CHAPTER 9
Summary
Five percent of newborns in Europe are born following assisted reproductive techniques (ART), a steadily increasing number. Consequently, development and health of children born following ART is of general significance. ART is associated with perinatal adversities such as low birthweight and preterm delivery. Nevertheless, ART has not been associated with adverse neurodevelopmental outcome during the first postnatal years. However, this does not preclude an effect of ART on long-term neurological development. Neurodevelopmental disorders may first emerge later in life, as a result of the continuing structural and functional development of the brain during childhood.

In this thesis the neurodevelopmental outcome of children born following ART is evaluated up until 4 years of age by exploring the influence of specific factors involved in assisted conception, such as ovarian hyperstimulation, the in vitro laboratory procedures and subfertility-related aspects. The subfertility aspects are threefold: the presence of a history of subfertility, the underlying cause of subfertility and the duration of subfertility in terms of time to pregnancy (TTP), as a proxy for the severity of subfertility.

Chapter 1 gives a general and concise overview of the literature available on child health and developmental outcome after ART. The overview indicates that ART does not seem to be associated with adverse neurodevelopmental outcome during the first postnatal years. However, insufficient information is available on the long-term consequences of assisted conception and subfertility-related aspects on a child’s neurodevelopment. Chapter 1 also describes the concept of brain development and vulnerability and introduces the applied fertility treatment techniques in the studies of the thesis. Finally the chapter introduces the two projects, the Groningen ART cohort study and the PGS (preimplantation genetic screening) study, on which the studies of the thesis are based.

Part I: The Groningen ART cohort study
Part I evaluates primarily the potential effects of ovarian hyperstimulation, the in vitro laboratory procedures, or a combination of both on neurodevelopmental outcome in preschool-aged children. Secondly, part I evaluates the potential effects of subfertility on neurodevelopmental outcome.

In Chapter 2 the potential effect of ART on neurodevelopmental outcome, expressed in terms of movement variation, was evaluated in the children of the Groningen ART cohort at the ages of 4, 10 and 18 months. All children were singletons, born following in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) with conventional controlled ovarian hyperstimulation (COH-IVF, n=68), after IVF in a modified natural cycle (MNC-IVF, n=57) or after natural conception born to subfertile couples (Sub-NC, n=90). Neurodevelopmental outcome was measured with the Infant Motor Profile (IMP), resulting in a total IMP score and five domain scores: variation, variability, symmetry, fluency and performance. Primary outcome was the domain score variation (i.e. movement repertoire size), a parameter reflecting the integrity of cortical connectivity. The main result of the study was that COH-IVF children had a significantly lower mean variation score than MNC-IVF children. However, a similar difference was not observed between the two IVF groups.
and the Sub-NC group. The study concludes that no clear effect of ovarian hyperstimulation and the in vitro procedure on movement variation throughout infancy could be demonstrated.

**Chapter 3** describes the neurological condition of the children of the Groningen ART cohort at the age of 2 years in terms of neurological optimality (neurological optimality score [NOS], fluency score) and the prevalence of minor neurological dysfunction (MND). Special attention was paid to potential effects of the presence and the underlying cause of subfertility on the outcome parameters. Singleton born following COH-IVF (n=66), MNC-IVF (n=56), Sub-NC (n=87) and after natural conception to fertile couples (reference group, n=101) were assessed with the Hempel examination. Primary outcome was the fluency score, as fluency of movements is easily affected by subtle dysfunction of the nervous system. The main results of the study were similar fluency scores, NOS and MND prevalence in the groups. However, the fluency score and NOS of the three subfertile groups were higher, and the prevalence of MND was lower than those in the reference group. The study concludes that the neurological condition of 2-year-olds born after ART is similar to that of children of subfertile couples conceived naturally. Moreover, subfertility does not seem to be associated with a worse neurological outcome.

**Chapter 4** describes the effect of the severity of subfertility, in terms of TTP, on the neurological condition of the children of the Groningen ART cohort at 2 years in terms of MND, assessed with the Hempel examination. Participants were singleton children born to subfertile couples (n=209). The main result of the study was that TTP of children with MND was significantly longer than that of children without MND. After correction for gestational age, parental age and parental level of education (multivariable logistic regression analyses), the association remained statistically significant. The study concludes that increased TTP was associated with suboptimal neurological development in 2-year-olds. This suggests that subfertility and its determinants are involved in the genesis of neurodevelopmental problems.

**Chapter 5** describes the effect of both ART-related aspects and the three previously mentioned subfertility aspects on the neurological condition of the children of the Groningen ART cohort at the age of 4 years, again, in terms of neurological optimality (NOS, fluency score) and the prevalence of complex MND. Specific attention was paid to the possibility of sex-specific effects of ART and subfertility. Four-year-old singletons born to subfertile parents (subfertile group, n=195), including singletons born following COH-IVF (n=63), MNC-IVF (n=53) and Sub-NC (n=79) and newly recruited 4-year-old singletons born to fertile parents after natural conception (reference group, n=98) were studied. Neurological development was evaluated with the Hempel examination, with the fluency score being the primary outcome parameter. The main results of the study were that the fluency score, NOS and the prevalence of complex MND were similar in COH-IVF, MNC-IVF and Sub-NC children. Neurological condition of children born to subfertile parents was similar to that of children of fertile parents and was independent of the underlying cause of subfertility. No statistically significant associations were found between TTP and the fluency score.
score and NOS. However, a positive correlation was found between TTP and the prevalence of complex MND; a correlation which could be attributed to girls, in whom an evident positive correlation was present. A similar association was absent in boys. The study concludes that ovarian hyperstimulation, the in vitro procedure and the combination of both, a history of subfertility per se and the underlying cause of subfertility were not associated with worse neurological outcome in 4-year-old singletons. However, increased TTP was associated with an increased risk for the complex form of MND, especially in girls. This suggests that rather the severity of subfertility than its presence or IVF-components affects neurological outcome. Moreover, girls may be neurologically more vulnerable for the effect of severity of subfertility than boys.

**Chapter 6** describes an explorative approach on the effect of both the ART-related aspects and the three subfertility aspects on the cognitive and behavioural development of the children of the Groningen ART cohort at the age of 4 years. Four-year-old singletons born to subfertile parents (subfertile group, n=195), including singletons born following COH-IVF (n=63), MNC-IVF (n=53) and Sub-NC (n=79) and newly recruited 4-year-old singletons born to fertile parents after natural conception (reference group, n=98) were studied. The primary cognitive outcome parameter was a total intelligence quotient (IQ), measured with the Kaufman Assessment Battery for Children, second edition (K-ABC-II); the behavioural outcome parameter was the total problem T-score, measured with the Child Behavior Checklist (CBCL). Causal inference search algorithms and structural equation modelling were applied as statistical tools. Unlike traditional statistics these methods are able to unravel underlying causal mechanisms and distinguish between confounders and intermediate effects. The main results of the study were the absence of direct or indirect causal effects of ovarian hyperstimulation or the in vitro procedure on cognitive and behavioural outcome in 4-year-old singletons born to subfertile couples. However, direct negative causal effects were found of the severity of subfertility in terms of time to pregnancy (TTP) on cognition and of the presence of subfertility on behaviour. Both effects were confounded by maternal age at child conception and maternal educational level. Additionally, cognition and behaviour were directly related to one another. The study concludes that suffering from subfertility per se, and especially from more severe subfertility – which by itself is associated with higher age and high educational level of the mother – negatively affects the child’s cognitive and behavioural outcome.

**Part II: The Groningen PGS study**

Part II evaluates the effect of preimplantation genetic screening (PGS) on neurodevelopmental outcome, in terms of neuromotor, cognitive and behavioural development of 4-year-old children.

**Chapter 7** describes the results of this prospective, assessor-blinded follow-up study on children born to women who participated in a multicentre randomized clinical trial (RCT) on the effect of IVF with or without PGS. Forty-nine children (31 singletons, 9 sets of twins) were born following IVF with PGS and 64 children (42 singletons, 11 sets of twins) were born
following IVF without PGS (controls). Assessments were the Hempel examination, resulting in a fluency score (primary outcome parameter), NOS and the prevalence of complex MND; the K-ABC-II, resulting in a total IQ score and the CBCL, resulting in a total problem T-score. The main result of the study was that neurodevelopmental outcome of PGS children was similar to that of controls. However, PGS affected neurodevelopmental outcome of twins in a different way than that of singletons. The fluency score and the NOS of PGS twins were significantly lower than those of control twins. In addition, PGS in twins was associated with a higher sequential IQ score. The study concludes that PGS does not seem to affect neurodevelopmental outcome of 4-year-old singletons, however, our data suggest that it may be associated with altered neurodevelopment in twins.

Chapter 8 discusses the findings of the studies in this thesis. The thesis concludes that ART-related aspects such as ovarian hyperstimulation, the in vitro laboratory procedures, or a combination of both were not associated with worse neurodevelopmental outcome – in terms of movement variation, neuromotor function, cognition and behaviour – in singleton children aged 4 months to 4 years. Additionally, a history of subfertility per se and the underlying cause of subfertility were not associated with worse neurological outcome in 2 and 4-year-old singleton children. However, increased TTP was associated with an increased risk for MND at 2 years and an increased risk for complex MND at 4 years. Moreover, subfertility per se, and especially more severe subfertility – which by itself is associated with higher age and high educational level of the mother – negatively affected the child’s cognitive and behavioural outcome. Furthermore, neurodevelopmental outcome in singletons born following IVF with PGS was similar to that of singletons born after IVF without PGS at age 4. PGS in twins was associated with a negative effect on neuromotor condition and a positive effect on sequential processing IQ. This may point to the possibility that the embryo biopsy inherent to PGS is associated with differences in brain function at a later age.

The major strengths of the Groningen ART cohort study is its prospective design, the unique composition of the study groups and the use of standardized, age-specific and sensitive tools used to evaluate neurodevelopmental outcome. Additional strengths are the blinding of our assessors to the mode of conception of the Groningen ART cohort children and the less than 10% post-natal attrition rate throughout the years. A limitation of the Groningen ART cohort study is the composition of the fertile reference group which does not match the way in which we recruited the children of the Groningen ART cohort. Moreover, the results cannot be generalized to multiples, as we studied singletons only.

The major strengths of the PGS study are the random assignment of the participants, the blinding of the assessors to the mode of conception and the prospective design of the study. The PGS study is the first follow-up study of a RCT on PGS in which children were followed as long as 4 years. Limitations are the relative small size of our study groups and the selective drop-out in both groups.
Future research on long-term health and development of children born to subfertile couples, with or without ART, has to continue. Health and neurodevelopmental follow-up has to be extended to school-aged children and adolescents to elucidate the long-term effects of ART and subfertility on the developing brain and body against the light of puberty-onset. Subsequently, it is also interesting to investigate the association between ART and parental subfertility and the offspring's fertility potential. Attention should also be paid to the effectiveness and safety of new techniques in assisted conception. At present, new techniques are being developed to improve the accuracy of PGS in terms of the timing of the biopsy (i.e. cleavage or blastocyst stage), the material used for screening (embryonic cells, the polar body or trophectoderm tissue) and the number of chromosomes that are needed to be screened. Another upcoming technique is frozen embryo replacement (FER). As single embryo transfer (eSET) is currently increasingly often applied, more embryos are available for freezing and a growing number of children are already born following FER. Health and development of these children needs to be monitored, as concerns have been raised about an increased risk of being large for gestational age (LGA) after FER in comparison to both fresh embryo transfers and spontaneous conception.

The results of this thesis underline the importance of long-term follow-up of health and development of children born to subfertile couples, without and with the application of ART. Monitoring and improving the safety and health of subfertile couples and their children is necessary, especially in today’s society were maternal age at child birth, subfertility and the application of ART are increasing and fertility treatment techniques are continuously being developed and renewed.