

Summary and general discussion



Summary

During the past decades, in vitro fertilization (IVF) has become a routine procedure in reproductive medicine to overcome subfertility problems in couples all over the world. Since the first IVF birth in 1978, an estimated three million children have been born worldwide after IVF or related assisted reproductive technologies (ART). In 2005, ~2.3% of all Dutch babies were born after assisted reproduction. Nowadays, it is increasingly acknowledged that the IVF procedure may affect the vulnerable processes occurring during conception and early embryonic development. There is a substantial body of evidence that IVF children are at increased risk for adverse perinatal outcome. In addition, various studies suggested an increased incidence of congenital abnormalities and rare imprinting diseases among IVF children. However, due to the lack of systematic follow-up of these children during the past years, it is largely unclear whether IVF treatment in humans is associated with substantial developmental consequences in later stages of life in conceived offspring. Therefore, the present thesis addressed several aspects of postnatal growth and development in 246 8- to 18-year-old IVF singletons and 233 spontaneously conceived controls born to subfertile parents. The aims and outline of the thesis were described in *Chapter 1*.

Chapter 2 presented an extensive review of the current literature about growth and development of children born after IVF treatment. Perinatal outcome of IVF pregnancies, the occurrence of congenital anomalies, imprinting disorders, malignancies and postnatal growth characteristics in IVF offspring were thoroughly discussed. Several meta-analyses and other well-designed studies provided substantial evidence that IVF children are at increased risk for adverse perinatal outcome, congenital malformations and rare epigenetic defects. There is still no consensus on whether observed health problems are related to the IVF procedure itself and/or the underlying subfertility problems of the parents. Studies examining postnatal growth, development and morbidity rates are scarce with conflicting results and other areas of long-term research in IVF children are still in its infancy.

The objective of *Chapter 3* was to investigate several cardiometabolic measures in 8- to 18-year-old IVF singletons and spontaneously conceived controls born from subfertile parents. Previous studies have demonstrated that adverse conditions during early prenatal life are associated with cardiometabolic dysfunction in postnatal life. Blood pressure was examined in 225 IVF children and 225 age- and gender-matched spontaneously conceived control children. Several indicators of insulin resistance were studied in a pubertal subpopulation. Systolic and diastolic blood pressure levels were higher in IVF children than in controls (109 ± 11 vs. 105 ± 10 mm Hg, $P < 0.001$; 61 ± 7 vs. 59 ± 7 mm Hg, $P < 0.001$, respectively). IVF children appeared ~2 times more likely to be in the highest systolic and diastolic blood pressure quartiles. Moreover, significantly higher fasting glucose levels were found in pubertal IVF children (5.0 ± 0.4 vs. 4.8 ± 0.4 mmol/l in controls, $P = 0.005$). Differences in blood pressure and fasting glucose could neither be explained by current body size, birth weight and other early life factors nor by parental characteristics including

cause of subfertility. Therefore, we hypothesized that the IVF procedure might contribute to the programming of cardiometabolic physiology and function in conceived offspring. The findings described in this chapter highlight the importance of continued cardiometabolic monitoring of IVF-conceived children in order to evaluate the clinical consequences in later life.

In **Chapter 4**, body composition in 233 IVF singletons and 233 spontaneously conceived controls born from subfertile parents was examined by anthropometry. In addition, body composition was also assessed in a pubertal subpopulation by means of dual energy X-ray absorptiometry (DXA). Other studies indicated a link between adverse stimuli during periconception versus disturbed adipose tissue development and obesity in postnatal life. Children born after IVF had a significantly lower subscapular-triceps skinfold ratio and a significantly higher sum of peripheral skinfolds, peripheral body mass, and percentage of peripheral body fat compared with controls. Although not reaching statistical significance, both DXA and skinfold measurements indicated that total body fat in IVF children is increased. The differences in peripheral fat assessed by anthropometry between IVF and control children could neither be explained by current and early risk factors nor parental factors like cause of subfertility. No differences in bone mineral composition between IVF children and controls were demonstrated. Since these observations suggest that body fat composition in IVF children is disturbed, follow-up of IVF children to monitor body fat pattern and potentially related health problems from adolescence into adulthood is of great importance.

Chapter 5 focused on several anthropometric, radiological and biochemical characteristics of pubertal development in IVF children and spontaneously conceived control children. Pubertal stage by Tanner's classification, age at menarche and menstrual cycle characteristics were examined in 115 IVF-conceived boys and 118 IVF-conceived girls, each with age-matched comparison groups. Bone age and sex hormone levels were studied in two distinct pubertal subpopulations. Pubertal stage and age at menarche were not significantly different between IVF and control children. Advanced bone age and higher DHEAS and LH levels were observed in pubertal IVF girls in comparison to controls. However, since these findings did not follow from a specific hypothesis being tested, multiple statistical significance testing could have led to chance findings.

Numerous studies have demonstrated a relationship between early postnatal growth and blood pressure and body fat in children and adolescents. Since little is known about postnatal growth in IVF offspring and its effects on blood pressure and body fat composition during childhood and adolescence, these aspects were studied in **Chapter 6**. Data from original growth charts up to four years of age were used to study early postnatal growth in 193 IVF and 199 control children born to subfertile parents. Significantly lower weight, height and BMI standard deviation scores (SDSs) at 3 months and weight SDS at 6 months of age were found in IVF children compared with controls. Likewise, IVF children demonstrated a greater gain in weight SDS, height SDS and BMI SDS during late infancy (3 months to 1 year) versus controls. Weight gain during early childhood (1–3 years) was related to blood pressure in IVF children but not in controls. Growth during late

infancy was not related to skinfold thickness in IVF children, unlike controls. Growth during early childhood was associated with skinfold thickness in both IVF and controls. It was concluded that growth during early childhood is related to several cardiovascular risk factors in IVF offspring during late childhood and adolescence.

The aim of the study in *Chapter 7* was to examine the school functioning of 8- to 18-year-old IVF children and spontaneously conceived controls born to subfertile parents. Measures of education level, school performance (need for extra help, repeating a grade, special education), and rates of learning and developmental disorders were compared in 233 IVF children and 233 controls. General cognitive ability was examined in a pubertal subpopulation. None of the measures concerning school functioning differed between IVF and control children. Children and adolescents born after IVF demonstrated good academic achievement and general cognitive ability. Furthermore, they did not experience more educational limitations than spontaneously conceived children and adolescents. These results suggest that school functioning of children and adolescents born after IVF treatment appears to be normal.

General discussion

The goal of the general discussion is to address methodological issues, pathophysiological considerations, potential implications of our findings and recommendations for future research.

Methodological considerations

The methodological issues of the studies presented in this thesis have been discussed in the separate chapters. The strengths of our study include the relatively large study size and the comparison group consisting of spontaneously conceived children born from subfertile parents. Furthermore, the IVF singletons and controls were carefully matched according to age and gender. All children were examined in a standardized manner and data regarding a wide range of developmental aspects were collected. In the following section, methodological considerations regarding the comparison group used, the potential for selection bias, the assessment of outcome variables, and confounding and the statistical analyses are described in more detail.

Comparison group

In order to be able to adequately examine postnatal growth and development in IVF children, an appropriate comparison group of unexposed children is needed. Ideally, the unexposed group resembles the exposed group with respect to all factors related to the outcomes of interest, except for the exposure under investigation. Although from a scientific point of view randomization of