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Pre-, Peri- and Postnatal Influences on Ophthalmologic Outcome

a study on children born after intracytoplasmic sperm injection (ICSI) and children born preterm

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Göteborg 2009

A doctoral thesis at a University in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarises the accompanying papers. These have already been published or are in a manuscript at various stages (in press, submitted or in manuscript).

ISBN 91-628-7774-3
ISBN 978-91-628-7774-3

Print: Vasatryckeriet 2009
Illustration: Made by Anna-Karin Larsson by the idea of Ann Hellström, based on a photograph by Lennart Nilsson.

To my darling Gerdt

ABSTRACT

The aims of the present study were to investigate the effects of prenatal factors in children born after intracytoplasmic sperm injection (ICSI) and peri- and postnatal factors in children born preterm on visual function and ocular fundus morphology at school age. In the children born preterm the ophthalmologic outcomes, including optic nerve morphology were analysed in relation to gestational age (GA), birth weight (BW) standard deviation score (SDS), serum levels of insulin-like growth factor I (IGF-I), weight at week 32 (SDS), and weight, length and head circumference (SDS) at school age. We found that there was no significant difference in visual function between children born after ICSI (n=137) and matched control children (n=159). Furthermore, we found that boys born after ICSI (n=35) had slightly abnormal retinal vascularisation with significantly fewer central retinal vessel branching points in comparison with the control group (n=203). Among the preterm children (n=66), with a mean GA at birth of 27.5 weeks, 74 % had some kind of ophthalmologic abnormality, and 17 % had visual impairment. Early as well as later growth was closely related to visual acuity and perception at school age. In addition low IGF-I levels and poor growth during the first weeks/months of life were correlated with small head circumference and refraction anomalies at school age. We also found an association between a small neuronal rim area in the optic disc and low BW and poor early growth, indicating the importance of early weight gain for neural development in children born preterm.

A gender specific effect of the ICSI procedure on vascular development in the eyes of boys cannot be excluded. In the preterm child the early postnatal growth and the growth factor IGF-I seem of importance for optimal development of visual functions, refraction and for head circumference at school age.

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, published or in manuscript, which will be referred to by their Roman numerals:

I. **Hök Wikstrand M**, Strömland K, Flodin S, Bergh C, Wennerholm UB, Hellström A. Ophthalmologic findings in children born after intracytoplasmic sperm injection. *Acta Ophthalmologica Scandinavica 2006;84:177-181.*

II. **Hök Wikstrand M**, Niklasson A, Strömland K and Hellström A. Abnormal vessel morphology in boys born after intracytoplasmic sperm injection. *Acta Paediatrica 2008;97:1512-1517.*

III. **Hök Wikstrand M**, Hård A-L, Niklasson A and Hellström A. Postnatal growth variables are related to ophthalmologic outcome at school age in very preterm children. *Submitted*

IV. **Hök Wikstrand M**, Hård A-L, Niklasson A and Hellström A. Birth weight deviation and early postnatal growth are related to optic nerve morphology at school age in very preterm children. *Submitted*

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LIST OF ABBREVIATIONS

AGA	Appropriate for gestational age
ART	Assisted reproduction technique/technology
BP	Branching points
BW	Birth weight
BPD	Broncho-pulmonary dysplasia
CPAP	Continuous positive airway pressure
CT	Computed tomography
FSH	Follicle stimulating hormone
GA	Gestational age
GW	Gestational week
HCG	Human chorionic gonadotropin
ICSI	Intracytoplasmic sperm injection
IGF-I	Insulin-like growth factor I
ITA	Index of tortuosity for arteries
ITV	Index of tortuosity for veins
IVF	In vitro fertilisation
IVH	Intraventricular haemorrhage
LBW	Low birth weight
LGB	Lateral geniculate body
MRI	Magnetic resonance imaging
NEC	Necrotizing enterocolitis
PCA	Postconceptional age
PMA	Postmenstrual age
PR	Percentile rank
PVL	Periventricular leucomalacia
RB	Retinoblastoma
ROP	Retinopathy of prematurity
SDS	Standard deviation score
SGA	Small for gestation age
SS	Sum of scaled scores
TNO	De Nederlandse Organisatie voor toegepast-natuurwetenschappelijk anderzoek
TVPS-R	Test of Visual-Perceptual Skills (Non-Motor)-Revised
VA	Visual acuity
VEGF	Vascular endothelial growth factor
VLBW	Very low birth weight
WHO	World Health Organization
WMD	White matter damage

INTRODUCTION

Modern technology has made possible the birth of children to previously infertile couples and the survival of very immature babies. These children are subjected to unnatural influences during different time periods of the first nine months normally spent intrauterine. In vitro fertilisation exposes the egg, sperm and embryo to an environment normally not present at conception, and in intracytoplasmic sperm injection (ICSI) to non-physiologic selection of sperms. Children born after ICSI have an increased risk of preterm birth, and implantation of more than one embryo increases the risk of multiple pregnancies, which further increases the risk of preterm birth. Other causes of preterm birth such as infection/inflammation and placental dysfunction may have a negative impact on the foetus. In addition, adaptation to extra uterine life during the third trimester demands intensive care which, although advanced, is far from creating a normal milieu for the infant.

The prevalence of preterm birth has continued to increase since the late seventies. This increase is associated with increasing prevalence of multiple births as well as changing maternal characteristics (more mothers older than 35 years, more mothers with high risk pregnancies, and more very young mothers).

Preterm birth may affect the visual system in several ways. Firstly, the premature exteriorisation removes the visual system from the nurturing intrauterine environment during a period of rapid maturation. Secondly, the overall immaturity of vascular and neural tissues makes the infant prone to develop lesions of the eyes and posterior visual pathways. In addition, the preterm child is exposed to light and visual stimulation at a time period naturally spent in the dark.

This thesis explores the effects of the prenatal influences in children born after ICSI and peri- and postnatal influences in children born preterm with GA <32 weeks, on visual outcome and eye morphology at school age. Approximately 20 % of the ICSI children were born preterm (GA <37 weeks) and 15 % were born small for gestational age (SGA). Although most children born very preterm will develop vision in the normal range it is well documented that very preterm birth is associated with visual impairment, and even modest degrees of low BW and prematurity may be associated with increased ophthalmic morbidity.

Development

The visual system

The development of the eye and the visual system will be reviewed with special emphasis on time-periods of importance for children born after ICSI and children born preterm.

The anterior segment

The eyelids are fused until 24-25 weeks postconceptional age (PCA) (Robinson J, 1989).

The corneal diameter is 6.2 mm in week 25 and increases linearly 0.5 mm every 15th day to 9.0 mm week 37 (Tucker et al., 1992). The cornea undergoes structural changes and flattens, but it has been documented that infants born preterm, at term equivalent, have more highly curved corneas and shallower anterior chambers than full term babies (Cook et al., 2003; Fledelius, 1982).

The pupils are large (mean diameter 4.7 mm) at 26 weeks and become smaller and have by 29 weeks a mean diameter of 3.4 mm. The pupils do not constrict to light until 30.6 weeks ±1 week (Isenberg et al., 1990).

The sclera is developed and formed of 50 cell layers by 24 GW, and no further mitoses are seen thereafter (O'Connor and Fielder, 2007).

The lens increases its proportion of gamma crystalline throughout gestation, and alters its shape from elongated to spherical form at term (O'Connor and Fielder, 2007).

The posterior segment of the eye

The neural retina

The development of the neural retina and visual system is complex. Several genes, like PAX-2 and PAX-6 are involved (Strachan and Read, 1994). Recent advances have shed light on the interplay between numerous transcriptional networks and growth factors that are involved in the specific stages of retinogenesis, the optic nerve formation and topographic mapping (Harada et al., 2007; Hatakeyama and Kageyama, 2004; Holt et al., 1988; Marquardt and Gruss, 2002; Turner and Cepko, 1987; Wetts and Fraser, 1988). The retina is composed of six types of neurons and one type of glia (Müller glia), which constitute three nuclear layers. Retinal ganglion cells are situated in the ganglion cell layer, horizontal, amacrine, bipolar and Müller glial cells in the inner nuclear layer, and the outer nuclear layer contains the photoreceptors (cones and rods). During retinogenesis, these seven cell types derive from a common population of retinal progenitor cells residing in the inner layer of the optic cup. Müller cells carry out many of the functions provided by radial glia, astrocytes and oligodendrocytes in the central nervous system (Harada et al., 2000). Retinal development is centred in the macula and proceeds to the periphery. Mitotic activity in the central retina stops at 14 weeks and in the periphery at 24 weeks (Provis et al., 1985). The photoreceptors begin to develop during the 5th month. The cones differentiate during the sixth month followed by rods, about a month later. Even at the earliest stages of foetal development only cones are found in the most central part of the retina (Hollenberg and Spira, 1972). The outer plexiform layer has reached the mid-periphery by 24 weeks when both cones and rods have inner segments and the photoreceptors in the central retina have rudimentary outer segments (Johnson et al., 1985). By 28 weeks outer segments, and outer plexiform layer are present throughout the retina (Birch and O'Connor, 2001).

At 22 weeks of gestation the area of the future fovea contains a cone photoreceptor layer and a layer of ganglion cells (Hendrickson, 1994). The ganglion, amacrine, bipolar, horizontal, and Müller cells move away from the fovea, while the cones move toward the fovea. The first sign of a foveal depression is detected at 25 weeks.

The foveal area is never vascularised during the development, and an inhibiting factor has been proposed (Provis et al., 2000). In the retinal pigment layer the melanosomes develop until the 27th week of gestation.

The retinal surface area is expanding by growth and maturation of individual cells until three weeks after birth (Provis et al., 1985) and its size is doubled from 24 weeks to term (O'Connor and Fielder, 2007).

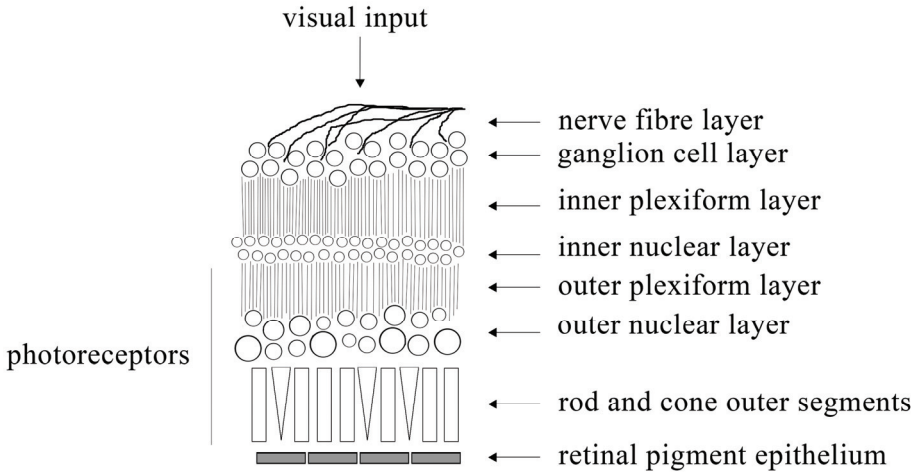

Figure 1. The retinal layers

The retinal vasculature

Retinal vasculogenesis, i.e. vessel formation by differentiation and migration of large numbers of spindle shaped mesenchymal precursor cells (angioblasts) from the optic disc, commences around 14 GW. The retinal vasculogenesis is replaced at 21 GW by angiogenesis, i.e. formation of vessel by budding and sprouting from already existing vessels. The angiogenesis is completed by term (Hughes et al., 2000). Around 25 GW the vessels from the upper and lower temporal vasculature meet along the horizontal meridian temporal of fovea. The retinal vessel formation is promoted by the increased metabolic demands of growing neurons which results in a local hypoxia. The progressing vasculature is accompanied by astrocytes that lie just ahead of the advancing endothelial cells. These astrocytes have processes that extend into the avascular retina (Ling and Stone, 1988; Provis et al., 1997), and sense the relative

hypoxia which promotes production of vascular endothelial growth factor (VEGF) within the astrocytes. VEGF stimulates endothelial cell proliferation at the vascular front. It has been shown that insulin-like growth factor 1 (IGF-I) also influences angiogenesis and acts as a critical permissive factor for normal vascularisation through interaction with locally produced VEGF (Hellstrom et al., 2002; Hellstrom et al., 2001; Smith et al., 1999). The proliferation of the endothelial cells causes capillaries to grow into the previously hypoxic retina, resulting in a local decrease in hypoxia, and a down regulation of the VEGF in nearby astrocytes. The process is iterated as the astrocytes and vascular endothelial cells migrate towards the periphery.

Astrocytes also play a role in endothelial cell differentiation and blood barrier function (Chan-Ling and Stone, 1992). In addition, astrocytes along with microglia contribute to the perivascular glia limitans, important for vessel integrity (Provis, 2001).

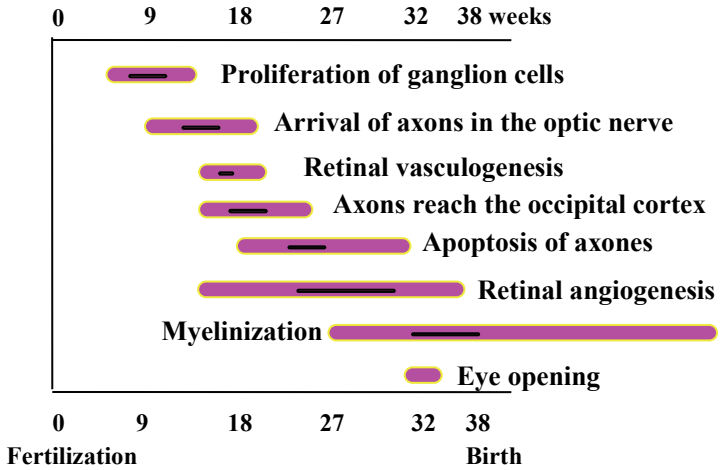

Figure 2. Overviews of the important events in the development of the final appearance of the ocular fundus. The bars indicate the approximate duration of the event. Black portions denote peaks of development. By courtesy of Ann Hellström.

The optic nerve, chiasm, lateral geniculate nucleus and optic tract

The retinal ganglion cells are seen as early as week five. They begin to penetrate the disc at 8 weeks and the optic tract fibres begin to reach the lateral geniculate body (LGB) around GW 11. Between GW 22 and 25 the characteristic six layers of the LGB develop (Hitchcock and Hickey, 1980). The number of axons peaks at 3.7 million by 17 weeks, and is thereafter reduced by apoptosis to 1.1 million by eight months. The process of apoptosis continues to about 30 GW, but is most intense between GW 16 to 20 i.e. before survival after preterm birth is possible (Provis et al., 1985). Simultaneously with apoptosis there is an increase in the number of glial cells and in the collagen content of the optic nerve. In an autopsy study it was demonstrated that 50 % of the growth of the optic disc and nerve was completed after 20 weeks of gestation and 75 % at term (Rimmer et al., 1993).

Myelination, which is performed by oligodendrocytes, begins at GW 20 in the LGB and proceeds anteriorly through the optic tracts around GW 24, continues through the chiasm, and reaches the optic nerve at 32 weeks (Ali et al., 1994; Takayama et al., 1991). Normally myelinisation stops at the lamina cribrosa, and no oligodendrocytes are seen anterior to this structure (Miller, 1982).

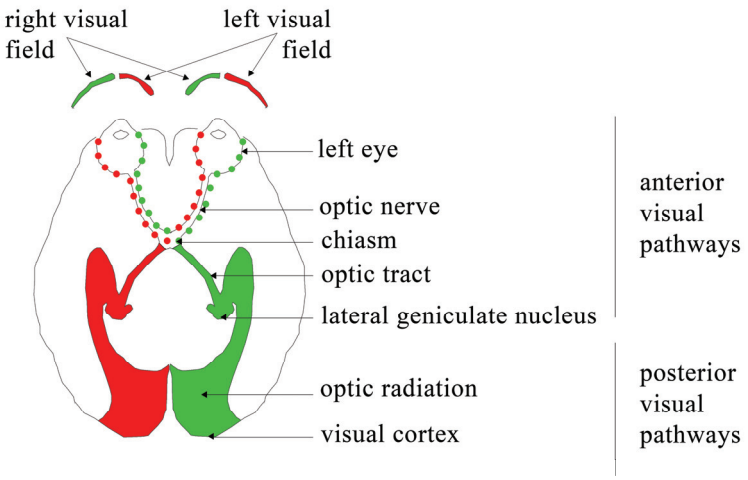

Figure 3. The visual pathways.

The brain

The cortex in the mature brain has six layers (I-VI). Layer I is the outermost layer next to pia mater. In the foetus the forming layers I-VI are called the cortical plate, and the layer below layer VI is a transient foetal structure called the cortical subplate.

Neurogenesis, proliferation of neurons, starts in GW five and is essentially completed by weeks 20 to 24 (Bystron et al., 2008; Volpe, 2001b). Simultaneously, radial glial cells produce systems of filaments which serve as guides for neurons in their migration from their sites of origin in the ventricular and later subventricular place to their target places in the cortical plate (Rakic, 1971; Watson, 1974). The radial glia cells also facilitate the development of columnar organisation of the neurons in the cortex (Rakic, 1988) which has a peak time from approximately the fifth month of gestation to several years after birth. During the organisational period the subplate neurons are established and differentiated, the dendrites and axons are ramified, synaptic contacts occur, apoptosis, proliferation, and differentiation of glial cells take place (Volpe, 2001b). The subplate neurons are important for the formation of connections between thalamus and cortex (Kanold, 2004; Kostovic and Judas, 2002; Volpe, 1996). The transient subplate neurons, which are the major neuronal type in the cerebral white matter, and the subplate region reaches its maximum thickness between 22 and 34 weeks of gestation (Ghosh and Shatz, 1992; Kostovic and Jovanov-Milosevic, 2006).

Apoptosis of the subplate begins late in the third trimester, and at about six months of postnatal age approximately 90 % of the subplate neurons have disappeared. In preterm babies the time course, when the subplate neurons are active in the developing brain, corresponds closely to when periventricular haemorrhages and ischemic lesions occur that may disrupt the subplate neurons, or their axonal collaterals to the subcortical, or cortical sites (Volpe, 2001b). The neurite development with ramifications is a very active process and a great number and variety of dendritic spines appear (i.e. sites of synaptic contact) in the cortex during the third trimester (Paldino and Purpura, 1979; Takashima et al., 1990).

The increase in cortical volume is particularly rapid between approximately postconceptional weeks 28 and 40, which has been documented by quantitative MRI measurements of cortical gray matter volumes in preterm infants during this period (Huppi et al., 1998; Kapellou et al., 2006; Kostovic and Judas, 2002).

The glial cell proliferation and differentiation are important in the developing brain, and there are many more glial cells than neurons in the CNS (Kinney and Back, 1998). Radial glia produces astrocytes, which play an important role for nutrition and support of neurons in reaction to metabolic and structural insults. Oligodendrocyte proliferation and differentiation proceed in four stages, from oligodendroglial progenitor, to preoligodendrocyte, to immature oligodendrocyte and finally to a mature oligodendrocyte that can produce myelin. The myelin producing mature oligodendrocytes are not abundant in the white matter until after term. During GW 24 to 32 there are mostly oligodendrocyte progenitors in the white matter with a peak in number at GW 28, when 90 % are oligodendrocyte progenitors, while by term 50 % are immature oligodendrocytes (Back et al., 2001). The immature oligodendrocytes are especially vulnerable to ischemia and inflammation, which lead to excitotoxicity and generation of free radicals that are produced by microglia. Microglia is involved during brain development involving apoptosis, vascularisation, and axonal development and the microglia reach a peak abundance in cerebral white matter in the third trimester (Billiards et al., 2006). Myelin provides insulation and speed up nerve conduction.

The myelination process slowly starts in the second trimester and continues into adulthood. Fifty percent of the oligodendrocytes are lost in apoptosis during their development (Barres et al., 1992).

Cerebellum develops rapidly during the last half of gestation and a volumetric study of premature infants has documented an approximately three-fold increase in volume from 28 to 40 GW (Limperopoulos et al., 2005).

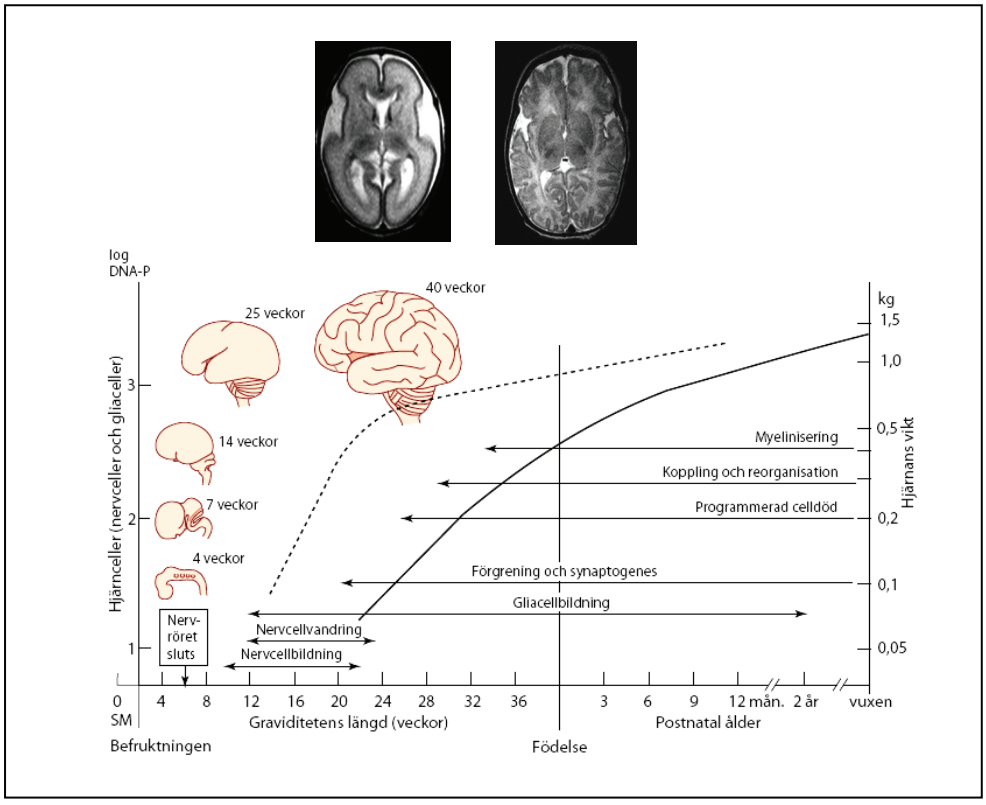

Figure 4. The development of the brain (by courtesy of Hugo Lagercrantz). Above MRI at gestational week 25 and 40 (by courtesy of M Rutherford).

IGF-I - neural and retinal vascular development

Insulin-like growth factor 1 (IGF-I) is a polypeptide, which resembles insulin in its molecular structure. In humans IGF-I is primarily produced by hepatocytes in the liver and the production is regulated by pituitary growth hormone (Holly and Perks, 2006). IGF-I exists extra-cellular and is bound to and controlled by six insulin-like growth factor binding proteins (Holly and Perks, 2006). Seventy-five percent of the IGF-I is bound to insulin-like growth factor binding protein 3 (IGFBP-3) together with an acid labile subunit (Jones and Clemmons, 1995). The insulin-like growth factor binding proteins can either inhibit, or potentiate cellular IGF-I responses, and influence distribution and elimination of IGF-I. The cellular actions of IGF-I are mediated through binding of IGF-I to the IGF-I receptor, which is located on the surface of different cell types in all tissues. IGF-I can also bind to the insulin receptor, but at a much lower affinity than insulin (Jones and Clemmons, 1995).

IGF-I is of major importance for foetal growth and is synthesized by all foetal tissues early in gestation, and the placenta is actively involved in regulating circulatory foetal levels of IGF-I (Gluckman and Pinal, 2003). Concentrations of foetal IGF-I are closely related to placental transfer of nutrients. The disruption of placental nutrient supply as well as amniotic supply (Han et al., 1996) at birth is followed by a rapid decline in levels of IGF-I. During pregnancy thyroxine plays a more important role than pituitary growth hormone in the regulation of foetal IGF-I (Deayton et al., 1993), but after birth IGF-I is mainly regulated by pituitary growth hormone.

IGF-I is related to nutrition, BW (Giudice et al., 1995) and gestational age (Hellstrom et al., 2003; Lineham et al., 1986; Smith et al., 1997b). At very preterm birth the IGF-I levels of the newborn decrease abruptly, and do not reach normal intrauterine values for several weeks/months (Engstrom et al., 2005; Lineham et al., 1986), in contrast to in term infants, in whom serum levels of IGF-I are restored in a few days (Engstrom et al., 2005; Kajantie et al., 2002; Lo et al., 2005). A recent study found a dramatic decrease in the circulating serum levels of IGF-I and its major binding protein, IGFBP-3 in very preterm infants, and that inflammation at birth with increased cord levels of pro-inflammatory cytokines was associated with a decrease in IGF-I

(Hansen-Pupp et al., 2007). The important role of nutrition for the foetal IGF-I levels was demonstrated in an animal study of foetuses of pregnant rats, who were fasted during the last days of gestation, and the serum IGF-I levels were 30 % lower than in the control foetuses (Davenport et al., 1990).

IGF-I acts directly on the brain and promotes differentiation, proliferation and maturation of progenitors of neural stem cells, and has anti-apoptotic properties (Hodge et al., 2007; McDonald et al., 2007; Ye and D'Ercole, 2006). Oligodendrocyte maturation is crucial for myelination as mentioned above, and several studies on mice and other rodents have shown an important role of IGF-I on differentiation of oligodendrocyte progenitor cells (D'Ercole et al., 1996; Lin et al., 2005; Wilson et al., 2003). In vitro, IGF-I has been found to promote remyelination (Mason et al., 2003), and cerebellar Purkinje cell development (Fukudome et al., 2003). In addition, a relationship has recently been shown in preterm infants between low cerebellar volume and decreased serum IGF-I levels (Hansen-Pupp et al., 2009).

IGF-I may also play an important role in the stimulation of postnatal brain growth. Over-expression of IGF-I in mice stimulated the brain growth and ameliorated the brain growth even in the face of under-nutrition (Lee et al., 1999), and IGF-I protected myelination in cases with under-nutritional insults (Ye et al., 2000). A relationship between low circulating levels of IGF-I, the development of ROP, and poor development of head circumference in preterm infants has also been documented (Lofqvist et al., 2006b). IGF-I is essential for the development of normal vascularisation of the human retina as mentioned above (Hellstrom et al., 2002; Hellstrom et al., 2001; Smith et al., 1999), and promotes the angiogenesis in the brain (Lofqvist et al., 2007; Lopez-Lopez et al., 2004). In the study by Lopez-Lopez and co-workers systemic injections of IGF-I in adult mice increased the brain vessel density.

A gender difference in IGF-I levels, where boys had lower levels than girls, has been shown in preterm (GA < 32 weeks) infants (Engstrom et al., 2005). In addition, in singleton ICSI boys, serum IGF-I was found to be lower than that of normally conceived boys (Kai et al., 2006).

IGF-I has also been shown to promote longitudinal postnatal growth (Fant and Weisoly, 2001). In addition, ocular growth is influenced by IGF-I and treatment with

IGF-I increases the ocular axial eye length in patients with short axial lengths due to growth hormone insensitivity (Laron syndrome) (Bourla et al., 2006).

Intracytoplasmic sperm injection technology

The ICSI procedure is recommended to couples who have failed to achieve fertilisation following standard in vitro fertilisation (IVF) treatment, when the male has abnormal sperm parameters (low count, poor motility, abnormal sperm forms and high levels of antibodies in the semen), and when the male must have his sperm surgically retrieved from the epididymis or testis because of lack of sperms in the ejaculate (azoospermia) due to for example congenital absence of both vasa deferentia. The treatment involves several stages. At first the woman is given a Gonadotrophin Releasing Hormone analogue (nasal spray or subcutaneous injection) to "shut down" the "normal" ovarian function. To ensure that the ovaries are inactive an ultrasound is performed. She is then given Follicle Stimulating Hormone (FSH) on a daily basis, which stimulates the ovaries to produce multiple follicles. The follicles will be assessed in number and size by ultrasound scans. When at least 3 follicles at $\geq$ 18 mm can be observed, indicating that there may be a mature egg, the egg collection is scheduled. Now another injection is administered, Human Chorionic Gonadotrophin (HCG). This injection helps to mature and release the eggs in the follicles for the egg collection. Approximately 36 hours after the HCG injection the oocyte retrieval takes place under sedation or general anaesthesia. A vaginal probe with a needle guide is under ultrasound guidance passed through the vaginal wall into each ovary. The follicles are drained and the collected follicular fluid is searched for eggs. Once the eggs are retrieved they are examined under the microscope for assessment of quality. The eggs are placed in an incubator for some hours and after that the cells that surround the egg are stripped off to assess the maturity of the egg, as ICSI can only be performed on mature eggs. When the eggs have been selected, a chosen sperm is rendered immotile, then sucked into the tip of a very fine glass needle and injected directly into the egg under the microscope (Figure 1). The eggs will then be placed in an incubator, and checked the following day for fertilisation. After 2-3 days following egg collection the embryo replacement takes place, usually without sedation. Under

ultrasound guidance a fine catheter, loaded with the embryo, is passed through the vagina, the cervix and into the uterus. Progesterone helps to maintain the thickness of the lining of the uterus to aid implantation, and is given from the day of the embryo transferral. A pregnancy test should be performed 14 days after the embryo transfer.
This procedure has raised concerns about risks for adverse outcome in children born after ICSI as it includes artificial induction of ovulation with a possibility of changes in follicle milieu and oocyte structure, the use of a sperm that cannot conceive naturally and exposure of the egg, sperm and embryo to the artificial in vitro environment including chemicals, freezing and mechanical manipulations.

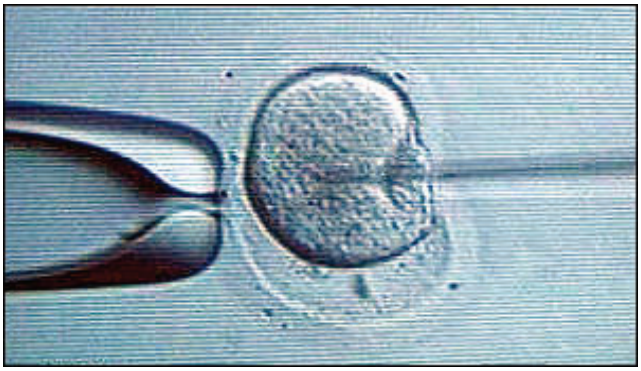

Figure 5. Intracytoplasmic sperm injection, (by courtesy of Ulla-Britt Wennerholm).

ICSI AND PRETERM OUTCOME

Pre- and perinatal factors

Maternal

Women undergoing IVF/ICSI and women giving birth to a preterm child have increased frequency of morbidity both before and during pregnancy. Diabetes mellitus (Yeshaya et al., 1995), inflammatory intestinal disease (Bradley and Rosen, 2004) and thyroid dysfunction (Poppe et al., 2007) have been associated with infertility. Maternal medical disorders, such as thyroid disease, asthma, diabetes and hypertension are associated with increased rates of preterm delivery (Goldenberg et al., 2008). Other studies have also demonstrated that ICSI/IVF mothers had diabetes mellitus (pre-existent) significantly more often than spontaneously conceiving mothers (Kapiteijn et al., 2006; Katalinic et al., 2004). Diabetes is a known risk factor for preterm delivery (Lepercq et al., 2004; Matsushita et al., 2008; Melamed et al., 2008).

Mothers undergoing ICSI/IVF more often use drugs as treatment for diseases such as diabetes, hypo/hyperthyroidism, and inflammatory disease than other mothers, beside the drug therapy associated with assisted reproduction. In addition, an increased use of heparin, heparin-like substances, and thrombocyte aggregation inhibitors has been related to conditions associated with subfertility (Kallen et al., 2005b).

All pregnant women in Sweden during a period of almost 10 years were studied, and maternal use of thyroid hormones during pregnancy had an increased rate of pre-eclampsia and diabetes (pre-existing or gestational), but the risk for preterm birth was only marginal (Wikner et al., 2008). An increased risk of deep venous thrombosis after single embryo transfer compared with spontaneously conceived pregnancies has been reported (Poikkeus et al., 2007), and a history of deep vein thrombosis has also been shown to be an independent risk factor for spontaneous preterm delivery (Ben-Joseph et al., 2008). The aetiology of preterm birth is complex and involves environmental and genetic factors, and the underlying mechanisms are not fully understood. In comparison with all women giving birth in Sweden during 1982 - 2001, the women who underwent IVF/ICSI during the same time period (n = 12 186) were older, more

often of first parity, smoked less, were more overweight and worked less outside home. Their use of medication in early pregnancy was nearly three times higher than among other pregnant women. In contrast, underweight body mass index in the pre-pregnancy health status in mothers with spontaneous conception is a risk factor for preterm delivery (Haas et al., 2005). Smoking is a risk factor for preterm delivery, but women who gave birth after IVF/ICSI smoked less than other pregnant women (Kallen et al., 2005b). Some studies have shown that older mothers have an increased risk of preterm delivery (Cnattingius et al., 1992; Cnattingius and Haglund, 1992; Prysak et al., 1995), while other authors found no increased risk of preterm delivery in older mothers, but an increased risk of obstetric complications (Berkowitz et al., 1990; Mbugua Gitau et al., 2009). Interestingly, it has been shown that mothers under the age of 20 years also have an increased risk of preterm birth (Stevens-Simon and McAnarney, 1991).

Many obstetric characteristics are similar in pregnancies of both women who are pregnant after IVF/ICSI and women who deliver preterm. The pregnancies are associated with an increased risk of gestational hypertension (Jackson et al., 2004; Johnson et al., 2009; Kallen et al., 2005c). Women who have undergone IVF/ICSI have increased risk of pre-eclampsia, placental abruption, placenta previa, preterm premature rupture of membranes and gestational diabetes which are all risk factors for preterm delivery (Ananth et al., 2001; Bonduelle et al., 2005; Covarrubias et al., 2008; Goldenberg et al., 2008; Hossain et al., 2007; Jackson et al., 2004; Krupa et al., 2006; Nygren et al., 2007; Poikkeus et al., 2007; Romundstad et al., 2006; Sutcliffe and Ludwig, 2007). A study of all women known to have had IVF/ICSI in Sweden between 1982 and 2001 showed, that the impact of the maternal obstetric characteristics on preterm delivery was rather small (Kallen et al., 2005c). The authors suggested that the reason for the increased risk of preterm delivery must be sought in the infertility status of the women. Multiple births account for a substantial risk of preterm delivery, and multiple births is a complication in pregnancies following IVF/ICSI as a result of transferral of more than one embryo. The twinning rate was about 23 % in Europe 2002, but lower in Sweden where the twinning rate after IVF/ICSI was 18.5 % 2001. After a recommendation from the Swedish Medical

Association in 2003 only one embryo is transferred and the frequency of multiple births had fallen to approximately 5 % in 2004 (Nygren et al., 2007).

Intrauterine infection is a frequent and important cause of preterm delivery, and microbiological studies suggest that intrauterine infection might account for 25-40 % of premature births (Goldenberg et al., 2000). The infection may activate the innate immune system, and release chemokines and cytokines which stimulate inflammatory mediators including prostaglandins which stimulate uterus contractility that can lead to preterm premature ruptures of membranes (Romero et al., 2006). The earlier in pregnancy a women presents with preterm labour, the higher is the frequency of intrauterine infection (Mueller-Heubach et al., 1990). Intrauterine inflammation and placental dysfunction are the most common causes of delivery before the 28th week of gestation (McElrath et al., 2008).

Genetics

Paternal genetics in ICSI

The impact of sperm quality on the outcome after assisted reproduction, especially after ICSI, which is the treatment for male factor infertility, has been discussed. It has been found that infertile men with sperm abnormalities (numerical and structural) have more chromosome aberrations (Aittomaki et al., 2005; Bonduelle et al., 2002; Lundin et al., 1998; Van Assche et al., 1996).

Microdeletions in a region on the long arm of the Y chromosome were found in 7.3 % of infertile men, and the majority of these deletions were seen in the azoospermic men. Once a deletion has occurred, it will be inherited by all male offspring if infertility is treated with ICSI, and it has been found that 9 % of sons born after ICSI had Y chromosome microdeletions (Kent-First et al., 1996). An increased frequency of hypospadia, a condition that might be associated with male infertility, has been reported in the ICSI male offspring compared with the general population (Bonduelle et al., 2005; Ericson and Kallen, 2001; Fedder et al., 2007; Katalinic et al., 2004; Wennerholm et al., 2000). An increased rate of chromosomal aberrations and sex chromosome abnormalities, compared with an expected rate, (Jacobs et al., 1992;

Nielsen and Wohlert, 1991) has been recorded in pregnancies after ICSI (Bonduelle et al., 2002; Wennerholm, 2004). These aberrations are most probably linked to maternal age, or directly to the characteristics of the infertile men being treated, rather than to the ICSI procedure itself (Wennerholm, 2004).

Genomic imprinting diseases

Genetic imprinting is a process, which occurs early in development and silences the copy of a gene inherited from either the mother or the father. By abnormal methylation pattern of an imprinted gene the gene expression may be altered. Oligozoospermia (< 20 million/ml) itself increases the risk for genetic imprinting disorders (Marques et al., 2004). Sutcliffe and co-workers found an association between ICSI/IVF and Beckwith-Wiedemann syndrome. Children born after IVF/ICSI with Beckwith-Wiedemann syndrome and Angelman syndrome showed loss of maternal allele methylation at a critical imprinting control region (Sutcliffe et al., 2006). An increased risk of imprinting diseases may be caused by the in vitro embryo culture (Maher, 2005), or some factor associated with infertility per se. A susceptibility to epigenetic imprinting diseases may be due to factors causing the infertility and the ovarian stimulation as part of the infertility treatment may play a role. Children with Angelman syndrome born after infertility treatment with induced ovulation showed increased frequency of imprinting defects (Ludwig et al., 2005). In the Swedish cohort of children born after ICSI one child had Silver Russel syndrome and one had Prader Willi syndrome, and both syndromes are associated with imprinting anomalies (Kallen et al., 2005a). In the Danish national IVF/ICSI cohort study no child was found to have imprinting anomalies (Lidegaard et al., 2005). Retinoblastoma (RB) is commonly caused by a mutation of one allele of the tumour suppressor gene RB1, combined with chromosomal loss, or deletion of the other allele (Thompson and Williams, 2005). Hypermethylation of the RB gene that inactivates the tumour suppressor function may play a role in the development of retinoblastoma (Ohtani-Fujita et al., 1997). Five children with retinoblastoma, associated with IVF/ICSI have been reported from the Netherlands (Moll et al., 2003). Imprinting

disorders are rare conditions, and large prospective multi-centre cohort studies are needed in order to conclude if there is a true increased prevalence in infants born after IVF/ICSI (Manipalviratn et al., 2009).

Genetics and preterm delivery

There is a familial pattern of preterm delivery indicating a genetic predisposition. Women who are born preterm are more likely to have a preterm delivery themselves (Porter et al., 1997; Wilcox et al., 2008), as are sisters of women who have had a preterm delivery (Winkvist et al., 1998). In a population-based prospective study sisters of an affected individual had higher risk of preterm delivery than sisters of unaffected individuals. The preterm deliveries were the result of premature rupture of membranes, placental abruption, and pre-eclampsia and the results suggest that the adverse pregnancy outcomes that aggregate in families may in part be explained by genetics (Plunkett et al., 2008).

Studies for evaluating the genetic influence while attempting to avoid confounding environmental influences have been performed on twins, and the genetics contribution to preterm delivery has been estimated to approximately 30 % (Clausson et al., 2000; Kistka et al., 2008; Lunde et al., 2007). In addition, racial disparities exist which suggest a genetic contribution, where African-American mothers are more prone to give birth prematurely (Palomar et al., 2007). Genetic studies have identified markers, which more accurately predict preterm birth than currently known risk factors e.g. proteins, and/or pathways involved in the disorder. Several genes have been reported to be associated with preterm delivery, although inconsistency between the studies has been problematic (Plunkett and Muglia, 2008).

Many of the genetic studies on preterm birth have focused on genes involved in inflammation, like the gene for tumour necrosis factor-α, a pro-inflammatory cytokine. However, studies of polymorphism in this gene in the mothers have been inconclusive.

Short term consequences

Malformations

Many studies have addressed the risk of congenital malformations in children born after IVF/ICSI. In large meta-analyses increased rates of up to 30-40 % risk of congenital malformations after IVF/ICSI compared with children born after spontaneous conception has been noted (Hansen et al., 2005; Kallen et al., 2005a; McDonald et al., 2005; Rimm et al., 2004). The children born after ICSI in Papers I and II were part of a larger multi-centre study, demonstrating malformations more often in singleton children born after ICSI (6 %) than in the spontaneously conceived group (3 %) at the age of 5 years ($p = 0.037$) (Bonduelle et al., 2004). In the large Swedish registry study on ICSI/IVF children by Källén and co-workers (Kallen et al., 2005a) the increase of malformations was 42 %, and was higher for singleton than for multiple births in comparison with normally conceived singletons and twins, respectively. After adjustments for year of birth, maternal age, parity, years of known childlessness, and smoking, the risk increase disappeared, and the authors concluded that the observed malformations mainly were due to maternal characteristics associated with subfertility. A Danish registry-based study compared the malformation rate in singletons born to fertile couples (time to pregnancy interval ≤ 12 months) and singletons born to infertile couples (time to pregnancy interval of > 12 months) who either conceived naturally, or with infertility treatment. Singletons born to infertile couples had a higher rate of congenital malformations, independently of whether the children were conceived spontaneously or after infertility treatment (Zhu et al., 2006). The findings suggest that the increased risk of congenital malformations in children born after IVF/ICSI is mostly a result of the underlying parental characteristics, rather than the assisted reproduction techniques themselves.

The specific types of malformations observed are neural tube defects, cardiovascular defects, choanal atresia and alimentary tract atresia (Kallen et al., 2005a). Similar results with regard to malformations have been found after standard IVF and ICSI. However, an increased risk of defects in the urogenital system in ICSI children e.g.

hypospadia has been found in several studies (Bonduelle et al., 2005; Ericson and Kallen, 2001; Fedder et al., 2007; Kallen et al., 2005a; Wennerholm et al., 2000).

Major congenital malformations increase the risk for preterm birth. In a population-based study, where chromosomal abnormalities were excluded, an increased risk for malformations like cleft lip and palate, diaphragmatic hernia, urogenital anomalies, heart defect, spina bifida, omphalocele/gastroschisis, tracheoesohageal anomalies and renal agenesis was found in infants born preterm. The risk of preterm birth was higher with multiple malformations, and the risk varied inversely with GA (Purisch et al., 2008). Cardiovascular malformations have been found in twice as many preterm children as in children born full term (Tanner et al., 2005). In addition, two other population-based studies found that significant malformations were more common in prematurely born babies than in infants born at term (Holmgren and Hogberg, 2001; Rasmussen et al., 2001). A recent study showed a high risk of preterm birth in babies with brain defects. Although the brains of preterm infants are particularly vulnerable to injury, the brain defects in that study developed in utero, and not at birth, or after birth. The authors speculated that it is either the brain defects themselves, or the underlying cause of the defect that result in preterm birth. Another possible mechanism may be that coagulopathy, which is associated with congenital brain defects, is involved in the induction of the preterm delivery (Brown, 2009).

Neonatal morbidity

It is clear that preterm delivery and low BW, as well as perinatal morbidity, occur in a higher frequency related to IVF/ICSI pregnancies than in naturally conceived pregnancies. The increased risk of low BW associated with assisted reproduction has been attributed largely to the higher rate of multiple gestations associated with the technique, and a multiple pregnancy is a well-established risk factor for adverse outcomes including preterm delivery, low BW and neonatal morbidity (Liu and Blair, 2002). There is no data suggesting that multiple births after IVF/ICSI have more adverse perinatal outcomes than multiple births after spontaneous conception (Helmerhorst et al., 2004).

However, several meta-analyses on singleton children born after IVF/ICSI have found similar results, reporting an approximately two-fold risk of being born preterm, and an increased risk of having a low, or very low BW, being born small for gestational age, being admitted to neonatal intensive care unit, as well as having higher perinatal mortality (Helmerhorst et al., 2004; Jackson et al., 2004; McDonald et al., 2005). In a large Swedish study by Källen and co-workers the odds risk ratio of adverse outcomes were reduced and not significant, when adjusting for year of birth, maternal age, parity, smoking and number of years of involuntary childlessness. They also reported a higher risk for cerebral haemorrhage, need for mechanical ventilation, use of continuous positive airway pressure (CPAP), neonatal sepsis, neonatal convulsions and respiratory problems after IVF/ICSI. The main reason for the increased risk the authors concluded was the high rate of multiple births in the study group (Kallen et al., 2005c). Significantly lower rates of premature delivery, low BW and paediatric complications requiring neonatal intensive unit care following births after transferral of only one embryo compared to dual-embryo transfer have been reported (Kjellberg et al., 2006). Vanishing twins after multiple embryo replacement has been considered to be one of the causes of the adverse neonatal outcome in singletons born after IVF/ICSI (Pinborg et al., 2007; Pinborg et al., 2005; Schieve et al., 2004), and that the infertility itself may play a causative role (Draper et al., 1999; Pandian et al., 2001; Schieve et al., 2002).

The more immature the baby, the greater is the risk of morbidity. The major disorders associated with preterm birth are respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), necrotising enterocolitis (NEC), sepsis, intraventricular haemorrhage (IVH), periventricular leucomalacia (PVL) and retinopathy of prematurity (ROP). Increased survival of infants born before 25 GW, during the last decade, has resulted in an increased prevalence of BPD as the tiniest infants require longer time with mechanical ventilations and CPAP, and are prone to develop sepsis (Lundqvist et al., 2009; Ronnestad et al., 2005). The disorders of the brain associated with prematurity and ROP are discussed below.

Retinopathy of prematurity (ROP)

Although most cases of ROP are self limiting it may be a serious threat to vision.

In infants born prematurely the retina is not fully vascularised (see page 11). The more premature the child, the larger is the avascular area. The sudden loss of nutrition and growth factors necessary for normal growth at preterm birth causes the vascular growth, that would normally occur in utero, to slow down or cease. In addition, the relative hyperoxia of the extra-uterine milieu together with supplemental oxygen cause a regression of already developed retinal vessel. As discussed earlier, IGF-I is necessary for normal development of retinal blood vessels (Hellstrom et al., 2002). Preterm birth is associated with a rapid fall in IGF-I, and the baby often suffers from immaturity, poor nutrition, acidosis, hypothyroxemia and sepsis which all may further reduce the IGF-I levels. When the neural elements of the retina mature and need more oxygen, poor vascularisation leads to hypoxia and production of vascular endothelial growth factor (VEGF). If sufficient IGF-I is not available VEGF is accumulated, as a minimum level of IGF-I is required for VEGF to induce vessel growth. When the IGF-I levels slowly increase when the infant matures, and IGF-I reaches the minimum level for VEGF to promote vessel growth, an excessive and uncontrolled neovascularisation may take place. However, in most cases vessel growth retardation is not severe enough to cause the proliferative stages of ROP. The serum levels of IGF-I during the first weeks of life in the babies are inversely correlated with the severity of ROP (Hellstrom et al., 2003).

The international classification of the stages of ROP (Committee for the Classification of Retinopathy of Prematurity Revisited, 2005) is presented in Figure 6 below. Stages 1 and 2 are considered as mild, while stages 3 to 5 are severe. The extraretinal neovascularisation in stage 3 may lead to retinal detachment. If the detachment involves the macula the child becomes blind. Dilatation and tortuosity of the central vessels are called plus disease or pre-plus disease, and are signs of a progressive disease. Aggressive posterior ROP is a virulent form of ROP seen in the tiniest babies (Committee for the Classification of Retinopathy of Prematurity Revisited, 2005).

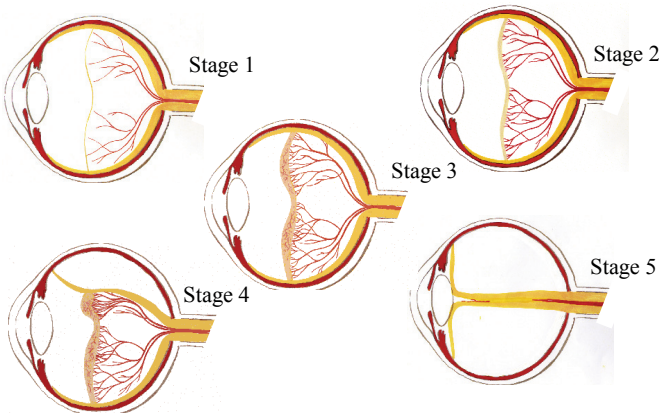

Figure 6: The stages of ROP. Stage 1: Demarcation line, Stage 2: Ridge, Stage 3: Ridge with extraretinal fibrovascular proliferation, Stage 4: Partial retinal detachment, Stage 5: Total retinal detachment (Drawings by Lisa Hård af Segerstad).

Risk factors for ROP are low GA/BW and oxygen supplementation (Lutty et al., 2006; Smith, 2003; Tasman et al., 2006). Hyperglycemia has also been reported to be associated with ROP (Ertl et al., 2006; Garg et al., 2003). An association between poor postnatal growth and later development of ROP has been reported (Allegaert et al., 2003; Wallace et al., 2000). A recent study has even shown that monitoring postnatal weight development can predict proliferative ROP and may thus modify and reduce traditional screening (Hellstrom et al., 2009).

The incidence in Sweden of ROP has been reported to be 36.4 % in infants with a BW of 1500 g or below. Mild ROP occurred in 18.2 % of the children and 18.2 % progressed to severe ROP. Twelve percent of the infants were treated with laser therapy of the peripheral avascular retina (Larsson et al., 2002). Even though ablation treatments have reduced the incidence of blindness by 25 % in the children with severe ROP, some cases still progress to retinal detachment and blindness (Chen and Smith, 2007). Advanced care is presently saving many critically ill, extremely premature infants with high risk of developing severe ROP needing treatment. Two recent studies report that the proportion of screened infants who needed ROP treatment has doubled in the last ten years (Schiariti et al., 2008; Slidsborg et al., 2008).

Periventricular leucomalacia and neuronal/axonal disease - "Encephalopathy of prematurity"

The encephalopathy of prematurity includes periventricular leucomalacia (PVL) and accompanying neuronal and axonal deficits that involve the cerebral white matter, thalamus, basal ganglia, cerebral cortex, brain stem and cerebellum (Volpe, 2009). The initiating mechanisms in PVL, both focal and diffuse, are ischemia and inflammation (Hagberg and Mallard, 2005). The focal PVL consists of microscopic areas of necrosis, which give rise to cystic lesions. The diffuse PVL, which is much more common, is characterised by marked astrogliosis and microgliosis, and a decrease in premyelinating oligodendrocytes (Haynes et al., 2003; Robinson et al., 2006). Between weeks 24 and 32 the periventricular white matter is in a watershed area between the cortex and the subcortical areas as the end capillaries from the cortex have not reached the periventricular area. Thus, there is a markedly low basal blood flow to the cerebral white matter (Borch and Greisen, 1998; Khwaja and Volpe, 2008). In addition to the low blood flow, the immature vessels lack smooth muscles, which interfere with the ability to change diameter in response to changes in blood pressure (Soul et al., 2007). Hypocarbia is a potent vasoconstrictor, and fluctuations in arterial carbon dioxide tension are common in the preterm infant who needs mechanical ventilation (Wiswell et al., 1996). Altogether, these mechanisms make cerebral white matter in the periventricular area especially vulnerable to ischemic lesions. Inflammation is often due to maternal intrauterine infection or postnatal sepsis, and results in excitotoxity and free-radical attack, causing destruction and/or dysfunction of oligodendrocytes and axons (Dammann et al., 2001). The white matter damage is accompanied by cerebral cortex and deep matter gray abnormalities (Kostovic and Judas, 2006; Volpe, 2005), involving excess apoptosis (Robinson et al., 2006) without replacement resulting in disturbances in the synaptogenesis and the connectivity (Ben-Ari, 2006; Kesler et al., 2006). The neurons migrate from the germinal matrix (ventricular/subventricular zones) through the white matter to the cortex when the white matter is most vulnerable. Especially the pre-oligodendrocytes are prone to ischemic-hypoxic insults and hypomyelination may occur (Segovia et al., 2008). The

vulnerability of pre-oligodendrocytes has been shown in humans in several studies (Back et al., 2005; Haynes et al., 2003; Robinson et al., 2006).

MRI studies have documented white matter damages (Counsell et al., 2008; Dyet et al., 2006; Inder et al., 2003), and diffusion-based MRI studies have shown abnormalities consistent with axonal degeneration and impaired development in children born preterm (Counsell et al., 2003; Counsell et al., 2007).

Axonal degeneration, studied by using apoptotic markers, has recently been found in children with PVL (Haynes et al., 2008).

Several regions of the brain have been found to be affected in children born preterm e.g. thalamus (Pierson et al., 2007), cerebellum (Limperopoulos et al., 2005) and the brain stem (Pierson et al., 2007).

Long term consequences

Children born after IVF/ICSI

Regarding general health up to five to eight years of age including vision and hearing, reassuring results have been found with no differences between singletons born after IVF/ICSI and children born after spontaneous conception except for more medical care in the IVF/ICSI group of children (Basatemur and Sutcliffe, 2008; Bonduelle et al., 2004; Bonduelle et al., 2005; Knoester et al., 2008; Ludwig et al., 2009; Sutcliffe and Ludwig, 2007). In the European study by Bonduelle and co-workers male infants after ICSI needed more urogenital surgery (5 %) in comparison with 1 % among the children born after spontaneous conception (Bonduelle et al., 2005). The systolic and diastolic blood pressure were significantly higher in the eldest ICSI children being followed up than in children born after spontaneous conception at the same age (Belva et al., 2007). Low BW may be associated with later development of cardiovascular disease (Barker et al., 1990; Lackland et al., 2003). IVF/ICSI singleton children have an increased risk for cerebral palsy which has been associated with preterm birth (Lidegaard et al., 2005; Stromberg et al., 2002). Several studies have shown no difference regarding the neurodevelopmental and cognitive outcome, including visual perception, at follow-up to five to ten years between children born

after IVF/ICSI and naturally conceived children (Belva et al., 2007; Leunens et al., 2008; Ponjaert-Kristoffersen et al., 2005; Ponjaert-Kristoffersen et al., 2004; Steel and Sutcliffe, 2009; Wagenaar et al., 2009).

Children born preterm

There are many studies that have documented neurodevelopmental disturbances in preterm children that affect vision, cognition (Allen, 2008; Cheong et al., 2008; Hack et al., 2002; Patra et al., 2006; Woodward et al., 2006), and motor functions (Allen, 2008; Inder et al., 2005; Larroque et al., 2008; Woodward et al., 2006), as well as behaviour (Bhutta et al., 2002; Cheong et al., 2008; Stjernqvist and Svenningsen, 1999). These disturbances can mostly be related to the "encephalopathy of prematurity" discussed above (Volpe, 2009). Other morbidities associated with prematurity like necrotising enterocolites and bronchopulmonary dysplasia have been found to be associated with adverse neurodevelopmental outcome (Anderson and Doyle, 2006; Rees et al., 2007).

Visual outcome

Ocular morphology

In children born after IVF/ICSI there are only few reports on ocular morphology. Retinoblastoma has been found in five children born after IVF/ICSI (Moll 2003). One study of 47 children born after IVF/ICSI, who were referred to an eye clinic, reported serious eye disorders, i.e. Coats disease, optic nerve hypoplasia/atrophy and coloboma with microphthalmus (Anteby et al., 2001).

In prematurely born children both ROP and brain lesions may influence the forthcoming visual outcome. In the macula an absent or reduced avascular zone has been found in children born at GA of 30 weeks or less (Mintz-Hittner et al., 1999). In children with a history of ROP increased central foveal thickness and preservation of inner retinal layers within the fovea have been described (Recchia and Recchia, 2007), indicating disturbed retinal development. In addition, it has been reported that

children born prematurely had a thicker central macula and thinner peripapillary nerve fibre layer compared with children born at term. The lower the BW and the smaller the head circumference the thicker central macula and the thinner the peripapillary nerve fibre layer (Wang et al., 2006). Another study, of six year old children found that the axial length of the eye and corneal radius were shorter in children born with low BW and those with a small head circumference at birth. The effects on the size of the eye lasted up to at least six years of age, however, no lasting effect on spherical equivalent of refraction was seen (Ojaimi et al., 2005). These results emphasise the importance of normal intrauterine growth for the eye development.

In the eye, the rod sensitivity as measured using electroretinography is reduced in preterm infants and this reduction has been attributed to factors like insufficient nutrition and retinal vascularisation and ROP (Fulton et al., 2008; Hamilton et al., 2008). The retina is a controller of the refractive development (Troilo and Wallman, 1991), and an association between reduced rod sensitivity and early ametropia has been found in children with ROP (Moskowitz et al., 2005).

Visual function

Children born after IVF/ICSI have only been tested for visual outcome in a few studies and no difference in visual acuity, stereopsis and eye movements in comparison with children born after spontaneous conception has been found (Belva et al., 2007; Bonduelle et al., 2005; Wikstrand et al., 2006).

Several long-term population-based studies up to adolescence have shown that children born preterm have reduced visual acuity, an increase in refractive errors, strabismus, visual field defects and reduced contrast sensitivity (Cooke et al., 2004; Fledelius, 1996; Hellgren et al., 2007; Holmstrom and Larsson, 2008; Holmstrom et al., 2006; Holmstrom and Larsson, 2005; Larsson et al., 2006; Larsson and Holmstrom, 2006; Larsson et al., 2003; Larsson et al., 2005; Lindqvist et al., 2007; O'Connor et al., 2007). Following laser ablation for ROP the visual fields of the prematurely born children may be peripherally constricted, and a decreased sensitivity within the central visual field unrelated to the history of ROP, has been found

(Larsson et al., 2004). Inferior visual field defects have been documented due to damage in the cerebral posterior superior white matter which is often seen in prematurely born children (Dutton et al., 2004; Jacobson et al., 1996). Poor visual perception has been found in several studies (Hard et al., 2000; Hellgren et al., 2009). Two studies on the visuo-cognitive development at six to seven years in children born before GW 32 compared with children born full term found that spatial function, selective attention and executive control were most affected. These three areas of functions have been linked to the dorsal visual cortical stream even though they involve other areas of the brain, suggesting vulnerability for the dorsal steam, which undergoes a longer developmental time course than the ventral stream (Atkinson and Braddick, 2007; Santos et al., 2008).

The more immature the child is, the greater the risk for visual impairment which has been shown in a recent Swedish follow-up study on children born before 25 GW. In the study ROP was found in 97 %, 75 % developed proliferative ROP, and 63 % needed laser ablation. A significant gender difference was found, where 32 % of the boys were blind or visually impaired compared with 9 % of the girls (Jacobson et al., 2009).

General outcome

Recent reports on long-term medical, psychiatric and social consequences in Swedish and Norwegian national cohorts of adult people born preterm showed an increased risk of having cerebral palsy, mental retardation and psychiatric disorders and the risks increased with the degree of prematurity. Disability pension from the society was several times higher in adults born preterm than in the group born at term. Preterm birth was also associated with a lower chance of completing a university education, and hence these individuals had a lower net salary (Lindstrom et al., 2009; Lindstrom et al., 2007; Moster et al., 2008). However, the majority of adults who were born very preterm lived an independent and self-supportive life and a large proportion completed higher education and "seemed to live a satisfying life" (Lindstrom et al., 2007; Moster et al., 2008).

Growth

The vast majority of studies which have examined growth in children born after IVF/ICSI from the neonatal period up to eight years of age have not found any differences regarding weight, stature and head circumference compared with spontaneously conceived children (Belva et al., 2007; Bonduelle et al., 2004; Bonduelle et al., 2005; Knoester et al., 2008; Ludwig et al., 2009). Few studies have reported differences in growth parameter between ICSI/IVF children and naturally conceived control children (Kai et al., 2006; Miles et al., 2007). The study of Danish children born after ICSI found that they were significantly smaller than their target height (SDS) compared with normally conceived children at three years of age, but at five years of age there was no difference between the groups in stature. The study included both twins and singletons born preterm and SGA after ICSI which may have influenced the results (Kai et al., 2006). Another study of children born after IVF/ICSI that excluded children born preterm and SGA found that the children born after ICSI were taller, most evident in girls, than the normally conceived control children after adjustment of parental height and age, and the IVF/ICSI children also had higher serum levels of IGF-I. The authors proposed that the in vitro manipulation had resulted in persistent metabolic alterations in the IVF/ICSI children they studied (Miles et al., 2007).

Studies on growth in preterm children born before GW 29 have demonstrated an initial decrease in weight during the first months of life, followed by an increase in weight gain approximately weeks 36 to 40 PMA (first peak). After a period of slower weight and length growth, a second increase in growth velocity, was demonstrated from 6 months to 2 years of corrected age (second peak). The initial transient period of slower weight gain may be ascribed to the discontinuation of parenteral nutrition and the beginning of full enteral feeding. The infants born very preterm remained growth retarded during the first 1-2 years, and they were growth retarded during a postnatal period corresponding to the last trimester (Bertino et al., 2006; Niklasson et al., 2003).

One study found that for children born very preterm it took up to seven years to catch up in weight and stature (Niklasson et al., 2003), and another study by Cutfield and co-workers found that five to ten year old children born with a very low BW (<1500 g) did not achieve their genetic height potential, and that the combination of SGA and prematurity was more likely to be associated with short stature than prematurity alone (Cutfield et al., 2004). A study that followed children born very preterm up to eight years of age showed that weight was lower at all ages, although not significantly lower at the age of eight years. However, the head circumference was smaller at birth and fell from birth to two years of age, and no catch up was found in head circumference between two and eight years of age (Kan et al., 2008).

Studies have shown that infants with very low BW with major morbidities grow less than infants with very low BW without morbidities (Bertino et al., 2006; Ehrenkranz et al., 1999). The protein loss in infants with extremely low BW is two-fold higher than in normal term infants, and protein and energy requirements are not being met in most extremely low BW infants (Denne, 2001). It has been shown that an aggressive enteral and parenteral nutritional approach reduced the growth retardation rate in weight, length and head circumference (Wilson et al., 1997). In addition, undernutrition may result in growth failure in infants with BPD, in which the breathing requires extra energy (Biniwale and Ehrenkranz, 2006). Nutritional status has been shown to regulate IGF-I (Fliesen et al., 1989; Isley et al., 1984). The gastrointestinal tract of premature newborns is immature and the shorter the GA, the less developed the gut function is. Premature infants often encounter problems with enteral feeding after birth, and enteral feeding is a natural stimulus for intestinal growth (Broussard, 1995; Kelly and Coutts, 2000; Teitelbaum and Allan Walker, 2005).

IGF-I is important for postnatal growth and serum levels IGF-I are positively associated with rapid postnatal weight gain and growth (Chellakooty et al., 2006; Fliesen et al., 1989; Ong et al., 2002). One study, which compared longitudinal circulating levels of IGF-I in preterm babies (GA 26 -37 weeks) born AGA and SGA, and full term babies born AGA found an immediate fall after birth from the mean foetal IGF-I levels (cord values) to a basal postnatal circulating level of IGF-I. The

basal level of IGF-I was related only to GA, and increased slowly from 25 weeks of gestation until four weeks after full term equivalent, and was independent of time of birth. It was found that full term infants had a prominent surge and rapidly increased the circulating IGF-I levels, and a less apparent increase was noted in the infants born between 34-37 weeks, but in the infants born before GW 33 there was a slow increase and they lacked the sharp postnatal increase of serum IGF-I levels seen in the more mature infants (Lineham et al., 1986). Another study compared plasma levels of IGF-I and its binding protein IGFBP-3 at the age of 5 to 10 years in children born SGA or AGA, preterm (GA<32 weeks) and at term (>36 weeks). The preterm children irrespective of whether they were born SGA or AGA had low plasma levels of IGF-I and IGFBP-3 while children born SGA at term had elevevated levels of these substances. The authors concluded that premature birth plays a more dominant role than SGA for the IGF-I and its binding protein axis (Cutfield et al., 2004). In addition, full term SGA children, who had lower levels of IGF-I at birth than full term AGA children, more rapidly increased their levels of IGF-I after birth, and had similar mean length and weight and higher levels of IGF-I at three years of age compared with the full term AGA children, indicating a relative IGF-I resistance in the SGA children. A gender difference was shown in both the SGA and AGA children, where the girls raised their levels of IGF-I more rapidly than the boys (Iniguez et al., 2006).

RATIONALE

Optimal growth is of critical importance for the developing foetus and infant. The children born after ICSI have increased risk for low BW, preterm birth and a moderate but significantly increased risk for being malformed**.**

Survival rates of preterm infants have improved with increased risks for peri- and postnatal morbidities. "Encephalopathy of prematurity" gives rise to impairments in vision, cognition, as well as in motor and psycho-neurological development and behaviour with long-term consequences.

In order to understand the role of pre- peri- and postnatal factors on the development of the visual system in children born after ICSI and in prematurely born children the following studies were conducted.

AIMS

The overall aim of the present study was to investigate the effects of prenatal factors in children born after ICSI and peri- and postnatal factors (BW, GA, IGF-I and growth) in children born preterm on visual function and ocular fundus morphology at school age.

The central hypothesis was that ICSI, early and late postnatal growth as well as early serum levels of IGF-I in preterm children influence the development of the visual system and visual functions during childhood.

Paper I

To investigate visual function in children born after ICSI and compare the results with those of a group of matched control children born after spontaneous conception.

Hypothesis: Visual function in children born after ICSI is different from visual function in children born after spontaneous conception.

Paper II

To investigate the ocular fundus morphology in children born after ICSI, and compare the results with those of a control group of children born after spontaneous conception.

Hypothesis: Ocular fundus morphology in children born after ICSI is different from ocular fundus morphology in children born after spontaneous conception.

Paper III

To evaluate the association between GA, BW, early (including IGF-I) and late postnatal growth variables and ophthalmologic outcome at school age in children born before 32 GW.

Hypothesis: Low GA, low BW and poor early and late postnatal growth variables have an adverse influence on ophthalmologic outcome at school age in children born before 32 GW.

Paper IV

To compare ocular fundus morphology in children born preterm with a control group and to evaluate the influence of GA, BW, early (including IGF-I) and postnatal growth variables on the ocular fundus morphology in the children born preterm.

Hypothesis: Ocular fundus morphology is different at school-age in children born before 32 GW from ocular fundus morphology in a control group. Low GA, low BW, poor early and late postnatal growth have an adverse influence on ocular fundus morphology at school age in children born before 32 GW.

METHODOLOGICAL CONSIDERATIONS

MATERIAL

All children (Papers I-IV) were examined in the Eye Clinic at the Queen Silvia Children's Hospital, Gothenburg. The ICSI children born between 1994 and 1996, and the preterm children born between December 1999 and April 2002 were examined at a median age of 5.4 years as were the matched control children in the ICSI studies. The examiners were blinded to the mode of conception in Papers I and II. All investigations were performed by three experienced ophthalmologists and experienced orthoptist from the same clinic have performed the orthoptic evaluations. All fundus photographs have been analysed by the same ophthalmologist.

Ninety-nine of the ICSI children participated in an international, multidisciplinary, multi-centre study (Bonduelle et al., 2004).

ICSI children

Paper I

Children consecutively born after ICSI from 1994 to 1996 in Gothenburg comprised the study group. The ICSI was performed at the Department of Reproductive Medicine at the Sahlgrenska University Hospital in Gothenburg and the Fertility Centre Scandinavia at the Carlanderska Hospital, Gothenburg. A total of 160 ICSI children were invited to participate in the study. The only exclusion criterion of the study was inability to speak Swedish. The dropout rate because of refusals was 23/160 (14.4 %), 15 of 114 singletons and 8 out of 46 twins. Hence the participation rate was 85.6 %. There was no significant difference regarding gender, BW or GA between participants and non-participants. Written consent was received from the children's parents.

Thus, the study population comprised 137 ICSI children (67 boys, 70 girls) (Figure 7). Thirty-eight (27.7 %) children were twins. Baseline characteristics of neonatal data are presented in Table 1. One boy had Marcus Gunn syndrome, one had Goldenhar syndrome, and one twin boy had been treated for ROP. In order to adjust for gender and differences in GA at birth a reference was used for evaluation of BW SDS (Marsal et al., 1996).

Table 1. Baseline characteristics for the ICSI children participating in Papers I and II (for retinal vessel analysis).

Characteristics	Paper I (n=137)	Paper II (n=57)
GA weeks, mean (SD)	38.6 (3.0)	38.4 (3.3)
GA weeks median (range)	39.6 (24.7-42.9)	39.3 (24.7-42.7)
Preterm (<37 GW)), n (%)	27 (19.7)	10 (17.5)
BW grams, mean (SD)	3096 (792)	3088 (874)
BW grams median (range)	3190 (650-4830)	3335 (650-4830)
SGA (≤ -22%), n (%)	20 (14.6)	7 (12.3)
Male sex, n (%)	67 (48.9)	35 (61.4)
Twins, n (%)	38 (27.7)	16 (28.1)

GA, gestational age; GW, gestational weeks; BW, birth weight; SGA, small for gestational age (≤ 22% compared with expected birth weight)

Paper II

At the examination of the 137 ICSI children fundus photographs through dilated pupils were taken for digital image analysis. Only photographs of satisfactory quality were analysed and 82 ICSI children (46 boys, 36 girls) fulfilled the criteria (Figure 7). The remaining 55 children refused or cooperated poorly. Among the children with fundus photographs 57 (35 boys, 22 girls) had photographs that allowed evaluation of the retinal vessel morphology as well as of the optic nerve head. The photographs must be focused with the optic disc centred (within half a disc off centre). Baseline characteristics of neonatal available data in the children with images analysed for the central retinal vessels, as well as for the optic nerve head, are presented in Table 1.
There were no differences in GA or BW between the ICSI children with fundus photographs, and the ICSI children without fundus photographs.

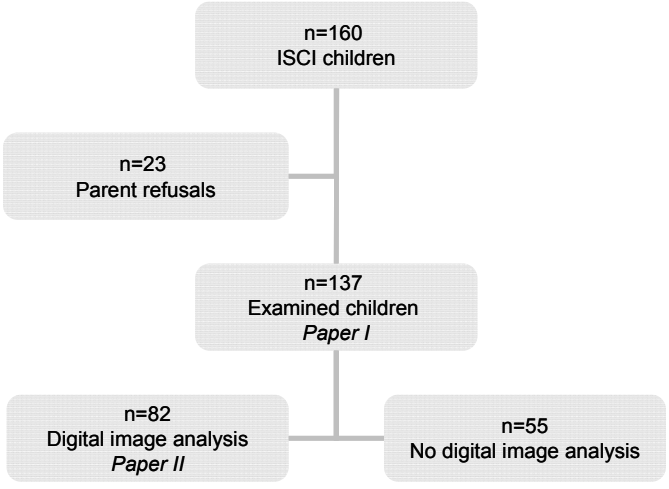

Figure 7. Flow chart of the ICSI children participating in Papers I and II.

Children born preterm

The participating children had a median corrected age of 5.4 years (range 4.8–6.1) at follow up examination.

Paper III

The study group consisted of children born between December 1999 and April 2002 with a GA of < 32 weeks, who were previously examined with regard to postnatal serum IGF-I levels in relation to ROP. A detailed description of the initial study group (n=70) is presented elsewhere (Hellstrom et al., 2003). In this study we included two children, who were earlier excluded because of syndromes. One child had died before school age, and hence 71 children were available for the follow-up study. Five children did not participate because of parental refusal.

Thus, 66 children, 30 boys and 36 girls were studied. There were no significant differences in GA or BW between the study group and the five drop-outs (3 boys, 2 girls) (Figure 8).

Files from hospitals and child health-care centres were reviewed with regard to health status and neurological development before the eye examination. For three children, one of whom had a mildly retarded development, results of neuro-imaging were lacking. The remaining 63 children had undergone ultrasound and ten of them were also examined using computed tomography (CT) and/or magnetic resonance imaging (MRI) (CT n=5, MRI n=3, CT & MRI n=2). One girl had Rett syndrome, one girl had Bartter syndrome and one boy had Prader-Willi syndrome. Baseline characteristics are given below in Table 2.

After laser ablation one girl developed cataracts and underwent cataract extraction, and later secondary implantation of intraocular lenses. One girl had bilateral corneal clouding of unknown aetiology, and one boy had a small anterior polar cataract in one eye.

Paper IV

The 66 children born preterm described above (Paper III) had fundus photographs taken at the time of the eye examination at school-age. Fifty-three children (24 boys, 29 girls) had fundus photographs, which allowed evaluation of the optic nerve head and the central retinal vessel morphology, and were included in the study. All children were examined with ophthalmoscopy (Figure 8). Baseline characteristics are presented in Table 2. Visual acuity of the best eye ranged from 0.05 to 1.25 (median 0.8, mean 0.7, SD 0.2). Spherical equivalent of refraction ranged from +8.6 to -2.8 dioptres. Four children were more than 4 dioptres hyperopic.

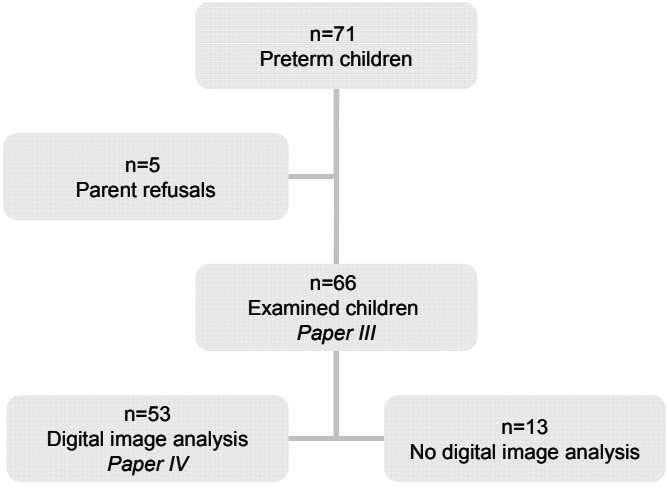

Figure 8. Flow chart of the children born preterm in Papers III and IV

Table 2. Baseline characteristics and morbidity in the preterm children participating in Papers III and IV.

Characteristics	**Paper III (n=66)**	**Paper IV (n=53)**
GA weeks, mean (SD)	27.5 (2.1)	27.6 (2.1)
GA median (range)	27.1 (23.4.4-31.1)	27.1 (23.4-31.1)
BW grams, mean (SD)	1025 (332)	1063 (342)
BW median (range)	948 (530-2015)	970 (530-2015)
SGA (≤ -2 SDS), n (%)	16 (24.2)	9 (17.0)
Male sex, n (%)	30 (45.5)	24 (45.2)
Singletons, n (%)	49 (74.2)	41 (77.4)
***Other morbidity**:*		
ROP stage <3, n (%)	30 (45.5)	25 (47.2)
ROP stage 3 total, n (%)	12 (18.2)	9 (17.0)
ROP treated, n (%)	7 (10.6)	4 (7.5)
BPD n (%)	13 (19.7)	10 (18.9)
NEC n (%)	4 (6.1)	2 (3.8)
Brain lesion, n (%)	10/63 (15.9)	6 (11.3)

Definition brain lesion: IVH ≥ grade III, Hydrocephalus, WMD/PVL, CP

GA, gestational age; BW, birth weight; SGA, small for gestational age; BPD, broncho-pulmonary dysplasia; NEC, necrotizing enterocolitis; IVH, intraventricular haemorrhage; WMD, white matter damage; PVL, periventricular leucomalacia; CP, cerebral palsy.

The children without fundus photographs

The 13 children (seven boys and six girls) who lacked fundus photographs of sufficient quality had significantly lower serum IGF-I levels, and a lower weight week 32 (SDS) than the children included in the fundus evaluation ($p=0.021$ and $p=0.0086$, respectively). There were no significant differences in GA and BWSDS between the groups. Visual acuity of the best eye among the children without photographs was significantly lower (median 0.4, range 0 to 0.9) than in the study group (median 0.8, range 0.05 to 1.25), $p<0.001$. Their HCSDS (n=10) was significantly more negative (median -0.6, range -3.1 to 0.3) in the comparison with HCSDS of the study group (median 0.3, range -1.2 to 2.0), $p<0.001$. From ophthalmoscopy and evaluation of photographs of poor quality only three children had a fundus considered without clinical remarks. Of the remaining nine children three had large cupping, and/or tortuous vessels (n=4), macula hypoplasia (n=3), or temporal dragging of retinal vessels after laser ablation (n=1). One girl had drusen in the optic nerve diagnosed on ultrasound. Four of the dropout children had brain lesions, three of them had WMD with PVL, one boy was operated on for hydrocephalus with shunt, and two had cerebral palsy. In addition, the group included the three children with syndomes, and one girl with pseudohakia and one with corneal clouding.

Table 3. Baseline characteristics and morbidity in the children without photographs in Paper IV.

Characteristics	**Children without photographs Paper IV (n=13)**
GA mean (SD)	27.1 (2.0)
GA median (range)	27.1 (24.3-30)
BW grams, mean (SD)	871 (227)
BW median (range)	790 (535-1335)
SGA (≤ -2SDS), n (%)	6 (46)
Male sex, n (%)	6 (46)
Singletons, n (%)	0 (0)
Other morbidity	
ROP stage <3, n (%)	5 (38.5)
ROP stage 3 total, n (%)	3 (23.1)
ROP stage 3 treated, n (%)	3 (23.1)
BPD n (%)	3 (23.1)
NEC n (%)	2 (15.4)
Brain lesion, n (%)	4 (30.8)

Definition brain lesion: IVH ≥ grade III, Hydrocephalus, WMD/PVL, CP

GA, gestational age; BW, birth weight; SGA, small for gestational age; BPD broncho-pulmonary dysplasia; NEC, necrotizing enterocolitis; IVH, intraventricular haemorrhage; WMD, white matter damage; PVL, periventricular leucomalacia; CP, cerebral palsy.

Control groups

Paper I

The control children born after spontaneous conception were matched for gender, age (+/3 months) and mother's age (+/- 3 years). They were recruited from the Swedish Medical Birth Registry. The exclusion criteria were inability to speak Swedish, and residency outside the western region of Sweden at the time of the examination from 1999 to 2002. A total of 208 controls were invited to participate in the study. Among the control children the refusals were 49 (23.6%) and the participation rate was 76.4 %.

Thus, the control group comprised 159 children (77 boys, 82 girls) born after spontaneous conception (Figure 9). There were two twins in the control-group (1.3 %). Six out of 159 controls (3.8 %) had been born preterm (<37 GW), none before 32 GW. Eleven out of 159 controls (6.9 %) had been born SGA.

The matching variables did not differ statistically between the ICSI and control groups, and their distribution was very similar. Therefore no adjustments for these variables were needed in the analysis.

In the following this control group, used for comparison with the ICSI children in Paper I, is referred to as the *matched control group*.

Papers II and IV

Among the matched control children, for the ICSI children in Paper I, 104 children (41 boys, 63 girls) had photographs of their fundus taken. In addition, a group of 99 subjects (53 boys, 46 girls) born at term after spontaneous conception, with BW appropriate for GA and no history of congenital, chronic or other serious disorder were included to increase the control group. This group was described in a previous study (Hellstrom and Svensson, 1998).

Thus, the control group comprised 203 children (94 boys, 109 girls) analysed for optic nerve variables, and 181 of the controls (83 boys, 98 girls) had photographs of satisfactory quality for retinal vessel analysis (Figure 9). In the following this group is referred to as the *control group*.

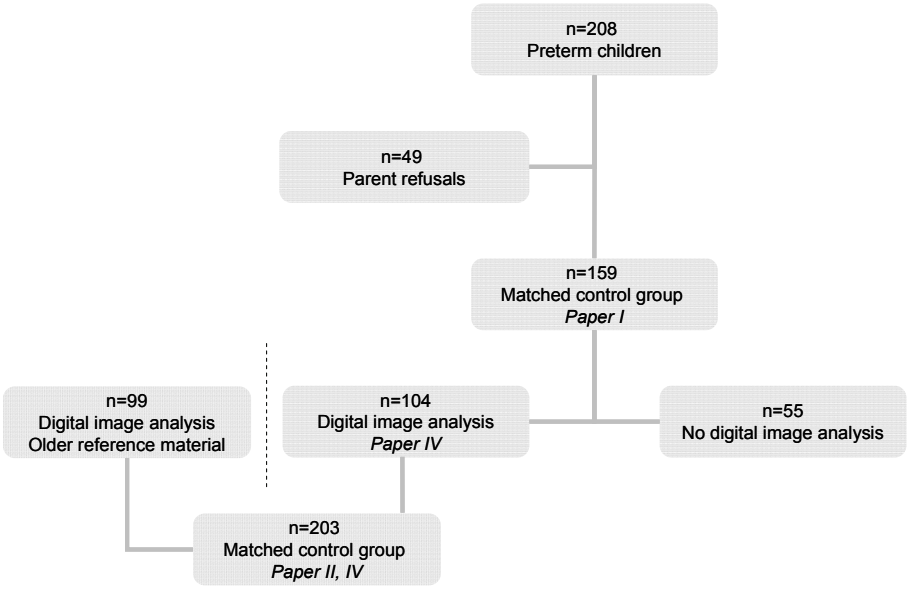

Figure 9. Flow chart over matched control group (Paper I) and control group (Papers II and IV)

Methods

Ophthalmic evaluation

In the eye clinic, monocular visual acuity (VA) was tested at a distance of 3 metres with the Lea Symbols Chart (Hered et al., 1997) in the ICSI children and the matched control group (Papers I and II). The Lea Symbols Chart is linear with five optotypes and in order to pass a line, four out of five optotypes have to be correctly recognized. One ICSI child was unable to perform a visual acuity test, and one ICSI child only allowed a binocular test. The child can name the symbols and do not need to hold a chart in their hands and point. When the preterm children (Papers III and IV) were examined we used the KM visual acuity chart, which by then had been evaluated and considered to be more appropriate for children in the age group 5-7 years (Moutakis et al., 2004). For seven children it was not possible to use the KM chart. Three children born preterm were tested with the HVOT chart, and one with the Lea Symbols Chart. Three children with a VA below 0.1 (logMAR 1.0), could only perform binocular tests with less demanding charts (Cardiff cards n=2 and Kays pictures n=1) and their binocular vision was valued as VA of the best eye in the analysis. One boy born preterm had only light perception and was considered as blind in accordance with the WHO definition (WHO, 2007). Four ICSI children, six matched control children and sixteen children born preterm were tested with their prescribed glasses. The orthoptic examination included cover testing at near and distant fixation, stereoacuity with the Lang and/or TNO test, and evaluation of ocular motility and convergence. The anterior segment of the eye was inspected using a slit-lamp. The eyes were examined in cycloplegia, 45 minutes after administration of a single drop (cyclopentholate 0.85 % and phenylephrine 1.5 %), with autorefraction, and/or retinoscopy and ophthalmoscopy. Fundus photographs were taken for later analysis (Papers II and IV). If the child/parents refused installation of cycloplegic drops the refraction was not measured and the fundi were examined through undilated pupils. This was the case in four ICSI children and three matched control children. Refraction was not performed in the pseudophakic preterm girl, who was operated on for bilateral cataract.

In Paper I significant refractive errors were defined as a spherical equivalent (SE) of myopia ≥0.5 diopters (D), hyperopia ≥3.5 D, astigmatism ≥1.0 D and anisometropia ≥1.0 D (De Becker et al., 1992). In Paper III we used another reference (Robaei et al., 2005) for significant refractive error which had been used in another follow up study of preterm children at the eye clinic (Hard et al., 2000). The only difference between the two references for clinical significant refractive error was for hyperopia which in Paper III was considered to be significant if SE was ≥3 D.

Visual perception

Visual perception (Paper III) was examined with the TVPS-R (Test of Visual Perceptual Skills-Revised) (Gardner, 1996). This is a non-motor test for children aged between 4 and 12 years and 11 months. It consists of seven subtests (1) discrimination: identification of forms; (2) memory: ability to remember single forms individually; (3) spatial relations: determination of correct direction of forms; (4) form constancy: recognition of the same form when it varies in size from the stimulus; (5) sequential memory: ability to remember a number of forms in a series; (6) figure ground: finding a form when it is hidden among other forms; (7) closure: determination of a whole form from parts of this form. Each subtest consists of 16 items and the number of correct responses yields a 'raw score' from 0 (all failed) to 16 (all correct). Based on this raw score, scaled scores are given for each subtest and age (at 3-months intervals). The scaled scores are then added to give a scaled score for the whole test which was used for analysis. A percentile rank (PR) based on the scaled scores of an American reference group of children is also provided.

Digital image analysis of fundus photographs

In Papers II and IV the ocular fundus photographs were taken through dilated pupils and analysed quantitatively, using a specially designed computer-assisted digital mapping system (Stromland et al., 1995).

The mean value of the two eye measurements was calculated for each studied fundus structure. If the fundus photograph of only one eye was of sufficient quality, that eye's photograph was used.

Digital mapping

The optic disc area was measured by marking the outlines with a cursor. The inner border surrounding the nerve tissue defined the optic disc; care was taken not to include the white peripapillary scleral ring. The cup was defined by contour, and its definition was facilitated by the course of the vessels and its pallor. The neuronal rim area was obtained by subtraction of the cup area from the disc area. The indices of tortuosity for arteries and veins were defined as the path length of the vessel divided by the linear distance from the vessel origin to a reference circle 3 mm from the centre of the optic disc. The same person, who was masked to the mode of conception in Paper II, performed all measurements, which eliminates inter-individual differences in the measurements. As the optic disc size may vary systematically with the method of measurement it is strength that all photographs in these studies are measured by the same person using the same digital analysing program.

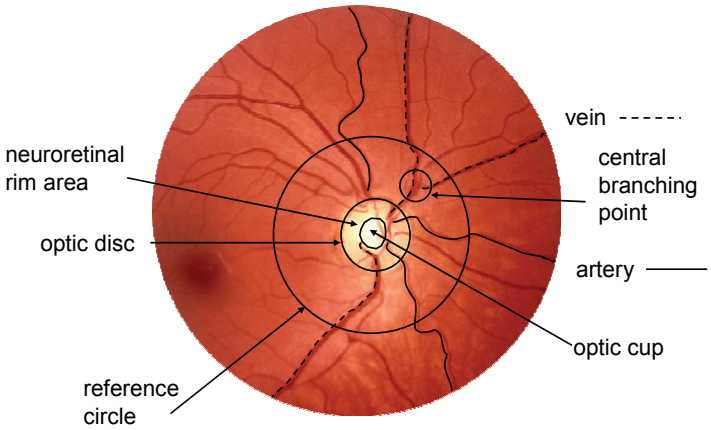

Figure 10. Digital mapping of ocular fundus

However, in order to see if there were magnification factors due to high refractive errors that influenced the results of the analyses of the optic disc variables in the children born preterm (Paper IV) compared with the control group, we analysed the optic disc and retinal vessel variables in all the children with photographs as well as excluding the four children with hyperopia exceeding +4 dioptres. The results did not change and therefore we have presented the result of all children (n=53).

It is a challenge to take photographs of young children. They have to focus on a small object and the luminance is high. Especially for the ICSI children there was a large proportion without photographs qualified for digital analyses (n=80 of 137, 42 %). The children often refused or cooperated poorly. The ICSI children had earlier on the same day as the photographs were taken been examined by a cardiologist and a paediatrician before the ophthalmologic investigation. Hence, many of them were tired when it came to the photograph procedure. It is understandable that it was too demanding with so many examinations in one and the same day. However, many of the children did not live in Gothenburg and to minimise the days for the parents and the children in Gothenburg all the medical examinations were performed in one day and the neuropsychological examination in another day. There were statistically significant more boys than girls among the 57 ICSI children with photographs (61.4 %

boys and 38.6 % girls) compared with the ICSI children without fundus photographs (non-participants n=80, 40 % boys and 60 % girls) ($p = 0.021$). In addition, the percentage of boys with photographs of quality to be analysed for retinal vessels was higher in the ICSI group (61 %) than in the control group (46 %) that may be a bias negative for the ICSI boys in our study. Among the preterm children we did not receive fundus photographs from nine children, and the photographs were of a poor quality in four children. Unfortunately, the sickest children born preterm were unable to cooperate in the photography procedure and we investigated their fundi by indirect ophthalmoscopy according to the study protocol. In addition, the sickest children have regularly been checked at the eye clinic, and we scrutinized the files for information of earlier or later fundus inspections. The photographs of the children born preterm were taken with a different angle than those of the children born after ICSI and the controls, which made the placement of the reference circle uncertain, and thus, a comparison of number of branching points with the controls impossible. While the area of the small structure of the optic nerve head could be transformed using a magnification factor, the vessels subtend a larger and curved area making such a transformation inappropriate, because of substantial risk for magnification errors. In Paper IV we therefore chose to present only the optic nerve variables, but in the thesis findings of the tortuosity index and central retinal branching points in relation to growth variables will be presented in the results below.

Measurements of peri- and postnatal growth variables including IGF-I levels

In Papers III and IV we set out to evaluate the association between GA, BW SDS, early (including IGF-1) and late postnatal growth variables and ophthalmologic outcome at school age in children born before 32 GW.

Birth weight and weight at PMA 32 weeks were measured, and standard deviation scores (BWSDS) and weight at 32 weeks (PMA) (SDS), were calculated for all children (Marsal et al., 1996). At the examination at school age head circumference (Fredriks et

al., 2000), weight and length (Wikland et al., 2002) were measured according to clinical praxis, and were expressed as standard deviation scores (SDS).

In the neonatal period venous blood samples (0.5 mL) had been taken weekly, and analysed using a procedure described in the original paper (Hellstrom et al., 2003). The mean values of serum IGF-I during postmenstrual age (PMA) 30 to 33 weeks (30.0 – 33.9) were used for analyses. We have chosen to analyse mean serum IGF-I levels during PMA 30-33 weeks because earlier studies have shown that during this time period the discrepancy in IGF-I levels is at its peak between children with and without morbidity (Hellstrom et al., 2003). However, we do not know whether this is the most optimal time period to measure serum IGF-I levels, and further analyses will be performed.

Statistics

Descriptive statistics are given as mean, standard deviation, median and range. Fisher's exact test was used for comparison of proportions between groups, and Fisher's non-parametric permutation test was used for continuous and ordered variables (Good, 2000a).

Mann-Whitney U test was performed to test differences between groups for continuous and ordered variables (Papers II and III). Spearman's rank correlation coefficient r_s was used for correlation analysis in Paper II. In Paper IV correlation was estimated with Pearson correlation coefficient and analysed with Pitman's non-parametric permutation test (Good, 2000b). All tests were two sided and conducted at 5 % significance level.

SUMMARY OF RESULTS

Paper I

Visual function in ICSI children was not different from visual function in children born after spontaneous conception

There was no significant difference between the ICSI children and the matched control group in visual acuity, although a slightly increased proportion of the ICSI children (9.6%) in comparison with the matched control group (6.3%) had a VA below 0.8 (Table 4). Neither did we find significant differences between the two groups concerning significant refractive errors (Table 4). The orthoptic evaluation (strabismus, ocular motility, stereopsis and convergence) did not show any significant differences between the ICSI and the matched control children.

Table 4. Visual acuity (VA), inter-eye differences, refractive errors (spherical equivalent) and anisometropia in ICSI children and children born after spontaneous conception.

	ICSI twins	**ICSI all children**	**Controls**	**ICSI vs controls OR (95% CI)**
Visual acuity	*n=37[a] n (%)*	*n=136[a] n (%)*	*n=159 n (%)*	
VA best eye	36 (97.3)	123 (90.4)	149 (93.7)	0.6 (0.2-1.6)
VA diff ≥ 2 lines	2 (5.4)	2 (1.5)	3 (1.9)	0.8 (0.1-6.9)
Refractive error	*n=38 n (%)*	*n=133[b] n (%)*	*n=156[b] n (%)*	
Hyperopia ≥3.5 D	3 (7.9)	6 (4.5)	4 (2.6)	1.8 (0.4-8.8)
Myopia ≥0.75 D	1 (2.6)	3 (2.3)	1 (0.6)	3.6 (0.3-189.0)
Astigmatism ≥1.5 D	1 (2.6)	2 (1.5)	4 (2.6)	0.6 (0.1-4.1)
Anisometropia ≥1 D	1 (0.7)	3 (2.3)	2 (1.3)	1.8 (0.2-21.5)
Children with refractive errors	4 (10.5)	9 (6.8)	8 (5.1)	1.3 (0.4-4.1)

[a] One ICSI twin child was unable to perform a *VA* test and one ICSI child only allowed a binocular test.

[b] Not all the children were examined in cycloplegia because some parents/children refused installation of eye drops

Paper II

The median number of retinal vessel branching points was lower in ICSI children than in the control group

Thirty-seven of the children born after ICSI (67%) had retinal vascular branching points below the median of the control group. The 95% confidence interval for the mean of the number of branching points among the ICSI children was in the range 24.6-26.2 and for the control children 26.9-28.0. Tortuosity index for arteries and veins did not differ between children born after ICSI and children born after spontaneous conception.

ICSI boys differ from the ICSI girls and from a control group in the retinal vessel morphology with less central retinal vessel branching points

A gender difference was found. The ICSI boys had significantly fewer branching points compared with the girls (p=0.022). There was no difference between the genders in the control group regarding the number of branching points. The ICSI boys had fever branching points compared with the boys in the control group, but the ICSI girls compared with the girls in the control group showed no difference regarding the number of branching points (Table 5).

Table 5. Cross-sectional data of retinal vascular branching points in 35 boys born after ICSI and 83 control boys and in 22 girls born after ICSI and 98 control girls.

Variable	**Boys**		
Branching points	*ICSI n=35*	*Control n=83*	*ICSI vs. Controls, p-value*
Mean (SD)	24.6 (2.8)	27.6 (3.6)	
Median Range)	24 (19-29.5)	27.5 (20-37)	<0.0001
Variable	**Girls**		
Branching points	*ICSI n=22*	*Control n=98*	*ICSI vs. Controls, p-value*
Mean (SD)	27.6 (3.6)	27.3 (3.7)	
Median Range)	27.5 (20-37)	27 (19-40)	0.45

There was a correlation between the number of branching points and tortuosity index for arteries (r_s=-0.27, p=0.043) and veins (r_s=-0.26, p=0.049). The lower the number of branching points, the higher the tortuosity index for arteries and veins.

We also found a relation between BW deviation and number of branching points within the ICSI children. With a low BW deviation there was a tendency to have a reduced number of branching points (p=0.058).

No difference in optic disc morphology between the ICSI children and the children born after spontaneous conception

Table 6. Cross-sectional data of optic disc variables and index of tortuosity for arteries and veins in ICSI children and in a control group.

Variable	ICSI children n=82	Controls n=203	ICSI vs. Controls p-value
Optic disc area, mm²			
Mean (SD)	2.4 (0.4)	2.4 (0.5)	
Median (Range)	2.4 (1.5-3.7)	2.3 (1.6-5.3)	0.34
Cup area, mm²			
Mean (SD)	0.4 (0.3)	0.3 (0.2)	
Median (Range)	0.3 (0-1.8)	0.3 (0-1.6)	0.34
Rim area, mm²			
Mean (SD)	2.08 (0.3)	2.07 (0.4)	
Median (Range)	2.1 (1.3-2.8)	2.1(1.3-3.9)	0.36
Variable	**ICSI children n=57**	**Controls n=181**	**ICSI vs. Controls p-value**
ITA			
Mean (SD)	1.09 (0.04)	1.09 (0.03)	
Median (Range)	1.08 (1.03-1.2)	1.08 (1.04-1.2)	0.49
ITV			
Mean (SD)	1.06 (0.02)	1.06 (0.02)	
Median (Range)	1.06 (1.03-1.1)	1.06 (1.02-1.2)	0.98

ITA, index of tortuosity for arteries; ITV, index of tortuosity for veins

Paper III

Children born very preterm had affected visual capacity

Eleven of 66 (17 %) children had visual impairment (VA ≤0.3), one of whom was blind (VA <0.05) and 28 children (42 %) had mildly reduced vision. Good VA was found in 27 (41 %) children.

In all infants with ROP stage 3 (n=12), the retinopathy regressed either spontaneously or after treatment, thus in no case was visual impairment due to retinal detachment. Among the visually impaired children there was one girl with Rett syndrome, one pseudophakic and one with cloudy corneas. Five of 11 visually impaired children had been diagnosed with brain lesions. The other children with brain lesions had mildly reduced or good vision.

A low visual acuity was associated with low gestational age, poor early and late growth

Low VA of the best eye was correlated with low GA, and low weight week 32 (SDS), but not to BW (SDS). Visual acuity was also correlated with low weight, length and head circumference (SDS) at follow-up (Table 7).

Refractive errors were associated with low gestational age, negative birth weight deviation and poor early as well as late growth

Significant hyperopia was associated with low neonatal serum levels of IGF-I, and to a small head circumference (SDS) at follow-up. Significant myopia (only four children were myopic) was related to low GA. Four of the hyperopic children were diagnosed with brain lesions, and two of the myopic children were treated for ROP.

Significant astigmatism was associated with both low BW (SDS) and low weight at week 32 PMA (SDS) (Table 7).

Anisometropia was found in 10/65 (15 %) of the children and nine (14 %) of the children had strabismus. Two children with strabismus had brain lesions and one had Rett syndrome. Anisometropia and strabismus showed no correlation to any of the measured growth variables.

Poor visual perception was correlated with poor early postnatal growth and a small head circumference at school age

Of the 58 children, who were capable to perform the TVPS-R test, a majority 33/58 (57%) had results below the third percentile of the American reference group and 30 children had a percentile rank of 1. Six children with brain lesions performed the test and three of them had a percentile rank of 1. Poor results were correlated with low weight at week 32 (SDS) and small head circumference (SDS) at follow-up (Table 7).

Table 7. Results of statistical analysis regarding visual outcome and postnatal growth variables - early and at follow-up.

Early growth variables	Visual acuity	Hyperopia[a]	Myopia[ab]	Astigmatism[a]	TVPS-R SS
IGF-I mean	n.s	p=0.0096	n.s	n.s	n.s
Gestational age	r_s= 0.29*	n.s	p=0.027	n.s	n.s
Birth weight SDS	n.s	n.s	n.s	p=0.047	n.s
Weight week 32 SDS	r_s =0.30*	n.s	n.s	p=0.0041	r_s =0.38**
Growth variables at follow-up					
HC SDS at 5 years	r_s =0.55***	p=0.022	n.s	n.s	r_s =0.39**
Weight SDS at 5 years	r_s =0.37**	n.s	n.s	n.s	n.s
Length SDS at 5 years	r_s =0.41***	n.s	n.s	n.s	n.s

[a] Clinically significant as defined in the Methods section, [b] n=4

TVPS-R SS, Test of Visual Perceptual Skills-Revised, sum of scaled scores; n.s., non significant, * p<0.05, ** p<0.01, *** p<0.001, Mann-Whitney U-test and Spearman's correlation coefficient r_s were used

Small head circumference at school age was correlated with low GA, poor early growth and low serum IGF-I levels

Head circumference (SDS) at school age was significantly correlated to GA (r_s=0.40, p=0.0012), neonatal serum levels of IGF-I (r_s=0.37, p=0.0031) and to weight at week 32 (SDS) (r_s=0.27, p=0.035). There was no correlation between early growth variables and length and weight at follow-up.

Paper IV

Smaller areas of the optic nerve head and rim, and larger cup area were found in children born very preterm compared with the control group

We found reduced areas of the optic nerve head, the neuronal (axonal) rim as well as a larger cup area in the children born preterm compared with the control group. The children born preterm also had an increased index of arteriolar tortuosity (Table 8, Figure 11).

Table 8. Ocular fundus variables in children born preterm and the control group.

Ocular fundus variable	Preterm group n=53	Control group n=203	Preterms vs Controls p-value and mean 95 % CI
Optic disc area, mm²			
Mean (SD)	2.2 (0.3)	2.4 (0.4)	
Median (range)	2.2 (1.3-2.7)	2.3 (1.6-5.3)	p<0.001
Mean (95 % CI)	2.16 (2.09; 2.26)	2.40 (2.34; 2.46)	-0.24 (-0.35; -0.12)
Cup area, mm²			
Mean (SD)	0.4 (0.3)	0.3 (0.2)	
Median (range)	0.4 (0-1.4)	0.3 (0-1.6)	p=0.0027
Mean (95 % CI)	0.43 (0.35; 0.52)	0.30 (0.27; 0.34)	0.13 (0.03; 0.22)
Rim area, mm²			
Mean (SD)	1.7 (0.4)	2.1 (0.4)	
Median (range)	1.7 (1.1-2.4)	2.1(1.3-3.9)	p<0.001
Mean (95 % CI)	1.73 (1.63; 1.83)	2.07 (2.02; 2.12)	-0.33 (-0.45; -0.22)
Tortuosity arteries			
Mean (SD)	1.1 (0.05)	1.09 (0.03)	
Median (range)	1.1 (1.05-1.3)	1.08 (1.04-1.2)	p<0.001
Mean (95 % CI)	1.13 (1.12; 1.14)	1.09 (1.08; 1.09)	0.04 (0.03; 0.06)
Tortuosity veins			
Mean (SD)	1.07 (0.02)	1.07 (0.02)	
Median (range)	1.07 (1.0-1.1)	1.06 (1.0-1.2)	0.19
Mean (95 % CI)	1.07 (1.06; 1.07)	1.06 (1.06; 1.07)	0.004 (-0.002; 0.011)

Fisher's non-parametric permutation test

Low birth weight and poor early growth were related to large cupping of the optic nerve head and a small neuronal rim area

A low BW (SDS) and weight at 32 weeks PMA (SDS) were associated with a reduced neuronal rim area and a large cupping (Table 9). There was no correlation between either, sex, GA, neonatal serum IGF-I levels, length, weight, or head circumference at follow-up and the ocular fundus structures at school age. Neither was there any relationship between degree of arteriolar tortuosity, or number of retinal branching points and any of the studied growth variables. At school age there was a tendency of an association between visual acuity of the best eye and optic disc area (p=0.054).

Table 9. Correlations between optic nerve head variables and perinatal growth variables

Perinatal growth variables	Optic disc area	Cup area	Rim area
IGF-I mean	n.s	n.s	n.s
Gestational age	n.s	n.s	n.s
Birth weight SDS	n.s	r=-0.37**	r=0.01*
Weight week 32 SDS	n.s	r=-0.38**	r=0.02*

Test of correlation with Pitmans's non-parametric permutation test (r)

* p<0.05, ** p<0.01

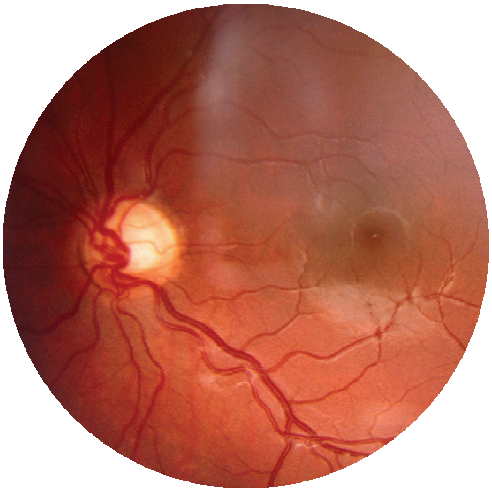

Figure 11. A boy born in GW 30 with poor birth weight (-1.7 BWSDS) and perinatal weight growth (-3.1 w32 WSDS), and with white matter damage. The disc cup is large (1.0 mm2) and the neuronal rim is small (1.3 mm2) in comparison with the median for disc cup (0.3 mm2) and rim areas (2.1 mm2) for the reference group

Larger cupping of the optic nerve head was found in children born preterm with known brain lesion

Preterm children with known brain lesions (n=6) had a significantly larger cupping of the disc (mean 0.7, SD 0.2, median 0.7, range 0.5-1.0 mm^2) than the preterm children without known brain lesions (n=47) (mean 0.4, SD 0.3, median 0.4, range 0-1.4 mm^2), p=0.046. Among the children without photographs that could be evaluated there were four children with known brain lesions, and in three of them a large cup of the optic nerve head was noted at ophthalmoscopy according to the study protocol.

GENERAL DISCUSSION

Modern assisted reproduction techniques have given many infertile couples an opportunity to become parents, and approximately one million children are born after IVF/ICSI worldwide. The proportion of children born after assisted reproduction now exceeds 5 % in the Scandinavian countries, and approximately half of them are born after ICSI (Nyboe Andersen et al., 2008). Especially ICSI, which is an invasive method, has raised worries about the outcome of the children. Infants born after ICSI have an increased risk of being born prematurely, both singletons and those born after multiple births, and account for a not negligible proportion of the increasing number of babies born preterm.

Advanced neonatal care has increased the survival rate of the tiniest children born prematurely, and there is also concern about the outcome of these small immature babies. As many as up to 75 % of infant deaths can be attributed to preterm birth, as can 42-47 % of children with cerebral palsy, and 23 to 37 % of the preterm population has significant cognitive, visual and hearing impairments in USA (Behrman and Stith Butler, 2007).

This thesis aims to evaluate effects of prenatal factors in children born after ICSI and peri- and postnatal factors in children born very preterm on visual function and ocular fundus morphology at school age. In the children born very preterm the ophthalmologic outcomes, including optic nerve morphology, were analysed in relation to GA, BW (SDS), serum levels of IGF-I, weight at week 32 (SDS), and weight, length and head circumference (SDS) at school age.

In the first two Papers children born after ICSI were studied regarding visual function and ocular morphology including the ocular fundus.

Regarding visual acuity in the children born after ICSI we found no difference compared with spontaneously conceived children, and similar results have been reported in other studies (Belva et al., 2007; Bonduelle et al., 2005; Knoester et al., 2008). We found no significant difference in the rate of refractive error between the ICSI children born at term, and the children born after spontaneous conception at term, which also is in accordance with other studies of refractive status in children

born at term after ICSI (Knoester et al., 2008). There were 38 children born after multiple births, and 27 of 137 children were born preterm (three born before GW 32), and it is known that children born premature have more refractive errors than children born at term (Larsson et al., 2003; O'Connor and Fielder, 2008). However, the preterm children born after ICSI in our study did not differ regarding refractive errors from the control group.

In our study one twin boy had Goldenhar syndrome, and one had Marcus Gunn syndrome. In addition to our findings ocular anomalies have also been reported in other studies (Anteby et al., 2001; Bonduelle et al., 2005; Knoester et al., 2008). In the study by Anteby and co-workers optic nerve head hypoplasia and atrophy, coloboma with microphthalmus, Coats disease, congenital glaucoma, congenital cataract and retinoblastoma were found among 47 children referred to an eye clinic born after IVF/ICSI. However, in the two later studies the anomalies were not specified (Bonduelle et al., 2005; Knoester et al., 2008). Goldenhar syndrome has previously been documented with an increased incidence among children, born after IVF/ICSI compared with a group of naturally conceived children, and the authors speculate that over-ripeness of the egg may affect epigenetic processes in the early zygote (Wieczorek et al., 2007). It has been found that epigenetic dysregulation occurs in some patients with Goldenhar syndrome (Fischer et al., 2006).

An increased risk for cerebral palsy has been reported in children born after IVF/ICSI (Hvidtjorn et al., 2009; Lidegaard et al., 2005; Stromberg et al., 2002). In the present study no participating child, but two dropouts born after ICSI had a diagnosis of cerebral palsy. This may erroneously have led to too favourable results of the ICSI children, since CP is associated with reduced vision, strabismus and refractive errors (Kozeis et al., 2007).

The participation rate in our study, 85.6 % in ICSI children and 76.4% in naturally conceived children, was not optimal, but comparable with several other studies (Cederblad et al., 1996; Leslie et al., 2003; Morin et al., 1989). There was no difference regarding variables such as gender, GA and BW between participants and non-participants, making the study population reliable concerning these variables.

In Paper II we examined the ocular fundus in children born after ICSI and the results were compared with those of a control group. This is, to our knowledge, the first morphometric study of the ocular fundus in children born after ICSI. We found that the boys, but not the girls, had less central retinal vessel density as demonstrated by fewer retinal vessel branching points. Since the retina is part of the central nervous system, and the vascularisation is adapted to the needs of the neural elements, one might speculate that the reduced number of vessel branching points reflects abnormalities in the neural retina as well as in the brain. Large registry-based studies with long follow-up period show evidence of an increase in neurological problems after IVF (Hvidtjorn et al., 2009; Lidegaard et al., 2005; Stromberg et al., 2002).

The reason for our finding we do not know. Unfortunately, we lack data of serum IGF-I levels in our study group, but one may speculate that lower serum levels of IGF-I in the boys, compared to the girls, may have contributed to the reduced number of vascular branching points. IGF-I is important for normal retinal vascularisation (Hellstrom et al., 2002), and persistent low serum levels of IGF-I after premature birth are associated with later development of ROP (Hellstrom et al., 2003). It has been found that children born preterm with low GA, and/or low BW have abnormal retinal vascularisation with fewer central retinal branching points (Hellstrom et al., 2000). Several studies on children born SGA have shown that these children have lower concentrations of IGF-I both in intrauterine life and post-partum than do children born AGA (Arosio et al., 1995; Ostlund et al., 1997). Even though seven boys of 35 (20 %) born after ICSI were prematurely born, and five of 35 (14 %) were born SGA, there was still a significant difference in number of vascular branching points when the premature and SGA born boys were excluded. Interestingly, lower serum levels of IGF-I in boys born after ICSI at three months of age in comparison with boys born after spontaneous conception have been reported. The difference in IGF-I levels had disappeared at the age of five years (Kai et al., 2006). In preterm infants male gender has been associated with lower levels of IGF-I at birth and in the neonatal period compared to girls (Engstrom et al., 2005). The reason for this gender difference is not clear, but it has been suggested that differences in sex steroids could influence the secretion of IGF-I in utero (Barrios et al., 1996). In addition, one study

in full term Caucasian babies has shown that the mean cord plasma concentrations of IGF-I in males were significantly lower than in the female babies suggesting "a sexual dimorphism in the GH-IGF axis" (Geary et al., 2003).

Remarkably, other studies have shown increased growth after in vitro fertilisation. One study on IVF/ICSI at the age of 7 years, in which twins and children born prematurely were excluded, found higher levels of IGF-I and IGF-II, and the children born after IVF/ICSI were taller compared with a control group (Miles et al., 2007). The authors speculated that the in vitro fertilisation process leads to epigenetic alterations of imprinted genes expressed either from the allele from the mother or the father. The authors also suggested that the children born after IVF/ICSI, with higher serum IGF-I and II, may have mild phenotypic features of Beckwith-Wiedemann syndrome with a normal BW and a taller stature in childhood (Miles 2007). Such features have been found in relatives of children with Beckwith-Wiedemann syndrome (Elliott and Maher, 1994). The overgrowth syndrome Beckwith-Wiedemann has been reported to occur up to nine times more commonly after in vitro conception than in the general population (DeBaun et al., 2003; Halliday et al., 2004; Maher, 2005). There are over 50 known imprinted genes in the human, and many of these are involved in cellular proliferation and growth. The IGF-II gene is imprinted and expressed only from the paternal gene in the placenta and foetus (DeChiara et al., 1991). In animal studies it has been shown that IGF-II plays a role for placental growth. Epigenetic modification of imprinted genes (via changes in DNA methylation) may provide a mechanism linking environmental cues to placental phenotype, with consequences for development both before and after birth. Therefore, changes in expression of imprinted genes have major implications for developmental programming (Fowden et al., 2006).

One might speculate that the fertilisation procedure may have influenced imprinting of genes in the children born after IVF/ICSI, and led to the alterations of retinal vascular development found in the boys in this study. One case-control study of children born after IVF/ICSI found an increased placenta/BW ratio in children born after assisted reproduction (Koudstaal et al., 2000). Infants born small with a large placenta has been associated with increased risk of hypertension and cardiovascular

disease in adulthood (Barker et al., 1990). One follow-up study of ICSI children at the age of eight years (the eldest children born after ICSI being examined) found higher diastolic and systolic blood pressure compared with a control group (Belva et al., 2007). In the international multi-centre study, in which the ICSI children in our study participated, blood pressure was similar in the children born after ICSI and in the spontaneously conceived children (Bonduelle et al., 2004).

Another possible mechanism behind our finding of fewer retinal branching points among the ICSI children could be maternal factors such as diabetes mellitus, and an increased use of heparin-like substances and thrombocyte aggregation inhibitors indicating a coagulopathy disorder in the mother (Kallen et al., 2005b). However, this is not likely in our study as only boys were affected.

The retinal vasculature is adapted to meet the needs of the neural retina, and one might anticipate a correlation between few vascular branching points, and reduced neuronal rim area of the optic disc. However, we did not find a relation between number of branching points and the neuronal rim area. Neither did we find any gender difference in neuronal rim area. We have only measured the branching pattern of the largest vessels in the most central part of the retina, and do not know whether a reduced number of branching points may be compensated by increased branching in the periphery, or by an increased density of the capillary bed. The development of the central retinal vessels takes place between 14 and 21 GW and we do not know what factors are involved in branching. Thus, neither the cause nor the clinical significance can be clarified from the present study.

When we eliminated factors earlier shown to be associated with a low number of central vascular branching points, i.e. low BW and preterm birth, the difference in vascular pattern still remained. This suggests other mechanisms (e.g. infertility, genetics of infertility, and/or the reproduction technology) responsible for the somewhat reduced number of vascular retinal branching points demonstrated in boys born after ICSI in the present study.

This is a limited group of children born after ICSI, and we cannot exclude a selection bias due to the low number of children with photographs (42 %), and the higher proportion of boys among the children with photographs, although there were no

differences in BW, or GA among the children with photographs and those without photographs. Larger numbers of patients would be welcome, but adequate statistics were obtained with this limited group.

The main limitation with Papers I and II is that our study groups are small, and when large enough studies become available the results concerning visual defects might be different. When studying relatively rare events, small studies can never demonstrate absence or presence of a difference. To detect a genuine association between assisted reproductive techniques and imprinting defects in children born after ICSI much larger studies are needed since these conditions are rare (Basatemur and Sutcliffe, 2008).

Papers III and IV have evaluated the association between GA, BW, early (including IGF-I) and late postnatal growth variables and ophthalmologic outcome including optic nerve morphology at school age in children born very preterm.

We found that low GA and poor early postnatal growth as reflected in low weight week 32 (SDS), but not BW (SDS), were correlated with reduced visual acuity of the best eye, and that poor early weight gain was correlated to poor visual perceptual skills at follow-up at five years of age. We also found that low weight (SDS), short stature (SDS), and small head circumference (SDS) at follow-up were strongly correlated with low visual acuity of the best eye. Interestingly, neither reduced visual acuity nor poor perceptual skills were related to earlier ROP. Although ROP had been found in 64 % of the children, none had retinal detachment.

In addition, we found that both low BW (SDS) and low weight at 32 weeks (SDS) were associated with reduced neuronal tissue of the optic nerve head and a large cup.

The correlations may reflect the strong influence on the visual system of the catabolic state, experienced by many very preterm infants during the neonatal period, with low weight and small head circumference persisting later in childhood.

In this study, 90 % of the children born preterm had a BW below 1500 g, and 17 % had a weight SDS below -2 at birth while a majority (64 %) had a weight SDS below -2 at 32 weeks. It should also be noted that all children had a lower weight (SDS) at 32

weeks, compared with their BW, indicating a postnatal period of poor weight gain. Children born very prematurely have problems with feeding in the neonatal period, the gastrointestinal tract is immature, and the nutrient intakes of protein and energy do not reach their required levels (Embleton et al., 2001). In addition, it is known that IGF-1 serum levels fall rapidly after preterm birth (Lineham et al., 1986), and poor nutrition and poor growth are associated with lower levels of IGF-I in tissues and serum in preterm children (Engstrom et al., 2005; Smith et al., 1997a). In utero the IGF-1 levels rise during the third trimester and children with poor BW and poor weight gain have lower levels of IGF-1 (Chellakooty et al., 2006; Giudice et al., 1995; Langford et al., 1998). IGF-1 is related to GA, BW and nutrition. As have been mentioned earlier a recent study reported that a long duration of low levels of serum IGF-1 was associated not only with ROP, but also with increased frequency of other morbidities in premature infants, such as IVH, NEC and BPD (Lofqvist et al., 2006a). Intra-uterine infection is a common cause of preterm delivery, and the immature immune system of the preterm baby makes sepsis a common complication. In both clinical and experimental studies inflammation has been shown to increase the risk of neurological injury (Hagberg and Mallard, 2005). It has been proposed, in a recent study on sheep foetuses, that prenatal endotoxin exposure through intrauterine infections may be harmful to the developing retina and optic nerve, and may influence the development of visual acuity (Loeliger et al., 2007).

Infections in very preterm infants with elevated levels of inflammatory cytokines in cord and neonatal blood have been associated with white matter brain damage (Hansen-Pupp et al., 2005; Yoon et al., 1996). The pre-myelinating oligodendrocytes are vulnerable not only to ischemia, but also to inflammation (Khwaja and Volpe, 2008). Inflammation at birth in very preterm infants has been associated with a decrease in IGF -I (Hansen-Pupp et al., 2007).

Reduced visual acuity has been reported in many follow-up studies of preterm children (Cooke et al., 2004; Hard et al., 2000; O'Connor and Fielder, 2007), and 11 % of visually impaired children in Sweden are born before GA 37 weeks (Blohme and Tornqvist, 1997). Infants with a BW below 1000 g are more than three times more

likely to have a visual acuity below 0.1 (logMAR 1.0) at the age of eight years than those born at term (Hack et al., 2005).

Decreased visual acuity may be the result of suboptimal development, or lesions to different parts of the visual system, i.e. the eyes, the visual pathways and different cerebral cortical areas involved in processing of the visual input. Thus, measuring visual acuity is a simple test to assess the health of the eyes, pathways to the brain, and the parts of the brain involved in processing the visual input. The strong correlation we found between low visual acuity and both low GA and low weight at week 32 (SDS) may illustrate the influence of the prematurity on different parts of the visual system, as both eyes and the brain were found to be affected. In our study eight of the 39 children with impaired or subnormal visual acuity had known brain lesions, which may have contributed to the low visual acuity. Furthermore, 10 of 39 children with visual acuity below 0.8 (logMAR 0.1) had findings on ultrasound with IVH grade 2 or below or a minor or suspicious ventricular enlargement. These ultrasound investigations were sometimes inconclusive and have therefore not been included in the analyses.

One might speculate that different structures in the eye and brain may have suffered form the lack of nutrients and growth factors experienced by most very preterm infants (Denne, 2001; Embleton et al., 2001; Lineham et al., 1986), and this lack may have contributed to the reduced visual function in the preterm children found in this study. The rods are during the third trimester in a phase of rapid development, with an increasing energy demand (Barishak, 1992). Parafoveal rods mature later and are more affected by ROP than the peripheral ones (Barnaby et al., 2007). The retinal function and vascularisation are closely related, and even though the photoreceptors are supplied by the choriocapillaris and not by the retinal circulation, a mediating role for the post-receptor retina has been proposed (Akula et al., 2007). In a mouse model of ROP, IGF-I was expressed throughout the retina while IGF-II and the IGF-I receptor were expressed almost exclusively in the photoreceptors and retinal vessels, suggesting a communication between the photoreceptors and the vasculature (Lofqvist et al., 2009b).

The optic disc is the only neuronal structure of the central nervous system that may be viewed, photographed, and analysed without invasive methods. We found that the children born very preterm at the age of five years had a smaller optic disc and smaller neuronal rim area than those of the control group. This is in accordance with the findings of a previous study of children born with GA <29 weeks (mean 27 weeks) in which a smaller disc and neuronal rim were found at school age (Hellstrom et al., 2000). In another study of preterm children born later (GA < 32 weeks, mean 29 weeks) there were no differences in the optic disc variables at the age of five years in comparison with a full-term control group (Hellstrom et al., 1997). In an epidemiologic study of Swedish visually impaired children, those who were born preterm and those born small for gestational age had an increased risk for optic nerve hypoplasia (Tornqvist et al., 2002). The examinations in our study were performed at the age of five years, and histologic and clinical studies have shown that there is no measurable growth of the optic nerve head after the age of three years (Hellstrom and Svensson, 1998; Rimmer et al., 1993).

The finding of reduced neuronal rim area and small optic disc area indicate a reduction of the axonal volume or a reduced number of axons. This may reflect a more global effect on the neuronal growth of the brain.

It has been shown that IGF-I is important for neurogenesis, but also for angiogenesis in the brain, and for repair after an insult (Lopez-Lopez et al., 2004; McDonald et al., 2007). Injury to the periventricular white matter (Volpe, 2001a) and corpus callosum (Johnston et al., 2001) has been linked to injury to oligodendrocyte precursors, which are important for myelination (Khwaja and Volpe, 2008), and it has been shown that IGF-I protects oligodendrocyte progenitor cells in the neonatal rat brain (Lin et al., 2005).

We did not find a correlation between serum levels of IGF-I, measured during GW 30 to 33 and the optic nerve morphology. Instead, we found that a large negative weight deviation at 32 weeks, that may reflect earlier growth retardation and growth factor deficiency, was strongly correlated to poor vision and visual perception. However, we did find an association between low early IGF-I serum levels and a small head at school age. We chose to analyse mean serum IGF-I levels during PMA 30-33 weeks

because earlier studies have shown that during this time period the discrepancy in IGF-I levels peaks between children with and without morbidity (Hellstrom et al., 2003).

Unfortunately, 13 children (20 %) could not cooperate well enough to provide satisfactory photographs. Fundus photography demands cooperation, concentration and fixation, and it is not surprising that a majority of the 13 children who lacked fundus photographs were more disabled than the children with fundus photographs. In addition, the 13 children who lacked fundus photographs had significantly lower serum IGF-I levels than those whose photographs were analysed. Four of these 13 children had known brain lesions, and three of them had large optic cups at ophthalmoscopy. If we had received photographs of sufficient quality from all the children, the results might have been different.

We found that low serum IGF-I levels during postmenstrual weeks 30-33 were associated with hyperopia, which was found in 36 % of our children. Hyperopia is normally associated with short axial lengths. Ocular growth is influenced by IGF-I, and treatment with IGF-I increases the ocular axial length in patients with short axial lengths due to growth hormone insensitivity (Laron syndrome) (Bourla et al., 2006). Cook et al compared premature children born before 32 GW without ROP with full-term infants, and at term the preterm group had shorter axial lengths, shallower anterior chambers, and more highly curved corneas. There was less hyperopia than expected in the premature group despite shorter axial lengths due to shallower anterior chambers and more curved corneas (Cook et al., 2003). It has been reported that infant hyperopia is associated with mild delays across many aspects of visual cognitive and visual motor development (Atkinson et al., 2007). This was reflected in our study where 25 % of the hyperopic children had known brain lesions.

Astigmatism was found in 35 % of the children and was associated with a low BW and poor early growth (low weight at weeks 32). Astigmatism has previously been linked to preterm birth and low BW (Hebbandi et al., 1997; Larsson and Holmstrom, 2006; O'Connor et al., 2006). The reason for the excess rate of astigmatism in children born preterm is not fully understood. The development of the anterior segment may be arrested as a result of preterm birth, and therefore the growth of the eye may be

insufficient and the emmetropisation process of the eye disturbed (Cook et al., 2003; Fledelius, 1990; O'Connor et al., 2006).

We did not find any gender differences in this study, although there are many studies reporting on the disadvantage of the male sex both regarding risk of obstetric complications (Demissie et al., 1999; Elsmen et al., 2004; Elsmen et al., 2006; Ingemarsson, 2003), neonatal mortality (Ingemarsson, 2003; Stevenson et al., 2000), and neonatal morbidity showing that boys needed more ventilatory and circulatory support than girls (Elsmen et al., 2004; Stevenson et al., 2000). Both white matter injury and IVH are more common in preterm boys than in preterm girls (Edwards, 2004). Significantly reduced white matter volumes have been found at the age of 8 years in preterm boys compared with boys born at term, but not in the girls born preterm compared with girls born full term (Reiss et al., 2004). Preterm birth adversely affects the development of the corpus callosum, especially in boys, and this may impair verbal skills in boys (Nosarti et al., 2004). A reduction of cortical development in prematurely born children, and more pronounced in boys than in girls, has been found at the age of 2 years (Kapellou et al., 2006). In addition, cognitive deficits and poor motor outcome are more common in prematurely born boys than in girls (Fily et al., 2006; Hack et al., 2002; Hindmarsh et al., 2000; Johnson and Breslau, 2000; Marlow et al., 2005; Ment et al., 2006; Wood et al., 2005). A recent study of children born before GA 25 weeks showed that the frequency of visual impairment and blindness among the boys was three-fold that of girls (Jacobson et al., 2009). A study by Barrenäs and co-workers demonstrated that men born small for gestational age without catch-up growth had an increased risk for sensorineural hearing loss, and suggested that this could originate from low IGF-I concentrations during foetal life (Barrenas et al., 2005). As discussed regarding the findings of lower number of retinal vascular branching points in boys born after ICSI (Paper II), it has been reported in a larger study group of very preterm children, in which the children in the present study participated, that the circulating serum IGF-I levels in the neonatal period were lower in the boys than in the girls (Engstrom et al., 2005). Our limited study group of children born very preterm may be one factor explaining the lack of sex differences found in this study.

Low weight (SDS) at week 32 and head circumference (SDS) at follow-up were also related to poor results on the test of visual perception. Poor performance on the visual perception test in prematurely born children in comparison with a Swedish reference group of full term children has previously been reported (Hard et al., 2000). Impaired visual perception indicates abnormal cerebral functions. Poor postnatal growth, especially of the head, in children born preterm (GA <32 weeks), has previously been found to be associated with cognitive impairment, including visual spatial skills at the age of seven years (Cooke and Foulder-Hughes, 2003). The children in that study were born with appropriate weight for age and there was a normal distribution of head circumference, and the authors suggested that postnatal growth restriction was a likely explanation to their findings (Cooke and Foulder-Hughes, 2003). Small head circumference at two and eight years of age in very preterm children has been reported to be strongly associated with motor and cognitive impairments at the age of eight years, but head circumference at birth was not related to school-age outcome. The authors also concluded that postnatal events soon after birth, and certainly before 2 years of age, have an effect on brain growth and function (Kan et al., 2008). A recent study following preterm infants up to two years of age found that at birth 7.5 % of 227 infants born very preterm had microcephaly (head circumference below -2 SDS) at term equivalent, and at the age of two years 28 % were microcephalic. The poor postnatal head growth became more evident at two years of age, and it was strongly associated with poor neurodevelopmental outcome and cerebral palsy. They also found a strong correlation between head circumference and brain volume at term on MRI (Cheong et al., 2008). In our study 15 % had been diagnosed with brain lesions, which may have influenced the postnatal head growth. However, in the study by Cheong and co-workers no significant association was found between head size and head growth with qualitative white and gray matter abnormalities on MRI, but the authors proposed a more global disturbance in brain growth not reflected in the tissue-specific grading scales they used in analysing their MRIs (Cheong et al., 2008).

During the time period of very preterm births, the cerebral blood flow to the white matter is low in the premature infant. In addition the auto-regulation of cerebral blood

flow in response to changes in blood pressure is impaired which can cause ischemia (Back et al., 2007; Khwaja and Volpe, 2008). The periventricular white matter is especially vulnerable during the period between 24 and 32 postmenstrual weeks, i.e. the time period when our study group was born, and the infants are prone to both focal and diffuse lesions in the periventricular white matter (Leviton and Gressens, 2007; Volpe, 2009). Injury to this area is associated with an increased risk of visual impairments, as the posterior visual pathways pass through this region of the brain. IGF-I has been shown to promote angiogenesis of the brain by inducing growth of endothelial cells, and protects neural tissue from ischemic lesions (Lopez-Lopez et al., 2004).

Hansen Pupp et al recently presented data demonstrating a relationship between cerebellar volume and serum IGF-I in very preterm infants, and suggested that cerebellar growth may be influenced by low levels of IGF-I during early postnatal catch-up, and that supplementation with IGF-I during early postnatal period may be of benefit especially in growth restricted infant, and needs to be further studied (Hansen-Pupp et al., 2009).

CONCLUDING REMARKS

Paper I

In this limited study group no significant difference in visual function was found between children born after ICSI and matched control children at the age of 5 years.

Paper II

Our findings showed that ICSI boys have abnormal vessel morphology as demonstrated by a reduced number of central retinal branching points. This difference still remained after elimination of factors earlier shown to be associated with a low number of vascular branching points, i.e. low BW and preterm birth, suggesting other mechanisms (e.g. infertility, genetics of infertility, and/or the reproduction technology) responsible for the low number of branching points.

Paper III

In very preterm children, independent of earlier development of ROP, poor early as well as later growth was closely related to reduced visual acuity and poor perception at school age. In addition, low IGF-I levels and poor growth during the first months of life appeared to adversely influence head circumference and refraction at school age in children born very preterm.

Paper IV

Preterm birth was associated with a reduced neuronal area of the optic disc. The association found between both low BW and poor early growth and later reduced optic nerve area indicate the importance of early weight gain for optimal neural development in preterm children.

FUTURE PERSPECTIVES

In vitro fertilisation including ICSI are now well recognized techniques for reproduction, helping many infertile couples to conceive, but the medical needs of children born after IVF/ICSI are greater than for normally conceived children. The number of children born after IVF/ICSI is increasing as the techniques are now available in most countries (Adamson et al., 2006). Multiple pregnancy due to transfer of more than one embryo is the major risk factor for adverse outcome after IVF/ICSI, as it may lead to low BW and/or preterm birth. The frequency of high order pregnancies differs between countries with approximately 5 % in Sweden and as much as 40 % in e.g. Italy and Greece (Andersen et al., 2008). Recent studies have shown a dramatic decline in the frequency of twin pregnancies as a result of single embryo transfer without a substantial change of the overall pregnancy rate (Bergh, 2005; Gordts et al., 2005). World wide the future parents and children born after ICSI would benefit from single embryo transfer.

Further studies are needed concerning the causes of infertility/subfertility of the parents and the genetics. The field of epigenetics may provide tools to study the consequences of expression of genes from both parents. The boys born after ICSI whose fathers have Y-chromosome abnormalities will inherit the chromosome defect, but the impact on the fertility status of the children born after IVF/ICSI is unknown.

Whether assisted reproductive techniques with ovarian stimulation, manipulation of the oocyte and sperm and the exposure to culture media affect gene expression is not known.

The increased rate of survival of children born very and extremely preterm has resulted in an increased population of children with a high frequency of disabilities. Many follow-up studies on children born preterm have been presented in this thesis showing deficits that engage visual, cognitive, motor and behavioural abilities often in combination. Besides the strain on the child and its family, it constitutes an economic problem since many of these children need more resources from the public medical and social welfare system than children born at term. Research in different disciplines is needed.

Habilitation

There is a need to develop tools to identify the special visual problems of preterm infants which may consist of reduced visual acuity and contrast sensitivity in combination with visual perceptual deficits and poor visuo-constructive ability. There is also a need for the development of habilitation strategies to ameliorate the child's ability to function in society and this has to be performed in a multidisciplinary way.

Obstetrics

Prevention of preterm birth would eliminate associated health problems and possible preventative measures have to be explored. Implantation of only one embryo in IVF is known to reduce multiple pregnancies and preterm birth but is not implemented everywhere yet. Infection and/or inflammation are common causes of preterm delivery which possibly could be reduced by early diagnosis and treatment. Other factors responsible for preterm delivery have to be elucidated.

Paediatrics and ophthalmology

In neonatology much research is focused on creating an optimal environment for the most immature babies. Although most very preterm infants receive supplemental oxygen the optimal oxygen saturation levels is not known, and at present is the subject of a large multicentre study (BOOST). Nutrition is a problem in these immature individuals and poor growth is associated with increased morbidity.

We found a strong correlation of early and late growth on the visual function at follow-up in children born very preterm. In addition, one recent study with the aim of identifying the prematurely born infants at risk of needing treatment for ROP, showed that by using an algorithm (Weight IGF-I, neonatal, ROP –WINROP™) factoring postnatal development of serum IGF-I levels and/or development of weight early prediction of ROP needing treatment was provided (Hellstrom et al., 2009; Lofqvist, 2009).

For decades, neonatal intensive care has focused on survival of the most immature babies. Time has come to find methods to ameliorate the nutrition for the children

born very preterm in order to optimize normal growth and development of essential structures such as vessels and neurons.

It has been shown that preterm infants who develop ROP and other severe postnatal morbidities, i.e. bronchopulmonary dysplasia and necrotizing enterocolitis, have low persisting levels of the growth factor IGF-I after birth and do not reach the corresponding age-matched foetal levels (Hellstrom et al., 2003). In addition, it is known, as discussed in this thesis, that IGF-I is essential for growth and development of the immature brain, as well as for the vasculature of the eye and the brain. Intervention with substitution of IGF-I to the very preterm babies to raise IGF-I up to normal intrauterine levels may thus be beneficial. Studies in this field are ongoing (Lofqvist et al., 2009a).

SAMMANFATTNING PÅ SVENSKA

Prevalensen av för tidig födelse har ökat sedan senare delen av 1970-talet och sjukvården räddar idag mycket och extremt för tidigt födda barn till livet i större utsträckning än tidigare.

För tidig födelse orsakas av många, delvis okända, faktorer. Kända orsaker är bl.a. infektion/inflammation och otillfredsställande funktion hos moderkakan.

Med modern teknik för assisterad befruktning, som IVF (provrörsbefruktning) inklusive intracytoplasmatisk spermieinjektion (ICSI), föds barn som annars inte hade kunnat bli till. Vid ICSI utsätts ägg, spermie och embryo för hantering som skiljer sig från det fysiologiska skeendet vid normal befruktning. Barn födda efter ICSI föds ofta för tidigt. Om flera embryon återförs till mamman leder det till flerbördsgraviditet vilket ytterligare ökar risken för för tidig förlossning.

De mycket för tidigt födda barnen kräver neonatal intensivvård och utsätts för extern påverkan under en period som de under normala betingelser skulle ha tillbringat i livmodern. Dessa barn måste, under det som skulle ha varit sista tredjedelen av graviditeten, anpassa sig till ett liv i en miljö som skiljer sig kraftigt från den i livmodern.

För tidig födelse innebär förlust av den närande och tillväxtfrämjande intrauterina miljön under en tid då det normalt sker en snabb mognad och tillväxt av vävnader. De omogna kärl- och nervvävnaderna hos det mycket för tidigt födda barnet har en ökad sårbarhet och skador kan därför uppstå i ögonen och i synbanorna i hjärnan.

Den aktuella undersökningen har gällt effekterna av prenatala faktorer på barn födda efter ICSI samt peri- och postnatala faktorer på barn födda mycket för tidigt. Vår studie avsåg att belysa hur dessa faktorer kan påverka barnens synfunktion och ögonbottenmorfologi vid 5-6 års ålder.

Utfallet av ögonundersökningen inklusive ögonbottenfynden av de för tidigt födda barnen analyserades i relation till gestationsålder, födelsevikt, nivåer i blodet av tillväxtfaktorn IGF-I, vikt vecka 32 och vikt, längd och huvudomfång vid 5 års ålder.

Barn födda efter ICSI (n=137) och barn födda med gestationsålder <32 veckor (n=66) genomgick en undersökning av ögon och synfunktion (synskärpa, och visuell perception) vid 5 års ålder. Barnen födda efter ICSI jämfördes med en matchad kontrollgrupp med barn födda efter normal befruktning (n=159).

Synnervens morfologi undersöktes med hjälp av digital bildanalys av ögonbottenfotografier hos 82 barn födda efter ICSI och hos 53 för tidigt födda barn. Dessutom undersöktes morfologin hos näthinnans centrala kärl hos 57 barn födda efter ICSI. Resultaten av ögonbottenundersökningarna jämfördes med resultaten hos en kontrollgrupp (n=203).

Vi fann ingen signifikant skillnad i synfunktionen mellan barn födda efter ICSI och barn födda efter spontan befruktning när barnen var 5 år. Pojkarna (inte flickorna) födda efter ICSI hade en avvikande kärlmorfologi med signifikant färre förgreningar av blodkärlen centralt på näthinnan i jämförelse med kontrollgruppen. För pojkar födda efter ICSI kvarstod skillnaden efter att vi uteslutit de pojkar som var för tidigt födda och de med låg vikt för sin gestationsålder. Detta kan tala för att det reducerade antalet kärlförgreningar, som tidigare setts hos prematurfödda barn och barn med låg vikt relativt födelseålder, kan orsakas även av andra faktorer, t.ex. genetiska faktorer eller faktorer relaterade till infertiliteten och befruktningsmetoden.

En klar majoritet (74 %) av de för tidigt födda barnen hade någon form av synavvikelse och 17 % av dem var synskadade (synskärpa ≤0.3), jämfört med 0.03% i normal befolkningen. Låg synskärpa och nedsatt visuell perceptionsförmåga vid 5 års ålder korrelerade med låg vikt i vecka 32 och låg vikt, kortvuxenhet och litet huvudomfång vid 5 års ålder. Refraktionsfel och litet huvudomfång vid 5 års ålder

hade ett samband med låg nivå av IGF-I i blodet under nyföddhetsperioden. De för tidigt födda barnen hade en mindre synnervsarea och arean av den del av synnerven som upptogs av nervvävnad var mindre än hos barnen i kontrollgruppen. Dessutom konstaterades ett samband mellan en liten synnervsarea och en liten area av nervvävnad med både låg födelsevikt i förhållande till gestationsålder och dålig tidig viktökning. Dessa fynd talar för att god tidig tillväxt är viktig för utvecklingen av nervvävnad hos för tidigt födda barn.

Vi har med detta arbete visat att synfunktion hos femåriga barn födda efter ICSI inte skiljer sig från synfunktionen hos barn födda efter spontan befruktning. En annan slutsats är att det kan finnas en könsspecifik påverkan på kärlutvecklingen i ögat hos pojkar födda efter ICSI.

Vi har även visat att dålig tidig tillväxt, mätt som vikt i vecka 32, och låg nivå av IGF-I i blodet har betydelse för den normala utvecklingen av synen och refraktionen liksom för huvudomfånget vid 5 års ålder hos mycket för tidigt födda barn. Studierna belyser betydelsen av den tidiga postnatala tillväxten och tillgången på tillväxtfaktorn IGF-I för optimal ögon- och synutveckling.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all of those who have made this work possible and have helped me during these years. In particular, I would like to thank:

Ann Hellström, "Knattan", my supervisor and friend, for your generosity, patience, reliable scientific competence, encouraging spirit, and concern! I would never have managed this thesis without your inspiration, warm guidance and strong and genuine support. I'm happy to know you and your family by now! Thanks to your husband Mikael for his good comments on some of the articles.

Anna-Lena Hård, my dear clever "co-supervisor" and co-writer, colleague and friend, for your enthusiastic commitment, competent advice, thorough eye and mind, for opening your house for us to write, discuss, having fun and walk in the beautiful nature of Bohuslän!

Aimon Niklasson, my co-supervisor for constructive criticism, scientific advice, nice cooperation examining all the children on Saturdays and sending me gorgeous pictures of flowers.

Birgitta Melander, our research coordinator and friend, for practical help with the patients, for generously helping me through the years from small to big matters, being a nice, service-minded, strong and reliable rock!

Chatarina Löfqvist, nice research collegue for analyses of neonatal data, but mostly for being my very helpful with mathematical formula- and all the digital work, without you and all your work I do not want to think of "the out-fit" of this thesis.

Ulla-Britt Wennerholm and **Christina Bergh**, co- authors and colleagues, working in the field of infertility, for sharing your scientific knowledge with me, letting me see the reproduction process "live" at the Fertility laboratory.

The members of the "POF" group – **Marita Andersson**, **Susann Andersson**, **Sonia Grönqvist,** and **Eva Aring,** for your support, friendship and encouragement. I am grateful for all the laughter that made our struggles easier.

Kerstin Strömland, my former supervisor, for enthusiastically introducing me to research in pediatric ophthalmology and showing me that it is "never too late".

The staff at the Department of Pediatric Optthalmology, **Eva Rudholm, Jonna Johansson**, **Lena Kjellberg, Melanie Baumann** and **Jennifer Andersson** for your interest, support and patience, helpfulness and many good laughs!

Ulf Stenevi, professor in Ophthalmology and former chairman of the Department of Ophthalmology, **Bertil Lindblom** professor in Ophthalmology, **Gunnar Ladenvall,** former chairman of the Department of Ophthalmology, and **Mats Tullberg,** former chairman of the Department of Ophthalmology, for providing working conditions that made this work possible.

All my colleagues at the Department of Ophthalmology in Mölndal Hospital for creating a nice and friendly working environment, especially **Lena Sjödell,** who has had a hard workload, when I have been involved in research.

Nils-Gunnar Pehrson, Emma Jeansson, Gunnar Ekeroth, Mikael Holtenman and **Per-Erik Ekman,** at the Statistiska Konsultgruppen i Göteborg, for helping me with statistical analyses with patience, and special thanks to **Nils- Gunnar** who has been with me all the way, explaining and teaching me a bit of your deep knowledge in the mysteries of statistics.

Sara Flodin, orthoptist and co-author for many weekends, when we were examining so many children and keeping me and the children in a good mood with "saft och bullar".

Eva Engström, collegue, and **Anne Rosenkvist**, study nurse at the Neonatology Department for collection of neonatal data

All the **children** and their **parents** for participating in the study.

And above all, my dear husband **Gerdt,** thank you for standing by all the time, supporting, taking good care of our household, treating me with good dinners and wine now and then, putting up with me even when I have been in my "thesis world".

Lisa, my "bonus-daughter", for skillful help me with figures and pictures.

Financial support

This thesis has been supported by grants from Göteborgs Läkaresällskap, the Swedish Research Council, the ”Föreningen De Blindas Vänner”, the ”Wilhelm och Martina Lundgrens Vetenskapsfond”, the Bertarelli Foundation, the ”Margit Thyselius fond för Blind Ungdom”, the ”Carmen och Bertil Regnérs Stiftelse for Forskning inom området ögonsjukdomar”, and the ”Stiftelsen Handlanden Herman Svenssons fond för blinda och synsvaga”.

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