Prospective follow-up study of 877 children born after intracytoplasmic sperm injection (ICSI), with ejaculated epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI

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A prospective follow-up study of 877 children born after ICSI was carried out. The aim of this study was to compile data on karyotypes, congenital malformations, growth parameters and developmental milestones so as to evaluate the safety of this new technique. The follow-up study included agreement to genetic counselling and prenatal diagnosis and was based on a physical examination at the Centre for Medical Genetics (Dutch-speaking Brussels Free University, Brussels, Belgium) at 2 months, 1 year and 2 years, when major and minor malformations and a psychomotor evolution were recorded. Between April 1991 and July 1995, 904 pregnancies obtained after intracytoplasmic sperm injection (ICSI) led to the birth of 877 children (465 singletons, 379 twins and 33 triplets). Prenatal diagnosis determined a total of 486 karyotypes, of which six were abnormal (1.2%) and six (1.2%) were familial structural aberrations, all transmitted from the father. This slight increase in de-novo chromosomal aberrations and the higher frequency of transmitted chromosomal aberrations are probably linked directly to the characteristics of the infertile men treated rather than to the ICSI procedure itself. In all, 23 (2.6%) major malformations were observed in the children born, defined as those causing functional impairment or requiring surgical correction. No particular malformation was disproportionately frequent. Compared with most registers of children born after assisted reproduction and with registers of malformation in the general population, the figure of 2.6% was within the expected range. These observations should be further completed by others and by collaborative efforts. In the meantime, patients should be counselled about the available data before any treatment: the risk of transmitted chromosomal aberrations, the risk of de-novo, mainly sex chromosomal, aberrations and the risk of transmitting fertility problems to

the offspring. Patients should also be reassured that there seems to be no higher incidence of congenital malformations in children born after ICSI.
Key words: children/congenital malformation/ICSI/pregnancy outcome/prenatal karyotypes

Introduction

Even if initially there was major concern about the safety of the newly introduced in-vitro fertilization (IVF) technique, it gradually became accepted that artificial reproductive technology did not involve a higher risk of congenital malformation. Only a few controlled studies have been performed on a limited number of children (Morin *et al.*, 1989; Ron-El *et al.*, 1994; Verlaenen *et al.*, 1995). In the few studies where paediatric follow-up and morphological investigations were carried out, results were reassuring, indicating no more problems than in the general population (Andrews *et al.*, 1986; Morin *et al.*, 1989). Four studies have dealt with the development (studied between the ages of 12 and 37 months) of children born after IVF, and have found no identifiable influence on the development of these children (Mushin *et al.*, 1986; Yovich *et al.*, 1986; Morin *et al.*, 1989; Ron-El *et al.*, 1994). Most of the information came from retrospective studies (Cohen *et al.*, 1988; Beral and Doyle, 1990; Rizk *et al.*, 1991; Medical Research International *et al.*, 1992; NPSU and the Fertility Society of Australia, 1992; Rufat *et al.*, 1994), where the incidence of malformation was compared with national registers and found not to be increased. Initial concern also arose when an increased incidence of some specific malformations [transpositions of the great vessels and neural tube defect (NTD)] was reported (Saunders and Lancaster, 1989). These data led Lancaster (1987) to suggest a possible relationship between NTD and ovulation induction agents; this was not confirmed by larger case control studies (Mills *et al.*, 1990) and no further questions were asked. When assisted fertilization and intracytoplasmic sperm injection (ICSI) were introduced, there were again new reasons for concern. ICSI is indeed a more invasive procedure, because one sperm cell is injected through the oocyte membrane and fertilization can be obtained from spermatozoa that could never have been used before in fertility treatment.

The safety of this novel procedure of assisted fertilization should therefore be assessed carefully (Palermo *et al.*, 1993; Van Steirteghem *et al.*, 1993a,b,c). In previous publications, we have described the first 55 children born after subzonal insemination (SUZI) and ICSI and subsequently 423 children born after ICSI; we failed to find any increased incidence of major congenital malformations compared with the general population (Bonduelle *et al.*, 1994, 1996). In another article we evaluated the results of the ICSI procedure 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 during the same time period and after the same ovarian stimulation and in-vitro culture conditions

(Bonduelle *et al.*, 1995a). To evaluate the safety of the ICSI procedure, we compared the data on karyotypes, congenital malformations, growth parameters and developmental milestones in the two groups of children and found no statistically significant differences. Thus we concluded from a limited number of children that when ICSI was carried out and compared with a standard IVF procedure, no additional risk was observed.

Here we evaluate further the safety of the ICSI procedure by studying the same parameters in a larger cohort of 877 children born after ICSI. Children born after SUZI or SUZI together with ICSI are no longer included in this series, although they were included in our first study of 55 children (Bonduelle *et al.*, 1994).

Materials and methods

Data from 904 pregnancies from April 1991 onwards, leading to the birth of 877 liveborn children before July 1995, have been described by Wisanto *et al.* (1996). The follow-up of this cohort of children was carried out by the Centre for Medical Genetics (Dutch-speaking Brussels Free University, Brussels, Belgium). Part of this cohort has been described previously (Bonduelle *et al.*, 1994, 1995a, 1996).

The design of the prospective follow-up study was as follows. Before starting ICSI, couples were asked to adhere to the follow-up conditions of our study. These conditions included genetic counselling and agreement to prenatal karyotype analysis, as well as participation in a prospective clinical follow-up study of the children. Couples also had to complete a standardized questionnaire, as described previously by Wisanto *et al.* (1995), returning it to the research nurse and, where possible, visiting the Centre for Medical Genetics (Dutch-speaking Brussels Free University, Brussels, Belgium) with the child after birth.

All couples referred for assisted fertilization were evaluated for possible genetic problems, either before starting in cases of maternal age >35 years, a positive family history or a chromosomal aberration carried by a parent, or at 6–8 weeks of pregnancy. A history, including a pedigree, was obtained to identify genetic risks or possible causes of congenital malformation. This history included details of medication, alcohol abuse and environmental or occupational risk factors and socio-economic status. A karyotype was routinely performed for the couple. In view of the possible risk factors of the new techniques of assisted fertilization, the couple was counselled to have a prenatal test (Bonduelle *et al.*, 1996). The advantages and disadvantages of the different types of prenatal diagnosis were discussed in detail at ~6–8 weeks of gestation; amniocentesis was suggested for singleton pregnancies, while chorionic villus sampling (CVS) was proposed for multiple pregnancies (De Catte *et al.*, 1995). Chromosome preparations were obtained from cultured amniocytes according to a modified technique of Verma and Babu (1989). Chromosome preparations from non-cultured and cultured chorionic villus cells were obtained using the techniques

described by Gibas *et al.* (1987) and Yu *et al.* (1986) respectively. If indicated, prenatal tests for other genetic diseases were planned.

The follow-up study of the expected child was explained further. It consisted of a visit to the clinical geneticist at 2 and 12 months of age, and after that once a year.

For all pregnancies, written data concerning 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, the 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 carried out at birth, looking for major and minor malformations and including an evaluation of neurological and psychomotor development.

For babies born elsewhere, written reports were obtained from gynaecologists as well as paediatricians, while a detailed morphological examination by a clinical geneticist from our centre was carried out at 2 months whenever possible. Additional investigations were performed if the anamnestic data or the physical examination suggested them.

At the follow-up examinations at 12 months and 2 years, the physical, neurological and psychomotor tests were repeated by the same team of clinical geneticists. At ~2 years or more, a Bayley test was performed to quantify the psychomotor evolution of the children. If parents did not come spontaneously to the follow-up consultations, they were reminded by telephone to make an appointment.

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 to be minor. A minor malformation was distinguished from normal variation by the fact that it occurs in $\leq 4\%$ of the infants of the same racial group. Malformations or anomalies were considered to be synonymous with structural abnormality (Smith, 1975; Holmes, 1976).

Results

For 859 of the 877 children we had complete information at birth; for 18 files the data were incomplete, even after several attempts to obtain it.

In the group of 877 children studied, 465 were singletons, 379 were from twin pregnancies and 33 were from triplet pregnancies; 448 were male and 429 female, excluding 11 cases of stillbirth (defined as fetal death ≥ 20 weeks or ≥ 400 g) (Table I). The sex ratios in the different subgroups are indicated in Table II.

Of the 877 children, 797 were born after a cycle using fresh embryos obtained by ICSI with ejaculated spermatozoa, 29 after ICSI using epididymal spermatozoa obtained after microsurgical epididymal sperm aspiration (MESA), 29 after ICSI

Table I. Singletons, twins and triplets born after replacement of embryos using intracytoplasmic sperm injection (ICSI)

	Ejaculated spermatozoa	Epididymal spermatozoa	Testicular spermatozoa	Transfer of frozen–thawed supernumerary embryos	Total
Singleton	419	17	11	18	465
Twin	357	6	12	4	379
Triplet	21	6	6	0	33
Total	797	29	29	22	877

Table II. Children born of male and female sex after intracytoplasmic sperm injection (ICSI)

	Male	Female	Total
Ejaculated spermatozoa	405	392	797
Epididymal spermatozoa	19	10	29
Testicular spermatozoa	12	17	29
Replacement of frozen–thawed ICSI embryos	12	10	22
Total	448	429	877

using testicular spermatozoa obtained by testicular sperm extraction (TESE) and 22 after replacement of the cryopreserved embryos obtained after ICSI (Table I) (Tournaye *et al.*, 1994, 1995; Van Steirteghem *et al.*, 1994). Overall, the mean maternal age with regards to the children born was 32.2 years (range 21.4–45.0); the mean maternal age was 32.3 years for the singleton pregnancies, 32.1 years for the twin pregnancies and 29.7 years for the triplet pregnancies. For the pregnancies obtained after ICSI in combination with MESA, TESE or frozen embryo transfer, the mean maternal ages were 31.3, 32.3 and 31.2 years respectively. Overall, the mean paternal age was 35.0 years (range 25.5–64.7).

We obtained data from the physical examination at birth for all the children. We compiled this information from the medical records and from careful questioning of the parents during follow-up consultations. For the children living further away, or where the parents were no longer willing to come to the clinic, detailed histories (except for one major malformation where we were given only the name of the malformation) were obtained from the paediatrician if any problem was mentioned in answer to the questionnaire.

During the follow-up at 2 months, 700 of the 877 (80%) children were examined by one of the geneticists; 194 (22%) children have so far been examined a second time at 1 or 2 years. Most of the children are still <2 years of age.

Genetic counselling

At the genetic counselling session we saw 596 (89%) of the 668 parents (465 parents of singletons, 192 parents of twins, of which five parents of twins had one liveborn and one stillborn child, and 11 parents of triplets). We concluded that for 422 children there was an increased risk because of genetic or

Table III. Genetic and environmental problems encountered in the genetic counselling sessions for parents of 877 children

Parameter	Number	Parameter	Number
Maternal or paternal age	163	Consanguinity	5
Maternal age ≥35 years	154	Third degree	1
Paternal age ≥50 years	9	Fifth degree	4
Chromosomal aberrations	13	Environmental problems	208
45,XY,t(14q21q)	One father	Smoking <10 cigarettes/day	21
45,XY,t(13q14q)	Two fathers	Smoking >10 cigarettes/day	9
45,XX,t(13q15q)	One mother	Drugs	174
45,XY,t(14q15q)	One father	Insulin	2
46,XX,t(11;17)(q23;q25)	One mother	Asthma medication	6
46,XY,inv(1)(p22p23.3)	Two fathers	Antihypertensive	7
46,XY,inv(5)(p13q13)	One father	Antibiotics	34
47,XY,+invdup(15p)	Two fathers of two twins: 4	Thyroid medication	8
		Benzodiazepines	1
Monogenic disease	13	Tocolytica	35
Adult polycystic kidney disease	3	Corticoids	4
Fragile X permutation	One mother of twin	Acetylsalycilic acid	2
		Multiple drugs	75
Duchenne muscular dystrophy carrier	One mother	Transfusion	1
		Infection	3
X-linked ichtyosis	One father	Varicella	1
X-linked mental retardation	One father	Cytomegalovirus	1
CBAVD and cystic fibrosis carrier	Six fathers	Toxoplasmosis infection	1
		Problems arising during pregnancy	4
Multifactorial disease	16		
Cleft lip and palate (recurrence risk >1%)	1	Oligohydramnios	1
		Toxoplasma infection of the mother	1
Neural tube defect	8		
Recurrence risk <1%	3	Positive triple test	1
Recurrence risk >1%	5	Intrauterine growth retardation	1
Epilepsy in father	3		
Diabetes type I in parent	2		
MODY diabetes in maternal line	1		
Bechterew HLA B27	1		

CBAVD = congenital bilateral absence of the vas deferens.

environmental problems (Table III). Each problem was counted once, twice or three times, corresponding to the number of fetuses at risk at the time of counselling. For 210 children there was an increased risk of a genetic complication as a result of: maternal age, 154; paternal age, 9; chromosomal aberrations, 13; monogenic disease, 13; multifactorial disease, 16; and consanguinity, 5. For 208 children we concluded that there was a non-genetic environmental problem (smoking, drugs, maternal infection). For four children there was an unforeseen problem (Table I).

Prenatal diagnosis

In total, 491 prenatal tests were performed, five of which were control samples for a previous test (Table IV): 110 patients had a CVS, 377 had an amniocentesis

Table IV. Prenatal diagnosis in singletons, multiples and unborn children[a] (total number of samples = 491)[b]

	Chorionic villus sampling	Amniocentesis	Cord blood
Singleton pregnancies			
Normal	18	295	4
Abnormal	2	1	
Transmitted	1	3	
Not confirmed	1	3	
Multiple pregnancies			
Normal	81	63	
Abnormal	1	0	
Transmitted	2	0	
Not confirmed	1	1	
Unborn children			
Normal	2	9	
Abnormal	1	1	
Not confirmed	1	1	
Subtotal	110	377	4

[a]Pregnancies aborted before 20 weeks of gestation or interrupted pregnancies.
[b]Five of the 491 samples were repeated analyses of previous tests.

Table V. Uptake of prenatal test and failure test results

	Singleton pregnancies	Multiple pregnancies	Total
Uptake (%)	333/465 (72)	153/412 (37)	486/877 (55)
Failure test results (%)	1	1	1

and four had a cord blood sampling. For 486 of the 877 fetuses a prenatal test was obtained (55%). In the singleton pregnancies, 72% (333/465) of the fetuses were tested, compared with 37% (153/412) of the multiple fetuses (Table V). In the tested singletons, 93% underwent an amniocentesis and 7% had a CVS, whereas in the tested multiples, 57% had a CVS and 43% underwent an amniocentesis. The failure rate of the prenatal tests was 1% in both singleton and multiple pregnancies. Five samples were taken as a control for a previous test: one fetus from a triplet pregnancy showed a 47,XX+20 karyotype by CVS, which was checked by amniocentesis (before selective fetocide which revealed a normal 46,XX karyotype); one twin with a 46XX/92,XXXX by CVS had a normal 46,XX karyotype from amniocentesis; and three singletons had an abnormal result on amniocentesis (a 46,XX/46,XY karyotype, a 46,XY/47XY,+5 karyotype and a 46,XX/46XXdel7 karyotype), which turned out to be respectively 46,XY, 46,XY and 46,XX normal on control cord blood punction.

Abnormal fetal karyotypes were found in six cases out of 486 tested fetuses (1.2%) (Table VI). In the singleton pregnancies these were a 47,XY,+21 karyotype (maternal age 41 years) shown by CVS, a 47,XXX karyotype (maternal age 32 years) shown by CVS, a 47,XYY karyotype (maternal age 25 years) shown by

Table VI. Abnormal results from 486 prenatal tests: 6/486 = 1.2%

Result	Method		Outcome	Maternal age (years)
47,XXX	Chorionic villus sampling	Singleton	Born	32
46,XX/47,XXX	Chorionic villus sampling	Singleton	Intrauterine death	44
47,XXY	Chorionic villus sampling	Twin	Born	28
47,XYY	Amniocentesis	Singleton	Born	25
47,XXY	Amniocentesis	Singleton	Interrupted	28
47,XY,t21	Chorionic villus sampling	Singleton	Interrupted	41

Table VII. Inherited structural aberrations: 6/486 = 1.2%

Karyotype	Type of pregnancy and mode of transmission
46,XY,inv(1)(p22p23.2)	Singleton, paternally transmitted
46,XY,inv(5)(p13q13)	Singleton, paternally transmitted
46,XY,+invdup(15p) (two)	Twin, paternally transmitted
45,XY,t(13;14)	Singleton, paternally transmitted
45,XX,t(14;15)	Singleton, paternally transmitted

amniocentesis, a 47,XXY karyotype (maternal age 28 years) shown by amniocentesis and a mosaicism 46XX/47,XXX shown by CVS (maternal age 44 years). This mosaicism in a child from an older mother was not checked at birth because the pregnancy ended in an intrauterine death and no further genetic analysis was performed. The parents chose to interrupt the pregnancy for trisomy 21 and for Klinefelter syndrome in a pregnancy that was obtained after the transfer of a cryopreserved embryo. One fetus from a twin pregnancy (maternal age 28 years) revealed an abnormal result: a 47,XXY karyotype shown by amniocentesis. This pregnancy was continued.

Six out of 486 cases (1.2%) were familial structural aberrations: 46,XY,inv(1)(p22p23.3), 46,XY,inv(5)(p13q13), 45,XY,t(13q14q), 45,XX,t(14q15q) and 47,XX,+invdup(15p) in twin pregnancies. All these structural aberrations were inherited from the fathers and were therefore already present before conception (Table VII).

In one twin pregnancy a prenatal DNA diagnosis for fragile X was performed on trophoblast cells because the mother was found to be a carrier of a permutation at the fragile X locus; both fetuses were female and carried the same permutation as the mother. Because there was no risk of mental retardation, the couple was reassured. For one mother a preimplantation diagnosis with amplification of the non-deleted region of the healthy allele was performed because she was a carrier of Duchenne muscular dystrophy and had chosen to undergo this treatment rather than a prenatal diagnosis for ethical reasons (Liu *et al.*, 1994, 1995). For one

Table VIII. Neonatal measurements in singletons, twins and triplets

	Singletons	Twins	Triplets	Total
No. of children	465	379	33	877
Mean birthweight (g)	3197	2436	1574	2807
Mean length (cm)	49.4	46.2	40.6	47.9
Mean head circumference (cm)	34.3	32.7	28.7	33.5

Table IX. Neonatal measurements in children born after the replacement of embryos using intracytoplasmic sperm injection

	Ejaculated spermatozoa	Epididymal spermatozoa	Testicular spermatozoa	Transfer of frozen–thawed supernumerary embryos	Total
No. of children	797	29	29	22	877
Mean birthweight (g)	2797	2969	2781	2982	2807
Range	550–4970	1280–4625	1220–4911	950–4030	550–4970
Mean length (cm)	47.9	48.4	49.2	48.7	47.9
Range	30–58	40.5–56	44–56	34–53.5	30–58
Mean head circumference (cm)	33.4	33.1	33.7	34.8	33.5
Range	21.5–39	28–36.5	29–37	31.5–37.5	21.5–39

other mother with a 1 in 4 risk of cystic fibrosis, a preimplantation diagnosis for cystic fibrosis was performed.

Neonatal data

Neonatal measurements for 877 liveborn children of >20 weeks gestation are listed in Table VIII for singletons, twins and triplets. Data in Table IX are for children born after ICSI, whether combined or not with MESA or TESE, and for children born after the replacement of frozen–thawed embryos obtained by ICSI. For the whole group, the mean birthweight was 2807 g, the mean body length was 47.9 cm and the mean head circumference was 33.5 cm.

Prematurity (birth at ≤37 weeks of pregnancy) was observed in 11% of singletons, 53% of twins and 100% of triplets (Table X). A birthweight <2500 g was observed in 11.7% of singletons, 50.0% of twins and 90.0% of triplets. A very low birthweight (<1500 g) was observed in 42 children.

Five children were resuscitated and 14 died during the neonatal period: three singletons, eight twins and three triplets [one triplet with holoprosencephaly on day 0; one twin with spina bifida and hydrocephaly on day 0; one twin born at 23 weeks on day 0; two twins born at 23 weeks on day 0 with birthweights of 500 and 550 g; two triplets born at 25 weeks with birthweights of 850 and 750 g on days 1 and 2; one twin born at 23 weeks on day 1; one twin born at 30 weeks with a birthweight of 1670 g died on day 7 with intracranial bleeding; one singleton died of a cardiac malformation (hypotrophy of the left ventricle) on day 7; one singleton born at 28 weeks with a birthweight of 1100 g died of

Table X. Neonatal problems in a total of 877 children

Problem	Numbers	%
Prematurity ≤37 weeks	287	
Singletons	52/465	11
Twins	202/379	53
Triplets	33/33	100
Dysmaturity birthweight <2500 g	326	
Singletons	48/465	10
Twins	147/379	39
Triplets	31/33	94
Very low birthweight <1500 g	42	
Singletons	10/465	2
Twins	14/379	4
Triplets	18/33	55
Resuscitation	5	
Neonatal death (first 30 days)	14	
Singletons	3	
Twins	8	
Triplets	3	
Hospitalization in a neonatal unit	348	
Observation	189	
Respiratory problems	64	
Intubation	33	
Pneumothorax	8	
Respiratory distress	10	
Transient respiratory problems	7	
Other problems	6	
Cerebral problems	17	
Intracranial bleeding	2	
Intraventricular bleeding	3	
Subendymal bleeding	6	
Other problems	6	
Feeding problems	37	
Total parenteral nutrition	19	
Tube feeding	18	
Sepsis	8	
Hyperbilirubinaemia[a]	65	
Other problems	10	

[a]From which 33 had only phototherapy.

renal insufficiency on day 9; one singleton born at 28 weeks with a birthweight of 1390 g died after an accidental detubation on day 12; and one twin born at 23 weeks died on day 16].

Karyotypes were obtained for four children in this group: two were normal and two were 47,XX+ marker (in the twins born at 23 weeks where the marker chromosome was inherited from the father, the pathological examination was normal for both).

Three other children died after day 30: one twin born at 32 weeks with a birthweight of 1940 g and a periventricular leucomalacia died on day 42; one twin born at 28 weeks with a birthweight of 610 g died on day 58; and one child died after sudden infant death syndrome.

Table XI. Major malformations in interruptions and stillbirths

Major malformation	Outcome
Gastroschisis, body stalk, malformation of lower limbs (amyoplasia)	Interruption at 12 weeks
Hygroma colli, karyotype 46,XX	Interruption at 13 weeks
Occipital encephalocoele	Selective interruption of one fetus from a twin pregnancy at 14 weeks
Dwarfism due to camptmelic dysplasie due to a de-novo mutation in the *SOX9* gene	
Trisomy 21	Interruption at 13 weeks
Anhydramnios	Interruption at 15 weeks
Hypoplastic left heart and hydrops	Stillbirth at 20 weeks
Gastrointestinal malformations	Stillbirth at 37 weeks

Neonatal complications were encountered for 348 children who were hospitalized in a neonatal unit (Table X).

Major malformations

Before birth, major malformations were found in four interruptions and two intrauterine deaths in a total of 11 stillbirths after 20 weeks (Table XI). No other malformations were detected prenatally, apart from one twin 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. As mentioned earlier, the child died at birth.

After birth, major malformations were found in nine of the 465 (1.9%) singletons, 13 of the 379 (3.4%) twins and one of the 33 (3.0%) triplets (Table XII). This was 2.6% (23/877) of all babies born alive. If we define the malformation rate as: (affected livebirths + affected fetal deaths + induced abortions for malformations)/(livebirths + stillbirths), then the figures are (23 + 2 + 6)/(877 + 6 + 11) = 3.5% (Eurocat, 1993).

In the singleton children

One child had a Pierre–Robin sequence with a pseudo-arthrogryposis and a pathology of the brain stem with deglutition problems in the first month and ocular movement problems still present at 1 month. One child had a cleft lip and palate and unilateral hand and foot malformations. One child had a hypotrophy of the left ventricle and died on day 7. One child had a hypospadias grade II with the meatus in the coronal sulcus and unilateral cryptorchidism; there was a slight ventral incurvation of the penis and a scrotum too proximately attached to the penis. Ultrasound of the kidneys was normal. Surgery is planned at the age of 2 years. One child had a scrotal hypospadias grade III, bifid scrotum, micropenis and bilateral cryptorchidism with the testes in the inguinal canal. Ultrasound of the kidneys was normal. One child had a malformation of the left hip and leg. One child had a urethral atresia and vesico-ureteral reflux. One child

Table XII. Major malformations in a group of 877 children[a]

Major malformation	Numbers	%
Singleton children	9/456	1.9
1. Pierre–Robin sequence with arthrogryposis		
2. Cleft lip and palate and unilateral hand and foot malformation[b]		
3. Hypotrophy of left ventricle		
4. Hypospadias II, unilateral cryptorchidism		
5. Hypospadias III, bifid scrotum and micropenis		
6. Malformation of the left hip and leg[c]		
7. Urethral atresia and vesico-ureteral reflux		
8. Bilateral hydronephrosis		
9. Preaxial polydactily of the first toe		
Twin children	13/379	3.4
1 and 2. Cleft palate in two non-identical twin children		
3. Tetralogy of Fallot		
4. Situs inversus and ventricular septum defect		
5. Diaphragmatical eventration		
6. Hypospadias with double meatus		
7. Femur–fibula–ulna syndrome		
8. Pes equinovarus and hip luxation		
9. Pes equinovalgus		
10. Spina bifida and hydrocephaly		
11. Aqueduct stenosis		
12. Craniostenosis		
13. Trisomy 21		
Triplet children	1/33	3.0
1. Holoprosencephaly		

[a]Pregnancies established after intracytoplasmic sperm injection (ICSI) with ejaculated spermatozoa unless otherwise indicated.
[b]Pregnancy was established after the replacement of frozen–thawed supernumerary embryos.
[c]ICSI was carried out with epididymal spermatozoa.

had a preaxial polydactily of the first toe, which is an extra digit with cartilage structure inserting at the level of the first phalanx of the first toe; this extra digit was surgically removed at the age of 1 year, leaving no further sequelae.

In the twin children

Two children from the same dizygotic twin pregnancy had cleft soft palates. Both had umbilical hernias and one had a cyst in the kidneys. No other malformations were found following an ultrasound examination of the brain, heart and kidneys. A DNA study proved that the children were dizygotic. At the age of 2 years surgery was performed and both are doing well. One child had a tetralogy of Fallot and was doing well at the age of 2 months. One child had a situs inversus and ventricular septum defect. One child had a diaphragmatic eventration, noticed at the age of 2 weeks, and was surgically treated. One child had a hypospadias with a double meatus, one at the normal position and one at the coronal sulcus. The father of the child had had exactly the same anatomical

aberration and a unilateral cryptorchidism which had been operated. At the age of 7 months this child presented a developmental delay. One child had femur–fibula–ulna syndrome, detected at birth with an absence of the fibular ray of the left leg and foot and of the two lateral rays of the right foot, which was in the equinovalgus position. One child had a pes equinovarus and hip luxation treated surgically. One child had a pes equinovalgus treated surgically. One child had spina bifida and hydrocephaly. One child had an aquaduct stenosis. One child had a craniostenosis. One child had trisomy 21, which was detected at birth. The mother was 36 years old and had declined a prenatal test.

In the triplet children

One child had a holoprosencephaly detected at the age of 15 weeks of pregnancy. Because of the multiplicity and severity of the malformation, the option of continuing the pregnancy was taken. Birth occurred after premature rupture of the membranes at 30 weeks. The affected child died immediately after birth. The two other children were normal.

Minor malformations

Minor malformations were found in 116 of the 877 children (13.2%) without major malformations (Table XIII). Moreover, two or more minor anomalies were found in 21 of the 877 children (2.4%). We found major and/or minor anomalies in 139 of the 877 (15.8%) children.

Surgery

Surgery was needed for 43 children. For the 23 children with major malformations, correction was needed for 17 of them (Table XIV). Another 25 children needed surgery for reasons other than major malformations: two for a closure of a ductus arteriosus, three for pyloric stenosis, one for a gastro-intestinal obstruction, 18 for an inguinal hernia and one for a circumcision.

At 2 months

At the age of 2 months, no new major malformations were found, but 45 minor malformations were noticed that had not been recorded at birth (Table XV).

Psychomotor and neurological developments

Psychomotor and neurological developments at the age of 2 months revealed problems in 18 of 700 children (2.5%) (Table XV). In all, 11 children had a developmental delay (six were twins). Of the five with a motor delay, two were normal at 1 year and two had delays on the Bayley test at 2 years, of 2 and 5 months; one has yet to be seen. Of the six children with a psychomotor delay or borderline development, one was normal, one was still delayed at 1 year, one had a delay on the Bayley test at 2 years of 1 month, and three have yet to be seen. Nine infants had neurological problems: six had hypotonia (two of whom also had a developmental delay and three were twins) and will have follow-up

Table XIII. Minor malformations in a group of 877 children born after intracytoplasmic sperm injection

Minor malformation	Number	Minor malfomation	Number
Cardiac problems	33	Skeletal problems	26
Atrium septum defect	12	Clinodactily fifth finger	1
Atrium septum defect + ventricular septum defect	2	Congenital hip luxation	5
		Uni- or bilateral simina crease	6
Atrium septum defect + ductus arteriosus	1	Bilateral pes equinovalgus	1
		Bilateral pes equinovarus	2
Ventricular septum defect	8	Syndactily toes	3
Tricuspid insufficiency	1	Overriding toes	4
Ductus arteriosus	10	Discrete hemihypertrophy leg	1
Urogenital problems	14	Torticollis	3
Cryptorchidism	3	Tegumental problems	29
Bifid scrotum	2	Congenital naevus	24
Coronal hypospadias	3	'Café au lait' spot	1
Vesico-ureteral reflux	3	Parotic angioma	1
Duplicated pyelum	1	Major angiomata	1
Adherences between labia majora of vagina	2	Cavernous angioma of nose wing	1
		Rudimentary nipple	1
Facial problems	12	Other problems	5
Ear: preauricular tag	5	Single umbilical artery	4
Mouth: short tongue frenulum	1	Ovarian cyst	1
Nose: broad nasal bridge	5		
Neck: excessive neck fold	1		
Cranio-facial problems	5		
Prominent occiput	1		
Flat occiput	1		
Asymmetrical ventricles	2		
Metopic suture open to bregma	1		

consultations. One child with clonal movements and one child with quadriplegia will have follow-up consultations. One child (birthweight 2560 g, APGAR 7/8/10) developed hydrocephalus at the age of 2 months and was treated surgically; at the age of 2 years he is doing well.

At the age of 1 or 2 years, 194 children were examined (22% of the children born at the time of investigation). No new major or minor malformations were found. For 41 of the children at the age of 2 years, a Bayley test was performed and the mean results (age of test results compared with chronological age) were +1.3 months in singletons (28 tested), +1.8 months in twins (10 tested) and −1.3 months in triplets (three tested).

Discussion

From the beginning of our ICSI treatments, nearly all patients were seen at the Centre for Medical Genetics (Dutch-speaking Brussels Free University, Brussels, Belgium) either before becoming pregnant or at 6–8 weeks of pregnancy. Because more and more of our patients live abroad, they tend to leave the country early and do not attend the genetic counselling sessions. Nevertheless, we have been

Table XIV. Surgery in 25 children without major malformations

Malformation	Numbers
Heart, closure ductus arteriosus	2
Brain, periventricular brain	1
Gastro-intestinal	
Pyloric stenosis	3
Subobstruction	1
Inguinal hernia	18
Circumcision	1

Table XV. First follow-up visit at 2 months of 700 children

Condition	Numbers
New major malformation	0
New minor malformation	45
Developmental delay	11
Motor delay	5
Psychomotor delay	1
Psychomotor development borderline	5
Neurological problems	9
Hypotonia	6
Quadriplegia	1
Hydrocephaly	1
Clonal movements	1

able to see 90% of the couples, and have proposed an increased risk for 210 of the 877 children (24%).

A total of 13 structural chromosomal aberrations were seen in the routinely performed karyotypes for 569 couples (1.1%), 11 of which were aberrations of the karyotypes of 569 fathers (1.9%). This was higher than the expected figure of 0.5% (Nielsen and Wohlert, 1991; Jacobs *et al.*, 1992) in the general population and was associated with the severe male factor infertility often present in the ICSI patient population (Hens *et al.*, 1988; Moosani *et al.*, 1995; Vogt, 1995). The different possibilities for the offspring were explained to all the parents carrying a structural aberration, in terms of the specific chromosomal aberration and the sex of the parent. A normal karyotype is, of course, possible, as well as a higher miscarriage rate and perhaps a lower implantation rate, both leading to a lower success rate for the ICSI procedure, a risk of non-balanced offspring which can be detected by a prenatal diagnosis and a risk of transmitting exactly the same structural aberration as present in the parent. Apart from the risk of transmitting the same chromosomal abnormality to the offspring, leading to greater genetic risks for the latter, parents were also informed about the possible higher risk of infertility, mainly for their male children.

We think it necessary to continue to perform parental karyotypes because for the couples with a structural aberration the global chances of success of the treatment procedure, as well as the strict indications for a prenatal test and the risks to the offspring, should be explained. It would be of help for future

counselling if a chromosome analysis of the germ cells was possible on a routine basis.

Even if almost all patients agree to tests before the start of the procedure, only 55% of the fetuses were in fact tested, more singletons (72%) than twins (37%). More parents of a multiple pregnancy were wary of the test procedure so we counselled them to have a CVS rather than amniocentesis and attributed a higher risk (of 1%) of miscarriage to the latter. If 43% of the multiples had an amniocentesis, compared with 57% who opted for CVS, this is because of interference after the counselling session by other medical professionals who encouraged the patient to choose amniocentesis for its lower risk of 0.5%.

Most of the couples withdrew from testing once pregnant because the risk for miscarriage after a fertility treatment was considered to be too high or for ethical considerations. As we feel confident that the risk of a de-novo chromosomal aberration is no higher than the risk to 37 year old women in the general population based on Ferguson-Smith's (1983) calculation at 1.2%, we do not put moral pressure on the patients if they do not wish to take this added risk from the testing but we do try to give them non-directive counselling.

Abnormal fetal karyotypes were found in six out of 486 fetuses tested (1.2%). The mean maternal age of the mothers who conceived was 32 years, which does not explain the higher rate of chromosomal aberrations found. For a mean maternal age of 32 years we would expect a figure of ~0.3% chromosomal aberrations at the time of prenatal diagnosis (Hook and Hamerton, 1977; Ferguson-Smith, 1983) rather than 1.2%, 1% of which, or five out of six, were sex chromosome aberrations. The incidence of these sex chromosome aberrations at the time of prenatal diagnosis was comparable with the incidence at birth (because these aberrations are non-critical to survival) (Nielsen and Wohlert, 1991; Jacobs *et al.*, 1992; Ledbetter *et al.*, 1992). The figure of 1% of sex chromosome aberrations can thus be compared with the total newborn population and is approximately five times more elevated than the figures of 0.19% (Jacobs *et al.*, 1992), 0.2% (Hook and Hamerton, 1977) and 0.23% (Nielsen and Wohlert, 1991) found in an unselected newborn population. One hypothesis for the higher incidence of chromosomal aberrations could be that using spermatozoa from men with fertility problems may lead to a higher number of gametes with chromosomal abnormalities. Data from the literature suggest that men with oligozoospermia, asthenozoospermia or teratozoospermia may have an increased frequency of chromosomal abnormalities. Moosani *et al.* (1995) examined spermatozoa from five infertile men: two with oligozoospermia, one with asthenozoospermia and two with teratozoospermia. Of spermatozoa karyotyped by the hamster ovum–human sperm fusion technique, 1.2% had no sex chromosome. These data therefore predict a total of ~1.5% 45,X and 47,XXY zygotes. Similarly, fluorescent in-situ hybridization (FISH) revealed that 0.31% of patient spermatozoa had XY disomy (control mean 0.16%). Pang *et al.* (1995) studied nine men with oligoasthenoteratozoospermia (OAT) by two-colour two-probe FISH (Pang *et al.*, 1994; Hoegerman *et al.*, 1995). They estimated that the combined frequency of all sex chromosome disomies (XX, XY and YY) in the

OAT spermatozoa is 2.7%. If sex chromosome disomies and nullisomies occur at equal frequencies, then their combined frequency in the more severely affected OAT population would be ~5%. We controlled the sperm characteristics of those men whose children had sex chromosome aberrations. As expected in most ICSI-treated couples for the six fathers of children with de-novo chromosomal aberrations, we found that all had at least two abnormal semen parameters: two were oligoasthenozoospermic, two were oligoteratozoospermic and two were oligoasthenoteratozoospermic. Even if data from the literature are somewhat preliminary, we may take our own observations as agreement and conclude that the higher frequency of chromosomal aberrations in spermatozoa from men with OAT is probably a risk factor in ICSI treatment and is at the root of the higher observed percentage of chromosomal aberrations. On the other hand, there is experimental evidence that epigenetic development mechanisms are altered in this means of conception (Tesarik and Sousa, 1994; Tesarik, 1995), leading to a higher degree of aneuploidy because of errors of mitosis during the early cleavage divisions. This mechanism could lead to a higher number of post-meiotic errors, such as the one case of mosaicism (46,XX/47,XXX) from an older woman. We did not observe an abnormally high incidence of these post-meiotic errors, but we think it is interesting to follow this in compiled data from all laboratories because inter-laboratory differences may play a role in the occurrence of such problems.

If spermatozoa from infertile men contain a higher degree of aneuploidy, there should be a higher number of chromosomal aberrations in the fertilized oocytes, as reported by Martin *et al.* (1991). This is supposed to influence the rate of spontaneous abortion, but it is difficult to connect increased abortion rates with a specific fertilization protocol. In the article by Wisanto *et al.* (1995), the abortion rate after ICSI was similar to the abortion rate after IVF treatment cycles, so arguing against the hypothesis. This should be studied in more detail.

Six out of a total of 486 results (1.2%) were familial structural aberrations: 46,XY,inv(1)(p22p23.3), 46,XY,inv(5)(p13q13), 45,XY,t(13q14q), 45,XX,t (14q15q) and 47,XX,+invdup(15p) in twin pregnancies. They were certainly not induced by the microinjection technique because they were all detected in the infertile males before their treatment. Statistically, familial structural aberrations can lead to normal karyotypes, to exactly the same structural aberration as in the parent or to a percentage of from 0 to 50% of non-balanced karyotypes. In this limited group of parents carrying a structural aberration, however, no unbalanced fetuses were found, but most of the structural aberrations were not of the type that entail high risks for the fetuses, as may be the case for some reciprocal translocations. All the parents carrying a structural aberration opted for a prenatal test.

We also controlled the sperm characteristics of men whose children had a transmitted chromosomal aberration and found a mean of 18.1×10^6/ml sperm cells (from 0.0 to 88), with a progressive motility type A absent in six of the seven samples and a normal morphology present in 7.5% (from 0 to 20). These

Table XVI. An overview of literature data on chromosomal anomalies after intracytoplasmic sperm injection (ICSI)

Author		*n*	%	List
Bonduelle *et al.* (1995)	Abnormal	6/486	1.2	47,XXY (twice) 47,XXX 47,XYY 46,XX/47,XXX 47,XX,+21
	Inherited structural anomalies	6/486	1.2	46,XY,inv(1)(p22q23.1) 46,XY,inv(5)(p13q13) 47,XX,+invdup(15p) (twice) 45,XY,t(13;14) 44,XX,t(14;15)
Govaerts *et al.* (1995)	Abnormal	1/55	1.8	Triploidy
	Inherited structural anomalies	4/55	7.2	
In't Veld *et al.* (1995)	Abnormal	5/15	33.3	47,XXY (twice) 45,X (twice) 45,X/46,X.dic(Y)(q11)/ 46,X.del(Y)(q11)
	Inherited structural anomalies	0/15	0.0	
	Personal communication	6/35	17.1	
ICSI Task Force (1996)		8/361	2.2	

[a]Tarlatzis *et al.* (1996).

sperm parameters showed more variation in concentration but were still abnormal for motility and morphology.

Data from the literature reveal a variable number of chromosomal aberrations (Table XVI) (In't Veld *et al.*, 1995). Indications for a prenatal test were often maternal age (whereas in our cohort the mean maternal age of women who accepted the prenatal tests was 32.4 years). This could explain the variation by different selection, but as the other series in Table XVI are still very limited, it is too early to draw any conclusion.

A number of arguments exist to support the idea of an additional risk because of the ICSI procedure: selective mechanisms against physiologically or genetically abnormal spermatozoa might be bypassed; abnormal oocytes might be fertilized; the altered environment or mechanical or chemical damage to the oocyte might lead to perturbations of meiosis and mitosis; various chemical or environmental exposures might also lead to point mutations, resulting in genetic diseases visible at birth or later in life.

The 2.6% major malformation rate is similar to most of the general population (Table XVII), national registries (Office of Population Censuses and Surveys, 1987–88; National Perinatal Statistics Unit and Fertility Society of Australia, 1992) and the assisted reproduction surveys (Table XVIII). Here we considered the livebirth malformation rate because this is the most frequently used, rather than a more precise calculation of the ratio, taking fetal deaths and interruptions

Table XVII. Malformation rates at birth in the general population

Author	Major (%)	Minor (%)	Sample size
Leppig *et al.* (1987)	3.8	40.7	4305
Marden *et al.* (1964)	2.1	14.7	4412
Mehes (1983)	2.2	17.2	4589
Myrianthopoulos and Chung (1974)	7.1	7.3	53 257

Table XVIII. Malformation rates in assisted reproduction surveys

Country/author	Study period	No. of malformations/ total no. of children	%
UK			
Beral and Doyle (1990)[a]	1978–1987	35/1581[b]	2.2
Rizk *et al.* (1991) (single centre)	1978–1987	24/961[b]	2.5
Australia and New Zealand			
Lancaster *et al.* (1995)	1992–1993	247/9807[c]	2.5
France			
Rufat *et al.* (1994)	1987–1989	40/1669[d]	2.9
Bachelot *et al.* (1995)	1986–1993	337/13 380[e]	2.5
Israel			
Friedler *et al.* (1992) (national survey)	1982–1989	32/1475	2.2
USA			
Medical Research International *et al.* (1995)	1993	164/6870[f]	2.3
Schattman *et al.* (1995)		11/303[g]	3.6
World Collaborative Report	1991	165/8036	2.1

[a]MRC Working Party on Children Conceived by IVF.
[b]Malformation rate during the first week of life, including seven chromosomal anomalies and excluding pyloric stenosis, heart murmur, undescended testis, hydrocoele, positional talipes, congenital dislocation of the hip, malformations of skin and integument, anomalies of the abdominal wall and unspecified anomalies of the ears and nose.
[c]Malformation rate in infants and fetuses of at least 20 weeks gestation, excluding 31 abortions for fetal abnormality of gestational age of $\geq$16 weeks.
[d]Malformation rate including three therapeutic abortions and six malformations diagnosed after the first week of life during follow-up.
[e]Malformation rate in children born alive, therapeutic abortions and stillbirths, including 23 chromosomal anomalies.
[f]Birth defects per neonate after in-vitro fertilization treatment.
[g]Major anomalies within the first year of life, assessed by questionnaire (68% response), 2.6% minor anomalies.

of affected fetuses into account, which is used in only a very few malformation surveys. National registries most often register the anomalies at birth or during the first week of life, whereas in this study the follow-up is carried through to 2 years. Moreover, risk figures in the national statistics will probably be somewhat lower because it is unlikely that malformations are generally searched for as carefully as in this survey. Of the 23 reported major malformations in this study, 22 were noticed at birth and the diaphragmatical hernia was detected at the age of 2 weeks (this would reduce our figure to 22/877 or 2.5% in a national register).

Assisted reproduction surveys have their limitations too: data were obtained through standard data collection forms, most often completed at birth. The

Table XIX. Malformations in children born after intracytoplasmic sperm injection (ICSI)

Author	No. of major malformations/ no. of children born	
	n	%
Bonduelle *et al.* (1995c)	23/848	2.7
Bonduelle *et al.* (1995b)	6/273	2.2
Govaerts *et al.* (1995)	3/76	3.9
ICSI Task Force (Bonduelle *et al.*, 1995b)	18/763	2.3

children born after assisted procreation were not examined in a systematic manner and no follow-up was provided to detect congenital malformations or developmental problems which become manifest only later. There is no system in place to check the reported results and the missing data. This explains why we expect to find malformation rates to be lower in the reported surveys after IVF than in this detailed prospective follow-up study of children after ICSI.

A few smaller studies were carried out to compare the outcome of IVF with natural conception, as in the study by Morin *et al.* (1989) on 83 IVF children and 93 matched controls. In their study, a systematic examination for 130 major and minor malformations showed no difference between IVF and the control group. In a recent retrospective study by Schattman *et al.* (1995), 3.6% (11/303) of the children had major anomalies after regular IVF within the first year of life, attested by questionnaires (with a 68% response). These rates were considered to be comparable with those observed in the New York population (New York State Department of Health, 1990). Even if only a small number of valid studies on malformation rates after assisted reproduction treatment are available, it is generally accepted that there are no more malformations than in the general population.

We have already published a few articles based on our ICSI population. In our first article on 55 children born after SUZI and ICSI (Bonduelle *et al.*, 1994), we found one child with multiple congenital anomalies. We were unable to find any difference in the malformation rate between children born after IVF and ICSI in a larger but still limited group of 130 children compared with a group of 130 children born after IVF (Bonduelle *et al.*, 1995a). The current finding of 2.6% major malformations is comparable with the figures found in IVF surveys (Table XVIII) and the few reported ICSI surveys (Table XIX).

Major malformations were observed in low percentages in the different subgroups (Table XX), ranging from 0.0% (0/29) in the ICSI with testicular spermatozoa group to 4.5% (1/22) in the children born after the replacement of frozen–thawed supernumerary ICSI embryos. As the totals in the subgroups are low, it is too early to conclude any difference resulting from the origins of the spermatozoa or additional techniques.

We found one or more minor anomalies in 116 children of the 877 (13.2%). Of the minor heart problems, most were detected at routine heart ultrasonography

Table XX. Abnormal prenatal diagnosis and major malformations in subgroups of children born after intracytoplasmic sperm injection (ICSI) in combination or not with micro-epididymal sperm aspiration (MESA), testicular sperm extraction (TESE) or cryopreservation (FRET)

	Abnormal prenatal diagnosis (*n*)	Children with malformation	
		n	%
ICSI	5/432	21/797	2.6
ICSI + MESA	0/33	1/29	3.4
ICSI + TESE	0/8	0/29	0.0
ICSI + FRET	1/13	1/22	4.5
Total	6/486	23/877	2.6

performed for ICSI babies born *intra muros*. All were transient or not expected to need surgical intervention. Therefore they would not have been mentioned in a national register. A figure of 13% minor anomalies is in the same range as found in the literature, where in the normal population 13.4% of newborn infants have one minor anomaly, 0.8% have two anomalies and 0.5% have three or more anomalies (Marden *et al.*, 1964). In a survey of 4305 newborn babies, Leppig *et al.* (1987) found that 39.9% of the children had one or more minor anomalies (28.4% had one anomaly, 8.4% had two anomalies and 3.1% had three or more anomalies) (Table XVII). It is important to screen for minor anomalies because these may be markers for major anomalies. If three or more minor anomalies are present, the risk of a major anomaly is between 20 and 90% according to the literature (Marden *et al.*, 1964; Leppig *et al.*, 1987).

Surgery was required for 43 children, 17 of whom had a major malformation. In all, 26 underwent a surgical intervention, 18 for an inguinal hernia and three for pyloric stenosis (Table XIV). We counted inguinal hernias as a delay in normal development rather than as a major malformation, although surgical treatment was necessary; this is also the rule in national registers like the Congenital Malformation Statistics of England and Wales.

Although surgical treatment was necessary, we did not classify pyloric stenosis as a major malformation because it represents the commonest gastro-intestinal condition and has a genetic basis. Moreover, several national registers (e.g. Office of Population Censuses and Surveys, 1982–88) exclude pyloric stenosis from their congenital malformations. A periventricular drain was needed following a neonatal complication rather than a malformation.

Conclusions

In this follow-up study of 877 children born after ICSI, a slight increase in de-novo chromosomal aberrations of 1.2% is probably linked directly to the characteristics of the infertile men treated rather than to the ICSI technique itself. A higher frequency of transmitted structural chromosomal aberrations of 1.2% was a result of transmitted aberrations from the fathers. Major malformations

were found in an expected range of 2.6% of children, comparable with the figures from other studies after assisted reproduction treatment or in population registries. These observations should be completed further by others and by collaborative efforts. In the meantime, patients should be counselled about the available data before any treatment is decided, including details of the higher risk of transmitted chromosomal aberrations, the risk of de-novo, mainly sex chromosome, aberrations, and the risk of transmitting fertility problems to the offspring. They should also be reassured that there seems to be no higher incidence of congenital malformation in children born after ICSI.

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Discussion

Tarlatzis: We heard earlier from Lancaster that in his experience and the numbers he presented, the incidence of malformations after ICSI is higher. With similar numbers of offspring you said that the incidence after ICSI was in the same range as in the general population. What about these interpretations of the data? Would you enlighten us? What is the background incidence in the general population which one has to compare? That is an important issue when we counsel our patients for IVF.

When assessing the major and minor malformations, can you identify some kind of trend in the malformations? Do some malformations seem to be more frequent than others? Could you give us a hint on relative risks?

Bonduelle: We did not see any particular malformation that often. Urogenital and the cardiac malformations are relatively frequent, but these are also the most common malformations in the general population.

Anonymous: There were some skeletal malformations. Could this be related to the prenatal diagnostic procedures?

Bonduelle: The skeletal problems were all very different and from different origins. They are probably not related to the prenatal diagnosis since the malformations described after sampling of the chorionic villi were mostly amniotic band syndromes, which were not found in this study.

Camus: In the statement that we can tell the couples that 97% of the ICSI children are normal, you refer mainly to the major congenital malformations. You have to add the obstetrical problems and all the complications linked to multiple pregnancies etc. This would imply that there are more than 3% of pregnancies with problems.

Bonduelle: The 97% is in relation to the morphological malformations. Total morbidity is higher if one takes into account the multiple pregnancies.

Camus: In the presentation by Wisanto, about 50% of the cases of pregnancies after ICSI with testicular spermatozoa did not have prenatal diagnosis. It is just this group that may have more chromosomal abnormalities.

Bonduelle: If there is really a patient group at risk, it is certainly the cases with non-obstructive azoospermia. Many of these patients are from abroad and this may be a reason why we have less prenatal karyotypes.

Concerning the comparison of malformations in ICSI children and the general population. Our data were compared to surveys conducted in a similar way. This involved one team examining the children for major malformations. We did not observe any difference between ICSI and the general population or with children born after other procedures of ART.

Lancaster: The results of our study and yours are remarkably similar. We both found a major malformation rate of 3.6% when induced abortions, life-births and still-births are included. Like you, we did not find any pattern of malformations. We compared ICSI births with other IVF briths, not with the general population. The incidence of malformations among these IVF births was 2.7% but the confidence intervals from the two groups overlapped and are not significantly

different. Frequencies may be 3.6% this year. I think we have to conclude that the major malformation rate after ICSI is similar to that after conventional IVF.
Tarlatzis: My problem is, that with exactly the same figure, you concluded that after IVF or ART, the incidence of malformation is significantly higher than in the general population. That was not the case in the data of Bonduelle, and why I wanted a comment from your presentation.
Lancaster: Those were not the findings I presented. I said that the malformation rate was 3.6% in ICSI births and 2.7% in other IVF births. I did not mention the general population at all in presenting the ICSI results. It is not appropriate to try to compare these data with the general population. Simpson and I spent much time discussing the problems of the different methods of ascertaining malformed fetuses and infants. That is why the non-ICSI IVF group is the appropriate comparison group here.
Ron-El: You said that chromosomal abnormalities arise in 1.2% of children when the average age of the mother is 32 years. This data implies that in countries where public health organisations are funding prenatal diagnosis, we should counsel every couple entering the ICSI programme that it is advisable to have amniocentesis or CVS. Is it so?
Bonduelle: Yes indeed, we should counsel the couple that there is a risk which is similar to the risk of a mother of 36 years but it is to the couple to decide. In conventional IVF, we have a lot of women aged 36 or more, and some do not wish to take the test because of a poor fertility history. They prefer to take the risk of chromosomal abnormality rather than risk losing the pregnancy. We are now in a situation where the couple can decide, whereas we could not do so in the first couples treated by ICSI since we did not really know the risks involved.
Devroey: We reported six de novo chromosome abnormalities but only five of these six were X-linked abnormalities. The sixth one was a patient of 41 years old with a diagnosis of Downs syndrome. You mentioned more recent data, i.e. six de-novo chromosomal aberrations on 585 prenatal karyotypes, which is 1.0% of the total.
Bonduelle: Our figures have declined since we have had more data, with no abnormal results in the meantime. A definitive conclusion will only be reached when the data set is much larger.
Devroey: I agree with you. We need many more cases.
In't Veld: Perhaps I may give a short comment on the data that you presented and the six abnormalities we found in 37 prenatal diagnoses. It is important to know that these are data that are coming from a prenatal setting and that it is in effect a select sub-group of ICSI patients, probably difficult to compare to consecutive series like the one presented. The estimate of chromosomal abnormalities should come from such consecutive series which you presented. The point we think of interest in our series is the high proportion of sex-chromosomal abnormalities. We carried out additional work on these patients to identify the parental origin of the disorder. We have done this on four of five cases. Four are of paternal origin, meaning that either the additional chromosome is paternal or the deleted chromosome is paternal. As a more general comment, I believe that

assessments of chromosomal abnormalities in an ICSI setting would be of interest if the parental origin was included, in order to find the preponderance of paternal origin.

Bonduelle: We also plan to look at the fathers from which these children with sex-chromosomal aberrations were born, to see if they have any meiotic abnormality or have abnormally high numbers of sex-chromosomal anomalies in their sperm (as evidenced by FISH).

Silber: Camus started the discussion about whether a higher risk of sex-chromosomal abnormalities will arise in the non-obstructive cases with severely deficient spermatogenesis. Although it is certainly true that spending six hours looking for five spermatozoa, which may be the last time you will obtain spermatozoa from that patient, they do happen to get pregnant from such a procedure. It is almost impossible to talk them into having a prenatal amniocentesis. On the other hand, before all of these patients come to Brussels to undergo a TESE programme for non-obstructive azoospermia, samples of their blood should be sent to Page where cells are immortalized and many extensive studies can be carried out on these men. If they should produce offspring, and even though prenatal diagnosis always seems to be declined, we will be able to ascertain whether these cases present with a higher risk of sex-chromosomal problems in the offspring, or any other deletions on the Y-chromosome, than other ICSI cases.

Liebaers: Is a karyotype at birth carried out on these children? Because that would also provide one answer since many of the sex-chromosomal anomalies would lead to an abortion and not be seen at birth. It would help to have chromosomes at birth.

Silber: I am pushing them to do that. Prenatal methods are not welcomed, but we will have information soon. Page has studied the molecular biology and will study the chromosomes in these patients.

Liebaers: The procedure, the blood drawing and the handling of the sample are different, so you cannot go back to chromosomes when you are interested in molecular studies. Both should be done, to get the information we need.

Page: It is hard to know what the standard procedure ought to be at this point. We are trying to operate on a vigilant research basis. Perhaps you are addressing the question of routinely karyotyping the children. In the study I described earlier we had not been routinely karyotyping the infertile men. We may do this on a routine basis. We should make it clear that we are talking about a full alert research protocol which would not necessarily be justified in a general clinical setting.

The benign inherited chromosomal anomalies interest me, I am curious with what confidence you labelled them as benign and what criteria are used in considering them to be benign?

Bonduelle: We do not have labelled criteria. These were benign as the parents were completely healthy and normal, apart from their fertility problem which may not be so benign. There was no mental retardation or malformation. They

are not benign in that sense that they may induce fertility problems. As far as their own health is concerned, there is no problem.

Liebaers: It does not mean that the parents do not have a high risk of miscarriage or aneuploidy in their offspring. But at least, in these children they were benign because they did not have any phenotypic effect. That is what is meant by benign.

Page: That is what I understood you to mean. The reason why I ask is that it is interesting to consider what that number would have been in the general population. This may in fact tell us something about the basis of infertility and to what degree you would expect the offspring carrying these otherwise phenotypically benign balanced translocations to have fertility problems.

Liebaers: We do not have an answer to that.

Bonduelle: The frequency of structural chromosomal aberrations in the parents and the children is much higher than what we observe in the normal population.

Diedrich: When In't Veld examined four of your five sex chromosomal abnormalities, you quoted that these four were of paternal origin. What kind of consequences does this have for you? Do you now examine the fathers and do a chromosomal examination? What are the consequences?

In't Veld: We had already karyotyped the fathers. There was no indication of any mosaicism there and we are in the process of looking at any mosaicism in spermatozoa. That would be one way of looking at this problem and of course we are trying to determine the parent of origin of the other two chromosomal abnormalities detected in this set.

Liebaers: If I may add to that. It means that it has at this point no practical implications for routine ICSI patients. It is neccessary to try to understand why we have these sexual chromosomal aberrations and what the cause might be.