Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999)

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BACKGROUND: To evaluate the safety of ICSI, this study compared data of IVF and ICSI children by collecting data on neonatal outcome and congenital malformations during pregnancy and at birth. METHODS: The follow-up study included agreement to genetic counselling and eventual prenatal diagnosis, followed by a physical examination of the children after 2 months, after 1 year and after 2 years. 2840 ICSI children (1991–1999) and 2955 IVF children (1983–1999) were liveborn after replacement of fresh embryos. ICSI was carried out using ejaculated, epididymal or testicular sperm. RESULTS: In the two cohorts, similar rates of multiple pregnancies were observed. ICSI and IVF maternal characteristics were comparable for medication taken during pregnancy, pregnancy duration and maternal educational level, whereas maternal age was higher in ICSI and a higher percentage of first pregnancies and first children born was observed in the ICSI mothers. Birthweight, number of neonatal complications, low birthweight, stillbirth rate and perinatal death rate were compared between the ICSI and the IVF groups and were similar for ICSI and IVF. Prematurity was slightly higher in the ICSI children (31.8%) than in the IVF children (29.3%). Very low birthweight was higher in the IVF pregnancies (5.7%) compared with ICSI pregnancies (4.4%). Major malformations (defined as those causing functional impairment or requiring surgical correction), were observed at birth in 3.4% of the ICSI liveborn children and in 3.8% of the IVF children ($P = 0.538$). Malformation rate in ICSI was not related to sperm origin or sperm quality. The number of stillbirths (born ≥20 weeks of pregnancy) was 1.69% in the ICSI group and 1.31% in the IVF group. Total malformation rate taking into account major malformations in stillborns, in terminations and in liveborns was 4.2% in ICSI and 4.6% in IVF ($P = 0.482$). CONCLUSIONS: The comparison of ICSI and IVF children taking part in an identical follow-up study did not show any increased risk of major malformations and neonatal complications in the ICSI group.

Key words: children/congenital malformations/genetic counselling/ICSI or IVF/male and female infertility

Introduction

Even if great concern was voiced at the introduction of IVF, a formal and systematic evaluation of this technique was not carried out. Gradually IVF became accepted as a safe technique, mostly on the basis of the information in registries (Cohen *et al*., 1988; Beral and Doyle, MRC Working Party on Children Conceived by In vitro Fertilization, 1990; Medical Research International, Society for Assisted Reproductive Technology (SART) and The American Fertility Society, 1992; Bachelot, 1995; Lancaster *et al*., 1995). One study (Lancaster, 1987) mentioned a statistically relevant increase in two types of birth defects, i.e. neural tube defects and transposition of the great vessels, in IVF as compared with the general population. This report has not yet been confirmed by other studies. However, in one Swedish retrospective cohort study on 5856 IVF babies

(Berg *et al*., 1999), the risk of neural tube defects (hydrocephaly risk ratio 5.7, anencephaly risk ratio 12.9) and oesophageal atresia (risk ratio 3.9) was higher in the IVF group than in the control group.

On the other hand, it also became clear that there is a higher incidence of prematurity in IVF singleton pregnancies [13% of prematurity <37 weeks versus 6% (Doyle *et al*., 1992); 14 versus 8% (Tan *et al*., 1992); 11.1 versus 4.4–6.1% (Olivennes *et al*., 1993); 38.7 weeks of gestation versus 39.8 weeks (Verlaenen *et al*., 1995); odds ratio of prematurity <37weeks 1.6 (Buitendijk, 2000)], a higher rate of low birthweight <2500 g [11 versus 6% (Doyle *et al*., 1992); 14 versus 7% (Tan *et al*., 1992); 11.1 versus 6.5–3.6% (Olivennes *et al*., 1993); 3175 versus 3393 g (Verlaenen *et al*., 1995)] and small for gestational age [17 versus 10% (Doyle *et al*., 1992); 11.2

versus 5.9–10.6% (Olivennes *et al*., 1993); odds ratio of lower birthweight after adjusting for a number of variables 1.4 (Buitendijk, 2000)] and a higher perinatal mortality rate [2.1 versus 1.2% (Buitendijk, 2000)], compared with the general population. Whether these observations are linked to the IVF procedure in itself rather than to confounding factors such as maternal age, parity, chronic condition (maternal diabetes, hypertension), or differences in behavioural risk (smoking, drinking, drugs) was a question which was difficult to answer, since a number of confounding variables were not simultaneously studied. A recent study (Buitendijk, 2000) indicates that maternal age and parity (and a number of variables studied such as maternal height, ethnic origin, education, smoking habits, alcohol consumption etc.) do not explain the higher incidence of prematurity, low birthweight and small size for gestational age; it is therefore still not clear what is the cause of this inferior outcome. In the mean time, the overall short-term outcome of IVF was considered satisfactory and IVF became a widespread procedure, in which often no more questions were asked.

ICSI was considered from the start as a risky procedure. ICSI is a more invasive procedure than routine IVF, since one spermatozoon is injected through the oocyte membrane and since fertilization can ensue from sperm which could never have been used previously in fertility treatment. Even more questions were raised and concern was again expressed when ICSI with non-ejaculated sperm, either epididymal or testicular, was introduced (Tesarik, 1995; Vogt *et al*., 1995). Emphasis was placed on the fact that the risk of chromosomal aberration might be even higher in men with non-obstructive azoospermia (Van Assche *et al*., 1996). Moreover, it was suspected that imprinting might be less complete at the time of fertilization if testicular sperm were used (Tesarik and Mendoza, 1996). If this were so, it would be unlikely to impair fertilization and early development, but anomalies might become manifest at birth or even later in life.

IVF was introduced at our Centres in 1983 and, from the start, a prospective follow-up study of the pregnancies and children was planned. For couples with male-factor infertility, our group introduced in 1991 the new ICSI technique, which involved injection of a single spermatozoon through the zona pellucida directly into the oocyte, and the first pregnancies and births to result from replacement of embryos generated by ICSI were reported (Palermo *et al*., 1992). The experience of ICSI has been that the fertilization rate is considerably better than after other assisted fertilization procedures and that more embryos able to achieve implantation have been produced. Since July 1992, ICSI has been the only procedure used in our Centre when some form of assisted fertilization is necessary (Van Steirteghem *et al*., 1993a,b,c).

From the moment of the introduction of ICSI in our Centre, the treatment involved an informed consent to a prospective follow-up study, involving data on genetic counselling, pregnancy, prenatal diagnosis and child follow-up. Partial data on this cohort have already been published (Bonduelle *et al*., 1994, 1995a,b, 1996a,b, 1999). In these, we (and other groups) failed to find any increased risk of major congenital malformations as compared with the general population, but we did find

an increased risk of chromosomal aberrations, mostly sex chromosomal aneuploidies (Bonduelle *et al*., 1994, 1995a,b, 1996a,b, 1997; Palermo, 1996; Kurinczuk and Bower, 1997). The results of the ICSI procedure were also evaluated by looking at the first 130 children born after ICSI and comparing this group of children with a control group of 130 matched children born after IVF pregnancies in the same period of time and after the same ovarian stimulation and in-vitro culture conditions (Bonduelle *et al*., 1995a). In order to evaluate the safety of the ICSI procedure, we compared the data on congenital malformations, growth parameters and developmental milestones in the two groups of children and could find no statistically significant differences. We therefore concluded from a limited number of children that when ICSI was carried out and compared with standard IVF procedure, no additional risk was observed. In a subsequent article we described the separate groups of children born after the use of epididymal and testicular sperm as well as children born after replacement of cryopreserved ICSI embryos and compared these data with the previous findings in the ICSI group: in this total group of 141 children, we observed no increase in the malformation rate (Bonduelle *et al*., 1997). We further evaluated the safety of the ICSI procedure by studying data on karyotypes, congenital malformations, growth parameters and developmental milestones in a larger cohort of 1987 children born after ICSI (Bonduelle *et al*., 1999).

In the present study we focused on neonatal data and malformations of the children at birth in the cohort of IVF pregnancies starting in 1983 and compared them with the same data in the cohort of ICSI pregnancies starting in 1991. In the ICSI cohort, data were also analysed in terms of the origin and characteristics of the sperm used. All pregnancies were obtained in a single centre at the Dutch-speaking Brussels Free University.

Materials and methods

From 1990 onwards, 3073 pregnancies ensued after subzonal insemination (SUZI) or ICSI, leading to the births of 2889 children from June 1991 until December 1999. Twenty-one of these pregnancies were obtained after SUZI and 10 after SUZI or ICSI. The first baby after ICSI was born in January 1992; from July 1992 ICSI has been the sole procedure when assisted fertilization was needed. From 1982 onwards, 3329 pregnancies ensued after IVF, leading to the births of 2895 children from January 1983 until December 1999.

From the beginning of our programme, stimulation was performed with clomiphene citrate and HMG (Trounson *et al*., 1986). From 1987 onwards the association with GnRH agonists and HMG was introduced (Smitz *et al*., 1987; Albano *et al*., 1996). From 1996 the association of HMG/recombinant FSH (European Orgalutran[R] Study Group *et al*., 2000) and GnRH antagonist was used for a reduced number of patients (Albano *et al*., 1996; European Orgalutran Study Group *et al*., 2000)

Indications for IVF were especially tubal infertility, unexplained infertility, endometriosis and mild male factor infertility. Indications for ICSI were oligoasthenoteratospermia, obstructive and non-obstructive azoospermia and failed IVF (Devroey *et al*., 1995, 1996; Bonduelle *et al*., 1999)

Only children born after a replacement of fresh embryos, using IVF or ICSI (and 31 children born after SUZI) (Palermo *et al*., 1993)

were considered in this study. Children born after mixed ICSI–IVF procedures or after ICSI in combination with preimplantation genetic diagnosis were not taken into account (Liu *et al.*, 1994). Children born after ICSI were conceived using ejaculated, epididymal or testicular spermatozoa. In some cases sperm which had been frozen were used.

Before starting IVF or ICSI, couples were asked to agree to participate in a prospective clinical follow-up study of the children. For most IVF and ICSI couples a parental karyotype was routinely performed. ICSI couples were also asked to provide an informed consent, including genetic counselling and agreement to prenatal karyotype. All ICSI couples were evaluated for possible genetic problems. A history, including a pedigree, was obtained in order to identify genetic risks or possible causes of congenital malformations. This history included details of medication, smoking and environmental or occupational risk factors and of socio-economic status. In view of possible risk factors due to the new techniques of assisted fertilization and taking into account the results of prenatal diagnosis obtained in our ICSI patients, couples during the early years of our programme were counselled to have a prenatal test (Bonduelle *et al.*, 1996a). Gradually, patients were informed more precisely about our knowledge concerning the risk of ICSI and left free to opt for a prenatal test procedure or not. In general, an amniocentesis was suggested for singleton pregnancies and a chorionic villus sampling for multiple pregnancies (De Catte *et al.*, 1996; Aytoz *et al.*, 1997). If indicated, prenatal tests for other genetic diseases were planned. During this session we had our opportunity to emphasize the importance of the clinical follow-up study. IVF couples' histories with an emphasis on possible genetic problems were recorded by the fertility specialist and only risk situations were referred for further genetic counselling. The importance of the clinical follow-up study for all the couples was emphasized on several occasions: in the general information sessions, during the personal contact with the gynaecologist and during contact with the nurses.

The clinical follow-up study included the completion of a standardized questionnaire as previously described (Wisanto *et al.*, 1995), and its return to the research nurse and, where possible, the parents were asked to visit the Centre for Medical Genetics with the child about 2 months after the expected delivery date.

For all pregnancies, written data on pregnancy outcome with regard to the babies were obtained from the gynaecologists in charge. Perinatal data, including gestational age, mode of delivery, birthweight, Apgar scores, presence or absence of malformations and neonatal problems were registered. If any problem was mentioned, detailed information was also requested from the paediatrician in charge. For babies born in our university hospital, a detailed physical examination was done at birth, looking for major and minor malformations and including evaluation of neurological and psychomotor development. These children also had a routine ultrasound examination of the brain, kidneys and heart during the neonatal hospitalization. If no prenatal test had been performed, some ICSI children also had a routine karyotype at or after birth with their parents' consent. For babies born elsewhere, written reports were obtained from gynaecologists as well as from paediatricians, while a detailed morphological examination by a geneticist from our centre was carried out after 2 months whenever possible. Additional tests were carried out if the anamnestic data or the physical examination so indicated.

At the child's age of 2 months, the information filled in by the gynaecologist/paediatrician was double-checked with the parents and completed with additional information on illness and development, either at the consultation in the genetic department or over the phone by the research nurse. At this time and when possible, the babies had a detailed physical examination for major and minor anomalies and an evaluation of their neurological and psychomotor development. Data on major anomalies at birth not reported in the questionnaires were added to the neonatal files, minor anomalies were added to the files at the moment of physical examination at the Centre. For the children living further away, or where the parents were no longer willing to come to the clinic, detailed histories were obtained from the paediatrician if any problem was mentioned in response to the questionnaire. At the follow-up examinations after 12 months and 2 years, the physical, neurological and psychomotor examinations were repeated by the same team of geneticists–paediatricians (data not shown).

A widely accepted definition of major malformations was used, i.e. malformations that generally cause functional impairment or require surgical correction. The remaining malformations were considered minor. A minor malformation was distinguished from normal variation by the fact that it occurs in ≤4% of the infants of the same ethnic group. Malformations or anomalies were considered synonymous with structural abnormality (Smith, 1975; Holmes, 1976) and were recorded using a checklist constructed on the basis of the textbook by Aase (1990) and containing a list of 237 items (Appendix 1). For situations where it was not clear from follow-up data up to 1 year whether a malformation should be considered major, identical guidelines for both ICSI and IVF files were followed, classifying the malformations into major or minor categories. In these guidelines an atrial septum defect (ASD) type II (foramen ovale) was considered minor. ASD type I was considered major. If no follow-up data or further details were available, an ASD was considered major. Ductus arteriosus was considered major if still patent at 3 months for a child born at term or at 6 months for a preterm child ≤36 weeks. Inguinal hernia was considered minor in a preterm child ≤36 weeks and major for a child born after 36 weeks. Pyloric stenosis was counted as major. In a second classification, malformations were classified according to the *International Classification of Diseases and Health Related Problems*, 10th Revision (ICD Manual, 1992); all malformations were coded following the ICD Manual as malformations (Q00–Q99).

Statistical analysis

Statistical analysis was performed using the SAS statistical package version 6.12.

Statistical tests were applied two-tailed at the 5% level of significance. (Since this is a safety study, aiming to investigate the safety of the ICSI treatment as compared to the IVF treatment, no multiplicity correction was applied to the significance level. Indeed, the type II risk in the analysis, which is increased when applying a correction for multiplicity to the significance level, is considered more important than the type I risk.)

Comparisons of the treatment groups for discrete variables were performed using Fisher's exact test. The Wilcoxon two-sample test was used to compare the two treatments for ordinal variables. When the comparison was controlled for singleton/multiples, the Cochran–Mantel–Haenzsel test for discrete variables was applied. Two-way analysis of variance with treatment (ICSI/IVF), singletons/multiples and their interaction was used for the analysis of continuous variables (birthweight, gestational age, maternal age).

Definitions

Biochemical pregnancy: significant increases in HCG levels (>10 IU/ml) between day 10 and 20 after LH surge (Camus *et al.*, 1989). Miscarriage: loss of a fetus with a gestational age <20 weeks. Gestational age: calculated from the day of oocyte aspiration which was defined as day 14 of the cycle. Stillbirth: intrauterine or

Table I. Live births and stillbirths after ICSI and IVF

	ICSI			IVF		
	Live births no. (%)	Stillbirths no.	Total no.	Live births no. (%)	Stillbirths no.	Total no.
Singletons	1499 (52.8)	18	1517	1556 (52.7)	6	1562
Multiples	1341	31	1372	1399	34	1433
Twins	1228 (43.2)			1250 (42.3)		
Triplets	113 (4.0)			145 (4.9)		
Quadriplets	0 (0)			4 (0.1)		
Total	2840 (100)	49	2889	2955 (100)	40	2995
% of total births	98.3	1.7[a]	100	98.7	1.3[a]	100

[a]Fisher's exact test P = not significant (0.241).

intrapartum death of a child born with a gestational age of $\geqslant 20$ weeks or with a birthweight of $\geqslant 500$ g. Preterm birth: delivery before 37 completed weeks of gestation. Perinatal mortality: number of intrauterine or intrapartum deaths and neonatal deaths <7 days after birth per 1000 children born with a gestational age of $\geqslant 20$ weeks. Low birthweight: <500 g at birth. Very low birthweight: <1500 g at birth. Early neonatal death: death of a child before day 7. Late neonatal death: death of a child from day 7 until day 28 inclusive. Infant death: death of a child later than day 28. Total malformation rate (affected livebirths + affected fetal deaths + induced abortions for malformations) divided by (livebirths + stillbirths) (Eurocat, 1993; Lechat and Dolk, 1993).

Results

Evolution of pregnancies and number of children

Of the 3073 ICSI pregnancies, 8.2% (251) were biochemical pregnancies, 1.6% (49) were ectopic pregnancies, 13.9% (428) ended in miscarriage, 0.6% (18) ended in a termination and 2254 pregnancies led to the births of 2889 children; 2.4% (73) pregnancies were lost for follow-up.

Of the 3329 IVF pregnancies 11.8% (394) ended in biochemical pregnancies, 2.2% (74) in ectopic pregnancies and 13.2% (439) in miscarriage; 0.7% (22) ended in a termination and 2314 pregnancies led to the birth of 2995 children (liveborn or stillborn); 2.6% (86) pregnancies were lost to the follow-up study. A slightly lower percentage of pregnancies led to the birth of a child in the IVF pregnancies (69.5%) compared with the ICSI pregnancies (73.3%) (Fisher's exact test $P < 0.001$).

In the ICSI pregnancies, 69.5% (1973) of the children were born in Belgium and 30.5% (867) were born in other countries. In the IVF pregnancies, 84.5% (2412) of the children were born in Belgium and 15.5% (443) were born in other countries.

Of the 2889 ICSI children, 2840 were liveborn and 49 were stillborn (1.7%); 1499 children (52.8%) were from singleton pregnancies, 1228 (43.2%) were from twin pregnancies and 113 (4.0%) were from triplet pregnancies (Table I).

Of the 2995 IVF children, 2955 were liveborn and 40 were stillborn (1.33%); 1556 (52.7%) were singletons, 1250 (42.3%) were from twin pregnancies, 145 (4.9%) were from triplet pregnancies and four (0.1%) were from a quadruplet pregnancy (Table I).

Of the 2840 ICSI children, 2477 were born after a cycle

Table II. Origin of spermatozoa used for ICSI, resulting in the birth of children

	Children no. (%)	Spermatozoa	
		Fresh	Frozen
Ejaculated	2477 (87.2)	2448	29
Non-ejaculated	311 (11.0)	259	52
Testicular	206 (7.3)	201	5
Epididymal	105 (3.7)	58	47
Donor sperm	52 (1.8)	0	52
Total	2840 (100.0)	2707	133

using fresh embryos obtained via ICSI with ejaculated sperm (including 2448 with fresh sperm and 29 with frozen sperm), 105 were born after ICSI using epididymal sperm obtained after microsurgical epididymal sperm aspiration (including 58 with fresh sperm and 47 with frozen sperm), 206 after ICSI using testicular sperm (obtained by testicular fine needle aspiration in 51 cases, by surgical testicular biopsy in 154 cases and by both techniques in one case) (including 201 with fresh sperm and five with frozen sperm) and 52 ICSI children were born after the use of donor sperm (Table II).

Pregnancy data

ICSI and IVF maternal characteristics were comparable for medication taken during pregnancy [Fisher's exact test P = not significant (0.443)], pregnancy duration [Fisher's exact test not significant ($P < 0.72$)] and maternal educational level [Wilcoxon two-sample test Z = not significant (0.108)] although only a sample of 61.2% of the ICSI mothers and of 68.6% of the IVF mothers was interviewed as to their educational level (Tables III and IV). For gravidity and parity there was a significant difference with a higher percentage of first pregnancies and first babies born to the ICSI mothers (Fisher's exact test $P < 0.001$) (Table III). Smoking habits were worse in the ICSI mothers with significantly more mothers smoking (Fisher's exact test $P < 0.017$) (Table III). Mean (SD) maternal age was 32.7 (4.26) years in ICSI versus 32.2 (4.07) years in IVF, which was slightly higher again in the ICSI group (Fisher's exact test $P < 0.001$) (Table V). Mean maternal age was also significantly lower in multiple pregnancies (32.3 years in ICSI, and 31.7 years in IVF) than in singleton

Table III. Genetic counselling uptake and factors influencing pregnancy outcome

	Genetic counselling	Medication taken	Smoking <10 cigarettes/day	Smoking ≥10 cigarettes/day	Smoking total	Gravidity G0	Parity P0
ICSI							
%	58.3	34.8[a]	3.2	2.2	5.4[b]	60.8[c]	77.3[d]
n	2840	2309	1655	1655	1655	2244	2244
IVF							
%	62.5	33.7[a]	2.3	1.4	3.7[b]	50.1[c]	72.7[d]
n	2955	2401	1846	1846	1846	2146	2147

[a]Fisher's exact test P = not significant (0.443).
[b]Fisher's exact test P = 0.017.
[c]Fisher's exact test $P < 0.001$.
[d]Fisher's exact test $P < 0.001$.

Table IV. Maternal educational level of the ICSI and IVF mothers

	Level 1	Level 2	Level 3	Level 4	Level 5	Total
ICSI						
n	207	540	719	273	0	1739
%	11.9	31.1	41.2	15.7	0	
IVF						
n	176	630	848	374	0	2028
%	8.7	31.1	41.8	18.4	0	

Level 1: university degree; level 2: higher education, not a university degree; level 3: school until 18 years; level 4: school until 15 years; level 5: education until 12 years.
Wilcoxon two-sample test P = not significant (0.108).

Table V. Maternal age and term in ICSI and IVF in singleton pregnancies versus multiple pregnancies

	Maternal age (years) Mean ± SD	No. of observations	Term (weeks) Mean ± SD	No. of observations (pregnancies)
ICSI	32.7[a] ± 4.3	2134	37.9[b] ± 2.6	1870
Singletons	32.8 ± 4.3	1485	38.8 ± 2.0	1300
Multiples	32.3 ± 4.1	649	35.7 ± 2.6	570
IVF	32.2[a] ± 4.1	2196	37.9[b] ± 2.8	2026
Singletons	32.4 ± 4.2	1529	38.6 ± 2.3	1393
Multiples	31.7 ± 3.7	667	35.8 ± 2.8	633

Statistical analysis (two-way) ANOVA.
[a]$P < 0.001$ significant difference in maternal age for singleton and multiple pregnancies with lower maternal ages in multiples ($P < 0.001$) in ICSI as well as in IVF (interaction $P = 0.496$).
[b]P = not significant (0.172) for comparison of gestational age between ICSI and IVF, but significant difference ($P < 0.001$) between singletons and multiples in ICSI as well as in IVF (interaction $P = 0.077$).

pregnancies (32.8 years in ICSI and 32.4 years in IVF) but the decrease in maternal age in multiples was very similar between ICSI and IVF pregnancies (linear model procedure $P < 0.001$). The percentage of patients attending the genetic counselling session was 58.3% of the ICSI patients and 62.5% of the IVF patients (Table III).

In the ICSI pregnancies, 18 terminations were performed: nine after prenatal karyotyping, one after prenatal DNA diagnosis and eight after detection of ultrasound anomalies. In the IVF pregnancies, 24 terminations were performed: 10 after prenatal karyotyping, 11 after detection of ultrasound

anomalies, two for psychosocial reasons and one for an unknown reason (Appendix 2).

Stillbirth occurred in 49 ICSI fetuses (1.7%) and in 40 IVF fetuses (1.3%); this is not statistically different [Fisher's exact test P = not significant (0.241)] (Table I). The stillbirth rate was 1.7%, varying from 1.2% in the singletons to 2.3% in the multiples in the ICSI children, and was 1.3%, varying from 0.4% in the singletons to 2.3% in the multiples, in the IVF children. Abnormal data on physical examination at birth or autopsy were found in eight ICSI children and in two IVF children (Table VI) (Appendix 2). Major malformations in

Table VI. Stillborn children (≥20 week pregnancy) from a total of 2889 ICSI children and 2955 IVF children

	Singletons	Multiples	Total	%
ICSI				
No. of stillbirths	18	31	49	1.7[a]
Normal physical examinations or autopsies	15	25	40	
Abnormal physical examinations or autopsies	2	6	8	
No physical examination or autopsy	1	0	1	
IVF				
Number stillbirths	6	34	40	1.3[a]
Normal physical examinations or autopsies	5	29	34	
Abnormal physical examinations or autopsies	0	2	2	
No physical examination or autopsy	1	3	4	

[a]Fisher's exact test P = not significant (0.241).

these terminations and stillbirths were taken into account in the calculation of the total malformation rate.

Prenatal diagnosis

Abnormal fetal karyotypes were found in 42 cases in 1437 prenatally tested ICSI fetuses (2.9%); 23 anomalies were *de novo* [of these, nine were sex chromosomal anomalies and 14 were autosomal anomalies, either numerical (six) or structural anomalies (eight)] and 19 were inherited abnormalities (in 15/19 cases the chromosomal structural defect was inherited from the father). Nine of the 42 pregnancies with a chromosomal anomaly were terminated (two sex chromosomal anomalies, seven numerical anomalies), three stillbirths occurred and 30 children were born without clinical abnormality at birth.

Abnormal fetal karyotypes were found in 15 of the 493 prenatally tested IVF fetuses (3.0%): 10 were numerical anomalies (trisomy 13, 18 and 21), and five were structural anomalies. All of the pregnancies with a numerical anomaly were terminated. The children with structural anomalies were born without clinical abnormality at birth.

Neonatal data

For 2799 of the 2840 ICSI children (98.5%) we had complete information at birth (even if some data, such as the head circumference, were missing in 24% of ICSI children and 21% of IVF children). For 2920 of the 2955 IVF children (98.8%) we had complete information at birth.

The sources of information on the ICSI children were the parents for 357 children (12.6%), the paediatricians and gynaecologists for 771 children (27.1%), the paediatricians and gynaecologists or pathologists for 15 deceased children (0.5%) and the geneticists for 1656 children (58.3%). The sources of information on the IVF children were the parents for 220 children (7.4%), the paediatricians and gynaecologists for 657 children (22.2%), the paediatricians and gynaecologists or pathologists for 51 deceased children (1.7%) and the geneticists for 1992 children (67.4%). For 41 ICSI children and for 35 IVF children, data remained very incomplete, even after several attempts to obtain the information.

At 2 months, we collected information on 2303 of the 2840 ICSI liveborn children (81.1%) and of 2283 (77.3%) of the 2955 IVF liveborn children. Neonatal measurements for ICSI and IVF liveborn children of ≥20 weeks of gestation are listed in Table VII. For the total ICSI group, mean birthweight was 2807 g, mean length was 47.9 cm and mean head circumference was 33.4 cm; for the total IVF group, mean birthweight was 2765 g, mean length was 47.7 cm and mean head circumference was 33.4 cm. Birthweight of all children in the ICSI and IVF groups was not found to be statistically different [ANOVA P = not significant (0.060)]. A significant difference between singletons and multiples was observed (ANOVA $P < 0.001$), but this difference was comparable in ICSI versus IVF children: no significant difference in birthweight was observed between singleton ICSI and IVF children or multiple ICSI and IVF children [ANOVA not significant (P = 0.277)].

Low birthweight (<2500 g) was present in 26.7% of the ICSI children and in 26.5% of the IVF children, which was comparable [Fisher's exact test P = not significant (0.858)] with a higher rate of low birthweight in multiples in ICSI as well as in IVF (Table VIII). Very low birthweight rate (<1500 g) was higher in the IVF children (5.6%) than in the ICSI children (4.4%) (Fisher's exact test P = 0.031), due to a higher percentage in singletons as well as in multiples (nonzero correlation P = 0.030) (Table VIII).

Mean gestational age was the same in ICSI and IVF pregnancies [Fisher's exact test P = not significant (0.72)] (Table V).

Prematurity defined as birth before 37 weeks of pregnancy was significantly higher in ICSI children than in IVF children (Fisher's exact test P = 0.046) due to a significantly higher prematurity rate in ICSI multiples than in IVF multiples (χ^2-test P = 0.032) (Table VIII).

Hospitalization in a neonatal unit for simple observation or for more severe neonatal problems or surgical interventions occurred more in the IVF children (43.6%) than in the ICSI children (40.5%) (Fisher's exact test P = 0.017) (Table IX). This is apparently related to a higher (not significant) frequency of hospitalization of IVF children, of both singletons and multiples [Breslow–Day test P = not significant (0.233)]. However, the percentage of hospitalization in a neonatal unit for neonatal treatments or surgical interventions (list of complications in Appendix 3) of 19.1% of the ICSI children was not significantly different from the percentage of 20.5%

Table VII. Birthweight (W), length (L) and head circumference (HC) in ICSI and IVF children

	ICSI children			IVF children		
	W (g)	L (cm)	HC (cm)	W (g)	L (cm)	HC (cm)
Total group	$n = 2799$	$n = 2452$	$n = 2164$	$n = 2920$	$n = 2697$	$n = 2336$
Mean	2806.7[a]	47.9	33.4	2765.3[a]	47.7	33.4
Median	2850.0	48.0	34.0	2807.5	48.0	33.8
SD	719.1	3.76	2.31	725.03	3.722	2.309
Range	500–4970	30–59	20.5–44.0	375–4950	26–59	21.0–43
Singletons	$n = 1476$	$n = 1410$	$n = 1228$	$n = 1523$	$n = 1434$	$n = 1229$
Mean	3224.3[b]	49.6	34.3	3176.4[b]	49.3	34.2
Median	3150.0	49.0	34.0	3220.0	50.0	34.3
SD	581.9	3.06	1.84	582.61	2.966	1.936
Range	610–4970	31–59	21.5–43	460–4950	27–59	21.5–43
Twins	$n = 1211$	$n = 1072$	$n = 873$	$n = 1251$	$n = 1011$	$n = 1015$
Mean	2394.5[b]	46.2	32.5	2382.5[b]	46.0	32.6
Median	2440.0	46.5	33.0	2450.0	46.5	33.0
SD	522.0	3.26	2.14	560.9	3.47	2.26
Range	500–3870	30–53.30	20.5–44	375–4480	26–55	21.0–40.5
Triplets	$n = 112$	$n = 78$	$n = 86$	$n = 142$	$n = 102$	$n = 92$
Mean	1761.8[b]	41.7	29.7	1768.5[b]	42.9	30.5
Median	1780	42.0	31.0	1745.0	43.0	30.6
SD	548.8	4.38	3.01	497.72	3.87	2.31
Range	610–3100	31–49.5	22.0–34.0	630–3670	29–53	24–35
Quadruples				$n = 4$		
Mean				1372.5[b]		
Median				137.0		
SD				105.0		
Range				1250–1500		

[a]ANOVA P = not significant (0.060). No difference in birthweight for ICSI and IVF children.
[b]ANOVA $P < 0.001$. Difference in birthweight between singletons and multiples.
[b]ANOVA P = not significant (0.277). Difference between singletons and multiples is the same in ICSI and IVF.

Table VIII. Low birthweight, very low birthweight and prematurity in ICSI and IVF children

	ICSI		IVF	
	n	% children	n	% children
Birthweight <2500 g				
Total	760	26.7[a]	784	26.5[a]
Singletons	106	7.1	121	7.8
Multiples	654	48.6	663	47.6
Twins	593	48.1	568	45.1
Triplets	61	54.0	94	65.0
Quadruplets	0		1	25.0
Birthweight <1500 g				
Total	125	4.4[b]	167	5.6[b]
Singletons	22	1.5	28	1.8
Multiples	103	7.6	139	9.9
Twins	64	5.2	96	7.6
Triplets	39	34.5	40	27.6
Quadruplets	0		3	75.0
Prematurity <37 weeks				
Total	902	31.8[c]	867	29.3[c]
Singletons	126	8.4	140	9.0
Multiples	776	57.6	727	51.6
Twins	669	54.6	600	47.6
Triplets	107	94.7	123	84.8

[a]Fisher's exact test P = not significant (0.858).
[b]Fisher's exact test P = not significant (0.031).
[c]Fisher's exact test P = 0.046.

of the IVF children [Fisher's exact test not significant (P = 0.187)].

Neonatal and perinatal deaths were compared in the ICSI versus IVF group (Table X). Early neonatal death was 0.19% in ICSI versus 1.02% in IVF. Late neonatal death was 0.21% in ICSI versus 0.54% in IVF. Total infant death was 0.53%

(infant death was observed until the age of 2 months) in ICSI and 1.73% in IVF, which is significantly higher in IVF (Fisher's exact test $P < 0.001$). Total perinatal death rate was 1.87% in ICSI which was comparable with 2.33% in IVF [Fisher's exact test P = not significant (0.238)].

Sex ratio of male/female is 1.01 in the total ICSI group, 0.97 in the non-ejaculated, 0.83 in the testicular and 1.29 in the epididymal group. There is no significant difference in the ejaculated versus the testicular [Fisher's exact test P = not significant (0.181)] or epididymal group [Fisher's exact test P = not significant (0.264)]. In the IVF group, sex ratio was 1.11 (Table XI).

Malformations

Major malformations in fetuses after pregnancy termination, stillbirths and live births

Prenatally, major malformations were found in ICSI in 17 terminations and in eight out of 49 stillbirths (Table VI). No other malformations were detected prenatally, apart from one child with a holoprosencephaly detected at the age of 15 weeks of pregnancy, where the multiplicity and the risk involved in a selective abortion led to the option of continuing the pregnancy. This child died at birth. Major malformations were found in IVF in 21 interruptions and two out of 40 stillbirths after 20 weeks. No other malformations were detected prenatally.

The major malformation rate (Table XII) in live births is 3.38% (96/2840) in ICSI children, which is comparable with 3.79% (112/2955) in IVF pregnancies [Cochran–Mantel–Haenzsel test P = not significant (0.402)]. Individual malformations are listed in Appendix 4. No statistical difference was observed in major malformation rates between ICSI and IVF children. In order to detect an increase of 1.5% (on top of the 3% malformation rate estimated in the general population), with a power of 80%, alpha 5%, we needed 2511 observations. With the actual number of children evaluated, we reach 85% power to detect a difference of 1.5% and a 90% power to detect a difference of 2%.

Major malformations were found in ICSI in 3.06% (46/1499) of the singleton children and in 3.65% (50/1341) of the children from multiple pregnancies (Table XII). Major malformations were found in IVF in 3.21% (49/1556) of the singleton children and in 4.50% (63/1399) of the children from multiple pregnancies. The malformation rates in multiples were significantly higher than in singletons in ICSI as well as in IVF (Cochran–Mantel–Haenzsel test P = 0.046).

Logistic regression analysis showed that the major malformation rate (per pregnancy) was related to birthweight ($P < 0.001$), and to gestational age ($P < 0.001$), but not to maternal age (P = 0.538) or to procedure (ICSI or IVF).

The total malformation rate for ICSI was 4.22% [(96 + 8 + 18)/(2840 + 49)] and for IVF 4.66% [(112 + 2 + 21)/(2855 + 40)]. These values are not significantly different (Fisher's exact test P = 0.482).

Major malformations in ICSI in relation to sperm origin and sperm parameters

Major malformations in ICSI were also considered in relation to sperm origin and sperm parameters (Table XIII). No statistical

Table IX. Neonatal complications in ICSI and IVF children

	ICSI		IVF	
	n	*%*	*n*	*%*
Admission to neonatal unit (1)				
Total	1149	40.5[a]	1288	43.6[a]
Singletons	261	17.5	289	18.7
Multiples	888	66.0	999	70.9
Twins	782	63.4	853	67.7
Triplets	106	93.8	146	99.8
Neonatal problems (2)				
Total	542	19.1[b]	605	20.5[b]
Singletons	126	8.4	111	7.2
Multiples	416	30.9	494	35.1
Twins	344	27.9	387	30.7
Triplets	72	63.7	107	71.8

(1) Observation and/or treatment in a neonatal unit.
(2) Complications or surgical intervention and hospitalization in a neonatal unit; this group is a part of (1).
[a]Fisher's exact test (two-tailed) P = 0.017.
[b]Fisher's exact test (two-tailed) P = not significant (0.187).

Table X. Neonatal death and perinatal death rate in ICSI and IVF

	ICSI			Total	%	IVF			Total	%
	Singletons	Multiples				Singletons	Multiples			
		Twins	Triplets				Twins	Triplets + Quadruplets		
Live births	1494	1233	113	2840	98.3	1547	1259	145 + 4	2955	98.7
Stillbirths	19	26	4	49	1.70[a]	6	30	4	40	1.33[a]
Early neonatal up to day 6	0	4	1	5	0.18[b]	6	20	4	30	1.02[b]
Late neonatal day up to 28	1	5	0	6	0.21	5	6	5	16	0.54
Infant death from day 29	1	2	1	4	0.14	1	4	0	5	0.17
Total infant death	2	11	2	15	0.53[c]	12	30	9	51	1.73[c]
Total perinatal death	19	30	5	54	1.87[d]	12	50	8	70	2.33[d]
Total live + stillbirths	1513	1259	117	2889	100	1553	1289	153	2995	100

[a]No difference in stillbirth rate between ICSI and IVF. Fisher's exact test P = not significant (0.241).
[b]Higher early neonatal death rate in IVF compared with ICSI. Fisher's exact test P = 0 < 0.001.
[c]Higher total infant death rate in IVF compared with ICSI. Fisher's exact test P = 0 < 0.001.
[d]Higher total perinatal death rate in IVF compared with ICSI. Fisher's exact test P = not significant (0.238).

difference was observed in the frequencies of major malformations in children born after replacement of embryos fertilized with ejaculated sperm (3.39%) or non-ejaculated sperm (3.21%) [Fisher's exact test P = not significant (1.000)] and no statistical difference was observed in major malformations after use of testicular sperm (2.91%) [Fisher's exact test P = not significant (0.838)]. or epididymal sperm (3.80%) [Fisher's exact test P = not significant (1.000)].

No statistical difference was observed in the frequency of major malformations of 3.8% (or 49/1301) in children when a sperm concentration of <5 000 000 sperm/ml had been used as compared with 2.8% (or 47/1635) when a sperm concentration of ≥5 000 000 sperm/ml had been used [Fisher's exact test P = not significant (0.175)] or when sperm concentrations ≥20 or <20×10⁶/ml were evaluated [Fisher's exact test P = not significant (0.121)]. No statistical difference was observed on the basis of other sperm parameters such as sperm morphology (≥14 or <14% normal morphology) [Fisher's exact test P = not significant (1.0)] or volume (≥2 or <2 ml) [Fisher's exact test P = not significant (1.0)]. For ≥50% motile sperm cells (50% of the total number of sperm cells are motile) versus <50% (missing data to be considered <50% motility), a significant difference in malformation rates was observed between 1.86%

for motility in ≥50% sperm and 3.77% in <50% motile sperm (Fisher's exact test P = 0.028).

Malformations coded with ICD-10
ICD 10 codes were used to define a number of malformations, both major and minor, to which a code is attributed. We recorded 6.7% (189/2840) in the ICSI children and 9.0% (265/2955) in the IVF children (Table XIV). This percentage is significantly different between the two groups (Fisher's exact test P = 0.001). The difference is due to a higher malformation rate in the IVF twins than in the ICSI twins. When we excluded the children who had routine examinations at birth (two or more ultrasounds in 8.0% of ICSI children and 11.7% of IVF children), such as routine ultrasound scans for brain, heart and kidney, the statistical difference disappeared, giving 4.4% in the ICSI group versus 5.3% in the IVF group (Table XV).

Malformations in the different organ systems
All major malformations were classified as malformations of different organ systems, and frequencies were similar between ICSI and IVF for most organ systems (Table XVI). An apparently higher non-significant percentage of gastrointestinal problems was observed in the IVF group (0.33% in the IVF group versus 0.10% in the ICSI group) [Fisher's exact test P = not significant (0.092)].

Table XI. Sex ratio in ICSI and IVF live births

Sperm origin	ICSI					IVF				
	Male	Female	Unknown	Male/female	Total	Male	Female	Unknown	Male/female	Total
Ejaculated	1192	1171	10	1.02[a,b]	2373	1149	1040	5	1.11	2959
Non-ejaculated	146	151	0	0.97	29.7					
Testicular	89	107	0	0.83[a]	196					
Epididymal	57	44	0	1.30[b]	101					
Total	1338	1322	10	1.01	2670					

[a]Fisher's exact test P = not significant (0.181).
[b]Fisher's exact test P = not significant (0.264).

Table XII. Major and minor malformations

	No. of children	Major malformation n (%)	Minor malformation n (%)	Minor malformation only	Major with minor
ICSI					
Singletons	1499	46 (3.06[b])	97 (6.47)	84	13
Multiples	1341	50 (3.65[b])	83 (6.18)	76	7
Twins	1288	45 (3.49)	75 (5.82)	68	7
Triplets	113	5 (4.42)	8 (7.07)	8	–
Total	2840	96 (3.38[a])	180 (6.34[c])	160	20
IVF					
Singletons	1556	50 (3.21[b])	122 (7.84)	119	3
Multiples	1399	63 (4.50[b])	173 (12.36)	159	14
Twins	1250	55 (4.40)	138 (11.04)	129	9
Triplets	145	8 (5.51)	35 (24.13)	30	5
Total	2955	112 (3.79[a])	295 (9.98[c])	278	17

[a]Cochran Mantel–Haenzsel test P = not significant (0.402); not more major malformations in ICSI or IVF.
[b]Cochran Mantel–Haenzsel test P = 0.046; more major malformations in multiples versus singletons in ICSI and IVF.
[c]Fisher's exact test P < 0.001; more minor malformations in IVF than in ICSI.

Table XIII. Major malformations in ICSI children in relation to sperm origin

	Total no. of children	Major malformation	% Major malformation
Ejaculated	2477	84	3.39[a,b,c]
Non-ejaculated	311	10	3.21[a]
Testicular	206	6	2.91[c,d]
Fine needle aspiration (FNA)	51	2	
Surgical biopsy	154	4	
FNA and biopsy	1	0	
Epididymal	105	4	3.80[b,d]
Donor sperm	52	0	0
Total	2840	96	3.38

[a]Ejaculated compared with non-ejaculated Fisher's exact test (two-tailed) P = not significant (1.000).
[b]Ejaculated compared with epididymal Fisher exact test P = not significant (1.000).
[c]Ejaculated compared with testicular Fisher exact test P = not significant (0.838).
[d]Testicular compared with epididymal Fisher exact test P = not significant (0.738).

Table XIV. ICD 10 codes in ICSI and IVF children, singletons and multiples

	No anomaly	ICD 10 codes	Total
ICSI			
Singletons			
n	1401	98	1499
%		6.5[a]	
Multiples			
n	1250	91	1341
%		6.8[a]	
Total			
n	2651	189	2840
%		6.7[b]	
IVF			
Singletons			
n	1448	108	1556
%		6.9[a]	
Multiples			
n	1242	157	1399
%		11.2[a]	
Total			
n	2690	265	2955
%		9.0[b]	

[a]Cochran Mantel–Haenzsel P = 0.014; difference in frequency due to a high frequency of ICD 10 codes in the IVF multiples.
[b]Cochran Mantel–Haenzsel P = 0.001; difference in ICSI and IVF frequencies of ICD 10 codes statistically significant.

In Table XVII, a detailed comparison between urological and genital malformations in ICSI and IVF is presented, taking into account major and minor anomalies in each organ system, where no significant differences were found for urological, for genital or for the combination of urogenital anomalies

Minor malformations
Minor malformations were observed in 180 ICSI children, 20 of whom already had one or more major malformations and in 295 IVF children, 17 of whom already had one or more major malformations. The higher malformation rate in IVF is mainly

Table XV. ICD 10 codes in ICSI and IVF children, excluding children with screening investigations

	No anomaly	ICD 10 codes	Total
ICSI			
n	2498	114[a]	2612
%		4.4	
IVF			
n	2496	139[a]	2608
%		5.3	

[a]Fisher's exact test P = not significant (0.107).
8.0% of ICSI children and 11.7% of IVF children had systematic screening programme at birth, when these children were excluded, no difference in ICD 10 codes was observed.

due to a higher rate of malformations in the multiples, where 74 (5.2%) minor heart problems, for example, were detected in IVF multiples compared with 22 (1.6%) heart problems in the ICSI multiples. These heart problems were ductus arteriosus, type II atrial septum defects or foramen ovale, and very small muscular ventricular septum defects, all spontaneously closed according to the follow-up data.

Follow-up at 2 months
During the follow-up consultations of 2 months, 444 more minor malformations for a total of 322 ICSI children were detected and 193 more minor malformations for a total of 143 IVF children were detected. For ICSI children, the follow-up rate at 2 months was 81.2 % of the total number of children born (71.2% were examined by a geneticist, for 12.4% written information from the paediatrician and for 15.5% written information from the parents was obtained); 18.8% of the children were lost to follow-up even after several attempts. For IVF children, follow-up rate at 2 months was 77.4% (87.2% examined by a geneticist, for 3.5% written information from the paediatrician and for 9.6% written information from the parents was obtained); 22.6% of the children were lost to follow-up even after several attempts.

Discussion

Pregnancy data

Although more parameters concerning pregnancy as well as concerning the children born were recorded, only the data focusing on the children's health and malformations in the neo-natal period up to 2 months were analysed for this study.

Many confounding variables due to pregnancy complications and obstetric events are not studied here and could have an effect on the neonatal outcome of the children, but studying this relation falls beyond the scope of this study. The patient group attending our hospital for ICSI and IVF treatment was a homogeneous population, although from different countries and nationalities in which a number of characteristics, such as maternal educational level and medication taken during pregnancy, were similar. In both groups the same rates of multiple pregnancies were observed, so that we could evaluate different parameters in the whole cohort as well as in the singletons or multiple pregnancy cohorts. Some variables were slightly different such as the mean

Table XVI. Major malformation rate per organ system in ICSI versus IVF: in live births

Systems	ICSI			IVF		
	Major malformation per system $n = 2840$	% of total	% of malformation	Major malformation per system $n = 2955$	% of total	% of malformations
Cardiac	30	1.06	31.9	44	1.49	40.0
Cleft lip/palate	6	0.21	6.4	6	0.20	5.4
Ear, eye	1	0.03	1.1	1	0.03	0.9
Gastrointestinal	3	0.10	3.0	10	0.33	9.1
Genital	11	0.38	11.7	21	0.71	19.1
Metabolic	2	0.07	2.1	2	0.06	1.8
Musculoskeletal	23	0.80	24.5	12	0.40	11.0
Nervous	12	0.42	12.8	6	0.20	5.4
Respiratory	0	0	0	1	0.03	0.9
Urinary	7	0.24	7.4	7	0.24	6.4
Total	95		100	110		100

[a]Fisher's exact test comparing ICSI and IVF for each system were all not significant.

Table XVII. Major and minor urogenital malformations

	ICSI	IVF
Urological malformation		
Renal (major)	1	2
Renal (minor)	0	0
Pyelo-ureteral (major)	4	3
Pyelo-ureteral (minor)	5	12
Urethra (major)	2	1
Urethra (minor)	0	0
Total	12	18
%	0.42[a]	0.61[a]
Genital malformation		
tMale malformation	$n = 1338$	$n = 1540$
Hypospadias (major)	4	12
Hypospadias (minor)	4	2
Hypospadias (major) glandular + cryptorchidism	0	1
Cryptorchidism (major)	5	10
Spermatic duct cyst (minor)	1	0
Female malformation	$n = 1332$	$n = 1390$
Labia minora adhesions (minor)	4	0
Ovarian cyst (major)	1	0
Ovarian cyst (minor)	0	1
Total males	14	22
% in males	1.05[b]	1.43[b]
% of total	0.49	0.74
Total females	5	1
% in females	0.37[c]	0.07[c]
% of total	0.17	0.03
Total males + females	19[d]	23
%	0.67[d]	0.78[d]
Total		
Urological + genital	31	41
%	1.09[e]	1.38[e]

[a]Fisher's exact test P = not significant (0.363).
[b]Fisher's exact test P = not significant (0.403).
[c]Fisher's exact test P = not significant (0.117).
[d]Fisher's exact test P = not significant (0.646).
[e]Fisher's exact test P = not significant (0.363).

maternal age, which was slightly higher in the ICSI group, and gravidity and parity, where a higher percentage of primigravidity and primiparity was observed in the ICSI group. These variables, however, do not favour the outcome for the ICSI children above that for the IVF children and will be more likely to have a negative influence. One may observe that the IVF cohort was born partly prior to the ICSI cohort and that only since June 1991 were the two cohorts followed simultaneously, with similar stimulation protocols. The effect of this difference in treatment period is impossible to evaluate. Taking the above remarks into account, we can still consider that our ICSI and IVF cohorts provide a good opportunity to study possible additional risk factors directly linked to the ICSI procedure or indirectly to the genetic characteristics of the sperm used in the ICSI procedure.

Pregnancy outcome in terms of risk of total early pregnancy loss (biochemical, extra uterine pregnancy and miscarriage) was slightly better in ICSI than in IVF. From these data we can conclude that there is no bias in the ICSI pregnancies due to elimination in early pregnancy of a number of anomalies possibly linked to the ICSI technique. No more pregnancy terminations for malformations or karyotype anomalies were performed in the ICSI group compared with the IVF group. No difference was found in the follow-up rates, which were high for ICSI and for IVF [both in the pregnancy cohort (97.6% in ICSI versus 97.5% in IVF) and for the neonatal data (98.5% in ICSI versus 98.8% in IVF)], so that the data are not biased by differing quantities of missing data (Aylward *et al.*, 1985).

Mean maternal age was slightly higher in the ICSI group (32.7 years) than the IVF group (32.2 years), probably due to the fact that the newly introduced technique made it possible to offer treatment for couples who had had no chance of being treated before.

The stillbirth rate of 1.69% observed in the ICSI group versus 1.33% in the IVF group, is statistically the same in ICSI as in IVF and comparable with literature data from Denmark (Loft *et al.*, 1999) and Sweden (Wennerholm *et al.*, 2000a). However, definitions of intrauterine death used in these two publications were different; in the Danish study on 730 children born after ICSI, with a definition of stillbirth of $\geq$24 weeks of gestation, a 1.23% stillbirth rate was observed, while in the Swedish study on 1293 ICSI pregnancies a 0.50% of stillbirth rate was observed after $\geq$28 weeks of gestation.

Prenatal diagnosis

Only 1437 of the 2889 ICSI fetuses were prenatally tested; abnormal fetal karyotypes were found in 2.9% (42/1437) of the prenatally tested ICSI fetuses: 1.56% were *de novo* and 1.18% were inherited. Abnormal fetal karyotypes were found in 3.0% (15/493) of the smaller number of prenatally tested IVF fetuses and were mostly related to a higher maternal age.

As described earlier (Bonduelle *et al.*, 1999) ~5% of the ICSI children were at increased risk for chromosomal anomalies due to the chromosomal aberrations in their parents, most often the fathers with either sex chromosomal aberrations or structural anomalies. This percentage is much higher than the expected value of 0.5% in the general population (Nielsen and Wohlert, 1991; Jacobs *et al.*, 1992) and is associated with the severe male factor infertility often present in the patient population for ICSI (Moosani *et al.*, 1995; Meshede *et al.*, 1998; Peschka *et al.*, 1999). For these parents a prenatal test was recommended, and only one non-balanced karyotype was found, for which an abortion was performed whereas all children with a balanced karyotype were born and found to be phenotypically normal. The same recommendation of a prenatal test was made to all couples before starting IVF for whom a structural aberration or mosaicism of the parents to be was found on routine karyotype. No non-balanced karyotypes were found in the prenatally tested IVF pregnancies.

De-novo aberrations were found in 23 tested ICSI children (1.56%), which is statistically higher than in the general population (0.5%) (Jacobs *et al.*, 1992) and which led to the abortion of eight fetuses (six for autosomal and two for sex chromosomal, numerical aberrations) after extensive counselling of the couples (Bonduelle *et al.*, 1998). This higher percentage of chromosomal anomalies in ICSI children could be related to the higher aneuploidy rate in the sperm of their fathers (Pang *et al.*, 1999; Shi and Martin, 2001). More details about chromosomal anomalies found in ICSI will be discussed in a following publication.

Among the IVF children, only 16.5% had a prenatal diagnosis, which resulted in the abortion of 10 fetuses with trisomies. The difference in uptake rate of prenatal diagnosis did not result in a significantly lower percentage of phenotypically recognizable karyotype anomalies at birth in the ICSI group. There were three clinically recognizable chromosomal anomalies in the ICSI group detected at birth (of whom the mothers had had no prenatal test during pregnancy) and three chromosomal anomalies detected at birth in the IVF group. As only those karyotype anomalies which were clinically visible at birth were considered major malformations, this low figure in both groups did not influence the malformation rate among live births (1/96 in the ICSI children versus 3/112 in the IVF children). The ICSI children with sex chromosomal anomalies and structural chromosomal anomalies detected prenatally were not phenotypically abnormal at birth and were therefore not counted as major malformations.

In the light of the data on prenatal karyotypes already published in previous articles (Bonduelle *et al.*, 1999) and considering our further observations, we think it is necessary to continue to offer prenatal diagnosis to all ICSI couples and to IVF couples with known chromosomal anomalies.

Neonatal data

Neonatal measurements were the same in ICSI as in IVF children for singleton pregnancies as well as for multiple pregnancies. As described in the literature by several authors, there is a lower birthweight in IVF singletons than in a control population [a higher rate of low birthweight (Doyle *et al.*, 1992; Tan *et al.*, 1992; Olivennes *et al.*, 1993; Verlaenen *et al.*, 1995); small for gestational age (Doyle *et al.*, 1992; Olivennes *et al.*, 1993; Buitendijk, 2000); and lower mean birth weight after adjustment for different variables, such as maternal age and height, education, parity, smoking habits and infant gestational age at birth (Buitendijk, 2000)]. In our cohort of ICSI children, where most of the influencing factors of maternal origin were similar to, or slightly more unfavourable than, those in the IVF group, we found a similar birthweight in ICSI and IVF singletons. We would therefore expect that birthweight in ICSI children would be somewhat lower than in a matched group from the general population, even in the singletons. This assumption, however, has yet to be substantiated in a controlled study. On the other hand, there is no evidence that the ICSI technique adds to the risk of lower birthweight associated with IVF offspring.

Analysis of the data also indicates that low birthweight and very low birthweight are mainly due to multiple pregnancies. For singletons, the rates of low birthweight (8.2%) and of very low birthweight (1.8%) are comparable with the percentages described for ICSI children in the literature (low birthweight in singletons between 6.7 and 7.6% and very low birthweight between 1.4 and 1.7%) (Loft *et al.*, 1999; Wennerholm *et al.*, 2000a) and are even lower than the percentages described in the IVF population in some data in the literature [11% (Doyle *et al.*, 1991); 14% (Tan *et al.*, 1992); 10% (Verlaenen *et al.*, 1995; Buitendijk, 2000)], but not in all (Dhont *et al.*, 1997; Reubinoff *et al.*, 1997). Term was the same in ICSI as in IVF but the prematurity rate was higher in ICSI due to a higher prematurity rate in ICSI multiple pregnancies. This higher prematurity rate in ICSI multiples might be related to maternal factors such as greater maternal age and higher percentage of primigravidae, but we do not really know. On the other hand, it does not affect the rate of low birthweight or very low birthweight (which is higher in IVF), nor the percentages of neonatal complications and neonatal interventions (with risk of a worse outcome later in life) which is similar in ICSI and IVF. The fact that more ICSI multiple pregnancies lead to premature birth does not contribute to a higher percentage of neonatal observations in ICSI; the higher rate of neonatal observations in IVF is probably related to the higher low birthweight and very low birthweight rate. For neonatal observations, indeed, different subjective parameters, such as 'precious and high-risk children after a fertility treatment' might have played a part in the decision to make observations and these could vary with respect to time.

Perinatal death rate was compared in ICSI (1.87%) and IVF (2.33%) and was not statistically different, but like the stillbirth rate it was comparable with data in the literature (Loft *et al.*, 1999; Wennerholm *et al.*, 2000a), taking the different definitions used into account. In Loft's article (1999) on 730 children born after ICSI, a perinatal death rate of 1.37% was observed, while in Wennerholm's article (2000a) on 1293 ICSI pregnancies, a

1.17% perinatal death rate was observed. Total infant death was higher in IVF than in ICSI due to higher numbers of children from triplet pregnancies, who died in the late neonatal period.

The sex ratio of male/female is 1.01 in the total ICSI group, 0.97 in the non-ejaculated, 0.83 in the testicular sperm group and 1.29 in the epididymal sperm group, whereas in the general population the sex ratio is 1.06. These small differences between the groups are not significant and do not suggest different percentages of Y-bearing sperm as a result of the origin of the sperm. In the IVF group, the ratio of 1.11 is slightly higher than that of the general population (1.06) whereas in the ICSI group the ratio of 1.01 is slightly lower. These differences in sex ratios between ICSI and IVF were observed in another study (Ericson and Källen, 2001), where sex ratios of 0.96 in ICSI and of 1.14 in IVF were recorded. These small differences observed in ICSI will hardly influence the malformation rate.

Major malformations

We found the same malformation rate in ICSI as in IVF, applying the same methodology and definitions of major malformation for both study groups. For 85.9% of the ICSI children and for 91.3% of the IVF children we had a very reliable source concerning major malformations (medical reports or examination in our centre). There is, however, a difference in the percentage of children examined by the geneticists in the ICSI (58.3%) versus the IVF group (67.4%). This could have led to a higher detection rate of malformations in the IVF group as a whole and have masked an increased malformation rate in the ICSI group. However, malformation rates in the children examined by the geneticists were 3.49% for the ICSI children ($n = 1973$) versus 4.3% in the IVF group ($n = 2412$) which indicates that when examined applying exactly the same methodology there are no more malformations in the ICSI group. There is, however, underreporting in the group not examined by the geneticists of our Centre since we observed a lower reported major malformation rate in the children born abroad: 3.11% in the ICSI group versus 1.8% in the IVF group; but this observation did not advantage the ICSI group.

Higher maternal age might have had a negative influence on number of malformations in ICSI children, but not on the control IVF group. Other maternal variables, such as maternal drug intake and maternal educational level and pregnancy duration, were similar in both groups. We can therefore conclude from our finding a similar percentage of major malformations in ICSI and IVF that there are no more malformations in this ICSI cohort than in the IVF cohort. The finding that malformation rates were higher in multiples than in singletons in ICSI as well as in IVF is expected, as in most registries more malformations have been found in multiples. (Källen, 1986; Doyle et al., 1991; Westergaard et al., 1999; Wennerholm, 2000b; Ericson and Källen, 2001)

Malformation rates in this data set are maximum estimates, since the children were scrutinized for major (and minor) malformations. The main purpose was to have an identical methodology in order to be able to compare malformation rates in ICSI versus IVF. Therefore the figures obtained in ICSI and IVF are higher than the figures in registries such as the Australian register (Lancaster et al., 1995) or the Swedish register (Berg et al., 1999). Moreover definitions in those registers may differ and certain conditions (such as pyloric stenosis, inguinal hernia) are sometimes omitted.

No difference in malformation rate was observed in relation to different sperm parameters even if in literature higher gonosomal aneuploidy is found in sperm from men with severe male factor infertility (Bernardini et al., 1997). No differences in major malformation rates were observed in the different subgroups: 3.39% in cases with ejaculated sperm, 3.80% in cases of ICSI with epididymal sperm and 2.91% with the testicular sperm. No significant difference was observed here, but as the totals in the subgroups are low, it is too early to draw final conclusions. In order to detect an increase of 2% in malformation rate (from 3 to 5%), with a power of 80%, alpha = 5%, after use of either ejaculated or non-ejaculated sperm, we would need 3000 children and we have had only 311 children in the non-ejaculated group until now. There seems, however, to be no indication of particular reasons for concern when using sperm from different sources.

Rates of ICD 10 codes (ICD Manual, 1992) were higher in the IVF children, mostly as a result of a higher number of minor problems, such as minor heart problems seen at ultrasound, for which an ICD 10 code is attributed. When the children for whom at least two routine ultrasounds had been performed were omitted, then the difference between ICSI and IVF was no longer significant. This means that the higher number of malformations is related mainly to the higher proportion of minor problems detected at the neonatal unit where more IVF children, mostly twins (71% in IVF compared to 66% in ICSI) were hospitalized and where more routine investigations as well as specific screenings for assisted reproductive technology children born intra muros were performed. On the other hand, the higher proportion of very low birthweight in IVF children is also a factor that leads to a higher rate of minor anomalies such as patent ductus arteriosus, atrial septum defect type II, inguinal hernia and other minor problems.

The higher major and minor malformation rate in IVF children, expressed in numbers of ICD 10 codes, is due to a higher rate of malformations in the multiple pregnancies, where 74 (5.2%) minor heart problems were detected in IVF children, as compared to 22 (1.6%) heart problems in the ICSI children.

When all major malformations were classified as malformations of different organ systems, no significant difference of frequency of malformations was observed between the ICSI and the IVF groups. However, a tendency of (not significantly) higher frequency of digestive problems of 0.33% was observed in the IVF group, compared with the ICSI group. A higher frequency of oesophageal atresia and maternal infertility has been described (Robert et al., 1993). The observation of 3-fold higher risk for alimentary atresia has also been made (Ericson and Källen, 2001) in an IVF cohort of 9111 children compared to the general population.

In ICSI, a higher risk of hypospadias than in the general population has been observed, but this was not found in IVF (Wennerholm et al., 2000b; Ericson and Källen, 2001). In our data, no statistical difference for urogenital, or urological or genital anomalies between ICSI and IVF children was found, even if we also compared the total numbers of major and minor

anomalies in each system. In absolute rates for hypospadias (major and minor similar to the ICD 10 codes used by Wennerholm *et al*., 2000b), we found 0.28% (8/2840) in the ICSI group and 0.47% (14/2955) in the IVF group. Wennerholm *et al*. (2000b) found 0.69% (7/1008) among ICSI children, which was significantly higher than in the general population, and 0.23% (13/5446) among IVF children, which was not significantly higher than in the general population. In our data, however, this problem does not appear to be greater in the ICSI group than in the IVF group. Hypospadias has been associated with reduced parental fertility (Källen *et al*., 1991) and with paternal problems (Sweet *et al*., 1974). In our patient group, there is a mixed indication for ICSI, for which maternal indications too (such as failed IVF) are accepted and on the other hand there are also male fertility problems in the IVF group. This makes it difficult to evaluate whether male infertility is associated with a higher frequency of hypospadias in the children. ICSI as a technique is not related to a higher risk for hypospadias in our series.

Conclusions

This study shows that pregnancy outcome after ICSI is similar to that for IVF. No greater miscarriage rate, stillbirth rate or perinatal death rate occurred among the ICSI pregnancies. Neonatal outcome, health of the ICSI children and major malformation rates are comparable among both ICSI and IVF children. Among the ICSI children, we did not observe any increase in the general major malformation rates in liveborns, or in the total malformation rates, the ICD 10 codes or the minor malformation rates. No differences in major malformation rates were observed in the different organ systems in ICSI compared to IVF. Sperm quality or sperm origin does not appear to play a role in the outcome of ICSI children.

Long-term follow-up is needed to obtain information on further sexual development, on fertility and on psychomotor and intellectual development of ICSI children. Ideally this research should be performed in studies including control groups in ICSI, IVF and after natural conception.

Acknowledgements

References

Aase, J.M. (1990) *Diagnostic Dysmorphology*. Plenum Press, New York, pp. 000–000.

Albano, C., Smitz, J., Camus, M. *et al*. (1996) Hormonal profile during the follicular phase in cycles stimulated with a combination of human menopausal gonadotrophin and gonadotrophin-releasing hormone antagonist (cetrorelix). *Hum. Reprod*., **11**, 2114–2118.

Aylward, P., Hatcher, R., Stripp, B., Gustafson, N. and Leavitt, L. (1985) Who goes and who stays: subject loss in a multicenter, longitudinal follow-up study. *Dev. Behav. Pediat*., **6**, 3–8.

Aytoz, A., De Catte, L., Bonduelle, M., Camus, M., Van Assche, E., Van

Steirteghem, A. and Devroey, P. (1998) Obstetrical outcome after prenatal diagnosis in intracytoplasmic sperm injection pregnancies. *Hum. Reprod*., **13**, 2958–2961.

Bachelot, A., Thepot, F., Deffontaines, D. *et al*. (1995) Bilan FIVNAT 1994. *Contracept. Fert. Sex*., **23**, 7–8, 490–493.

Beral, V. and Doyle, P. (1990) Report of the MRC Working Party on Children Conceived by In Vitro Fertilization. Births in Great Britain resulting from assisted conception, 1978–87. *Br. Med. J*., **300**, 1229–1233.

Berg, T., Ericson, A., Hillensjo, T., Nygren, K.-G. and Wennerholm, U.-B. (1999) Deliveries and children born after in-vitro fertiisation in Sweden 1982–95: a retrospective cohort study. *Lancet*, **354**, 1579–1585.

Bernardini, L., Martini, E., Geraedts, J. *et al*. (1997) Comparison of gonosomal aneuploidy in spermatozoa of normal fertile men and those with severe male factor detected by in-situ hybridisation. *Mol. Hum. Reprod*., **3**, 431–438.

Bonduelle, M., Desmyttere, S., Buysse, A. *et al*. (1994) Prospective follow-up study of 55 children born after subzonal insemination and intracytoplasmic sperm injection. *Hum. Reprod*., **9**, 1765–1769.

Bonduelle, M., Legein, J., Derde, M.-P. *et al*. (1995a) Comparative follow-up study of 130 children born after ICSI and 130 children born after IVF. *Hum. Reprod*., **10**, 3327–3331.

Bonduelle, M., Hamberger, L. and Joris H. (ICSI Task Force) (1995b) Assisted reproduction by ICSI: an ESHRE survey of clinical experiences until 3 December 1993. *Hum. Reprod. Update*, **1**, May, CD ROM.

Bonduelle, M., Legein, J., Buysse, A. *et al*. (1996a) Prospective follow-up study of 423 children born after intracytoplasmic sperm injection. *Hum. Reprod*., **11**, 1558–1564.

Bonduelle, M., Willikens, J., Buysse, A. *et al*. (1996b) Prospective study of 877 children born after intracytoplasmic sperm injection, with ejaculated epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI. *Hum. Reprod*., **11** (Suppl. 4), 131–159.

Bonduelle, M., Devroey, P., Liebaers, I.A. and Van Steirteghem, A. (1997) Commentary: Major defects are overestimated. *Br. Med. J*., **7118**, 1265–1266.

Bonduelle, M., Aytoz, A., Van Assche, E. *et al*. (1998) Incidence of chromosomal aberrations in children born after assisted reproduction through intracytoplasmic sperm injection. *Hum. Reprod*., **13**, 781–782.

Bonduelle, M., Camus, M., De Vos, A. *et al*. (1999) Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum. Reprod*., **14** (Suppl. 1), 243–264.

Buitendijk, S. (2000) IVF pregnancies: outcome and follow-up. Doctoral thesis, University of Leiden.

Camus, M., Van den Abbeel, E., Van Waesberghe, L. *et al*. (1989) Human embryo viability after freezing with dimethylsulfoxide as a cryoprotectant. *Fertil. Steril*., **51**, 460–465.

Cohen, J., Mayaux, M.J. and Guihard-Moscarao, L. (1988) Pregnancy outcomes after *in vitro* fertilisation. A collaborative study on 2342 pregnancies. *Annals of the New York Academy of Science*, **541**, 1–6.

De Catte, L., Liebaers, I., Foulon, W., Bonduelle, M. and Van Assche, E. (1996) First trimester chorion villus sampling in twin gestations. *Am. J. Perinat*., **13**, 413–417.

Devroey, P., Liu., J., Nagy, Z., Goossens, A. *et al*. (1995) Pregnancies after testicular sperm extraction and ICSI in non-obstructive azoospermia. *Hum. Reprod*., **10**, 1457–1460.

Devroey, P., Nagy, P., Tournaye, H., Liu, J., Silber, S. and Van Steirteghem, A.C. (1996) Outcome of intracytoplasmic sperm injection with testicular spermatozoa in obstructive and non-obstructive azoospermia. *Hum. Reprod*., **11**, 1015–1018.

Dhont, M., Neubourg de, D., Elst van der, J. and Sutter de, P. (1997) Perinatal outcome of pregnancies after assisted reproduction. *J. Assist. Reprod. Gen*., **10**, 575–580.

Doyle, P., Beral, V., Botting, B. and Wale, C. (1991) Congenital malformations in England and Wales. *J. Epidemiol. Community Health*, **45**, 43–48.

Doyle, P., Beral, V. and Maconochie, N. (1992) Preterm delivery, low birthweight and small-for-gestational-age in liveborn singleton babies resulting from *in vitro* fertilization. *Hum. Reprod*., **7**, 425–428.

Ericson, A. and Källen, B. (2001) Congenital malformations in infants born after IVF: a population based study. *Hum. Reprod*., **16**, 504–509.

Eurocat (European Registration of Congenital Anomalies) (1993) *Report 5, Surveillance of Congenital Anomalies 1980–1990*. Edited by a Eurocat Working Group.

European Orgalutran[®] Study Group, Borm, G. and Mannaerts, B. (2000) Treatment with the gonadotrophin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating

hormone is effective, safe and convenient: results of a controlled, randomized, multicentre trial. *Hum. Reprod.*, **15**, 1490–1498.

Holmes, L.B. (1976) Congenital malformations. *N. Engl. J. Med.*, **295**, 204–207.

ICD Manual (1992) *International Statistical Classification of Diseases. Injuries and Causes of Death*. Based on the 10th Revision Conference. World Health Organization, Geneva.

Jacobs, P., Browne, C. Gregson, N., Joyce, C. and White, H. (1992) Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. *J. Med. Genet.*, **29**, 103–106.

Källen, B. (1986) Congenital malformations in twins: a population study. *Acta Genet. Med. Gemell. (Roma)*, **35**, 167–178.

Källen, B., Castilla, E., Kringelbach, M. *et al*. (1991) Parental fertility and infant hypospadias: an international case–control study. *Teratology*, **44**, 629–634.

Kurinczuk, J. and Bower C. (1997) Birth defects conceived by intracytoplasmic injection: an alternative interpretation. Bonduelle, M., Devroey, P., Liebaers, I., A., Van Steirteghem, A. Major defects are overestimated. *Br. Med. J.*, **7118**, 1260–1266.

Lancaster, P.A.L. (1987) Congenital malformations after in vitro fertilization. *Lancet*, **ii** (1392), 3.

Lancaster, P., Shafir, E. and Huang, J. (1995) *Assisted Conception Australia and New-Zealand 1992 and 1993*. AIHW National Perinatal Statistics Unit, Sydney, pp. 1–71.

Lechat, M.F. and Dolk, H. (1993) Registries of congenital anomalies: Eurocat. *Envir. Health Persect.*, **101** (Suppl. 2), 153–157.

Liu, J., Lissens, W., Silber, S. *et al*. (1994) Birh after preimplantation diagnosis of the cystic fibrosis deltaF508 mutation by the polymerase chain reaction in human embryos resultig from intracytoplasmic sperm injection with epididymal sperm. *J. Am. Med. Assoc.*, **272**, 1858–1860.

Loft, A., Petersen, K., Erb, K. *et al*. (1999) A Danish national cohort of 730 infants born after intracytoplasmic sperm injection (ICSI) 1994–1997. *Hum. Reprod.*, **14**, 2143–2148.

Medical Research International, Society for Assisted Reproductive Technology (SART) and The American Fertility Society (1992) Assisted reproductive technology in the United States and Canada: results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. *Fertil. Steril.*, **64**, 13–21.

Meschede, D., Lemcke, B., Exeler, B. *et al*. (1998) Chromosome abnormalities in 447 couples undergoing intracytoplasmic sperm injection\prevalence, types, sex distribution and reproductive relevance. *Hum. Reprod.*, **13**, 576–582.

Moosani, N., Pattinson, H.A., Carter, M.D. *et al*. (1995) Chromosomal analysis of sperm from men with idiopatic infertility using sperm karyotyping and fluorescence in situ hybridisation. *Fertil. Steril.*, **64**, 811–817.

Nielsen, J. and Wohlert, M. (1991) Chromosome abnormalities found among 34 910 newborn children: results from a 13-year study in Arhus, Denmark. *Hum. Genet.*, **87**, 81–83.

Olivennes, F., Rufat, P., Andre, B. *et al*. (1993) The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. *Hum. Reprod.*, **8**, 1297–1300.

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A.C. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340**, 17–18.

Palermo, G., Camus, M., Joris, H. *et al*. (1993) Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm injection. *Fertil. Steril.*, **59**, 826–835.

Palermo, G., Colombero, L., Schattman, G. *et al*. (1996) Evolution of pregnancies and initial follow-up of newborns delivered after intracytoplasmic sperm injection. *J. Am. Med. Assoc.*, **276**, 1893–1897.

Pang, M.G., Hoegerman, S.F., Cuticchia, A.J. *et al*. (1999) Detection of aneuploiy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in-situ hybridisation in spermatozoa from nine patients with oligoasthenoteratospermia undergoing intracytoplasmic sperm injection. *Hum. Reprod.*, **14**, 1266–1273.

Peschka, B., Leygraaf, J., Van der Ven, K. *et al*. (1999) Type and frequency of chromosome aberrations in 781 couples undergoing intracytoplasmic sperm injection. *Hum. Reprod.*, **14**, 2257–2263.

Reubinoff, B.E., Samueloff, A. and Ben-Haim, M. (1997) Is the obstetric outcome of in vitro singleton gestations different from natural ones? A controlled study. *Fertil. Steril.*, **67**, 1077–1083.

Robert, E., Mutchnik, O, Mastroiacovo, P. *et al*. (1993) An international collaborative study on the epidemiology of oesophageal atresia or stenosis. *Reprod. Toxicol.*, **7**, 405–421.

Shi, Q. and Martin, R.H. (2001) Aneuploidy in human spermatozoa: FISH analysis in men with constitutional chromosomal abnormalities, and in infertile men. *Reproduction*, **121**, 665–666.

Smith, D.W. (1975) Classification, nomenclature, and naming of morphologic defects. *J. Pediatr.*, **87**, 162–164.

Smitz, J., Devroey, P., Brackmans, P., Camus, M., Khan, I., Staessen, C., Van Waesberghe, L., Wisanto, A. and Van Steirteghem, A.C. (1987) Management of failed cycles in an IVF/GIFT programme with the combination of a GnRH analogue and HMG. *Hum. Reprod.*, **2**, 309–314.

Sweet, R., Schrott, H., Kurland, R. and Culp, O. (1974) Study of the incidence of hypospadias in Rochester, Minnesota, 1940–1970, and a case control comparison of possible etiological factors. *Mayo Clin. Proc.*, **49**, 52–58.

Tan, S., L., Doyle, P., Campbell, S. *et al*. (1992) Obstetric outcome of *in vitro* fertilization pregnancies compared with normally conceived pregnancies. *Am J. Obstet. Gynecol.*, **167**, 778–784.

Tesarik, J. (1995) Sex chromosome abnormalities after intracytoplasmic sperm injection. Letter to the editor. *Lancet*, **346**, 1095.

Tesarik, J. and Mendoza, C. (1996) Genomic imprinting abnormalities: a new potential risk of assisted reproduction. *Mol. Hum. Reprod.*, **2**, 295–298.

Trounson, A., Howlett, D., Rogers, P. and Hoppen, H.O. (1986) The effect of progesterone supplementation around the time of oocyte recovery inpatients superovulated for *in vitro* fertilization. *Fertil. Steril.*, **45**, 532–533.

Van Assche, E., Bonduelle, M., Tournaye, H. *et al*. (1996) Cytogenetics of infertile men. *Hum. Reprod.*, **11** (Suppl. 4), 1–24; disc. 25–26.

Van Steirteghem, A.C., Liu, J., Joris, H. *et al*. (1993a) Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum. Reprod.*, **8**, 1055–1060.

Van Steirteghem, A.C., Nagy, Z., Joris, H. *et al*. (1993b) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod.*, **8**, 1061–1066.

Van Steirteghem, A.C., Nagy, Z., Liu, J. *et al*. (1993c) Intracytoplasmic sperm injection. *Assist. Reprod. Rev.*, **3**, 160–163.

Verlaenen, H., Cammu, H., Derde, M.P. and Amy, J.J. (1995) Singleton pregnancy after *in vitro* fertilization: expectations and outcome. *Obstet. Gynecol.*, **86**, 906–910.

Vogt, P.H., Edelmann, A., Hirschmann, P. and Kohler, M.R. (1995) The azoospermia factor (AZF) of the human Y chromosome in Yq11: function and analysis in spermatogenesis. *Reprod. Fertil. Dev.*, **7**, 685–693.

Wennerholm, U.B., Bergh, C., Hamberger, L. *et al*. (2000a) Obstetric outcome of pregnancies following ICSI, classified according to sperm origin and quality. *Hum. Reprod.*, **15**, 1189–1194.

Wennerholm, U-B., Bergh. C., Hamberger, L. *et al*. (2000b) Incidence of congenital malformations in children born after ICSI. *Hum. Reprod.*, **15**, 101–105.

Westergaard, H.B., Tranberg Johansen A.M., Erb, K. and Nyboe Andersen, A. (1999) Danish National In-Vitro Fertilization Registry 1994 and 1995: a controlled study of births, malformations and cytogenetic findings. *Hum. Reprod.*, **14**, 1896–1902.

Wisanto, A., Magnus, M., Bonduelle, M., Liu, J., Liebaers, I., Van Steirteghem, A.C. and Devroey, P. (1995) Obstetric outcome of 424 pregnancies after intracytoplasmic sperm injection (ICSI). *Hum. Reprod.*, **10**, 2713–2718.

Appendix 1. Numbered list of minor malformations

Craniofacial

1. Flat occiput
2. Frontal bossing
3. Maxillary hypoplasia
4. Metopic fontanel
5. Metopic suture open
6. Metopic suture open to bregma
7. Micrognathia (moderate)
8. Parietal bossing
9. Parietal foramina
10. Prominent occiput
11. Scalp defects
12. Sloping forehead
13. Syndrome of asymmetric crying face (if not in combination with other problems)

Eyes

1. Almond-shaped eyes
2. Blepharophimosis
3. Brushfield spots
4. Corectopia
5. Distichiasis
6. Ectropion
7. Entropion
8. Epicanthal fold(s) after 2 years
9. Flat brow
10. Heterochromia iris
11. Hypertelorism
12. Hypotelorism
13. Lacrimal duct stenosis/atresia
14. Lateral slanting palpebral fissures upward
15. Lateral slanting palpebral fissures downward
16. Long eyelashes
17. Pterygium
18. Ptosis
19. Short palpebral fissures
20. Small coloboma of iris
21. Straight eyelashes
22. Synophrys
23. Tangled eyelashes
24. Telecanthus
25. Thinning of lateral eyebrow

Ears

1. Abnormal angulation
2. Absence of superior crus of anthelix
3. Asymmetrical size
4. Auricle abnormalities
5. Bilateral large ears
6. Crumpled ears
7. Cryptotia
8. Cup-shaped ears
9. Darwinian tubercle
10. Ear lobe bifid
11. Ear lobe cleft
12. Ear lobe crease
13. Ear lobe notched

14. Ear lobe perforated
15. Hairy ears
16. Indented upper helix
17. Lack of helical fold
18. Low set ears
19. Microtia
20. Overdevelopment of root helix
21. Overturned helix
22. Pointed ears
23. Preauricular pit/sinus
24. Preauricular tag
25. Prominent ears
26. Tragus absent
27. Tragus bifid
28. Triangular ears

Mouth

1. Ankyloglossia
2. Asymmetric tongue
3. Channel teeth
4. Flat philtrum
5. Gum broad alveolar ridge
6. Gum cleft
7. Hypodontia
8. Lip pits angular
9. Lip pits lower
10 .Lobulated tongue
11. Long philtrum
12. Macroglossia
13. Microglossia
14. Palatum gothic
15. Palatum pits
16. Palatum torus
17. Short philtrum
18. Supernumerary palatal teeth
19. Uvula bifid

Skin

1. Absence of skin
2. Angiofibromas
3. Café-au-lait spots
4. Capillary haemangioma
5. Cavernous haemangioma
6. Compound naevi
7. Congenital angioma: if >4 cm or multiple or cavernous
8. Congenital junctional naevi
9. Congenital naevus
10. Discrete amniotic bands
11. Epidermal naevi
12. Epidermal naevi
13. Epidermal naevi
14. Hypopigmented macula
15. Lentigines
16. Mongolian spot
17. Naevus flammeus
18. Naevus sebaceus of Jadassohn

Appendix 1 (*continued*). **Numbered list of minor malformations**

19. Port wine stain
20. Skin dimple over acromion
21. Skin dimple over lateral elbows
22. Skin tag over other location
23. Telangiectasias

Chest and heart

1. Atrial septum defect/ventricular septum defect: closed spontaneously
2. Atrial septum defect/ventricular septum defect: trivial
3. Atrial septum defect/ventricular septum defect: foramen ovale (ASD type II)
4. Atrial septum defect type II + ductus arteriosus
5. Beaded ribs
6. Gynaecomasty
7. Murmur
8. Nipple supernumerary left
9. Nipple supernumerary right
10. Nipples widely spaced
11. Pectus carinatum
12. Pectus excavatum
13. Ribs fused or bifid
14. Sternum cleft
15. Sternum short
16. Ventricular septum defect + ductus arteriosus
17. Ventricular septum defect muscular: minor
18. Hair
19. Absence of hair whorl
20. Alopoecia areata
21. Electric hair
22. Elongated sideburn of scalp hair
23. Excessively curly hair
24. Low anterior hairline
25. Low posterior hairline
26. Male pattern baldness
27. Poliosis
28. Supernumerary scalp
29. Upswept posterior hairline
30. Whisker hair
31. Widow's peak

Abdomen

1. Inguinal hernia if child born ≤36 weeks
2. Umbilical hernia >1 cm
3. Unusual umbilical position
4. Ventral hernia

Genitalia

1. Abnormal penile length
2. Abnormal testis
3. Adhesions between labia minora
4. Chordee
5. Clitoris hypertrophy
6. Cryptorchidism left
7. Cryptorchidism right
8. Glandular hypospadias
9. Hydrocoele >1 year

10. Hypospadias dorsal frenulum/abnormal meatus
11. Phimosis
12. Redundant foreskin
13. Shawl scrotum
14. Thelarche
15. Vaginal tag

Urinary

1. Renal problems: if no follow-up or if unknown
2. Vesico-ureteral reflux if <2 years

Anus

1. Anal stenosis
2. Anal tags
3. Haemorrhoids
4. Rectal polyps

Nails

1. Hippocratic nails
2. Koilonychia
3. Leukonychia punctate
4. Leukonychia striate
5. Leukonychia total
6. Longitudinally grooved nails
7. Narrow hyperconvex nails
8. Racquet nails
9. Small nails
10. Transversally grooved nails

Back

1. Hair over lower midline
2. Hair over lower spine
3. Pilonidal sinus
4. Sacral dimple/pit
5. Scapula high/small
6. Scoliosis moderate

Limbs

1. Genu varum
2. Genu valgum
3. Genu recurvatum
4. Hips: subluxation (therapy = abduction)
5. Segmental shortening of a limb (minimal)

Hands

1. Broad thumbs
2. Clinodactyly fingers
3. Cutaneous syndactyly
4. Fifth finger extra crease left
5. Fifth finger extra crease right
6. Fifth finger single crease left
7. Fifth finger single crease right
8. Fetal pads on fingertops
9. Macrodactyly of finger
10. Metacarpal shortening
11. Short ring finger

Appendix 1 (*continued*). **Numbered list of minor malformations**

12. Single transverse palmar crease bilateral
13. Small accessory digit
14. Tapered fingers

Feet

 1. Broad distal hallux
 2. Broad distal hallux
 3. Bullous toes
 4. Calcaneovalgus
 5. Clinodactyly of toes
 6. Clubfoot
 7. Dorsiflexed hammertoes
 8. Splinting or plastering
 9. Increased space toes 1–2
10. Irregularity of toe length
11. Macrodactyly 2–3

12. Macrodactyly others
13. Metatarsus adductus: if not treated or splinted
14. Overriding toes
15. Pes equinovarus: if not treated or splinted
16. Postaxial polydactyly
17. Prominent heel
18. Recessed toe
19. Syndactyly 2–3
20. Syndactyly others

General

 1. Periventricular cyst if no complications
 2. Macrosome

Metabolic

 1. Hypothyroidism: if <36 weeks

Appendix 2. List of congenital malformation in children after stillbirth and in fetuses after pregnancy termination

Malformations	No. of cases	Malformations	No. of cases
Stillbirths in ICSI		Molar	1
Hydrocephalus	1	Acardiacus in triple	1
Karyotype anomaly fetus	1	Total	18
Single umbilical artery	1	Stillbirths in IVF	
Cleft lip and palatum	1	Meckel–Gruber syndrome	1
Gastrointestinal malformation	1	Spina bifida	1
Malformation and intrauterine growth retardation	1	Total	2
Spina bifida	1	Terminations in IVF	
Trisomy 18	1	Anencephaly	3
Total	8	Botdysplasia	1
		Cloacal dysgenesis	1
Terminations in ICSI		Holopresencephaly	1
Amyoplasia	1	Intrauterine growth retardation	1
Anencephaly	1	Karyotype anomaly	4
Camptomelic dysplasia	1	Malformation	1
Fragile X mutation	1	Meningomyelocoele and polymalformation	1
Hygroma colli	2	Spina bifida	1
Intrauterine growth retardation	1	Trisomy 18	1
Karyotype anomaly	3	Trisomy 21	5
Karyotype mosaicism in twin pregnancy	1	Conjoined twin	1
Potter syndrome	1	Total	21
Trisomy 18	1		

IUGR = intrauterine growth retardation.

Appendix 3. List of neonatal complications

Neonatal interventions

Incubator
Resuscitation
Tube feeding
Total parenteral nutrition
Intubation
Antibiotics
Exsanguino transfusion
Perfusion

Neonatal problems

Neurological
    Asphyxia perinatal
    Convulsions
    Intracranial bleeding
    Hypotonia
    Leukomalacia periventricular
    Meningitis
    Myoclonics benign
    Near-miss
    Subarachnoidal bleeding
    Subependymal bleeding
    Subependymal and retinal bleeding
    Paresis of nervus brachialis
    Hydrocephaly
Urological
    Pyelonephritis
    Cystitis
Infection
    Infection
    Sepsis
    Fever
    Streptococcal infection, mother
    High C-reactive protein
Gastrointestinal
    Gastro-oesophageal reflux
    Deshydratation
    Meconium ileus
    Meconium plug and surgery
    Necrotizing enterocolitis
    Necrotizing enterocolitis and surgery

Oesophagitis
    Haemorrhagic gastritis
    Gastroenteritis
Respiratory
    Bronchopulmonal dysplasia
    Hyaline membrane
    Respiratory distress syndrome
Cyanosis
    Haemothorax
    Meconium aspiration
    Pneumothorax
    Pulmonal interstitial emphysema
    Tachypnoea transient
    Pleuritis
Weight
    Dysmature
    Macrosomia
Metabolic
    Acidosis
    Hypocalcaemia
    Hypoglycaemia
Circulatory
    Apnoea + bradycardia
    Bradycardia
    Ductus arteriosus
    Cardiopathy
    Patent ductus arteriosus
    Cardiopathy transient
    Feto-placental maternal haemorrhage
    Pulmonal hypertension
Acquired
    Fracture clavicula
    Trauma
Eye
    Conjunctivitis
    Retinopathy of prematurity stage II
    Retinopathy of prematurity stage III
Mors
    Mors *in utero*
    Early death
Skin
    Collodion baby

Appendix 4. List of major congenital malformations in liveborn children

		Malformation 1	Malformation 2	Malformation 3	Single/multiple
(MA) in ICSI					
1.	MA	Trisomy 21	Cardiopathy		2
2.	MA	Atrial septum defect			2
3.	MA	Atrial septum defect			2
4.	MA	Atrial septum defect			1
5.	MA	Atrial septum defect			2
6.	MA	Atrial septum defect?			2
7.	MA	Atrial septum defect–ventricular septum defect			1
8.	MA	Cardiac malformation			1
9.	MA	Coarctatio aorta			2
10.	MA	Dextrocardia			1
11.	MA	Ductus arteriosus			2
12.	MA	Ductus arteriosus			1
13.	MA	Hypopl. left ventricle			1
14.	MA	Ischaemic skin and muscle lesion			1
15.	MA	Part. atrioventricular canal			1
16.	MA	Pulmonalis stenosis			1
17.	MA	Pulmonalis stenosis			3
18.	MA	Tetralogy of Fallot			2
19.	MA	Tetralogy of Fallot			1
20.	MA	Transposition great vessel			1
21.	MA	Ventricular septum defect	Pulmonalis stenosis		2
22.	MA	Ventricular septum defect	Pulmonalis stenosis		2
23.	MA	Ventricular septum defect			1
24.	MA	Ventricular septum defect			2
25.	MA	Ventricular septum defect			1
26.	MA	Ventricular septum defect			2
27.	MA	Ventricular septum defect			2
28.	MA	Ventricular septum defect			2
29.	MA	Cleft palatum	Ovarian cyst		2
30.	MA	Cleft lip + cleft palatum			1
31.	MA	Cleft lip + cleft palatum			1
32.	MA	Cleft lip bilateral			3
33.	MA	Cleft palatum			2
34.	MA	Cleft soft palate			1
35.	2MA	Anus imperforatus + fistula + malformation	Cardiac malformation		2
36.	MA	Pylorus stenosis			2
37.	MA	Microphthalmia			2
38.	2MA	Hypospadias	Cryptorchidism unilateral		1
39.	MA	Cryptorchidism			2
40.	MA	Cryptorchidism			1
41.	MA	Cryptorchidism			1
42.	MA	Cryptorchidism			1
43.	MA	Cryptorchidism unilateral			2
44.	MA	Hypospadias			2
45.	MA	Hypospadias			1
46.	MA	Hypospadias			2
47.	MA	Ovarian cyst			1
48.	2MA	Respiratory chain (enzymatic) defect	Ventricular septum defect		1
49.	MA	Hypothyroidy			2
50.	2MA	Craniostenosis (scapocephaly)	Hip dysplasia congenital		2

Appendix 4 (*continued*). **List of major congenital malformations in liveborn children**

		Malformation 1	Malformation 2	Malformation 3	Single/multiple
51.	MA	Pes equinovarus bilateral	Hip luxation congenital		2
		Malformation 1	Malformation 2	Malformation 3	Single/multiple
52.	3MA	Hernia diafragmatica	Coarctation aorta	Pyloric stenosis	1
53.	MA	Beckwith–Wiedeman			1
54.	MA	Club foot			1
55.	MA	Craniostenosis			2
56.	MA	Craniostenosis			1
57.	MA	Diafrag. eventration			2
58.	MA	Femur–fibula–ulna syndrome			2
59.	MA	Hip dysplasia			2
60.	MA	Hip dysplasia			2
61.	MA	Hip luxation congenital			1
62.	MA	Hip luxation congenital			1
63.	MA	Hip luxation congenital			1
64.	MA	Hip and leg malformation			1
65.	MA	Pes equinovarus bilateral			1
66.	MA	Pes equinovarus bilateral			2
67.	MA	Pes equinovarus bilateral			2
68.	MA	Pes equinovarus bilateral			1
69.	MA	Polydactyly hand and foot			1
70.	MA	Polydactyly preaxial fingers			1
71.	MA	Polydactyly preaxial fingers			1
72.	A	Postaxial polydactyly bilateral fingers			2
73.	MA	Microcephaly	Renal hypo- and dysplasia bilateral		1
74.	MA	Anencephaly			2
75.	MA	Aqueductus stenosis			2
76.	MA	Holoprosencephaly			3
77.	MA	Hydrocephalus			2
78.	MA	Hydrocephalus			1
79.	MA	Microcephaly			1
80.	MA	Neural tube defect + Arnold Chiari			2
81.	MA	Periventricular leukomalacia			2
82.	MA	Spina bifida + hydrocoele			2
83.	MA	Leukomalacy discrete.			3
84.	MA	Periventricular leukomalacia			3
85.	MA	Mixoedema			1
86.	MA	Polymalformative syndrome			2
87.	MA	Situs inversus			2
88.	MA	Pierre–Robin syndrome			1
89.	MA	Hernia umbilical >1 cm			2
90.	MA	Naevus cong. junct. large			1
91.	MA	Hydronephrosis			2
92.	MA	Unilateral urether			1
93.	MA	Uretheral stenosis			1
94.	MA	Urethral valve			1
95.	MA	Urethral web			1
96.	MA	Vesico-uretheral reflux			2

Appendix 4 *continued*). **List of major congenital malformations in liveborn children**

		Malformation 1	Malformation 2	Malformation 3	Single/multiple
Major anomalies in IVF					
1.	MA	Trisomy 21	Cardiopathy		2
2.	MA	Trisomy 18			1
3.	MA	Trisomy 21	Truncus arteriosus		1
4.	MA	Aorta stenosis			1
5.	MA	Aorta stenosis			2
6.	MA	Aorta stenosis			2
7.	MA	Aortic valve stenosis			2
8.	MA	Atrial septum defect			2
9.	MA	Atrial septum defect			1
10.	MA	Atrial septum defect			2
11.	MA	Atrial septum defect			3
22.	MA	ASD type I large	Mitralis insufficiency		2
13.	MA	ASD type 1			2
14.	MA	ASD type II +ductus. arteriosus	Right ventricular hypertrophy		2
15.	MA	Atrial septum defect + ventricular septum defect			1
16.	MA	Atrioventricular canal			2
17.	MA	Coarctation aorta			2
18.	MA	Coarctation aorta			2
19.	2MA	Ductus arteriosus	Accessory digit small		2
20.	MA	Ductus arteriosus			1
21.	MA	Ductus arteriosus			1
22.	MA	Ductus arteriosus			1
23.	MA	Ductus arteriosus			2
24.	MA	Ductus arteriosus			3
25.	MA	Ductus arteriosus			3
26.	MA	Ductus arteriosus			3
27.	MA	Pulmonalis stenosis			1
28.	MA	Pulmonalis stenosis			2
29.	MA	Pulmonalis stenosis			2
30.	MA	Pulmonalis stenosis			1
31.	MA	Pulmonalis stenosis			1
32.	MA	Tachycardy junctional			1
33.	MA	Tachycardy supravent.			2
34.	MA	Tachycardy supraventricular			2
35.	MA	Tetralogy of Fallot			1
36.	MA	Transposition great arteries			1
37.	MA	Transposition great vessel			2
38.	2MA	Tricuspidalis insufficiency	Pylorus stenosis		2
39.	MA	Tricuspidalis insufficiency			3
40.	MA	Ventricular septum defect			1
41.	MA	Ventricular septum defect			1
42.	MA	Ventricular septum defect			2
43.	MA	Ventricular septum defect			2
44.	MA	Ventricular septum defect			2
45.	MA	Ventricular septum defect			3
46.	MA	Cleft lip			3
47.	MA	Cleft lip bilateral			1
48.	MA	Cleft palatum			1
49.	MA	Cleft palatum	Tracheo-oesophageal atresia		1
50.	MA	S. Pierre-Robin + cleft palatum	Ventricular septum defect		1

Appendix 4 (*continued*). **List of major congenital malformations in liveborn children**

		Malformation 1	Malformation 2	Malformation 3	Single/multiple
51.	MA	Duodenal atresia			2
52.	MA	Hirschsprung disease			1
53.	MA	Hirschsprung disease			1
54.	MA	Pyloric stenosis			1
55.	MA	Pyloric stenosis			2
56.	MA	Pyloric stenosis			2
57.	MA	Pyloric stenosis			2
58.	MA	Pylorus stenosis			2
59.	MA	Tracheo-oesophageal atresia + fistula	Ileal atresia + Meckels diverticle	Duodenal atresia	2
60.	MA	Cataract anterior bilateral			1
61.	2MA	Cryptorchidism	Tetralogy of Fallot	Hypospadias	1
62.	MA	Cryptorchidism bilateral			1
63.	MA	Cryptorchidism unilateral			1
64.	MA	Cryptorchidism unilateral	Hypospadias		1
65.	MA	Cryptorchidism unilateral			1
66.	MA	Cryptorchidism			1
67.	MA	Cryptorchidism			1
68.	MA	Cryptorchidism			1
69.	MA	Cryptorchidism			2
70.	MA	Cryptorchidism			2
71.	MA	Hypospadias glandular + curvature			2
72.	2MA	Hypospadias			1
73.	MA	Hypospadias			1
74.	MA	Hypospadias			2
75.	MA	Hypospadias			2
76.	MA	Hypospadias			2
77.	MA	Hypospadias			2
78.	MA	Hypospadias			2
79.	MA	Hypospadias			2
80.	MA	Hypospadias			3
81.	MA	In-utero torsio testis unilateral			1
82.	MA	Plexus choroideus bleeding			2
83.	MA	11β-Hydroxylase deficiency			1
84.	MA	Cystic fibrosis			2
85.	MA	Short chain acetyl A deficiency			2
86.	2MA	Arthrogryposis	Cleft palatum		1
87.	MA	Arthrogryposis			2
88.	MA	Craniostenosis			1
89.	MA	Hemivertebra	Rib agenesis	Scoliosis	1
90.	MA	Hernia diafragmatica			2
91.	MA	Hip dysplasia			1
92.	MA	Hip dysplasia bilateral			2
93.	MA	Hip dysplasia			1
94.	MA	Omphalocoele			2
95.	MA	Preaxial polydactyly hand			1
96.	MA	Werdnig–Hoffmann disease			1
97.	MA	Anencephaly			2
98.	MA	Myelocoele			2
99.	MA	Spina bifida + hydrocoele			2
100.	MA	Werdnig–Hoffmann disease			2
101.	MA	Sacrococcygeal teratoma			1
102.	MA	Hypomelanosis of Ito			1

Appendix 4 (continued). **List of major congenital malformations in liveborn children**

		Malformation 1	Malformation 2	Malformation 3	Single/multiple
103.	MA	Trachea obstruction			1
104.	MA	Peri-anal lipome			1
105.	MA	Umbilical vessel rupture			2
106.	MA	Duplication pyelum			2
107.	MA	Horseshoe kidney			2
108.	MA	Hydronephrosis unilateral			2
109.	MA	Pyelo-uretheral junction stenosis			1
110.	MA	Pyelo-uretheral junction stenosis			1
111.	MA	Urethral obstruction			2
112.	MA	Unknown			2