

University of Groningen

Neurodevelopmental outcome of children born following assisted reproductive technology

Middelburg, Karin Janette

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2011

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Middelburg, K. J. (2011). *Neurodevelopmental outcome of children born following assisted reproductive technology: 0-2 years*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Part IV
**General discussion
and future perspectives**

9



This thesis describes the neurodevelopmental outcome of children born following assisted reproductive technology. Series of sensitive, validated measures have been used to scrutinize neurodevelopment up to the age of two years. Overall, results of the studies in this thesis have been reassuring. In the Groningen ART cohort study, we found no differences in neurodevelopmental outcome - including mental, psychomotor, neurological and behavioural development - of children born following ART with and without ovarian hyperstimulation up to the age of two years. Additionally, follow-up of children born after ART with preimplantation genetic screening showed no association between PGS conception and impaired mental, psychomotor, and behavioural outcome at age two; however an association was found between PGS conception and lower neurological optimality scores.

The human central nervous system continues to develop after birth. Large part of myelination and synapse formation and elimination occurs throughout childhood and adolescence (Graaf-Peters and Hadders-Algra, 2006). Therefore, the age of two years is too early to draw pertinent conclusions on neurodevelopmental outcome of ART children. Continuation of follow-up and meta-analysis of follow-up studies is still needed to warrant the safety of assisted reproductive technology on long term.

Methodological considerations

True distinction between the effects of ART and the underlying indication for treatment can only be made in a trial with random allocation of assisted and natural conception, which is unethical (Buck Louis *et al.*, 2005; Knoester, 2007). In practice, the possibilities for follow-up studies are therefore limited. Best evidence comes from register based studies and prospective cohort studies. The former have proven to be very valuable when studying disorders of low prevalence, such as cerebral palsy (Ericson *et al.*, 2002; Stromberg *et al.*, 2002; Pinborg *et al.*, 2004; Klemetti *et al.*, 2006). However, register based studies do not allow for detailed study of mental, psychomotor, neurological and behavioural development. For this matter we need controlled studies. The systematic review taught us that the number of controlled studies with robust methodological quality is still limited, since follow-up studies are often hampered by practical difficulties. In the following paragraphs, the methodology of the studies described in this thesis will be addressed.

The Groningen ART-cohort study

This prospective, longitudinal cohort-study is unique for its study groups; children born after IVF/ICSI with controlled ovarian hyperstimulation (COH-IVF) are compared to children born after IVF/ICSI in a modified natural cycle (MNC-IVF), and a control group consisting of naturally conceived children born to couples who were waiting for subfertility work-up. With this design we aimed at disentangling the potential effects of controlled ovarian hyperstimulation and the *in vitro* procedure itself on neurodevelopmental outcome. Nevertheless, the medication used in MNC-IVF - although minimal - may have caused an overestimation of the effect of IVF or an underestimation of the effect of COH. In the interpretation of the results of our study we were not hampered by this minor confounding of MNC, since assisted reproduction was not associated with reduced neurodevelopmental outcome in any of the studies. An alternative method to analyze the effect of ovarian stimulation on neurodevelopmental outcome would be to compare outcome of naturally conceived children to children born following ovulation induction. A drawback of this method is that the latter procedure aims at obtaining only 1 or 2 follicles, while in COH-IVF the aim is to harvest up to 10 oocytes (Nargund *et al.*, 2007). Thus, studying children born following ovulation induction is not truly representative of the effects of ovarian hyperstimulation.

Another unique aspect of this study is its exceptionally high follow-up rate (97-98% at age two). Utmost effort was put into maintaining children in follow-up. Parents were involved in the study by means of regular examinations since birth and newsletters concerning the progress of the study. Furthermore, home-visits were made when parents were unable to visit the hospital. But most importantly, the children enjoyed the neurodevelopmental examinations, as these are very playful. Despite the high follow-up rate, sample size of the study is still relatively small, which is a limitation of the study. It precludes any definite conclusion on disorders of low incidence.

It may be assumed that the low attrition, together with good initial prenatal enrolment (63-76%) has yielded a representative sample of singleton ART children in Groningen. Generalisability to all ART children is limited, since we excluded twins. COH-IVF is associated with an increased prevalence of twins. We excluded twins from the analyses as we were unable to identify a sufficiently large group of twins born following MNC-IVF and naturally conceived twins born to subfertile patients. Selection bias was studied by comparing characteristics of participants and non-participants. Participation was largely non-selective, with the exception of maternal age in NC mothers (non-participating mothers were younger).

We applied detailed, standardised neurodevelopmental assessments at the corrected ages of 2 weeks, 3, 4, 10, 18 and 24 months. The instruments used allowed us to study subtle differences in neurodevelopmental outcome early in life. The advantage of assessing neurodevelopmental outcome at early age is the relatively limited influence of postnatal factors, such as social conditions, on developmental outcome. This allows for a closer linkage of findings to early ontogenetic events. Data on the long term predictive value of the neurodevelopmental instruments used in this thesis (GMs, TINE and Hempel) in groups of infants not considered at high risk for developmental disorder is scarce. However, reliability measured by inter- and intra-assessor agreement, of the instruments is good (Hempel, 1993; Hadders-Algra *et al.*, 2009; Bouwstra *et al.*, 2009; Middelburg *et al.*, 2010). Therefore the instruments can be used to compare groups; however, it is unknown what the exact significance of the current findings is for the children's long-term neurodevelopmental outcome.

Notably, the secondary analyses (the comparison between the subfertile group and fertile reference group) in the GM study and the studies concerning developmental follow up at age two are hampered by the fact that the fertile reference groups were recruited separately and postnatally. Another limitation of these comparisons was that the assessors were not blind to group allocation, which reduced internal validity, and with that, external validity of the comparison. In addition, for the reference infants in the GM-study no information was available on conception method. It is rather likely that some of the children were born following assisted reproduction. This may imply that actual differences in GM-quality between children of fertile and subfertile couples were larger than indicated in the study.

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

To our knowledge this study is the first prospective follow-up study of children born after IVF in which couples were randomised into IVF with or without PGS. Randomisation contributed to the comparability of the study and control group, it resulted in similar demographic characteristics in the two groups.

The main limitation of this study is its sample size. The power analysis was based on the number of women needed to detect an increase in ongoing pregnancy rate instead of children needed for follow-up. Unfortunately, the number of children available for follow-up was limited by the negative effect of PGS on ongoing pregnancies (Mastenbroek *et al.*, 2007). In the study reporting on neurodevelopmental outcome from 2 weeks to 18 months, sample size was even further reduced. Due to logistical reasons, only children living in the northern part of the Netherlands were studied at these ages.

Since both twins and singletons were included in this study, data on neurodevelopmental outcome are representative for all children born following PGS. Generalisability is also supported by the relatively high participation rates (~80%), which may likely be a result of the fact that couples were informed on the follow-up before start of the IVF-treatments. Prenatal or - even better - preconceptional inclusion of children helps to prevent selection bias, as later inclusion may be affected by the child's neurodevelopmental status. Parents of children with a neurodevelopmental disorder may be inclined to refrain from participation in follow-up research for two reasons. First, confrontation with the limitations of their child during assessment. Secondly, these parents frequently visit the hospital for follow-up of their child's disorder and therefore time constraints may play a role. On the other hand, parents who are concerned about their child's development may be eager to participate, with the intention of receiving a professional opinion on the child's development. Similarly, parents of children with advanced development are often willing to cooperate in our experience.

The influence of ovarian hyperstimulation and ART laboratory procedures on neurodevelopmental outcome

Factors that may affect perinatal outcome after ART may also influence development and health of children born after ART. The mechanisms underlying these relations are as yet unclear. Hypothetically, embryo development may be affected by one or more components of the ART-procedure. For instance, several natural selection procedures are bypassed in ART and culture conditions may cause disturbed genomic imprinting (Ceelen and Vermeiden, 2001; Young *et al.*, 2001; Khosla *et al.*, 2001). Furthermore, altered hormone levels in ART may interfere with nidation and placentation, leading to suboptimal uterine conditions (Ertzeid and Storeng, 2001; Ceelen and Vermeiden, 2001; van der Auwera and D'Hooghe, 2001). Research so far primarily focussed on perinatal outcome measures, such as birth weight and gestational age. Nevertheless, it is possible that consequences extend beyond the perinatal period (Barker, 1995).

In our studies, we chose to correct for birthweight and gestational age by means of multivariate statistics as our research question was whether ovarian hyperstimulation or the in vitro procedure affected outcome at 2 years, given the potential effect of assisted reproduction on perinatal outcome. One could also argue not to correct for these factors, since they are mediators on the pathway from assisted reproduction to neurodevelopmental outcome. Therefore we repeated the analysis without the mediating factors birth weight and gestational age. Overall, this did not affect the results (reported in Chapters 4 and 6).

The results of the systematic review showed no consistent differences in neuromotor, cognitive, language and behavioural development between children born after ART and naturally conceived children. Evidence is sufficient to conclude that gross developmental pathology in children born following ART is absent up to the age of three years. Specific gross effects of ovarian hyperstimulation or the ART laboratory procedures are therefore neither expected in this age period. Nonetheless, the measures used to assess developmental outcome in most studies (e.g. the Bayley Scales of Infant Development) are not capable of evaluation of neuromotor and cognitive functioning in a detailed sense. Subtle deviations may have little clinical relevance for individual functioning; however subtle deviations in a substantial subpopulation may have impact on society at large. For this reason, it is justified to scrutinise neurodevelopment of the continuously increasing percentage of children born following assisted reproduction.

The relation between subfertility and neurodevelopmental outcome: cause or consequence?

Subfertility itself may also contribute to outcome of ART children. It is known that subfertile couples have an increased risk of obstetric complications and adverse perinatal outcome, such as increased risk of preeclampsia, antepartum haemorrhage, caesarean section, preterm birth, low birth weight and perinatal death (Draper *et al.*, 1999; Pandian *et al.*, 2001; Thomson *et al.*, 2005).

In the Groningen ART-cohort, we found a remarkable high percentage of the naturally conceived pregnancies of subfertile couples to be complicated by pregnancy induced hypertension. Furthermore, the naturally conceived children of subfertile couples were relatively often born by caesarean section, showed more signs of foetal distress, and were more frequently admitted to neonatal intensive care than children of the ART-groups, even though birth weight and gestational age at birth were higher. Possibly, this was a chance-finding. Our study was not designed, nor powered for these outcome measures. But theoretically, these findings may also have been the result of an increased risk in pregnancies of subfertile couples in combination with less intensive obstetrical care than provided in ART-pregnancies. If the association between subfertility and perinatal outcome is clarified, this may help to provide customised obstetrical care to patients at risk (Thomson *et al.*, 2005).

At the age of three months, we saw a slightly reduced general movement quality in children born to subfertile couples compared to a reference group. Whereas, at the age of two years, we found better neurological outcome in the

subfertile group than in another reference group. The inconsistency of these findings may be the result of selection bias. The reference group at 3 months was representative for the general population. Children were recruited and assessed as part of a general health check-up provided to all Dutch children. At the age of two years, parents were invited to volunteer at the child-welfare-centre, but a separate appointment was made for the examinations. The total time scheduled for the assessments was nearly two hours per child. The effort that had to be taken may have led to selection of parents with concerns about their child's development.

The relation between subfertility and neurodevelopmental outcome was also studied in the Danish national cohort study. Sun *et al.* reported no increased prevalence of epilepsy and febrile seizures in children of untreated subfertile couples compared to children of fertile couples (Sun *et al.*, 2007) and Zhu *et al.* did not find an association between time to pregnancy and age of milestone achievement or risk for cerebral palsy (Zhu *et al.*, 2009; Zhu *et al.*, 2010a). Yet, Zhu *et al.* did find a modestly increased risk of developmental coordination disorder (Zhu *et al.*, 2010b). Altogether, solid evidence of an association between subfertility and neurodevelopment is absent. Nevertheless, it is conceivable that a non-optimal genetic make-up or hormonal condition results not only in subfertility, but also in less optimal neurodevelopmental outcome in offspring.

Preimplantation genetic screening and neurodevelopmental outcome

Theoretically, embryo biopsy as performed in PGS may induce damage which interferes with the embryo's further development. For instance, the use of laser or chemicals for opening the zona pellucida may induce thermal, mechanical or mutagenic side effects (Kanyo and Konc, 2003) with long-lasting consequences.

In the PGS-study we found a statistically significant difference between the PGS and control group on the neurological optimality score (NOS) at 2 years, but not on other psychomotor and neurological measures. This finding indicates that the difference found is rather subtle, since the range of optimal behaviour is narrower than the range of normal behaviour (Prechtel, 1980). Other groups who studied PGS-children reported similar outcome in study and control groups, except for lower scores on the loco-motor subscale of the Griffiths in children born after PGS (Banerjee *et al.*, 2008; Nekkebroeck *et al.*, 2008a; Nekkebroeck *et al.*, 2008b). Altogether, an increased risk for a less favourable neurological outcome after PGS cannot be excluded by evidence available.

Suggestions for future research

Neurodevelopmental disorders may emerge when children grow older and cognitive functioning evolves. The body of evidence of good methodological quality concerning neurodevelopment of children born after ART beyond pre-school age is still limited, therefore this should continue to be a focus of follow-up research. Future meta-analyses would be helpful to overcome sample size limitations in current follow-up studies. Crucial in this matter is uniformity of neurodevelopmental assessments. Louise Brown - the first and therefore eldest person born after IVF - has currently only reached her mid-thirties (Stephoe and Edwards, 1978). Thus, long-term or trans-generational consequences of assisted reproduction may still appear when the cohort of people born after ART grows older. In this aspect, reproductive functioning of people conceived with ART also deserves attention.

The most important factor influencing neurodevelopmental outcome after ART is twin-status. Twin pregnancies carry an increased obstetrical risk and therefore medical specialists consider them less desirable (Land and Evers, 2004). The majority of subfertile patients, however, prefer twins over singletons (van Wely *et al.*, 2006). Understandably, parents often focus on the higher pregnancy rates after double embryo transfer. Additional risks in terms of neurodevelopmental outcome of ART-twins compared to ART-singletons are only known to a limited extent. Information available is largely based on nation-wide register based studies. Remarkably, the reported prevalence of cerebral palsy in ART-singletons and ART-twins is not significantly different, which may be attributable to an increased risk in ART-singletons due to higher prematurity and low birthweight rates (Pinborg, 2005). Controlled studies showed that ART-twins had significantly lower scores on cognitive functioning than ART-singletons (Bonduelle *et al.*, 2003; Olivennes *et al.*, 2005). Information on minor neurological dysfunctioning of ART-twins is not available. The information may help caregivers to counsel patients on the risks of twins and, with that, the risks of treatment strategies that may result in birth of twins, such as ART with controlled ovarian hyperstimulation and double embryo transfer.

The influence of subfertility *per se* on developmental outcome in children may be further investigated by studying children of couples with different indications for assisted reproduction. For instance, tubal pathology is expected to have less effect than unexplained subfertility or subfertility due to male factor. Similarly, time to pregnancy may be related to developmental outcome.

Recently, a growing interest in cardiovascular outcomes following ART arose (Painter and Roseboom, 2007). It is known that the early environment of developing

organisms is important in determining later cardiovascular health (Barker, 1995). In animals, assisted reproduction results, for instance, in hypertension in mice and an increased cardiovascular risk in cattle (Rerat *et al.*, 2005; Watkins *et al.*, 2007). Moreover, preliminary evidence is provided that ART children are also more prone to obesity, hypertension and diabetes (Belva *et al.*, 2007; Ceelen *et al.*, 2008). These findings deserve further attention in future follow-up studies.

Technological possibilities in assisted reproduction develop continuously. New methods are often introduced in practice without a pre-clinical phase, as results in animal studies cannot easily be extrapolated to humans. Moreover, clinical studies in healthy volunteers are also impossible. Therefore, the only way to evaluate the safety of new assisted reproductive techniques is in retrospect. As yet, the knowledge on development of children born following preimplantation genetic screening, preimplantation genetic diagnosis, cryopreservation and in vitro maturation is limited at best. It is of utmost importance that the safety for offspring is evaluated before large scale implementation of these methods.

Concluding remarks

Results of the studies in this thesis have been reassuring. We found no differences in neurodevelopmental outcome - including mental, psychomotor, neurological and behavioural development - up to the age of two years in children born following ART with and without ovarian hyperstimulation and a naturally conceived control group born to subfertile parents. The follow-up study of children born after IVF with PGS showed similar mental, psychomotor and behavioural outcome in 2-year-old PGS and control children. Neurological optimality scores were lower in PGS-children. Long term follow-up is still needed to warrant safety of ART as neurodevelopmental disorders may emerge when children grow older.

