Neurodevelopmental outcome of children born following assisted reproductive technology

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1 General Introduction

BACKGROUND

The number of children born following assisted reproductive technology (ART) has shown a steady rise during the last decades. In 1978, Steptoe and Edwards reported the first pregnancy and live birth following *in vitro* fertilisation (IVF) (Steptoe and Edwards, 1978). Since then, numbers increased substantially. In 2010, Edwards was even awarded a Nobel Price, as his achievements in the development of ART are considered to represent a milestone in modern medicine. Meanwhile, up to 4% of children are born following ART in several European countries and it is likely that in the future this percentage will rise even further (Nyboe Andersen *et al.*, 2009).

As a consequence of the growing number of ART-children, health and development of these children has become of general importance. Over the years, it is well established that ART-children have an increased risk of being born preterm or with a low birth weight compared to their naturally conceived peers (Australian in vitro fertilisation collaborative group, 1985; Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004). This difference is partly the result of the relatively high incidence of twins among ART-children. Yet, poorer outcomes are also found in singleton pregnancies (Australian in vitro fertilisation collaborative group, 1985; Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004). The fact that preterm birth and low birth weight are strongly related to impaired neurodevelopment (Bhutta *et al.*, 2002) and diseases in adult life (Barker, 1995) necessitates careful follow-up of ART-children.

In this thesis the neurodevelopmental outcome of ART-children is evaluated up to the age of two years. An attempt is made to unravel the biological mechanisms that may underlie potentially poorer neurodevelopmental outcome. To clarify which mechanisms may be involved, I will shortly address the techniques used in conventional IVF, IVF in the modified natural cycle and IVF with preimplantation genetic screening.

Assisted Reproductive Technology

Since its introduction, ART has undergone several technological developments. These refinements were, in general, aimed at reducing side effects and improving success rate.

Conventional IVF consists of the following phases. First, the woman's monthly hormone cycle is suppressed with drugs (Gonadotrophine Releasing Hormone (GnRH)-agonists or antagonists) in order to avoid a Luteinising Hormone (LH)-surge, and untimely ovulation. In addition, the ovaries are stimulated to develop multiple follicles by administration of Follicle Stimulating Hormone (FSH). Growth of the follicles is carefully monitored with regular ultrasounds and measurement of estradiol levels. When several follicles have reached diameters of

approximately 18-20 mms and estradiol levels are sufficiently high, maturation of the follicles is boosted by supplementation of human Chorionic Gonadotrophin (hCG). Oocyte retrieval, by transvaginal ultrasound-guided follicle aspiration, is planned approximately 34 hours later. In the laboratory, the retrieved oocytes are then inseminated either naturally - by a selected sperm sample - or with intracytoplasmic sperm injection (ICSI). In the latter procedure, one spermatozoon is selected on its appearance and injected into the oocytes' cytoplasm (Palermo *et al.*, 1992). After *in vitro* fertilisation, the zygotes are cultured for 2 to 3 days. During this period, several cell cleavages take place, resulting in the origination of an early embryo. Hereafter, one or two of the morphologically best-looking embryos are transferred to the uterus. In case a woman achieves pregnancy, the endometrium may be supported by supplementation of progesterone or hCG. Surplus good-looking embryos may be cryopreserved and transferred in subsequent cycles if pregnancy does not occur (Zeilmaker *et al.*, 1984).

IVF in the Modified Natural Cycle

An alternative to conventional IVF is IVF in the modified natural cycle (MNC-IVF). The aim of this procedure is to use the one follicle that naturally develops to dominance (Nargund *et al.*, 2007). Like in conventional IVF, follicle size and estradiol levels are monitored closely. In contrast to conventional IVF, only when the lead follicle has reached a diameter of approximately 14 mms, GnRH- antagonists and FSH are started, mostly resulting in maturation of one single follicle (Rongières-Bertrand *et al.*, 1999;Pelinck *et al.*, 2005). With this approach, medication use is strongly reduced and the natural selection method for the dominant follicle is preserved. A resulting limitation, however, is a reduced pregnancy rate per cycle. Advantages of MNC-IVF above conventional IVF are a close to zero risk of multiple gestation and a negligible risk of ovarian hyperstimulation syndrome (Pelinck *et al.*, 2007).

Preimplantation Genetic Screening

The intention of IVF with preimplantation genetic screening (PGS) is to enhance the efficiency of assisted reproduction. An increase in numerical chromosomal abnormalities (aneuploidies) in ageing women may be one of the causes of a decrease in the chance of pregnancy with age (Wilton, 2002). Therefore, the concept of PGS is to identify and discard embryos with an abnormal chromosomal constitution. In the procedure, a hole is made in the zona pellucida of an embryo with laser or by chemical means. Subsequently, one or two blastomeres are aspirated so that copy numbers of several chromosomes can be determined with fluorescence in situ hybridisation (FISH). Theoretically, PGS should lead to higher ongoing pregnancy rates, however, in 2007 a randomised controlled trial on the

efficiency of PGS showed reduced instead of improved pregnancy rates following PGS (Mastenbroek *et al.*, 2007). This finding was confirmed in a recent meta-analysis (Mastenbroek *et al.*, 2008). For this reason, the technique, as described above, is no longer practiced on routine basis. Currently, feasibility of alternative forms of PGS (such as polar body biopsy and more comprehensive chromosome testing) is studied (Harper *et al.*, 2010;Geraedts *et al.*, 2010).

Mechanisms that may underlie poorer outcome following ART

It is conceivable that one or more components of the ART-procedure induce alterations in embryo development and in this way influence health and development of children born following ART. So far, the majority of studies focussed on the relation between ART and birth weight or other perinatal outcomes. From animal studies, it is well known that culture conditions affect birth weight, likely because of disturbed genomic imprinting in early embryo development (Young *et al.*, 2001;Khosla *et al.*, 2001;Ceelen and Vermeiden, 2001). In addition, hormonal hyperstimulation is proven to affect birth weight in animal models (Ertzeid and Storeng, 2001;Ceelen and Vermeiden, 2001;van der Auwera and D'Hooghe, 2001). Explanations that have been postulated for less optimal perinatal outcome are impaired endometrial receptivity because of altered hormone levels in ART or loss of natural selection of the dominant follicle, resulting in reduced oocyte quality (Ertzeid and Storeng, 2001;van der Auwera and D'Hooghe, 2001;Pelinck *et al.*, 2010). In studies concerning humans, type of culture medium (Dumoulin *et al.*, 2010) as well as ovarian hyperstimulation (Olivennes *et al.*, 1993;Ombelet *et al.*, 2006;Klemetti *et al.*, 2010) seem to affect birth weight. Remarkably, children born following frozen-thawed embryo transfer show higher birth weights and gestational ages than children born following fresh embryo transfer (Källén *et al.*, 2005;Pinborg *et al.*, 2009). Possibly, this is explained by transfer of the former group in naturally unstimulated cycles instead of hyperstimulated cycles with supra-physiological estradiol levels, which may effect endometrial receptivity. Alternatively, higher birth weights after freezing and thawing may be caused by selection of superior embryos by the cryopreservation procedure (Pinborg *et al.*, 2009; Källén *et al.*, 2005). However, since a recent study reported no linear relation between parameters of ovarian stimulation, such as doses of gonadotrophins used or duration of stimulation and birth weight (Griesinger *et al.*, 2008), it remains unclear to what extent ovarian hyperstimulation affects human birth weight.

In humans, subfertility itself is another factor suggested to be an important contributor to outcome of ART children. Subfertile couples are known to have an increased risk of obstetric complications and adverse perinatal outcome, including

increased risks of preeclampsia, antepartum haemorrhage, caesarean section, preterm birth, low birth weight and perinatal death (Thomson *et al.*, 2005;Pandian *et al.*, 2001;Draper *et al.*, 1999). Since some studies (Kapiteijn *et al.*, 2006;De Geyter *et al.*, 2006), but not others (Romundstad *et al.*, 2008) reported worse perinatal outcome in ART-children when compared to children of subfertile couples, it is still difficult to determine to what extent subfertility explains worse perinatal outcome following ART. Truly unravelling the effects of ART and the underlying indication for treatment can only be done by means of a trial with random allocation of assisted and natural conception, which is unethical and therefore impossible (Buck Louis *et al.*, 2005;Knoester, 2007).

Finally, vanishing twins may affect outcome following ART-pregnancies. Singleton pregnancies after ART are often the result of spontaneously reduced twin pregnancies and these so-called vanishing twins are related to preterm birth and low birth weight (Dickey *et al.*, 2002;Pinborg *et al.*, 2005;Pinborg *et al.*, 2007).

The above described factors that may influence perinatal outcome following ART may also influence development and health of ART-children. It would be of great interest to elucidate the mechanisms that underlie these associations. This could lead to the identification of at-risk subfertile couples and the provision of customised fertility, obstetrical or child-welfare care (Thomson *et al.*, 2005).

AIMS

The present thesis describes two projects. The aim of the first project is to disentangle the potential effects of controlled ovarian hyperstimulation and the *in vitro* procedure itself on neurodevelopmental outcome in infancy. In the second project, the aim is to study whether PGS in addition to 'conventional' IVF or ICSI affects neurodevelopmental outcome in infancy.

METHODS

Two separate projects are described in the present thesis. First, studies resulting from the data of the Groningen ART-cohort and secondly, studies on follow-up of children born after IVF with PGS. The two projects ran in parallel.

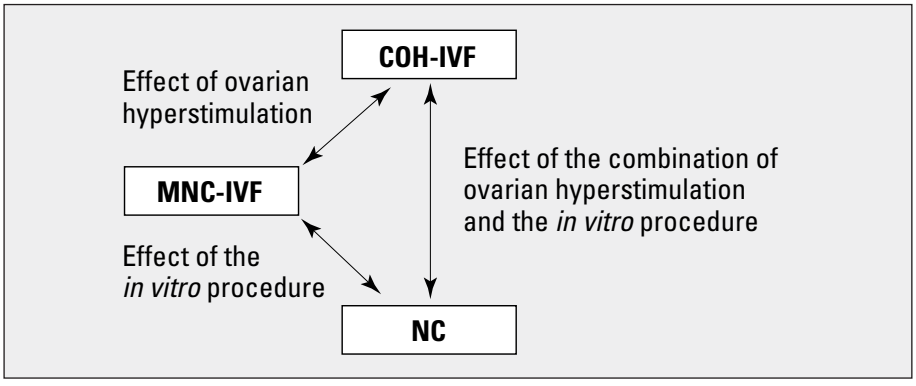
The Groningen ART-cohort study

The Groningen ART-cohort consists of three groups of prospectively recruited singletons. Couples were recruited at the Department of Reproductive Medicine of

the University Medical Center Groningen, a tertiary care centre in the Netherlands. All couples who were pregnant after IVF or ICSI, with a term date between March 2005 and December 2006 were asked to participate in a longitudinal study on neurodevelopmental outcome of IVF/ICSI children, during the third trimester of their pregnancy. This resulted in two groups; children born after 'conventional' IVF/ICSI with controlled ovarian hyperstimulation (COH-IVF) and children born after IVF/ICSI in the modified natural cycle (MNC-IVF). A third group was formed by children of couples who had a naturally conceived (NC), singleton pregnancy while on the waiting list for fertility evaluation or treatment during the same time frame. The couples on the waiting list had been subfertile for at least 1 year. The latter control cohort was chosen as we expected that parental characteristics like, for instance, parity and parental age of the subfertile couples would resemble the characteristics of IVF couples. Excluded from the study were twins and children born after cryopreserved or donated oocytes or embryos.

The Groningen ART-cohort is formed to disentangle the separate effects of ovarian hyperstimulation and the *in vitro* procedure. MNC-IVF differs fundamentally from COH-IVF in that the one oocyte that naturally develops to dominance is used. Minimal medication is supplemented to achieve this goal. Potential differences in neurodevelopmental outcome of COH-IVF and MNC-IVF children may, therefore, be attributed to ovarian hyperstimulation and/or the loss of natural selection of the dominant follicle. Likewise, potential neurodevelopmental differences between MNC-IVF and NC-children are attributable to the *in vitro* procedure (figure 1). When results of the Groningen ART-cohort study are interpreted, it should be noted that the medication used in MNC-IVF, although minimal, may cause an overestimation of the effect of the IVF procedure itself and an underestimation of the effect of ovarian stimulation. Since IVF is in general applied following ovarian hyperstimulation, we

Figure 1 - Diagram of the groups and effects studied in the Groningen ART-cohort study



also compared neurodevelopmental outcome of COH-IVF and NC children. The information obtained in this way is valuable for subfertile couples considering IVF treatment (figure 1).

Follow-up of children born after IVF with Preimplantation Genetic Screening

The PGS follow-up study consists of children of couples who participated in a double blind, two-centre, randomised controlled trial on the efficiency of PGS to improve ongoing pregnancy rates in IVF (Mastenbroek *et al.*, 2007). Women participating in the trial had to be between 35 and 41 years old. Exclusion criteria for women were previously failed IVF-cycles and objections against a possible double embryo transfer. Randomisation of women into IVF with or without PGS was performed centrally with minimisation for age (35-37 or 38-41 years) and reproductive technique (IVF or ICSI), with stratification according to study centre (Academic Medical Center, Amsterdam or University Medical Center Groningen) (Mastenbroek *et al.*, 2007). Couples were informed that a follow-up program was part of the PGS-trial and that children born to couples who were included in the trial would be invited for follow-up during the third trimester of pregnancy. For practical reasons, children born after treatment in Groningen were invited to participate in an extensive follow-up program, whereas children born after treatment in Amsterdam were only invited for assessments at the age of two years.

Table 1 - The assessment battery

Assesment age	Neurodevelopmental test used	Outcome measures
2 weeks	GMs	Quality of GMs
3 months	GMs	Quality of GMs
4 months	TINE	Clinical neurological classification
10 months	TINE	Clinical neurological classification
18 months	Hempel	NOS, fluency-score and clinical neurological classification
2 years	Hempel	NOS, fluency-score and clinical neurological classification
	BSID-II	MDI and PDI
	CBCL	Total problem scale, Internalizing scale and Externalizing scale

GMs: General Movements, TINE: Touwen Infant Neurological Examination, Hempel: Hempel's neurological examination for toddlers, NOS: Neurological Optimality Score, BSID-II: Bayley's Scales of Infant Development - second edition, MDI: Mental Developmental Index, PDI: Psychomotor Developemental Index, CBCL: Child Behaviour Check List

An overview of neurodevelopmental tests used in the present thesis

The ages of assessment and neurodevelopmental tests used in the present thesis are presented in table I. Age-specific testing is necessary, since an infant's functional repertoire expands rapidly due to abundant structural changes in the

developing nervous system in the first two years of life. In infancy, the emphasis of the assessments was on quality of neurological functioning, either expressed in the quality of General Movements (GMs) or in the occurrence of Minor Neurological Dysfunction (MND). Complementary, cognitive and behavioural function was assessed at the age of two years.

OUTLINE OF THIS THESIS

The studies in this thesis concern neurodevelopmental outcome of children born following ART. The thesis is divided into 4 parts;

Part I; Literature review
Chapter 2 provides a systematic overview of the literature on neuromotor, cognitive, language and behavioural outcome in children born following IVF or ICSI.

Part II; The Groningen ART-cohort study
In this cohort, we investigated the effect of ovarian hyperstimulation and the in vitro procedure on neurodevelopmental outcome in cohorts of children born following modified natural cycle IVF, controlled ovarian hyperstimulation IVF and children born to subfertile parents.
Chapter 3 describes early neuromotor development, measured by means of the quality of General Movements at 2 weeks and 3 months.
Chapter 4 documents the neurological condition of the children measured with the Touwen Infant Neurological Investigation (TINE) at 4 and 10 months and the Hempel examination at 18 months. Both measures, TINE and Hempel, focus on the presence of minor neurological dysfunction.
Chapter 5 presents the neurological condition of the children measured with the Hempel examination at 2 years.
Chapter 6 describes mental and psychomotor development and behaviour measured with, respectively, the Bayley Scales of Mental Development and the Child Behaviour Check List at the age of 2 years.

Part III; Follow-up of children born after IVF with Preimplantation Genetic Screening
This part addresses neurodevelopmental outcome of children born following IVF with PGS compared to children born after 'conventional' IVF. Neurodevelopmental assessments carried out in the PGS follow-up study are similar to the assessments in the Groningen ART cohort study.

Chapter 7 deals with neurodevelopmental outcome measured at 2 weeks, 3, 4, 10 and 18 months.
Chapter 8 reports mental, psychomotor, neurological and behavioural outcome in 2-year-old children.

Part IV; General discussion, future perspectives and summary
Chapter 9 contains the general discussion and future perspectives.
Chapter 10 summarizes the results of the studies in English and Dutch.