem scores; neither in boys nor in girls. The increased rate of multiples and preterm born children in the IVF-group did not affect the results. At 8-9 years of age, Colpin et al.[7] assessed problem behaviour in 27 IVF-children and 23 NC-controls, and showed no significant differences either. Only Montgomery et al.[16] found that IVF-children less often scored in the (borderline) clinical range, when assessing 494 IVF-children older than 4 years of age with the CBCL and comparing their scores with norm values.

The lack of stratification by gender in the majority of studies limits the comparison of the present study with the literature, as the difference that we found between ICSI and IVF was mainly attributable to a difference among the girls. As compared to the norm values, ICSI and NC-girls score slightly high in the present study, whereas IVF-girls score equal to the norm or slightly lower. When comparing our data with those of Colpin et al.[7] and Ponjaert-Kristoffersen et al.,[20] the crude problem scores of our NC-control group and theirs were very similar (Colpin – mother norms, considering that 96% of the forms had been filled out by the mother in our NC-group: internalising behaviour 5.1, externalising behaviour 7.9, and total problem behaviour 21.2; Ponjaert-Kristoffersen: internalising behaviour 4.3, externalising behaviour 8.2, and total problem behaviour 20.0). Apparently, the IVF-scores in the present study were particularly low rather than the ICSI and NC-scores being high.

It remains unclear why IVF-children would show less problem behaviour than their ICSI or NC counterparts. The majority of CBCL-forms were completed by the mother in the present study. Based on the differences in background of infertility, we could hypothesise that IVF-mothers – who will often have been subfertile – judge their child more positively as compared to ICSI and NC-mothers – who did not have fertility problems (themselves). Why this would particularly involve girls is unclear. We suggest further assessment of problem behaviour in ICSI, IVF, and NC-children in future research with stratification by gender of the child as well as by parent who completed the form.

Parenting stress

We showed that problem behaviour and parenting stress are positively correlated, with comparable regression coefficients in the three conception groups. However, the differences between the three groups regarding problem behaviour are not reflected in similar differences in parental stress. We would have expected to find the stress scores of ICSI and NC-parents higher than those of IVF-parents, but instead, ICSI and IVF were comparable and NC-parents indicated less stress.

Lower levels of stress on the child domain suggest that NC-parents experience less stress due to particular characteristics of the child (see subscales in Methods section) in the parent-child relationship. The stress levels of NC-parents were around the lower border of the norm. It is possible that the participating NC-parents have unwittingly been a selection of NC-parents who experience little stress. ICSI and IVF-parents may have been less prone to such a selection as they were more committed to the study and would have participated anyway. Selection bias based on the level of stress would explain the difference between our outcomes and previous reports: no difference in stress has been found between IVF and NC-parents[7,43] or ICSI, IVF and NC-parents.[19] Golombok et al.[10] have even shown lower stress rates in mothers of 4 - 8-year-old singletons born after artificial reproduction as compared to natural conception, results that have been supported by the follow-up study of ICSI and NC-children at age 5 of Ponjaert-Kristoffersen et al.[20] Whether ICSI-parents were indeed more tolerant or had answered the questionnaires in a more socially desirable way could not be distinguished.

For future research, we recommend measuring parenting stress in a survey separate from time-consuming examinations. This may prevent (mainly NC-) parents who experience a lot of stress from refusing to cooperate.

Quality of life

To our knowledge, (health-related) quality of life of ICSI-children has not been assessed previously. The lack of differences between ICSI and IVF-children or between ICSI and NC-children on quality of life as scored by the child and parent separately was very reassuring, as well as the lack of differences on health status and health-related quality of life.

Methodology

We carried out a follow-up study with matched controls, in a single centre. Participation rates in the ICSI and IVF groups were 73% and 79% and we therefore assume to have included a representative sample of these populations. To what extent the naturally conceived control group represents its reference population cannot be measured. Selection bias most likely occurred in the low socio-economic status group, as participation rates were low: from the nine schools with a low socio-economic status in the catchment area only seven NC-children applied.

As we did not succeed in finding preterm IVF-children to match all six preterm ICSI-children, we limited the ICSI-IVF comparison to children born at term. This did not conflict with our design, as we aimed to investigate the extra effect of ICSI as compared to IVF, both methods of artificial reproduction of which prematurity is a well-known complication.[44,45] Preterm children were indeed included in the ICSI-NC comparison as the total difference in psychological well-being between children born after ICSI and NC includes the negative consequences of the higher rate of prematurity.

We did not succeed in obtaining a 100% completion rate of the questionnaires. The large amount of forms to fill out may have played a role in parents accidentally skipping questions or pages. Besides, the CBCL, NOSI, and TACQOL are rather long forms that are not easy to complete for parents who are less familiar with the Dutch language (proportions of children with Dutch as the primary language spoken at home were similar in the three groups, data not shown). Because we handled missing values within questionnaires according to the test manuals and because the numbers of missing forms were fairly equally distributed between ICSI and IVF or ICSI and NC, we assume that our conclusions are not biased in these.

Although the results of IQ-testing of the children will be published separately, we chose to adjust for IQ differences between the groups if IQ was univariately associated with an outcome variable of the present study. This improved the precision of the results, but caused no material changes.

In summary, besides an unexpected increase in the prevalence of autism/ASD in ICSI-children, which is uncertain and will need more research, ICSI-children showed no rise in problem behaviour as compared to NC-children. IVF-children had lower behavioural problem scores than ICSI-children and less often scored in the (borderline) clinical range. An increase in parenting stress was found in ICSI versus NC-parents; however, this may have resulted from selection bias. (Health-related) quality of life was similar in the three conception groups. We conclude that the majority of ICSI-singletons assessed at 5-8 years of age show normal psychosocial well-being.

References

(1) Hansen M, Bower C, Milne E, De Klerk N, Kurinczuk JJ. Assisted reproductive technologies and the risk of birth defects--a systematic review. *Hum Reprod.* 2005; 20(2):328-338.

(2) Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. *Obstet Gynecol.* 2004; 103(6):1144-1153.

(3) Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born >=32 weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod.* 2007; 22(2):506-515.

(4) Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. Cognitive and motor development of 8-year-old children born after ICSI compared to spontaneously conceived children. *Hum Reprod.* 2006; 21(11):2922-2929.

(5) Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *JAMA.* 2002; 288(6):728-737.

(6) Cederblad M, Friberg B, Ploman F, Sjoberg NO, Stjernqvist K, Zackrisson E. Intelligence and behaviour in children born after in-vitro fertilization treatment. *Hum Reprod.* 1996; 11(9):2052-2057.

(7) Colpin H, Soenen S. Parenting and psychosocial development of IVF children: a follow-up study. *Hum Reprod.* 2002; 17(4):1116-1123.

(8) Gibson FL, Ungerer JA, McMahon CA, Leslie GI, Saunders DM. The mother-child relationship following in vitro fertilisation (IVF): infant attachment, responsivity, and maternal sensitivity. *J Child Psychol Psychiatry.* 2000; 41(8):1015-1023.

(9) Gibson FL, Ungerer JA, Tennant CC, Saunders DM. Parental adjustment and attitudes to parenting after in vitro fertilization. *Fertil Steril.* 2000; 73(3):565-574.

(10) Golombok S, Brewaeys A, Cook R, Giavazzi MT, Guerra D, Mantovani A et al. The European study of assisted reproduction families: family functioning and child development. *Hum Reprod.* 1996; 11(10):2324-2331.

(11) Golombok S, Brewaeys A, Giavazzi MT, Guerra D, MacCallum F, Rust J. The European study of assisted reproduction families: the transition to adolescence. *Hum Reprod.* 2002; 17(3):830-840.

(12) McMahon CA, Ungerer JA, Tennant C, Saunders D. Psychosocial adjustment and the quality of the mother-child relationship at four months postpartum after conception by in vitro fertilization. *Fertil Steril.* 1997; 68(3):492-500.

(13) Golombok S, MacCallum F. Practitioner review: outcomes for parents and children following non-traditional conception: what do clinicians need to know? *J Child Psychol Psychiatry.* 2003; 44(3):303-315.

(14) Hahn CS. Review: psychosocial well-being of parents and their children born after assisted reproduction. *J Pediatr Psychol.* 2001; 26(8):525-538.

(15) Van Balen F. Development of IVF children. *Dev Rev.* 1998; 18(1):30-46.

(16) Montgomery TR, Aiello F, Adelman RD, Wasylyshyn N, Andrews MC, Brazelton TB et al. The psychological status at school age of children conceived by in-vitro fertilization. *Hum Reprod.* 1999; 14(8):2162-2165.

(17) Levy-Shiff R, Vakil E, Dimitrovsky L, Abramovitz M, Shahar N, Har-Even D et al. Medical, cognitive, emotional, and behavioral outcomes in school-age children conceived by in-vitro fertilization. *J Clin Child Psychol.* 1998; 27(3):320-329.

(18) Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization in Sweden: child morbidity including cancer risk. *Fertil Steril.* 2005; 84(3):605-610.

(19) Barnes J, Sutcliffe AG, Kristoffersen I, Loft A, Wennerholm U, Tarlatzis BC et al. The influence of assisted reproduction on family functioning and children's socio-emotional development: results from a European study. *Hum Reprod.* 2004; 19(6):1480-1487.

(20) Ponjaert-Kristoffersen I, Tjus T, Nekkebroeck J, Squires J, Verte D, Heimann M et al. Psychological follow-up study of 5-year-old ICSI children. *Hum Reprod.* 2004; 19(12):2791-2797.

(21) Sutcliffe AG, Edwards PR, Beeson C, Barnes J. Comparing parents' perceptions of IVF conceived childrens behavior with naturally conceived children. *Infant Mental Health Journal.* 2004; 25(2):163-170.

(22) Batstra L, Neeleman J, Hadders-Algra M. The neurology of learning and behavioural problems in pre-adolescent children. *Acta Psychiatr Scand.* 2003; 108(2):92-100.

(23) Hadders-Algra M. Two distinct forms of minor neurological dysfunction: perspectives emerging from a review of data of the Groningen Perinatal Project. *Dev Med Child Neurol.* 2002; 44(8):561-571.

(24) Hadders-Algra M. Developmental coordination disorder: is clumsy motor behavior caused by a lesion of the brain at early age? *Neural Plast.* 2003; 10(1-2):39-50.

(25) Knoester M, Vandenbroucke JP, Helmerhorst FM, Van der Westerlaken LA, Walther FJ, Veen S. Matched follow-up study of 5-8 year old ICSI-singletons: comparison of their neuromotor development to IVF and naturally conceived singletons. *Hum Reprod.* 2007; 22(6):1638-46.

(26) Van Duijn C, Keij I. Sociaal-economische status indicator op postcodeniveau. *Maandstatistiek van de bevolking.* 2002; 2:32-35.

(27) Chen A, Schwarz D, Radcliffe J, Rogan WJ. Maternal IQ, child IQ, behavior, and achievement in urban 5-7 year olds. *Pediatr Res.* 2006; 59(3):471-477.

(28) Goodman R, Simonoff E, Stevenson J. The impact of child IQ, parent IQ and sibling IQ on child behavioural deviance scores. *J Child Psychol Psychiatry.* 1995; 36(3):409-425.

(29) Achenbach TM. *Manual for the Child Behavior Checklist/4-18 and 1991 Profiles.* University of Vermont, Department of Psychiatry, Burlington, Virginia, USA: 1991.

(30) Verhulst FC, Van der Ende J, Koot HM. *Handleiding voor de CBCL/4-18.* Afdeling Kinder- en Jeugdspsychiatrie, Sophia Kinderziekenhuis/Academisch Ziekenhuis Rotterdam/Erasmus Universiteit Rotterdam, The Netherlands: 1996.

(31) Verhulst FC, Van der Ende J, Koot HM. *Handscoringsformulier CBCL/4-18 Profiel voor Meisjes/Jongens - Probleemschalen.* Afdeling Kinder- en Jeugdpsychiatrie, Sophia Kinderziekenhuis/Academisch Ziekenhuis Rotterdam/Erasmus Universiteit Rotterdam, The Netherlands: 1997.

(32) De Brock AJLL, Vermulst AA, Gerris JRM, Abidin RR. *Nijmeegse Ouderlijke Stress Index.* Lisse, the Netherlands: Swets en Zeitlinger b.v.; 1992.

(33) Abidin RR. *Parenting Stress Index*: Manual. Charlottesville, Virginia, USA: Pediatric Psychology Press; 1983.

(34) Theunissen NC, Vogels TG, Koopman HM, Verrips GH, Zwinderman KA, Verloove-Vanhorick SP et al. The proxy problem: child report versus parent report in health-related quality of life research. *Qual Life Res.* 1998; 7(5):387-397.

(35) Verrips GHW, Vogels TGC, Koopman HM, Theunissen NCM, Kamphuis RP, Fekkes MINN et al. Measuring health-related quality of life in a child population. *Eur J Public Health.* 1999; 9(3):188-193.

(36) Vogels T, Verrips GH, Verloove-Vanhorick SP, Fekkes M, Kamphuis RP, Koopman HM et al. Measuring health-related quality of life in children: the development of the TACQOL parent form. *Qual Life Res.* 1998; 7(5):457-465.

(37) Williams JG, Higgins JP, Brayne CE. Systematic review of prevalence studies of autism spectrum disorders. *Arch Dis Child.* 2006; 91(1):8-15.

(38) Croughan MS, Schembri M, Bernstein D, Chamberlain N, Purcell N. Maternal and childhood outcomes following infertility and infertility treatments. *Fertil.Steril.* 86[Suppl 2], S4-5. 2006; Abstract.
(39) Henderson M. Infertility link to autism in children. *Times Newspapers Ltd., Times Online.* 26-10-2006; (Accessed January 24, 2007, at http://www.timesonline.co.uk).
(40) Reichenberg A, Gross R, Weiser M, Bresnahan M, Silverman J, Harlap S et al. Advancing paternal age and autism. *Arch Gen Psychiatry.* 2006; 63(9):1026-1032.
(41) Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: a call for investigation. *Am J Hum Genet.* 2004; 74(4):599-609.
(42) Schanen NC. Epigenetics of autism spectrum disorders. *Hum Mol Genet*. 2006; 15 Spec No 2:R138-R150.
(43) McMahon CA, Gibson F, Leslie G, Cohen J, Tennant C. Parents of 5-year-old in vitro fertilization children: psychological adjustment, parenting stress, and the influence of subsequent in vitro fertilization treatment. *J Fam Psychol*. 2003; 17(3):361-369.
(44) Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ*. 2004; 328(7434):261-264.
(45) Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol*. 2004; 103(3):551-563.

Chapter 6

General Discussion

6.

In this thesis we evaluated health and development of children born after ICSI-treatment in the Leiden University Medical Center. At 5-8 years of age we compared them with children born after IVF and children born after natural conception with regard to pregnancy course, perinatal outcome, congenital malformations, neuromotor development, cognitive development, general health, growth, medical consumption, behaviour, parenting stress, and (health-related) quality of life. Overall, the results were reassuring. ICSI-children showed no clear adverse outcomes as compared with IVF, except for an increase in problem behaviour as perceived by the parents. Thus, the ICSI-procedure seems as safe as the IVF-procedure. Nevertheless, when compared with naturally conceived (NC) children, ICSI-children had poorer perinatal outcomes (increased rate of prematurity, low birth weight, small for gestational age, as is the case with IVF-children), slightly lower cognitive development, and caused more parenting stress. Unmeasurable confounders may have affected our results on cognitive development; the decrease in parenting stress after natural conception may have been caused by selection bias; part of the effects may originate from infertility itself. However, based on our results we cannot exclude that part of the differences between the conception groups may be due to the ICSI-procedure.

The General Discussion will address subjects for debate that came forward during the study period. Additionally, recent developments and recommendations for future research are considered.

Selection of natural conception controls

As mentioned in the General Introduction, we had several options in selecting a group of naturally conceived control children. By enrolling children from regular pre-schools and primary schools, we inherently selected children who were sufficiently healthy and developed to follow mainstream education. No bias was introduced as in the ICSI-group only one child attended special education. Nevertheless, we excluded this child from the analyses of cognitive development (Chapter 3).

In our project, we did not aim at a comparison of an isolated ICSI-group with separate IVF or NC reference populations. Rather, we aimed at comparability between the groups and similarity in the measurements. Therefore, the IVF and NC-groups represented only that part of their source population that corresponded to the ICSI-group. The matching of NC to ICSI was not perfect on age and social class, but this was overcome by statistical adjustment. However, another problem appeared. On three main variables the NC-group deviated from the standardised norms of the reference population: they showed poorer neuromotor development, more problem behaviour, and less parenting stress. These deviations between the NC and reference population were larger than expected, taking into account the potential differences induced by the matching process. This raised questions about the representativeness of the NC-group. Poorer neuromotor development and more problem behaviour as perceived by the parents suggest a selection of children those are 'too pathological'. Their parents may have worried and therefore volunteered. The findings in the NC-group of low parental stress, scores above the mean on IQ-testing and an absence of adverse health outcomes seem to contradict this hypothesis.

Two more arguments against a selection bias on the basis of poor child development/health are: (i) parents knew that neuromotor development would be assessed, but the scale (minor neurological dysfunction) was too subtle to be recognised by the parents and to become a reason to volunteer, and (ii) we had asked the parents about their motivation to participate. With a response rate of 70%, five NC-parents declared to be interested in a specific part of the results, one of which (2%) involved intelligence testing for giftedness. One child (2%) participated because the parents questioned his motor development; behaviour was involved in three enrolments (5%): one child was lagging behind on behaviour and cognition, one had negative judgements of behaviour in school, and one had worrying behavioural problems. The remaining 92% of the children were enrolled by their parents for reasons of: helping other people, promoting scientific knowledge, and curiosity towards the project and their child's outcome.

If the NC-group has indeed been too pathological, the results of the ICSI-children are less reassuring than they seem. However, as argued above, we believe that the NC-group was not affected by this kind of selection bias. As described in Chapter 3 on cognitive development and in Chapter 5 on psychosocial well-being, we have also taken into account the inverse possibility, that the NC-group was a selection of children with higher IQs and with parents who experience less stress.

What then caused the high prevalence of MND and high problem behaviour scores in the NC-group? No evidence supports an increasing prevalence of MND since the '70s, in which the norm population was born. Other differences in characteristics between the norm population and the NC-group (e.g. parity) may indeed account for the difference in MND-prevalence. Yet, it is more likely that we interpreted the neurological examinations very (or even too) strictly, and thereby elevated the number of children with simple MND. This may be supported by the finding of coordination problems in 40% of the NC-children. However, the investigator had been trained and a sample of 32 children has been reviewed on videotapes by a specialist in neurodevelopment assessment, resulting in a rate of agreement of 0.94. For the present study it was most important that one investigator, who was blinded to the conception mode of the children, did all the assessments. Thereby the comparison of the three conception groups remained valid.

Regarding behaviour, it is not easy to find a likely explanation for the high scores of problem behaviour perceived by the parents. Differences in demographic factors between the NC-group and the norm population may play a role, such as age distribution. The norm scores are based on scores of children aged 4-11 years, in which the age category 5-8 years could hypothetically form a peak. Furthermore, taking part in a study such as ours, with the assessment of a wide scope of health and development parameters, may have induced parents to be more focused on their child and to complete the questionnaire more strictly than in the situation of a norm population. Both explanations would justify high problem scores in the NC-group. ICSI and NC-scores would stay comparable and would be appreciated as normal; IVF-scores would remain lower.

In- or exclusion of prematurely born children

Prematurity is a well-known consequence of artificial reproduction[1,2] and a risk factor for adverse health and developmental outcome both early and later in life.[3-5] Therefore, prematurity is an important variable to take into account when measuring effects of ICSI or IVF. Depending on the research question, the investigator will decide whether to include or exclude preterm children, and in case of inclusion, whether to adjust for prematurity in the analyses or not.

Originally, we had formulated two research questions. First, we aimed to compare ICSI and IVF-children to detect an effect of the ICSI-procedure per se. Children born after ICSI and IVF have backgrounds of parental subfertility, maternal hormonal stimulation, fertilisation in vitro, and an increased risk of prematurity and low birth weight. Except for the type of underlying subfertility, the only difference between ICSI and IVF is the procedure of actual fertilisation. Second, we compared ICSI and NC-children to assess the overall effect of ICSI versus natural conception. With the overall effect we meant the potential negative effect of ICSI through either prematurity or any unknown factor. This research question was based on the clinical question: will a child born after ICSI differ from a child born after natural conception, given similar parental characteristics up to the time of conception?

In the ICSI-IVF comparison, prematurity was not expected to disturb comparability as prematurity is more frequent after both ICSI and IVF. However, as our groups were small, we decided to match on gestational age [premature/at term] to ensure that the only difference between the two groups would be the conception procedure. By matching we lost the ability to investigate the effect of prematurity in the causal pathway from mode of conception to outcome, but this was not in conflict with our research question.

We experienced difficulties in finding matching premature IVF-controls in four out of the six cases. In our opinion, two ICSI-IVF pairs could not truly represent the premature ICSI and IVF-children, and we restricted the analyses to term-born children. The only consequence was that if we found an extra effect of ICSI over IVF, our conclusions were limited to children born at term. This approach was applied in the assessment of neuromotor development, health, and psychosocial well-being (Chapter 2, 4, and 5). Leaving in the two pairs of preterm children would not lead to material changes in outcome. However, in Chapter 3 on cognitive development, they were included mainly to support transparency and comprehensibility for the reader.

In the ICSI-NC comparison, the issue is more complicated. Our original investigation of the overall effect (i.e. the clinical question) indicated inclusion of prematurely born children, without statistical adjustment in the analysis. However, after several reviews of our paper we found that when a difference showed between ICSI and NC, readers were interested in the net effect of ICSI, i.e. the effect superimposed on that of prematurity that is already well known. To answer this question, which has a more biological character, premature children had to be excluded, or adjustment for prematurity was required.

Some reviewers argued that the latter question was the more clinically relevant one. We think that it is less useful to inform parents on the risk of adverse outcomes given their child is born at term, at a point of time when the risk of prematurity is still present, and high.

We decided to adopt a middle course. In every part of the study we focused on the original clinical question, but we added analyses of the net effect (i.e. the biological question) to the assessments of neuromotor development and cognitive development (Chapter 2 and 3). In the former we excluded preterm children, in the latter we adjusted for prematurity in the statistical analysis.

Multiple observers

A point of debate that came forward in the study on cognitive development (Chapter 3) was the use of multiple observers. We relied on nine trained investigators, who examined 255 children with the RAKIT-short version. Although a limitation of the number of investigators would have been preferable, we object to the criticism that the use of nine observers weakens our results. First, the RAKIT is a validated test instrument with an objective scoring system, if carried out by trained investigators. Second, the investigators were blinded to the child's conception mode, so they could not have been influenced by prior information. Third, the investigators were scheduled independently from the child's conception mode, so were equally distributed in the ICSI, IVF and NC-group (distribution in ICSI versus IVF $p=0.303$, distribution in ICSI versus NC $p=0.590$). Fourth, by performing an ANOVA, we found that the mean scores of the nine observers did not differ significantly (ICSI & IVF $p=0.877$, ICSI & NC $p=0.741$). Fifth, when we take a closer look at the most extreme mean IQ-scores assigned by the observers in combination with the distribution of observers, we see that the two investigators who assigned the highest mean scores (110, n=10 and 114, n=6) did not predominantly examine NC-children. Also in the opposite direction, the two investigators who assigned the lowest mean scores (104, n=53 and 105, n=35) did not examine mainly ICSI-children. Finally, the mean scores of each investigator were generally lower for ICSI-children than for IVF and NC-children, which decreased the likelihood of confounding by investigator.

Confounding

In the 'statistics' sections of Chapter 2 to 5, we describe how we dealt with potential confounders. A confounder is a factor that is related to the exposure (mode of conception) as well as the outcome (e.g. IQ) and may introduce or hide an association between those two. For example, ICSI-parents were of lower socio-economic status than NC-parents; low socio-economic status is associated with lower child IQ (an indirect effect), so without adjustment for socio-economic status, ICSI-children will have lower IQ-scores than NC-children even in the absence of an actual effect.

In Chapter 5 on psychosocial development we explained the different ways in which we handled potential confounding factors in the ICSI-IVF and ICSI-NC comparison. In short, in the ICSI-IVF comparison we adjusted for all the potential confounders to enhance comparability for all variables except the in vitro fertilisation

procedure. In the ICSI-NC comparison we adjusted for potential confounders that were not positioned in the causal pathway between conception mode and outcome. This approach served to answer our original clinical question of the overall effect of ICSI as compared to NC. As explained in the paragraph on prematurity, investigating the net effect of ICSI required an approach similar to the ICSI-IVF comparison (ICSI versus NC with adjustment for prematurity and all other potential confounders).

There are slight differences between the four studies in how we handled confounding. In the design of the study on neuromotor development (Chapter 2) we assumed that matching would prevent large differences between the groups. Using previous research reports, we identified parity, maternal age, prematurity, and low birth weight as confounding factors. When the groups appeared to differ on other variables (e.g. parental educational level), we additionally corrected for those factors, regardless of the presence of a univariate association with neuromotor outcome. In Chapter 3 on cognitive development, the method of adjustment was changed to correction for those variables in which the groups were different and that might have affected cognitive developmental outcome.

From Chapter 4 onwards, we refined the way in which we corrected for confounders. In the evaluation of child's health after ICSI-treatment (Chapter 4), many parameters were assessed. When differences in main outcome measures were detected, we investigated whether an association was present between the (demographic) variables in which the groups differed and the particular outcome measure, to explore the possibility of confounding. If an association was present and reasonable, the variable was entered as a covariate in a regression model or was used for stratification. In Chapter 5, this approach was extended to all outcome measures: each parameter of psychosocial development was adjusted for confounding factors that were identified by considering the plausibility and statistical proof of a univariate association between variables in which differences had existed between the groups (thus were associated with the exposure) and outcome.

In conclusion, during the study period the methods of adjustment have evolved. The final approach may be the most comprehensive, and at the same time prevents the possibility of introducing bias by adjusting for a factor that is associated with exposure, but not with outcome.

Recent developments

1. Causality

Although associations have been found between artificial reproduction and follow-up outcome parameters, little is known about causality[6] of these relationships. First, it is unknown whether adverse outcomes after ART, such as prematurity, low birth weight, and congenital malformations are the result of the ART-procedure itself or of the underlying infertility, given a singleton child and similar parental characteristics. Second, even if the main cause of adverse outcome would be identified (ART/infertility), the causal pathway may still be unclear. Currently, the first careful steps are taken in these areas of research.

1. a. Infertility as a causal factor

The majority of studies that investigate potential negative effects of ICSI, including the present study, use IVF-children or children born after natural conception as controls. With IVF-children, an effect due to the procedure can be measured given artificial reproduction. Nevertheless, the differences in type of underlying infertility may confound the results. With NC-children as controls, the effect of ICSI as a technique of artificial reproduction can be investigated versus natural conception. However, this effect is inseparable from the indication of treatment: infertility, and perhaps subclasses of maternal and paternal infertility. Only a trial with random assignment of conception mode would prevent these entanglements,[7] but this is not a realistic option. An alternative approach to unravel the effects of ART and infertility is to compare children born after ART with children born after natural conception from both subfertile (time to pregnancy >12 months) and fertile couples.[8]

In 2005, Thomson et al.[9] found a higher risk of obstetric and perinatal complications in singleton pregnancies from previously subfertile couples as compared with couples that were fertile without problems. Among subfertile couples, there were no differences in outcome between pregnancies with or without infertility treatment. Hence, subfertility and not infertility treatment seems to be responsible for poorer outcomes after artificial reproduction. On the contrary, De Geyter et al.[10] and Kapiteijn et al.[11] found that ART-children had lower birth weights and shorter pregnancy durations when comparing ICSI and/or IVF-children with naturally conceived children from previously subfertile couples. This indicates that subfertility alone does not explain poorer outcome after ART and that factors such as infertility treatment may also contribute.

In parallel with perinatal outcome, the hypothesis on separate effects of subfertility and infertility treatment has been tested on the end-points congenital malformations and epilepsy.[12,13] Zhu et al.[13] found an increased rate of congenital malformations in subfertile (regardless of treatment) versus fertile couples, and a higher prevalence of genital organ malformations in children born after ART as compared to children born after natural conception from subfertile couples. Of the various ART-treatments, ICSI had the largest adverse effect. Similarly, the increased risk of epilepsy in children born after ART was partially explained by subfertility, and partially by infertility treatment.[12]

1. b. The causal pathway from ART to outcome

The first hypotheses on causal pathways in artificial reproduction concern the influence of ART on *birth weight* in singletons.

First, double-embryo transfers (DET) may play a role. Singletons born after double-embryo transfer associated with a vanishing twin, appear to have a lower mean birth weight than singletons born after single-embryo transfer (SET).[14,15] Besides, birth weight after single-embryo transfer approaches birth weight after natural conception.[16] Although the couples who underwent SET may have been a selection of good-prognosis patients, the presence of a second fetal sac following

DET might apparently affect the implantation process, placental growth and/or foetal nourishment.

In the previous paragraph we concluded that subfertility as well as infertility treatment may negatively affect ART-outcome. Infertility treatment consists of controlled ovarian hyperstimulation (COHS) with or without intrauterine insemination (IUI), IVF, or ICSI. A second hypothesis on how ART may influence birth weight concerns an effect of COHS. Singletons born after COHS + IVF have shown comparable birth weight outcomes to singletons born after COHS only (or with IUI),[11, 17] while poorer birth weight outcomes have been found among singletons born after COHS (without IVF) as compared with natural conception.[11, 17-19] Kallen et al. and Kapiteijn et al. showed that this was not (solely) a confounding effect of subfertility.[11, 18]

Another indication for a negative effect of COHS is that low birth weight is less frequent after cryopreserved-thawed embryo transfer as compared to regular IVF,[20-22] with cryopreserved-thawed embryo transfer being mainly implemented in the natural instead of hormonally stimulated cycle.

Whether the association between ovarian stimulation and birth weight represents a causal relationship is yet unclear. An important criterion for causality is biological plausibility (Hill's criteria).[6] Ovarian stimulation may negatively influence fetal development by affecting oocyte quality or changing the maternal endocrine environment.[23] A biological mechanism has been identified in mice by Sibug et al. They showed that urinary gonadotrophins (but not recombinant gonadotrophins) reduced the expression of vascular endothelial growth factor (VEGF) in the uterus in the peri-implantation period.[24] VEGF is involved in angiogenesis during the early stages of blastocyst implantation.[25, 26] Although a biological mechanism in mice cannot be directly extrapolated to the human situation, it may give an indication.

Finally, the potential influence of genomic imprinting should be mentioned in the discussion of low birth weights following ART-pregnancy.[27-29] Epigenetic programming occurs during gametogenesis and allows differentiation of the germ cell. By methylation, the majority of genes are blocked. Of some genes, only one allele is methylated, a process that is called imprinting. After fertilisation, both the paternal and maternal genes are again demethylated, except for the imprinted genes. This conservation of programming might be disturbed by in vitro culture of embryos during ART. Khosla et al. have shown deregulation of imprinting after culture of preimplantation mouse embryos in serum-containing medium, with lower fetal weights on embryonic day 14.[30]

When more evidence is found for these theories, an effort can be made to extend them to other outcomes, such as prematurity, neuromotor and cognitive development.

2. Preimplantation genetic screening

With preimplantation genetic diagnosis (PGD), embryos are screened prior to implantation for single gene disorders, structural chromosomal abnormalities, and aneuploidy. PGD is carried out in fertile couples with a high risk of transmitting genetic defects, in order to decrease the risk of (i) abnormalities in the foetus, (ii) the potential need for termination of pregnancy after later prenatal diagnosis, and (iii) spontaneous abortion.[31] Preimplantation genetic screening for aneuploidies (PGS) is a nearly identical technique but aims to improve pregnancy rates in women undergoing ART, particularly in the cases of (i) advanced maternal age, (ii) repeated ICSI/IVF-failure, (iii) repeated miscarriage and (iv) testicular sperm extraction.[32] There is yet insufficient evidence to determine whether PGS positively affects live birth rates in women undergoing ICSI/IVF-treatment.[32] Nevertheless, the rate of spontaneous pregnancy loss may be reduced.[33] Follow-up studies are warranted to assess the health and development of live-born ICSI/IVF-children after PGS.

Suggestions for future research

For the design of future studies, we recommend the inclusion of a control group born after natural conception from previously infertile couples. These couples may be identified via former IVF waiting lists. In the Netherlands a similar cohort has been identified in the OMEGA study, including women diagnosed with subfertility in all 12 Dutch IVF-clinics between 1980 and 1995.[34] Additionally, a group of children born after COHS (preferably urinary gonadothrophins) may represent births after hormonal stimulation only.[11] In this way, outcomes can be compared after natural conception and normal fertility, natural conception in the presence of subfertility, natural conception with subfertility and hormonal stimulation, artificial conception with hormonal stimulation and in vitro fertilisation (IVF), and artificial conception with hormonal stimulation and in vitro fertilisation using a microinjection pipette (ICSI).[35] If sample sizes allow for the distinction between various types of sub-/infertility, this may promote clarification of cause and consequence in artificial reproduction.

Regarding the assessment of neuromotor development in ICSI-children, which will preferably be continued up to adulthood, it seems more important to focus on minor rather than on major deviations (provided that we all use the same definitions): the present data only show an increase in non-pathological neuromotor developmental delay (simple MND). Prospective, precise assessment of the intervention as well as control groups is required, using a test instrument that is capable of detecting such subtle deviations.

Cognitive development after ICSI should be reassessed at older ages, because our results contradict those of previous studies, and IQ may evolve until adulthood. It would be extremely helpful if parental IQs could be obtained.

The importance of continuous follow-up of health in ICSI-children focuses on the ability to identify rare diseases and diseases that occur later in life. Both the increasing number of children born after ICSI and the advancing age of the first cohort warrant, and will facilitate, the collection of this important information.

A remarkable finding of the present study was the high prevalence of autism/autistic spectrum disorders (ASD) among ICSI-children. We suggest further research in this field, especially as these disorders have been suggested to be associated with errors in genomic imprinting, and imprinting defects are thought to be associated with ART in various other ways too (e.g. low birth weight, Beckwith-Wiedemann syndrome).

Finally, an obvious suggestion for future research will be the follow-up of children born after ICSI into their reproductive stage. Children born after ART may inherit parental factors that initially caused the infertility. Additionally or alternatively, the procedure of artificial reproduction may leave its marks.[36] Reassuringly, in 2006 Louise Brown, the first human being born after IVF, gave birth to a naturally conceived, healthy baby boy: a good start!

Conclusion

In conclusion, the original concerns on the health and development of ICSI-singletons were in principle justified by the invasive character of the procedure. Although the results of follow-up studies are inconclusive on some outcomes, the majority of concerns that parents might have regarding health and development can be dispelled up to 8 years of age, in particular when ICSI is compared with IVF.

References

(1) Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ*. 2004; 328(7434):261-264.

(2) Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol*. 2004; 103(3):551-563.

(3) Aylward GP. Neurodevelopmental outcomes of infants born prematurely. *J Dev Behav Pediatr*. 2005; 26(6):427-440.

(4) Hack M, Flannery DJ, Schluchter M, Cartar L, Borawski E, Klein N. Outcomes in young adulthood for very-low-birth-weight infants. *N Engl J Med*. 2002; 346(3):149-157.

(5) Veen S, Ens-Dokkum MH, Schreuder AM, Verloove-Vanhorick SP, Brand R, Ruys JH. Impairments, disabilities, and handicaps of very preterm and very-low-birthweight infants at five years of age. The Collaborative Project on Preterm and Small for Gestational Age Infants (POPS) in The Netherlands. *Lancet*. 1991; 338(8758):33-36.

(6) Hill AB. The environment and disease: association or causation? *Proc R Soc Med*. 1965; 58:295-300.

(7) Buck Louis GM, Schisterman EF, Dukic VM, Schieve LA. Research hurdles complicating the analysis of infertility treatment and child health. *Hum Reprod*. 2005; 20(1):12-18.

(8) Rimm AA, Katayama AC, Diaz M, Katayama KP. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. *J Assist Reprod Genet*. 2004; 21(12):437-443.

(9) Thomson F, Shanbhag S, Templeton A, Bhattacharya S. Obstetric outcome in women with subfertility. *BJOG*. 2005; 112(5):632-637.

(10) De Geyter C, De Geyter M, Steimann S, Zhang H, Holzgreve W. Comparative birth weights of singletons born after assisted reproduction and natural conception in previously infertile women. *Hum Reprod*. 2006; 21(3):705-712.

(11) Kapiteijn K, De Bruijn CS, De Boer E, De Craen AJ, Burger CW, Van Leeuwen FE et al. Does subfertility explain the risk of poor perinatal outcome after IVF and ovarian hyperstimulation? *Hum Reprod.* 2006; 21(12):3228-3234.

(12) Sun Y, Vestergaard M, Christensen J, Zhu JL, Bech BH, Olsen J. Epilepsy and febrile seizures in children of treated and untreated subfertile couples. *Hum Reprod.* 2007; 22(1):215-220.

(13) Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ*. 2006; 333(7570):679.

(14) De Sutter P, Delbaere I, Gerris J, Verstraelen H, Goetgeluk S, Van der EJ et al. Birthweight of singletons after assisted reproduction is higher after single- than after double-embryo transfer. *Hum Reprod*. 2006; 21(10):2633-2637.

(15) Pinborg A, Lidegaard O, La Cour FN, Nyboe AA. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod*. 2005; 20(10):2821-2829.

(16) De Neubourg D, Gerris J, Mangelschots K, Van Royen E, Vercruyssen M, Steylemans A et al. The obstetrical and neonatal outcome of babies born after single-embryo transfer in IVF/ICSI compares favourably to spontaneously conceived babies. *Hum Reprod*. 2006; 21(4):1041-1046.

(17) Olivennes F, Rufat P, Andre B, Pourade A, Quiros MC, Frydman R. The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. *Hum Reprod.* 1993; 8(8):1297-1300.

(18) Kallen B, Olausson PO, Nygren KG. Neonatal outcome in pregnancies from ovarian stimulation. *Obstet Gynecol*. 2002; 100(3):414-419.

(19) Ombelet W, Martens G, De Sutter P, Gerris J, Bosmans E, Ruyssinck G et al. Perinatal outcome of 12,021 singleton and 3108 twin births after non-IVF-assisted reproduction: a cohort study. *Hum Reprod.* 2006; 21(4):1025-1032.

(20) Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. *Fertil Steril*. 2005; 84(3):611-617.

(21) Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. *Fertil Steril.* 2005; 83(6):1650-1658.

(22) Wennerholm UB, Bergh C, Hamberger L, Westlander G, Wikland M, Wood M. Obstetric outcome of pregnancies following ICSI, classified according to sperm origin and quality. *Hum Reprod.* 2000; 15(5):1189-1194.

(23) Ertzeid G, Storeng R. The impact of ovarian stimulation on implantation and fetal development in mice. *Hum Reprod*. 2001; 16(2):221-225.

(24) Sibug RM, De Koning J, Tijssen AM, De Ruiter MC, De Kloet ER, Helmerhorst FM. Urinary gonadotrophins but not recombinant gonadotrophins reduce expression of VEGF120 and its receptors flt-1 and flk-1 in the mouse uterus during the peri-implantation period. *Hum Reprod*. 2005; 20(3):649-656.

(25) Kapiteijn K, Koolwijk P, Van der Weiden RM, Van Nieuw AG, Plaisier M, Van Hinsbergh VW et al. Human embryo-conditioned medium stimulates in vitro endometrial angiogenesis. *Fertil Steril*. 2006; 85 Suppl 1:1232-1239.

(26) Smith SK. Angiogenesis and implantation. *Hum Reprod*. 2000; 15 Suppl 6:59-66.

(27) Horsthemke B, Ludwig M. Assisted reproduction: the epigenetic perspective. *Hum Reprod Update.* 2005; 11(5):473-482.

(28) Niemitz EL, Feinberg AP. Epigenetics and assisted reproductive technology: a call for investigation. *Am J Hum Genet*. 2004; 74(4):599-609.

(29) Nikolettos N, Asimakopoulos B, Papastefanou IS. Intracytoplasmic sperm injection--an assisted reproduction technique that should make us cautious about imprinting deregulation. *J Soc Gynecol Investig.* 2006; 13(5):317-328.

(30) Khosla S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol Reprod.* 2001; 64(3):918-926.

(31) Shahine LK, Caughey AB. Preimplantation genetic diagnosis: the earliest form of prenatal diagnosis. *Gynecol Obstet Invest*. 2005; 60(1):39-46.

(32) Twisk M, Mastenbroek S, Van Wely M, Heineman MJ, Van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. *Cochrane Database Syst Rev.* 2006;(1):CD005291.

(33) Munne S, Fischer J, Warner A, Chen S, Zouves C, Cohen J. Preimplantation genetic diagnosis significantly reduces pregnancy loss in infertile couples: a multicenter study. *Fertil Steril.* 2006; 85(2):326-332.

(34) Klip H, Burger CW, De Kraker J, Van Leeuwen FE. Risk of cancer in the offspring of women who underwent ovarian stimulation for IVF. *Hum Reprod*. 2001; 16(11):2451-2458.

(35) Wang JX, Norman RJ, Kristiansson P. The effect of various infertility treatments on the risk of preterm birth. *Hum Reprod.* 2002; 17(4):945-949.

(36) Jensen TK, Jorgensen N, Asklund C, Carlsen E, Holm M, Skakkebaek NE. Fertility treatment and reproductive health of male offspring: a study of 1,925 young men from the general population. *Am J Epidemiol*. 2007; 165(5):583-590.

Summary

Chapter 1. General Introduction

Intracytoplasmic sperm injection (ICSI) is the technique of artificial reproduction that was introduced in 1992, mainly to overcome male factor infertility. Follow-up studies showed that children born after ICSI might have a slightly increased risk of chromosomal aberrations, congenital malformations, prematurity, and low birth weight. Whether this effect is due to the ICSI-procedure itself or to related factors such as infertility and hormonal pre-treatment, is yet unclear. No delay has been reported in neuromotor or cognitive development until the age of 5 years. The aim of the current study was to investigate the potential negative effects of ICSI in 5 - 8-year-old singleton children on their neuromotor development, cognitive development, health and psychosocial well-being. We compared the ICSI-children with children born after the established procedure of in vitro fertilisation (IVF), and with children born after natural conception (NC). To that aim, we conducted a matched controlled follow-up of singletons born between June 1996 and December 1999 following ICSI-treatment in the Leiden University Medical Center. Participation rates were 79% in the ICSI-group (n=87), 73% in the IVF-group (n=83), and not ascertainable in the NC-group (n=85).

Chapter 2. Neuromotor Development

Neuromotor development was measured by physical examination (neurological examination according to Touwen, adapted by Hadders-Algra), by a single investigator blinded to the conception mode of the children. The prevalence of minor neurological dysfunction (MND) was similar for ICSI and IVF-children. ICSI-children showed a slight increase in MND as compared to NC-controls (crude PR 1.31 95%CI [1.02; 1.55]). This finding was partially explained by a difference in parity (adjusted PR 1.22 95%CI [0.86; 1.52]).

Chapter 3. Cognitive Development

Similarly, we compared ICSI-singletons at age 5-8 years with IVF and NC-singletons on cognitive development. Nine trained investigators blinded to conception mode measured the children's intelligence quotients (IQ) using the short version of the Revised Amsterdam Child Intelligence Test (RAKIT). ICSI-children had slightly lower IQ-scores than IVF-children (mean score: ICSI 103 versus IVF 107, adjusted difference 3.6 [-0.8; 8.0]). We divided IQ-scores in three categories: <85, 85-115, and >115; the distribution of IQ was similar for ICSI and IVF. The difference between ICSI and NC-children was more pronounced (mean score: ICSI 103 versus NC 110, adjusted difference between 5.6 [0.9; 10.3] and 7.1 [1.7; 12.5]) and we noted a shift to lower IQ-categories following ICSI as compared with NC. Despite statistical adjustment for education and socio-economic status, selection bias may have caused or may have increased the difference found between ICSI and NC, by potential self-selection of more intelligent parents and their children in the NC-group.

Chapter 4. Health

We studied various parameters of pregnancy, perinatal period, congenital malformations, general health, medical consumption, and growth in our cohort of 87 ICSI-singletons. Congenital malformations (dysmorphic features) and growth were assessed during a physical examination; the other variables were covered by questionnaires. We found all health parameters to be similar for ICSI and IVF-children. When comparing ICSI to NC, ICSI-children were more often born preterm (6 versus 0, p=0.014) and with low birth weight (7 versus 1, OR 7.4 [0.9; 62.5]). ICSI-mothers had more pregnancy complications (n=33 versus 18) and in-hospital deliveries (PR 1.36 [1.2; 1.5]). ICSI and NC-children were comparable for congenital malformations, general health, medical consumption, and growth. We concluded that beyond the period of pregnancy and birth, until age 5-8 years, ICSI-singletons were as healthy as singletons born after IVF and NC.

Chapter 5. Psychosocial Well-being

Behaviour, parenting stress, and (health related) quality of life of the ICSI-cohort were scored, to provide data on psychosocial well-being at 5-8 years of age. We used standardised questionnaires (Child Behaviour Checklist, Parenting Stress Index, Dux25 Parent, Dux25 Child, TACQOL) and retrieved additional information on behavioural disorders from the questionnaire 'child health'. Four questionnaires were completed by the parents and one by the child (Dux25 Child). We found the prevalence of behavioural disorders in ICSI, IVF, and NC-children to be similar, although our sample sizes were too small to draw firm conclusions. Remarkable was the rate of 3.4% (n=3) children with autism/Autistic Spectrum Disorders in the ICSI-group. Problem behaviour as measured with the CBCL was similar among ICSI and NC-children, but higher than reported for IVF. Scores of IVF-children were also less often in the (borderline) clinical range. We have no explanation for these differences between ICSI and IVF-children or for the observation that they were mainly found in girls. Levels of parenting stress were similar in the ICSI and IVF-group; scores were lower for parents of NC-children. As compared with normal values, NC-scores were low, which may indicate the selection of NC-parents who generally experience little stress. (Health-related) quality of life scores were similar in the three conception groups. We concluded that the majority of ICSI-singletons assessed at age 5-8 showed normal psychosocial well-being.

Chapter 6. General Discussion

In the General Discussion, we start out by discussing methodology issues: selection of NC-controls, in- or exclusion of prematurely born children, multiple observers, and confounding. We selected NC-controls from regular pre-schools and primary schools, which made the group liable to selection bias. We argue that the NC-group represented its reference population well, in spite of several limitations. The ICSI and IVF-groups were matched – amongst others – for prematurity, but in four out of six premature cases, no match could be found. We decided to exclude preterm children from the ICSI/IVF analyses, which was not in conflict with our study design. The use of multiple observers to perform the RAKIT might be seen

as a disadvantage, but we explain why our results were not affected. We also clarify the different approaches used in the separate articles (Chapter 2 to 5) to adjust for confounding factors. Adjustment should preferably be carried out when a factor is both associated with exposure and outcome and this association has both a statistical and biological character.

Recent developments in the exploration of the causal pathway from ART to outcome are described. Infertility and ART-procedures seem jointly responsible for adverse outcomes after ART. Double-embryo transfer, ovarian stimulation, and genomic imprinting are suggested to be intermediate factors following ART-procedure. Another recent development is the application of preimplantation genetic screening in ART, to improve pregnancy rates by selecting chromosomally normal embryos for implantation.

Suggestions for future research involve (i) the inclusion of a group of children born after natural conception from previously infertile couples; (ii) the inclusion of children born after controlled ovarian hyperstimulation only; (iii) focus on minor deviations in neuromotor development rather than major; (iv) reassessment of the cognitive development of ICSI-children up to age 20, with adjustment for parental IQ; (v) continuation of the follow-up on ICSI-children's health, until rare diseases and diseases that occur later in life can be detected; (vi) monitoring of the prevalence of autism and autism spectrum disorders in ICSI-children; (vii) assessment of the reproductive ability of ICSI-offspring.

Samenvatting

Hoofdstuk 1. Algemene Inleiding

Intracytoplasmatische sperma injectie (ICSI) is de methode van kunstmatige bevruchting die sinds 1992 wordt toegepast bij de behandeling van voornamelijk mannelijke infertiliteit. Studies waarin ICSI-kinderen werden vervolgd in de tijd hebben aangetoond dat deze kinderen een licht verhoogd risico lopen op chromosoomafwijkingen, aangeboren afwijkingen, prematuriteit en een laag geboortegewicht. Het is echter nog niet duidelijk of deze verhoogde risico's worden veroorzaakt door de ICSI-procedure zelf of door gerelateerde factoren als infertiliteit en hormonale stimulatie. De neuromotore en cognitieve ontwikkeling van ICSI-kinderen lijkt ongestoord, in elk geval tot de leeftijd van 5 jaar. Het doel van de studies beschreven in dit proefschrift was om te onderzoeken of ICSI negatieve effecten heeft op de neuromotore ontwikkeling, cognitieve ontwikkeling, gezondheid en het psychosociale welzijn van eenlingen in de leeftijdscategorie van 5 tot 8 jaar. Hiertoe ontwierpen we een vervolgstudie naar eenlingen geboren tussen 1-6-1996 en 31-12-1999 na ICSI-behandeling in het Leids Universitair Medisch Centrum. De ICSI-kinderen werden vergeleken met kinderen geboren na de gevestigde methode van in vitro fertilisatie (IVF) en met kinderen geboren na natuurlijke conceptie (NC); beide groepen werden via matching samengesteld. Deelname percentages waren 79% in de ICSI-groep (n=87), 73% in de IVF-groep (n=83), en niet te achterhalen in de NC-groep (n=85).

Hoofdstuk 2. Neuromotore Ontwikkeling

De neuromotore ontwikkeling werd bepaald middels een lichamelijk onderzoek (het neurologisch onderzoek volgens Touwen, aangepast door Hadders-Algra), uitgevoerd door één onderzoeker die geblindeerd was voor de wijze van conceptie van het kind. De prevalentie van minor neurological dysfunctions (MND) was gelijk voor ICSI en IVF-kinderen. ICSI-kinderen kregen iets vaker de score MND toegekend dan NC-kinderen (ruwe PR 1.31 95%CI [1.02; 1.55]); gedeeltelijk werd dit verschil verklaard door een verschil in pariteit tussen de groepen (gecorrigeerde PR 1.22 95%CI [0.86; 1.52]).

Hoofdstuk 3. Cognitieve Ontwikkeling

Op vergelijkbare wijze vergeleken we de ICSI-eenlingen op de leeftijd van 5-8 jaar met eenlingen geboren na IVF en NC op cognitieve ontwikkeling. Negen getrainde testassistenten die geblindeerd waren voor de conceptie-groep namen de geReviseerde Amsterdamse Kinder Intelligentie Test (RAKIT, verkorte versie) af bij de kinderen ter bepaling van het IQ. ICSI-kinderen hadden iets lagere IQ-scores dan IVF-kinderen (gemiddeld: ICSI 103 versus IVF 107, gecorrigeerde verschil: 3.6 [-0.8; 8.0]). We deelden de IQ-scores op in drie categorieën: <85, 85-115 en >115. De verdeling over deze categorieën bleek voor ICSI en IVF-kinderen gelijk. Het verschil tussen ICSI en NC-kinderen was meer uitgesproken (gemiddeld: ICSI 103 versus NC 110, gecorrigeerde verschil tussen 5.6 [0.9; 10.3] en 7.1 [1.7; 12.5]) en we zagen een verschuiving naar lagere IQ-categorieën bij ICSI vergeleken met NC. Ondanks de

statistische correctie voor opleidingsniveau en socio-ecomische status zou het verschil tussen ICSI en NC kunnen zijn ontstaan door de zelfselectie van meer intelligente ouders en hun kinderen in de NC-groep (selectie bias).

Hoofdstuk 4. Gezondheid

We onderzochten ons cohort van 87 ICSI-kinderen op parameters van zwangerschap en perinatale periode, aangeboren afwijkingen, algemene gezondheid, medische consumptie en groei. Aangeboren afwijkingen (dysmorfe kenmerken) en groei werden onderzocht tijdens het lichamelijk onderzoek; de overige variabelen werden verzameld via vragenlijsten. ICSI en IVF-kinderen waren zeer vergelijkbaar op al deze parameters van gezondheid. Bij het vergelijken van ICSI en NC bleek dat ICSI-kinderen vaker prematuur (<37 weken, n=6 versus 0, p=0.014) en met een laag geboortegewicht (<2500 gram, n=7 versus 1, OR 7.4 [0.9; 62.5]) werden geboren. ICSI-moeders hadden meer zwangerschapscomplicaties (n=33 versus 18) en ziekenhuisbevallingen (PR 1.36 [1.2; 1.5]). ICSI en NC-kinderen waren vergelijkbaar wat betreft aangeboren afwijkingen, algemene gezondheid, medische consumptie en groei. We concludeerden dat na de periode van zwangerschap en geboorte, tot de leeftijd van 5 tot 8 jaar, ICSI-eenlingen net zo gezond zijn als eenlingen geboren na IVF en NC.

Hoofdstuk 5. Psychosociaal Welzijn

In hoofdstuk 5 onderzochten we het gedrag, de ouderlijke stress en de (gezondheidgerelateerde) kwaliteit van leven van het ICSI-cohort, om inzicht te krijgen in het psychosociale welzijn. We hebben gebruik gemaakt van standaard vragenlijsten (Child Behaviour Checklist, Nijmeegse Ouderlijke Stress Index, Dux25 Parent, Dux25 Child, TACQOL) en verkregen extra informatie over het gedrag van het kind uit de vragenlijst 'gezondheid van het kind'. Vier lijsten werden ingevuld door de ouders en één door het kind (Dux25 Child). Wij vonden geen verschillen tussen ICSI, IVF en NC in gedragsproblemen gerapporteerd door de ouders, hoewel onze groepsgrootte niet voldoende was om harde conclusies te trekken. Opvallend was het percentage van 3.4% (n=3) kinderen met autisme/een autistisch spectrum stoornis in de ICSI-groep. Probleemgedrag van ICSI en NC-kinderen gemeten met de CBCL was gelijk, maar in beide gevallen hoger dan van IVF-kinderen. De scores van IVF-kinderen lagen ook minder vaak in het klinisch (grens) gebied. Wij hebben hiervoor geen verklaring, noch voor het feit dat de verschillen tussen ICSI en IVF-kinderen voornamelijk werden gevonden in meisjes. Ouderlijke stress was vergelijkbaar voor de ICSI en IVF-groep; de scores waren lager voor ouders van NC-kinderen. In vergelijking met de normwaarden waren de NC-scores laag. Dit kan wijzen op selectie van NC-kinderen waarvan de ouders weinig stress ervaren. Scores voor (gezondheidgerelateerde) kwaliteit van leven waren gelijk in de drie conceptiegroepen. We concludeerden dat de meerderheid van de ICSI-eenlingen onderzocht op 5-8 jarige leeftijd in psychosociaal welzijn niet afwijkt van IVF en NC-kinderen.

Hoofdstuk 6. Algemene Discussie

In de Algemene Discussie bespreken we eerst de methodologische onderwerpen: selectie van NC-controles, in- of exclusie van prematuur geboren kinderen, multipele onderzoekers en confounding. De NC-groep was samengesteld uit kinderen van reguliere kleuter- en basisscholen; dit maakte de groep vatbaar voor selectie bias. We beargumenteren waarom de NC-groep representatief is voor haar referentiepopulatie, op enkele beperkingen na. De ICSI en IVF-groep werden gematched onder andere op prematuriteit, maar voor vier van de zes premature kinderen vonden wij geen match. We besloten de premature kinderen uit te sluiten van de ICSI/IVF analyses, wat niet in conflict was met de studieopzet. Een nadeel van ons onderzoek zou kunnen zijn dat multipele onderzoekers verantwoordelijk waren voor de uitvoer van de RAKIT, maar in dit hoofdstuk leggen we uit waarom dit onze resultaten niet heeft beïnvloed. In de Algemene Discussie lichten we toe hoe we in de verschillende hoofdstukken (2 t/m 5) zijn omgegaan met correctie voor confounding. Correctie moet bij voorkeur worden toegepast wanneer een factor zowel is geassociëerd met de blootstelling als de uitkomst en wanneer deze associatie zowel een statistisch als biologisch karakter heeft.

Recente ontwikkelingen in de exploratie van het causale pad van ART naar uitkomsten worden besproken. Infertiliteit en ART-procedures lijken samen verantwoordelijk te zijn voor ongunstige uitkomsten na ART. Double-embryo transfer, hormonale ovariële stimulatie en genomic imprinting zouden hierin een intermediaire rol kunnen spelen. Een andere recente ontwikkeling is de toepassing van preimplantatie genetische screening bij ART. Deze techniek, waarbij specifiek embryo's zonder genetische afwijkingen worden geselecteerd voor implantatie, is gericht op het verhogen van de zwangerschapskans.

Tot slot stellen we voor om in toekomstig onderzoek (i) een groep kinderen te includeren geboren na natuurlijke conceptie uit ouders met vruchtbaarheids-problemen; (ii) een groep kinderen te includeren geboren na gecontroleerde ovariële stimulatie; (iii) de nadruk te leggen op kleine neuromotore afwijkingen, meer dan op grote; (iv) de cognitieve ontwikkeling van ICSI-kinderen te vervolgen tot de leeftijd van 20 jaar, met correctie voor ouderlijk IQ; (v) het vervolgonderzoek naar de gezondheid van ICSI-kinderen voort te zetten totdat zeldzame ziektes en ziektes die pas later in het leven tot expressie komen kunnen worden ontdekt; (vi) de prevalentie van autisme en autisme spectrum stoornissen in ICSI-kinderen te monitoren; en (vii) de vruchtbaarheid van ICSI-nakomelingen te onderzoeken.

List of Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
ADD	Attention Deficit Disorder
ANOVA	ANalysis Of VAriance
ART	Artificial Reproductive Techniques
ASD	Autistic Spectrum Disorder
ATZ	AsthenoTeratooligoZoospermia
BMI	Body Mass Index
CBCL	Child Behaviour CheckList
CBS	Central Bureau of Statistics
95%CI	95% Confidence Interval
COHS	Controlled Ovarian HyperStimulation
CP	Cerebral Palsy
DET	Double-Embryo Transfer
Dux25	Dutch Children TNO AZL Quality of Life questionnaire
FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
hCG	human Chorionic Gonadotrophin
HRQoL	Health-Related Quality of Life
ICSI	IntraCytoplasmic Sperm Injection
ICD-10	International Classification of Diseases and Related Health Problems, tenth revision
IQ	Intelligence Quotient
IUI	IntraUterine Insemination
IVF	In Vitro Fertilisation
MND	Minor Neurological Dysfunction
NC	Naturally Conceived/Natural Conception
NOSI	Nijmeegse Ouderlijke Stress Index (= PSI)
OR	Odds Ratio
PGD	Preimplantation Genetic Diagnosis
PGS	Preimplantation Genetic Screening for aneuploidies
PR	Prevalence Ratio
PSI	Parenting Stress Index (= NOSI)
RAKIT	Revised Amsterdam Child Intelligence Test
SES	Socio-Economic Status
SET	Single-Embryo Transfer
SOI	Standaard Onderwijs Indeling
SPSS	Statistical Package for the Social Sciences
TACQOL	TNO AZL Child Quality Of Life questionnaire
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organisation

Curriculum Vitae

Marjolein Knoester was born on November 20th, 1979 in Leidschendam, the Netherlands. From 1992 until 1998 she attended High School at the Marnix Gymnasium in Rotterdam. After passing her finals, she started her studies in the School of Biomedical Sciences at Leiden University. After passing the propaedeutic year cum laude in 1999, she switched to the study of Medicine at Leiden University. In 2000 she passed the propaedeutic year cum laude and she completed her master's degree in 2003.

From September 2003 until May 2007 she worked as a PhD-Student on the research project presented in this thesis in the Departments of Paediatrics and Gynaecology of the Leiden University Medical Center (promotores: F.M. Helmerhorst, J.P. Vandenbroucke, F.J. Walther, co-promotor: S. Veen). During her appointment at the Leiden University Medical Center, she also trained in Epidemiology. In June 2007 she began her internships to obtain her medical degree.

Curriculum Vitae

Marjolein Knoester werd op 20 november 1979 geboren te Leidschendam. Vanaf 1992 volgde zij haar middelbare schoolopleiding aan het Marnix Gymnasium te Rotterdam. In 1998 behaalde zij haar gymnasiumdiploma en begon met de studie Biomedische Wetenschappen te Leiden. Na het cum laude behalen van de propedeuse in 1999, stapte zij over naar de studie Geneeskunde, eveneens aan de Universiteit Leiden. De propedeutische fase rondde zij cum laude af in 2000, waarna in 2003 het doctoraal examen werd behaald.

Van september 2003 tot mei 2007 werkte zij als promovenda op de afdelingen Kindergeneeskunde en Gynaecologie van het Leids Universitair Medisch Centrum, waarvan dit proefschrift het resultaat is (promotores: F.M. Helmerhorst, J.P. Vandenbroucke, F.J. Walther, co-promotor: S. Veen). Tijdens haar werkzaamheden in het Leids Universitair Medisch Centrum ving zij aan met de opleiding ter registratie tot Epidemioloog B. In juni 2007 begon zij met de co-schappen tot het behalen van het arts-examen.

Appendix

Antwoordformulier

Naam ouder 1:
Naam ouder 2:
Zwangerschap door: **spontaan / IVF / ICSI**
Naam kind:
Zwangerschapsduur: maanden en weken
Uitgerekende datum:
Geboortedatum:
Geslacht: jongen / meisje
Geboortegewicht: gram
Het kind is de (1e, 2e, enz) uit een gezin van (1, 2, 3, enz) kinderen
Leeftijd vader bij begin zwangerschap:
Leeftijd moeder bij begin zwangerschap:
Opleiding vader:
Opleiding moeder:
Beroep vader:
Beroep moeder:
Adres: Postcode:
Plaats: Tel:
E-mail: Mobiel:

Wij willen **wel / niet** meewerken aan het vervolgonderzoek naar de gezondheid en ontwikkeling van kinderen geboren na een vruchtbaarheids-behandeling, in vergelijking tot kinderen geboren na spontane bevruchting.

Indien u niet wilt meewerken wilt u dan zo vriendelijk zijn de reden te vermelden?
Reden:

Vragenlijst voor de ouders

Algemene gegevens

Geachte mevrouw, mijnheer,

deze vragenlijst gaat over algemene gegevens. Dit houdt bijvoorbeeld in: uit welk land u komt, wat uw beroep is en naar welke school uw kind gaat.
We willen u vragen alle vragen zorgvuldig in te vullen en daarbij het hokje van uw keuze duidelijk zwart te maken.

Hartelijk dank

Waar verbleef uw zoon of dochter overdag meestal, voordat hij/zij naar de kleuterschool ging? (meerdere antwoorden mogelijk)

O op een kinderdagverblijf/crèche
O op de peuterspeelzaal
O bij een oppasgezin
O bij familie (als oppas)
O bij een au pair/oppas aan huis
O thuis

Op wat voor school zit uw kind nu?

O regulier onderwijs
O regulier maar eens blijven zitten/extra kleuteren
O regulier maar met bijles (remedial teaching)
O speciaal, nl .....

In welke groep? [...]

Wat is de **hoogst** afgemaakte opleiding van **vader**?

O geen
O speciaal lager onderwijs
O lager onderwijs
O lager beroepsonderwijs (LTS, ITO, LHNO, LAS, LEAO)
O mulo/mavo
O havo
O atheneum/gymnasium/vwo/hbs/lyceum
O middelbaar beroepsonderwijs (MBO)
O hoger beroepsonderwijs (HBO)
O universitaire opleiding

Wat is de **hoogst** afgemaakte opleiding van **moeder**?

O geen
O speciaal lager onderwijs
O lager onderwijs
O lager beroepsonderwijs (LTS, ITO, LHNO, LAS, LEAO)
O mulo/mavo
O havo
O atheneum/gymnasium/vwo/hbs/lyceum
O middelbaar beroepsonderwijs (MBO)
O hoger beroepsonderwijs (HBO)
O universitaire opleiding

Wat is het beroep van vader?*
* Indien de biologische vader niet meer de verzorger is van het kind, hier het beroep van de verzorger invullen
.....

Is vader nog werkzaam in dit beroep?
O ja, full-time
O ja, part-time
O nee

Wat is het beroep van moeder?*
* Indien de biologische moeder niet meer de verzorger is van het kind, hier het beroep van de verzorgster invullen
.....

Is moeder nog werkzaam in dit beroep?
O ja, full-time
O ja, part-time
O nee

Hoe is de verzorging van het kind verdeeld over beide ouders?
O allebei evenveel
O vader heeft een groter deel van de zorg dan moeder
O moeder heeft een groter deel van de zorg dan vader

Hoe is de gezinssituatie?
O ouders leven samen
O ouders leven gescheiden, het kind woont meestal bij moeder
O ouders leven gescheiden, het kind woont meestal bij vader
O ouders leven gescheiden, het kind woont bij beide ouders evenveel
O vanwege het overlijden van vader, woont het kind samen met moeder
O vanwege het overlijden van moeder, woont het kind samen met vader

Wat is het land van herkomst van vader?
O Nederland
O Turkije
O Marokko
O China
O Suriname
O anders, nl .....

Wat is het land van herkomst van moeder?
O Nederland
O Turkije
O Marokko
O China
O Suriname
O anders, nl .....

Welke taal wordt er thuis meestal gesproken?
O Nederlands
O Turks
O Marokkaans
O Chinees
O Surinaams
O Engels
O anders, nl .....

Eventuele bijzonderheden of toelichting: .....
.....
.....

Vragenlijst voor de ouders
Zwangerschap en geboorte

Geachte mevrouw, mijnheer,

deze vragenlijst gaat over de zwangerschap en de geboorte van uw zoon of dochter.
Wij willen u vragen deze lijst zo zorgvuldig mogelijk in te vullen, eventueel aan de hand van het kraamformulier en/of het groeiboekje.
Wanneer u ruimte tekort komt, kunt u op de laatste pagina verder schrijven.

Hartelijk dank

Gegevens moeder

Geboortedatum (dg/mnd/jr) [ ][ ]-[ ][ ]-[ ][ ]

Leeftijd moeder aan het begin van de zwangerschap [ ][ ]

De zwangerschap kwam tot stand:
O spontaan
O via IVF
O via ICSI

Aantal voorafgaande pogingen:
medicamenteus [...]
aantal pogingen IVF [...]
aantal pogingen ICSI [...]

Indien van toepassing: is er bij de moeder een oorzaak gevonden voor het uitblijven van een spontane zwangerschap?
O nee
O ja
Zo ja, welke? .....
.....

Ziekte en ziekenhuisopname moeder

Leed de moeder aan bepaalde ziekten *vóór* de zwangerschap (bijv. suikerziekte)?
O nee
O ja
Zo ja, welke?.....
.....

Leed de moeder aan bepaalde ziekten *tijdens* de zwangerschap?
O nee
O ja
Zo ja, welke?.....
.....

Is moeder opgenomen geweest in het ziekenhuis tijdens de zwangerschap *om een andere reden dan de bevalling?*

O nee
O ja

Indien 'ja': in welke week van de zwangerschap was de opname, in welk ziekenhuis en om welke reden?

week	reden	ziekenhuis
.....	.....	.....
.....	.....	.....
.....	.....	.....

Medicijngebruik

Gebruikte moeder medicijnen tijdens de zwangerschap?

O nee
O ja

Indien 'ja', welke? (geen ijzer, vitaminen en fluor vermelden)

.....
.....

Heeft de moeder foliumzuur gebruikt vanaf 4 weken voor de bevruchting tot 8 weken na de bevruchting?

O nee, helemaal niet
O wel gebruikt, maar korter: nl [...] wk voor en [...] wk na de bevruchting
O ja, die volledige periode

Roken/alcohol/drugs tijdens de zwangerschap

Rookte moeder tijdens de zwangerschap?

O nee
O ja, < 10 sigaretten per dag
O ja, > 10 sigaretten per dag

Dronk moeder alcohol tijdens de zwangerschap?

O nee
O 1-2 glazen per week
O 2-7 glazen per week
O >7 glazen per week
O incidenteel excessief drankgebruik (>10 glazen)

Bij 'incidenteel excessief drankgebruik', hoe vaak kwam dit voor tijdens de zwangerschap? [...] keer

Gebruikte moeder soft drugs tijdens de zwangerschap?

O nee
O ja

Zo ja, welke? .....
En hoe vaak? [...] keer, per

O week
O maand
O zwangerschap

Gebruikte moeder hard drugs tijdens de zwangerschap?

O nee
O ja

Zo ja, welke? .....
En hoe vaak? [...] keer, per

O week
O maand
O zwangerschap

Gegevens vader

Geboortedatum (dg/mnd/jr) [ ][ ]-[ ][ ]-[ ][ ]

Leeftijd vader aan het begin van de zwangerschap [ ][ ]

Indien van toepassing: is er bij de vader een oorzaak gevonden voor het uitblijven van een spontane zwangerschap?

O nee
O ja

Zo ja, welke?.....
.....

Roken/alcohol/drugs tijdens de zwangerschap

Rookte vader tijdens de zwangerschap?

O nee
O ja, < 10 sigaretten per dag
O ja, > 10 sigaretten per dag

Dronk vader alcohol tijdens de zwangerschap?
O nee
O 1-2 glazen per week
O 2-7 glazen per week
O >7 glazen per week
O incidenteel excessief drankgebruik (>10 glazen)

Bij 'incidenteel excessief drankgebruik', hoe vaak kwam dit voor tijdens de zwangerschap? [...] keer

Gebruikte vader soft drugs tijdens de zwangerschap?
O nee
O ja
Zo ja, welke? .....
En hoe vaak? [...] keer, per
O week
O maand
O zwangerschap

Gebruikte vader hard drugs tijdens de zwangerschap?
O nee
O ja
Zo ja, welke? .....
En hoe vaak? [...] keer, per
O week
O maand
O zwangerschap

Bevalling, geboorte van het kind

Wat was de datum (dg/mnd/jr) waarop moeder was uitgerekend?
[ ][ ]-[ ][ ]-[ ][ ]

Geboortedatum (dg/mnd/jr) kind [ ][ ]-[ ][ ]-[ ][ ]

Zwangerschapsduur [ ][ ] weken + [ ][ ] dagen

Geslacht
O jongen
O meisje

Wijze van geboorte
O vaginaal, hoofdligging
O vaginaal, stuitligging
O keizersnede

In geval van een keizersnede, wat was de reden?
O conditie moeder
O conditie kind
O zowel conditie moeder als conditie kind

Is *moeder* opgenomen geweest in het ziekenhuis in verband met problemen rondom de bevalling?
O nee
O ja

Zo ja, hoe lang? [ ][ ] weken + [ ][ ] dagen

Wat was de reden?.....
.....

De volgende vragen kunt u eventueel beantwoorden met behulp van het kraamformulier en/of het groeiboekje van uw zoon of dochter.

Wat was het geboortegewicht (in grammen)? [ ][ ][ ][ ]

Wat was de lengte bij de geboorte (in cm)? [ ][ ]

Wat was de schedelomtrek (in cm)? [ ][ ]

Hoe ging het met uw kind direct na de geboorte?
O direct goed
O goed na opstartproblemen
O niet goed, namelijk .....
.....

De start van uw baby is weergegeven in een Apgarscore, na 1, 5 en 10 minuten. Deze score staat op het kraamformulier genoteerd. Als u dit formulier niet meer heeft kunt u deze vraag overslaan.

Apgarscore na 1 minuut [ ][ ]

Apgarscore na 5 minuten [ ][ ]

Apgarscore na 10 minuten [ ][ ]

Aangeboren afwijkingen

Is uw kind geboren met een aangeboren afwijking (zoals bijvoorbeeld een hazenlip, een extra vinger of teen, onvolledige aanleg van de plasbuis, hart- en vaatafwijkingen, het syndroom van Down, een klompvoet, liesbreuk)?

O nee
O ja

Zo ja, kunt u de afwijking benoemen of beschrijven?.....
.....

Is uw kind geopereerd voor deze aandoening?

O nee
O ja

Komt deze *of een andere* aangeboren afwijking voor bij u in de familie?

O nee
O ja

Zo ja, welke afwijking?	En bij welk familielid?
.....	.....
.....	.....
.....	.....

Gezin

Hoeveel kinderen heeft u? [...]

Hoeveel kinderen zijn er geboren vóór het kind waarover u nu deze vragenlijst invult? [...]

Bent u vaker zwanger geweest dan van deze kinderen?

O nee
O ja

Hoeveel keer bent u *in totaal* zwanger geweest? [...] keer

Dit is het einde van de vragenlijst. Heeft u nog aanvullende opmerkingen, dan kunt u deze op de volgende pagina opschrijven. Hartelijk dank voor uw medewerking.

Vragenlijst voor de ouders
Gezondheid van het kind

Geachte mevrouw, mijnheer,

deze vragenlijst gaat over de algemene gezondheid van uw kind. Een deel van de vragen is met '[x] nee' of '[x] ja' te beantwoorden, een deel met [0]/[1]/[2] etc. en een deel van de vragenlijst bestaat uit open vragen (het gaat dan meestal om een toelichting bij een ja/nee antwoord). We willen u vragen alle vragen zorgvuldig te beantwoorden. Mocht u toch ergens, om welke reden dan ook, een antwoord niet invullen, wilt u dan zo vriendelijk zijn om aan te geven waarom?
Het invullen van deze vragenlijst kost ongeveer 10-15 minuten.

Dank u wel

Algemeen

Medische consumptie

Waar bent u bevallen?
O thuis
O ziekenhuis
In welk ziekenhuis? .....

Is uw kind direct na de bevalling opgenomen in het ziekenhuis?
O nee
O ja
Zo ja, hoelang? van [ ][ ]-[ ][ ]-[ ][ ] tot [ ][ ]-[ ][ ]-[ ][ ]
Reden:.....
.....
In welk ziekenhuis?.....

Is uw kind tot nu toe wel eens onder behandeling geweest van een medisch specialist?
O nee
O ja
Zo ja, welk specialisme?
O allergoloog
O cardioloog
O chirurg
O dermatoloog
O gynaecoloog
O huisarts
O internist
O kinderarts
O kno-arts, audioloog
O longarts
O mond- en kaakchirurg
O oogarts
O orthopeed
O revalidatiearts
O reumatoloog
O uroloog
O neuroloog
O psychiater
O plastisch chirurg
O radioloog
O anders, namelijk
.....
.....

En wat was de reden?
Reden specialist 1:
.....
.....

Reden specialist 2:
.....
.....

Reden specialist 3:
.....
.....

Reden specialist 4:
.....
.....

Hoe oud was uw kind toen?
1 [ ] jaar en [ ][ ] maanden
2 [ ] jaar en [ ][ ] maanden
3 [ ] jaar en [ ][ ] maanden
4 [ ] jaar en [ ][ ] maanden

Is uw kind tot heden wel eens opgenomen geweest in het ziekenhuis (bijv voor een operatie)?
O nee
O ja
Zo ja, hoe vaak? [ ] keer

Wat was de reden?
Opname 1:.....
Opname 2:.....
Opname 3:.....
Opname 4:.....
Opname 5:.....

Hoe oud was uw kind toen?
Opname 1 [ ] jaar en [ ][ ] maanden
Opname 2 [ ] jaar en [ ][ ] maanden
Opname 3 [ ] jaar en [ ][ ] maanden
Opname 4 [ ] jaar en [ ][ ] maanden
Opname 5 [ ] jaar en [ ][ ] maanden

En in welk ziekenhuis?
1.....
2.....
3.....
4.....
5.....

Hoe vaak heeft u de afgelopen twaalf maanden met uw kind de huisarts bezocht?
0 = nooit
1 = 1 à 2 keer
2 = 3 à 5 keer
3 = ongeveer 10 keer
4 = vaker [ ]

Is uw kind onder behandeling (geweest) van een logopedist?
O nee
O ja
Zo ja, wat was de reden?
.....
.....

Is uw kind onder behandeling (geweest) van een fysiotherapeut?
O nee
O ja
Zo ja, wat was de reden?
.....
.....

Voeding

Heeft uw kind voedingsproblemen gehad vlak na de geboorte?
O nee
O ja
Zo ja, was dit
1 = niet willen drinken
2 = wel willen, maar niet kunnen drinken
3 = spugen
4 = anders, [ ] namelijk.....
.....

Heeft uw kind borstvoeding gekregen?
O nee
O ja
Zo ja, hoe lang? [ ] maanden

Medicijngebruik

Gebruikte uw kind *in het verleden* medicijnen die zijn voorgeschreven door huisarts of specialist?
0 = nee, zelden of nooit [ ]
1 = ja, wel eens
2 = ja, regelmatig
Zo ja, wat voor soort medicijnen?
.....
.....
.....

Gebruikt uw kind *nu* medicijnen die zijn voorgeschreven door huisarts of specialist?
0 = nee, zelden of nooit [ ]
1 = ja, wel eens
2 = ja, regelmatig
Zo ja, wat voor soort medicijnen?
.....
.....
.....

De volgende vraag gaat over geneesmiddelen die je zonder doktersrecept kunt kopen bij de apotheek, drogist en sommige supermarkten. Dit zijn de 'vrij verkrijgbare geneesmiddelen'. Een voorbeeld van vrij verkrijgbare geneesmiddelen zijn aspirines.

Gebruikte uw kind *in het verleden* medicijnen zonder recept van een dokter?
0 = nee, zelden of nooit [ ]
1 = ja, wel eens
2 = ja, regelmatig
Zo ja, wat voor soort medicijnen?
.....
.....
.....

Gebruikt uw kind *nu* medicijnen zonder recept van een dokter?
0 = nee, zelden of nooit [ ]
1 = ja, wel eens
2 = ja, regelmatig
Zo ja, wat voor soort medicijnen?
.....
.....
.....

Specifiek

De ademhaling

1. Hoest uw kind meestal 's ochtends, in het najaar- en winterseizoen?
   O nee
   O ja
2. Hoest uw kind meestal overdag of 's nachts, in het najaar- en winterseizoen?
   O nee
   O ja

Als u ja heeft ingevuld bij vraag 1 of 2 (of bij beide) dan vraag 3 beantwoorden; bij nee door naar vraag 4.

3. Hoest uw kind zo *vrijwel dagelijks*, wel 3 maanden per jaar?
   O nee
   O ja
4. Heeft uw kind *wel eens* last gehad van kortademigheid bij het buiten spelen of bij het oplopen van de trap?
   O nee
   O ja

Indien u ja heeft geantwoord op vraag 4 dan vraag 5 beantwoorden; bij nee door naar vraag 6.

5. Heeft uw kind *in de afgelopen 12 maanden* last gehad van kortademigheid bij het buiten spelen of bij het oplopen van de trap?
   O nee
   O ja
6. Heeft uw kind *wel eens* last gehad van piepen op de borst?
   O nee
   O ja

Indien u ja heeft geantwoord op vraag 6 dan vraag 7 beantwoorden; bij nee door naar vraag 8.

7. Heeft uw kind *in de afgelopen 12 maanden* last gehad van piepen op de borst?
   O nee
   O ja
8. Heeft uw kind *wel eens* aanvallen gehad van kortademigheid met piepen?
   O nee
   O ja

Indien u ja heeft geantwoord op vraag 8 dan vraag 9 en 10 beantwoorden; bij nee door naar vraag 11.

9. Heeft uw kind *in de afgelopen 12 maanden* aanvallen gehad van kortademigheid met piepen?
   O nee
   O ja
10. Hoe vaak is dit voorgekomen *in de afgelopen 12 maanden*?
    [ ] keer

11. Heeft uw kind ooit gedurende langere tijd *vrijwel dagelijks* een verstopte neus of loopneus gehad?
O nee
O ja
Indien u ja geantwoord heeft op vraag 11 dan vraag 12 beantwoorden; bij nee door naar vraag 13.
12. Had uw kind *in de afgelopen 12 maanden* vrijwel dagelijks een verstopte neus of een loopneus?
O nee
O ja
13. Heeft een dokter *wel eens* astma vastgesteld bij uw kind?
O nee
O ja
Indien u ja heeft geantwoord bij vraag 13 dan vraag 14 en 15 beantwoorden; bij nee door naar vraag 16.
14. Hoeveel astma-aanvallen had uw kind *in de afgelopen 12 maanden*?
[ ]
15. Gebruikt uw kind *op dit moment* medicijnen tegen astma?
O nee
O ja
16. Heeft een dokter *wel eens* bronchitis vastgesteld bij uw kind?
O nee
O ja
Indien u ja geantwoord heeft op vraag 16 dan vraag 17 beantwoorden; bij nee door naar vraag 18.
17. Hoe vaak heeft uw kind bronchitis gehad *in de afgelopen 12 maanden?*
[ ] keer
18. Heeft een dokter *wel eens* longontsteking vastgesteld bij uw kind?
O nee
O ja
Indien u ja geantwoord heeft op vraag 18 dan vraag 19 beantwoorden; bij nee door naar vraag 20.
19. Hoe vaak heeft uw kind longontsteking gehad *in de afgelopen 12 maanden*?
[ ] keer
20. Hoest uw kind gewoonlijk - dat is wel 5 dagen per week - (bijv. bij het opstaan of overdag of 's nachts)?
O nee
O ja
20a. Zo ja; Hoest uw kind in perioden van 3 maanden achtereen?
O nee
O ja
21. Heeft uw kind gewoonlijk - dat is wel 5 dagen per week - last van volzitten op de borst?
O nee
O ja
21a. Zo ja; Heeft uw kind dit in perioden van wel 3 maanden achtereen?
O nee
O ja

22. Heeft uw kind gewoonlijk - dat is wel 5 dagen per week - last van opgeven van fluimen? (bijv. bij het opstaan of overdag of 's nachts)
O nee
O ja
22a. Zo ja; Heeft uw kind dit in perioden van wel 3 maanden achtereen?
O nee
O ja
23. Heeft uw kind in de afgelopen 12 maanden wel eens een periode gehad waarin het meer dan gewoonlijk hoestte *met* opgeven van fluimen (sputum)?
O nee
O ja
23a. Zo ja; Duurde zo'n periode *langer dan 3 weken*?
O nee
O ja
24. Heeft uw kind de afgelopen 12 maanden wel eens last gehad van piepen op de borst?
O nee
O ja
24a. Zo ja; Hoe vaak heeft uw kind last van piepen op de borst gehad de afgelopen 12 maanden?
[ ] keer
25. Heeft uw kind de afgelopen 12 maanden last gehad van aanvallen van benauwdheid *met* piepen op de borst (astma-aanvallen)?
O nee
O ja
25a. Zo ja; Hoe vaak heeft uw kind zo'n aanval (gemiddeld per maand)?
[ ] keer
26. Vindt u dat uw kind de afgelopen 12 maanden wel eens kortademig is geweest?
O nee
O ja
26a. Zo ja; Vindt u dat uw kind sneller kortademig is dan leeftijdgenootjes?
O nee
O ja
27. Heeft uw kind de afgelopen 12 maanden wel eens last gehad van kortademigheid bij traplopen of bij spelen?
O nee
O ja
27a. Zo ja; Heeft uw kind wel eens last gehad van kortademigheid bij lopen over vlak terrein in normaal tempo?
O nee
O ja
28. Heeft uw kind in de afgelopen 12 maanden vaak last gehad van een verstopte neus of een loopneus?
O nee
O ja

28a. Zo ja; Heeft uw kind dit wel in perioden van 3 maanden achtereen?
O nee
O ja

Zijn bij uw kind zijn/haar amandelen geknipt?
O nee
O ja

Rookt u en/of uw partner?
O nee
O ja, 1 van beiden
O allebei

Heeft uw kind 'taaislijmziekte' (CF)?
O nee
O ja

Urinewegen

Heeft uw kind wel eens blaasontsteking/urineweginfecties gehad waarvoor de huisarts medicijnen (antibiotica) heeft voorgeschreven?

0 = nee nog nooit
1 = ja, 1 keer
2 = ja, vaker dan 1 keer [ ]

Bij een zoon: waren de testes (ballen) direct ingedaald?
O nee
O ja
O eenzijdig
Zo niet, hoe oud was uw zoon toen ze uiteindelijk wel indaalden?
[ ][ ] maanden

Gebeurde dit spontaan of met een operatie?
O spontaan
O operatief

Gehoor

Heeft uw kind afwijkingen aan het gehoor?
O nee
O ja
Zo ja, is dit al sinds de geboorte?
O nee
O ja
Kunt u de afwijking benoemen of beschrijven:
.....
.....
.....

Was er sprake van een afwijkende gehoortest op het consultatiebureau of bij de schoolarts?
O nee
O ja
Heeft uw kind buisjes in de oren?
O nee
O ja
Heeft uw kind een gehoorapparaat?
O nee
O ja

Oog

Heeft uw kind afwijkingen aan de ogen?
O nee
O ja
Zo ja, is dit al sinds de geboorte?
O nee
O ja
Kunt u de afwijking benoemen of beschrijven: .....
.....

Is de oorzaak bekend van deze afwijking?
O nee
O ja
Zo ja, wat is de oorzaak?.....
.....

Hoe is het zicht van uw kind?
0 = uw kind ziet goed
1 = uw kind ziet matig, heeft een bril nodig
2 = uw kind is zeer slechtziend
3 = uw kind is blind aan 1 oog
4 = uw kind is blind aan beide ogen [ ]

Is uw kind kleurenblind?
O nee
O ja

Kijkt of keek uw kind scheel?
O nee
O ja
Zo ja, is uw kind hiervoor behandeld?
O nee
O ja
Zo ja, hoe?.....
.....

Overig

Heeft uw kind wel eens last gehad van
– stuipen bij koorts?
O nee
O ja
– stuipen zonder koorts?
O nee
O ja

Is uw kind bekend met epilepsie?
O nee
O ja

Heeft uw kind de afgelopen zes maanden wel eens last gehad van:
1 slaapstoornis
O nee
O ja
2 eczeem
O nee
O ja
3 eetproblemen
O nee
O ja

4 veel huilen
O nee
O ja
5 onrust
O nee
O ja
6 hoofdpijn
O nee
O ja
7 maagpijn
O nee
O ja
8 buikpijn
O nee
O ja
9 misselijkheid
O nee
O ja
10 overgeven
O nee
O ja

Is uw kind ergens allergisch voor, wat door de huisarts of een andere dokter is bevestigd?
O nee
O ja
Zo ja, waarvoor?
1.....
2.....
3.....

Heeft uw kind nog klachten of aandoeningen waar in deze vragenlijst niet naar is gevraagd?
.....
.....
.....
.....

Wat vindt u van de gezondheid van uw kind ten opzichte van andere kinderen?
0 = mijn kind lijkt *gezonder* dan andere kinderen
1 = mijn kind lijkt *even gezond* als andere kinderen
2 = mijn kind lijkt *minder gezond* dan andere kinderen [ ]

Hartelijk dank voor uw medewerking!