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Prospective follow-up study of 423 children born after intracytoplasmic sperm injection

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In order to evaluate the safety of the intracytoplasmic sperm injection (ICSI) procedure, a prospective follow-up study of 423 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. Before starting the infertility treatment, couples were asked to participate in a follow-up study including genetic counselling and prenatal diagnosis. The follow-up study of the child was based on a visit to the paediatrician–geneticist at birth or at 2 months of age, at 1 year and at 2 years of age when a physical examination for major and minor malformations and a psychomotoric evaluation were done. Between April 1991 and September 1994, 320 pregnancies obtained after ICSI led to the birth of 423 children (222 singletons, 186 twins and 15 triplets). Prenatal diagnosis determined a total of 293 karyotypes, one of which was abnormal (0.3%), and four were benign familial structural aberrations, all inherited from the paternal side. A total of 14 (3.3%) major malformations were observed, defined as those causing functional impairment or requiring surgical correction. Neurological or developmental problems at the age of 2 months were found in 14 children, four of whom were multiples. Compared to most registers of children born after assisted reproduction and to registers of malformations in the general population, the figure of 3.3% major malformations is within the expected range. Before drawing any firm conclusion, further careful evaluations of the available data are necessary.

Key words: congenital malformation/fetal karyotypes/intracytoplasmic sperm injection/outcome

Introduction

Several retrospective studies have shown that the overall risk of major malformation is not increased in infants born after in-vitro fertilization (IVF) as compared to the national registers (Cohen *et al.*, 1988; Beral and Doyle, 1990; Rizk *et al.*, 1991; Rufat *et al.*, 1994; Medical Research International, Society for Assisted Reproductive Technology and The American Fertility Society, 1992; National Perinatal Statistics Unit and Fertility Society of Australia and New Zealand, 1992). In the few studies where paediatric follow-up and morphological investigations were done, 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 development (studied between the ages of 12 and 37 months) of children born after IVF and have not identified any influence on the development of these children (Mushin *et al.*, 1986; Yovich *et al.*, 1986; Morin *et al.*, 1989; Ron-El *et al.*, 1994). Controlled studies performed on a limited group of children born after either IVF or natural conception are scarce, but these too conclude that there is no increase in the malformation rate (Morin *et al.*, 1989; Ron-El *et al.*, 1994; Verlaenen *et al.*, 1995). On the basis of these data it is assumed that children from assisted reproductive technology before the era of microinjection did not present congenital anomalies or developmental problems more frequently than children born from in-vivo pregnancies. Intracytoplasmic sperm injection (ICSI) has brought a new technology into the field of assisted reproductive technology. 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 a previous publication, we described our first 55 children born after subzonal insemination (SUZI) and ICSI and failed to find any increased incidence of major congenital malformations as compared to the general population (Bonduelle *et al.*, 1994). In a subsequent 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 to a control group of 130 matched children born after IVF pregnancies in the same time period and after the same treatment protocol (Bonduelle *et al.*, 1995). In order 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 could not find any statistically significant difference.

We thus concluded from a limited number of children that when ICSI was carried out in conjunction with standard IVF procedure, no additional risk was observed. In this article we evaluate further the safety of the ICSI procedure by studying the same parameters in a larger cohort of 423 children born after ICSI. Children born after SUZI or SUZI and ICSI are no longer included in this series, although they had been included in our first study of 55 children (Bonduelle *et al.*, 1994).

Materials and methods

The data from 320 deliveries between April 1991 and September 1994, leading to the birth of 423 children, were recently described by Wisanto *et al* (1995) The follow-up of this cohort of children was carried out by the Centre for Medical Genetics, Belgium. Part of this cohort has already been described in previous articles (Bonduelle *et al.*, 1994, 1995)

Before starting ICSI, couples were asked to adhere to the follow-up conditions of our study. These conditions include genetic counselling and agreement to prenatal karyotype analysis as well as participation in a prospective clinical follow-up study of the children. This included completing the standardized questionnaire as described in the article by Wisanto *et al.* (1995), returning it to the research nurse and whenever possible visiting the Centre for Medical Genetics with the child after birth.

All couples referred for assisted fertilization were evaluated for possible genetic problems A history, including a family tree, was obtained in order to identify genetic risks or possible causes of congenital malformations. 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 Pros and cons of prenatal diagnosis were discussed in detail at ~6–8 weeks of gestation in view of possible risk factors due to the new techniques of assisted fertilization. Amniocentesis was suggested for singleton pregnancies, while chorionic villus sampling (CVS) was proposed for multiple pregnancies 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 by means of the technique 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 further explained: it was to consist of a visit to the geneticist–paediatrician at 2 and at 12 months of age, and then 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, 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.

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

At follow-up examination at 12 months and 2 years, the physical, neurological and psychomotor examinations were repeated by the same team of geneticist–paediatricians. If parents did not come spontaneously to the follow-up consultations, they were reminded by phone to make an appointment

A widely accepted definition of major malformations was used, namely 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 racial group. Malformations or anomalies were considered synonymous with structural abnormality (Smith, 1975, Holmes, 1976).

Table I. Numbers of children for whom, after genetic counselling, there was perceived to be an increased risk of genetic and environmental problems

Genetic/environmental problem	No of children (parents)
Maternal (paternal) age	76
maternal age ≥35 years	75
paternal age	1
Chromosomal aberrations	8
45,XY,t(14q21q)	(one father)
45,XX,t(13q15q)	(one mother)
46,XX,t(11,17)(q23,q25)	(one mother)
46,XY,inv(1)(p22p23 3)	(two fathers)
46,XY,inv(5)(p13q13)	(one father)
47,XY,+inv dup(15p)	(one father of twins)
Monogenic disease	7
Adult polycystic kidney disease	(two fathers of one singleton and one twin)
Fragile-X syndrome	(one mother of twins with premutation)
X-linked mental retardation	(one mother)
Multifactorial disease	11
Cleft lip and palate [>1% recurrence risk (RR)]	2
Neural tube defect (<1% RR)	4
Neural tube defect (>1% RR)	2
Epilepsy in parent	1
Diabetes type I in parent	1
Bechterew HLA B27 + in parent	1
Consanguinity	5
Consanguinity 3rd degree	1
Consanguinity 5th degree	4
Environmental problems	68
Smoking <10 cigarettes/day	32
Smoking >10 cigarettes/day	6
Drugs	
insulin	1
asthma treatment	3
antibiotics	12
multiple drugs	13
Cytomegalovirus infection	1

Results

In the group of 423 children studied, 222 were singletons, 186 were from twin pregnancies and 15 were from triplet pregnancies; 210 were female and 213 male, including three cases of stillbirth. Overall, the mean maternal age was 32.1 years (range 22 6–43.9, SD 3.78); for singleton pregnancies the mean maternal age was 32.3 years (range 22.6–43.9, SD 3.94), and for twin pregnancies it was 31.8 years (range 23.8–40.6, SD 3.41). Overall, the mean corresponding paternal ages were 35.0 years (range 25.5–64.7, SD 5.91), 35 4 years (range 26.3–64.7, SD 6.26) and 34 3 years (range 25.5–48.9, SD 5.0) respectively.

For all the children, data from the physical examination at birth were compiled both from the medical records as well as 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, 322 of the 423 (76%) children were examined by one of the geneticists, at 1 year 70 (16%) children were examined and at 2 years 11 children

have attended the consultation so far. Indeed most of the children were <2 years of age.

Genetic counselling

After the genetic counselling session, we concluded that for 172 children there was an increased risk due to genetic or environmental problems. For 107 children there was an increased risk of a genetic complication: maternal (paternal) age, $n = 76$; chromosomal aberrations, $n = 8$; monogenic disease, $n = 7$; multifactorial disease, $n = 11$; consanguinity, $n = 5$. Three children had >1 source of risk. For 68 children we concluded that there was a non-genetic environmental problem (smoking, drugs, maternal infection) (Table I).

Prenatal diagnosis

In all, 49 patients had a CVS and 189 had an amniocentesis. For 238 of the 320 ongoing pregnancies a prenatal test was performed (74%). In the singleton pregnancies, 94% of the patients chose an amniocentesis and 6% chose a CVS, whereas 30% of the patients with a multiple pregnancy underwent an amniocentesis and 70% a chorionic villus biopsy. Among the 222 singleton pregnancies, of which 181 (81.5%) were tested, two failures of the culture occurred after amniocentesis and six mosaicisms (due to in-vitro artefacts or mosaicisms in the amniocytes and to maternal contamination in one case) were found; they were not confirmed in a control karyotype either by cordocentesis (done in one case) or at birth, where the karyotypes were all normal. In the multiple pregnancies, of which 57 (58%) were tested (55 twins and two triplets), one failure of the culture after CVS occurred; one mosaicism found via CVS, a 46,XX/92,XXXX karyotype on direct and long-term culture, was further investigated by amniocentesis where the karyotype was normal 46,XX and one mosaicism found on amniocentesis, a 45,X/46,XY karyotype, was further investigated by cordocentesis where the karyotype was also normal 46,XY. Abnormal fetal karyotypes were found in only one case out of 238 pregnancies or 297 fetuses (181 singletons, 110 twins and six triplets) tested and 293 results obtained: a mosaicism 46,XX/47,XXX (1/13) revealed by amniocentesis. This mosaicism occurred in a child of an older mother (43.9 years) and was not checked at birth since the pregnancy ended in an intrauterine death and no further genetic analysis was performed. One pathological karyotype was obtained out of a total of 293 results (1.3% failure of the culture), giving only 0.3% abnormality. Four cases out of a total of 293 results (1.3%) were benign familial structural aberrations: 46,XY,inv(1)(p22p23.3), 46,XY,inv(5)(p13q13) and 47,XX, +inv dup(15p) in twin pregnancies. All these structural aberrations were inherited from the fathers and were therefore already present before conception.

For one twin pregnancy, a prenatal diagnosis for fragile X was performed on trophoblast cells since the mother was found to be a carrier of a premutation at the fragile X locus; both fetuses were female and carrier of the same premutation as the mother. As there was no risk of mental retardation, the couple were reassured.

Table II. Neonatal measurements (mean ± SD) in 420 liveborn children
Values in parentheses are ranges

	Weight (g)	Length (cm)	Head circumference (cm)
Singletons ($n = 213$)	3223 ± 623 (2372–3960)	49.4 ± 3 4 (35–55)	34.3 ± 2.0 (23–38)
Twins ($n = 186$)	2459 ± 493 (1320–3400)	47 ± 2.7 (43–50)	33 ± 0.5 (26 5–36)
Triplets ($n = 15$)	1310 ± 387 (690–1817)	39 ± 3 94 (33–44)	29± 2.0 (25 5–30 5)

Neonatal data

Neonatal measurements for 420 liveborn children are listed in Table II. Major neonatal complications were encountered in 10 singletons and 17 children from multiple pregnancies: sepsis ($n = 2$), meningitis ($n = 1$), major pulmonary problems ($n = 20$) and intracranial bleeding ($n = 4$). Five children died in the early neonatal period (one triplet with a holoprosencephaly at day 0, two triplets born at 25 weeks with birthweights of 850 and 750 g at day 1 and 2, one twin born at 23 weeks at day 1 and one twin born at 30 weeks with a birthweight of 1670 g died at day 7 with an intracranial bleeding) and four other children died between day 9 and day 42 (one singleton born at 28 weeks with a birth weight of 1100 g died of renal insufficiency at day 9, one singleton born at 28 weeks with a birthweight of 1390 g died after an accidental detubation at day 12, one twin born at 23 weeks died at day 16, one twin born at 32 weeks with a birthweight of 1940 g died with a periventricular leucomalacia at day 42). Of these nine children, seven were therefore multiples. 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).

Major malformations

In this series, selective reduction was performed for one child from a twin pregnancy, with a major occipital encephalocoele, at the 12th week of pregnancy. One other pregnancy was terminated at 15 weeks because of an anhydramnios. No other malformations were prenatally detected, apart from a 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 above, the child died at birth.

Major malformations were found for four singleton children, nine twin children and one triplet child. This was 3.3% (14/420) of all babies born alive. Defining the malformation rate as (affected livebirths + affected fetal deaths + induced abortions for malformations) divided by (livebirths + stillbirths) the figures were (14 + 0 + 2)/(420 + 3) = 3.7% (Eurocat, 1993).

In the singleton children

One child had a Pierre–Robin sequence with a pseudoarthrogryposis and a pathology of the brainstem with deglutition problems in the first month and ocular movement problems still present at 1 month. One child had a preaxial polydactily

Table III. Minor malformations found in 84 children

Malformation	No of children showing anomaly
Cardiac anomalies (n = 19)	
Atrium septal defect type II	7
Atrium septal defect type II and inguinal hernia	1
Atrium septal defect type II and ductus arteriosus	1
Ventricular septum defect and pre-auricular tag	1
Ventricular septum defect and congenital angioma	1
Ventricular septum defect and sacral dimple	1
Ductus arteriosus	6
Tricuspidal valve insufficiency	1
Urogenital anomalies (n = 17)	
Coronal hypospadias	1
Cryptorchidism	1
Inguinal hernia	7
Hydrocoele	7
Duplication pyeleum	1
Facial anomalies (n = 4)	
Epicantal folds	2
Pre-auricular tag	1
Ear pit	1
Limb anomalies (n = 8)	
Unilateral simian crease	3
Bilateral simian crease	1
Clinodactuly fifth finger	1
Overriding toes	2
Metatarsus adductus	1
Dermatological anomalies (n = 25)	
Angioma of the nose	1
Major angiomata	1
Angioma	20
Congenital naevus	20
Cutis marmorata congenita	1
Other (n = 11)	
Pilonidal sinus	1
Umbilical hernia	10

of the first toe, i.e. 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. 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 a bilateral cryptorchidism with the testes in the inguinal canal. Ultrasound of the kidneys was normal.

In the twin children

Two children of the same dizygotic twin pregnancy had a cleft soft palate. Both had an umbilical hernia and one a cyst in the kidneys. No other malformations were found by ultrasound of the brain, heart and kidneys. DNA study proved the children were dizygotic. At the age of 2 years surgery was performed and they are doing well. One child had a tetralogy of Fallot and was doing well at the age of 2 months. One child had a diaphragmatic eventration, noticed at the age of 2 weeks and 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 exactly the same anatomical aberration and a unilateral cryptorchidism which had been surgically treated. At the age of 7 months this child presented a developmental delay. One child had a 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 equino-valgus position. One trisomy 21 was detected at birth. The mother was 36 years old and had declined a prenatal test. One child was born with a situs inversus and ventricular septum defect (no information is available yet for this child). One child had a soft tissue syndactylism of the full length of the third and fourth finger. This malformation was operated on to allow normal growth and use of the fingers.

In the triplet children

One child had a holoprosencephaly detected at the age of 15 weeks of pregnancy, but because of the multiplicity as well as the severity of the malformation the option to continue 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 were found in 84 children (Table III). Moreover minor anomalies were also found in an additional three children who also had a major malformation. Therefore, in all, one or more minor anomalies were found in 87 children.

Surgery was needed for 21 children: for the children with major malformations, correction was needed for 11 of the 14 children. Another 10 children needed surgery for reasons other than major malformations: two for a pyloric stenosis, seven for an inguinal hernia and one child needed a periventricular drain.

The neurological and psychomotor development at the age of 2 months revealed problems in 14 children: axial hypotonia (n = 2), general hypotonia (n = 5, two associated with psychomotoric delay), hypertonia (n = 2, associated with psychomotoric delay), clonal movements (n = 1), bilateral central hearing loss of 50 decibels (n = 1) and a borderline psychomotoric development (n = 3). Four of these children (three of whom were twins) had a birthweight <2500 g and four were from multiple pregnancies. One child with hypertonia had a normal examination at the age of 1 year, the others are doing well (information from the parents) but have to be followed further

At the age of 1 year, 70 children were examined [this is 16.5% of the number of children born at the time of investigation and 65% (70/108) of the children reaching 12 months at the time of investigation]: one twin child (birthweight 2560 g, Apgar 7/8/10) developed hydrocephaly after the age of 2 months. At 2 years only 11 children have so far been seen; they did not present any problem.

Milestones were checked for the different groups: smiling was present on average at 7.1 weeks (5 3 weeks in singletons and 8.1 weeks in twins); sitting was achieved on average at 6.8 months in singletons and at 7.3 months in twins; walking was performed on average at 12.5 months in singletons and at 13.8 months in twins.

Discussion

From the beginning of our ICSI programme, nearly all patients were seen at the Genetic Department either before starting (in cases of maternal age >35 years, positive family history or a

chromosomal aberration carried by a parent) or at 6–8 weeks of pregnancy, and counselled to have a prenatal test. Even if almost all patients agree to have the test before the start of the procedure, ~25% refuse the test once pregnant because the risk for miscarriage after a fertility treatment is considered too high or because of ethical considerations. As mentioned in the article by Wisanto *et al.* (1995), three late abortions (two after amniocentesis and one after CVS) on 238 tested pregnancies (1.2%) could be attributed to a prenatal diagnostic procedure. This is approximately what we would expect from the general population as regards singleton (Hanson *et al.*, 1985; Tabor *et al.*, 1986; Jahoda *et al.*, 1991) or twin pregnancies (Pergament *et al.*, 1992; Wapner *et al.*, 1993; De Catte *et al.*, 1995).

On one hand, the expected risk of the test procedure from our own limited data and from figures in the general population is 0.5–1%, and on the other hand the risk for a chromosomal abnormality in the ICSI population in this study was 1/293 (0.3%). This was approximately what would be expected from a mean maternal age of 32.1 years (range 22.6–43.9). Moreover, since the one benign mosaicism 46,XX/47,XXX occurred in a child of an older mother (43.9 years), there is no indication from our results of a higher incidence of chromosomal abnormality in this group of children. Numbers, however, are still too small to draw firm conclusions.

The four benign familial structural aberrations: 46,XY, inv. (1)(p22p23.3), 46,XY,inv.(5)(p13q13) and 47,XX+inv. dup.(15p) in twins were all inherited from their infertile fathers and were therefore certainly not induced by the microinjection technique. Statistically, familial structural aberrations can lead either to normal karyotypes or exactly the same structural aberration as in the parent, or to unbalanced karyotypes in 0–50% of cases. In this limited group of parents carrying a structural aberration, however, no unbalanced fetuses were found, and most of the structural aberrations were not of the type that involve high risks for the fetuses, as may be the case for some reciprocal translocations.

As we routinely screen for the parental karyotypes, one might argue that the option of performing prenatal tests only in cases of parental structural aberration could avoid the additional risk from test procedures in these precious pregnancies. If any structural anomaly were found, the risk for a non-balanced aberration would be real (depending on the type of structural anomaly) and selective counselling would be possible. However, we are aware of more abnormal results in our own actual data of prenatal testing in ICSI pregnancies, where a higher risk of sex chromosome anomalies is found (~1.0% chromosomal anomalies of which five out of six are sex chromosome anomalies). On the other hand, in a very limited series described by In't Veld *et al.* (1995), five sex chromosomal anomalies were found in 15 pregnancies tested because of advanced maternal age. We think that these data do not correctly represent the true situation and are rather the result of small numbers; however, we would still discuss with our patients a higher risk figure of ~1% in our ICSI couples (Liebaers *et al.*, 1995).

In all, eight instances of structural aberrations were found in the routinely performed karyotypes for the 320 couples (1.2%) giving birth to the 423 children, six of which were aberrations occurring in the paternal karyotypes (1.9%). This is higher than the expected figure of 0.5% in the general population and is probably associated with male infertility (Hens *et al.*, 1988; Moosani *et al.*, 1995). We therefore think it necessary to continue to perform paternal karyotypes, since for the couples with a structural aberration, a discussion of the total chances of success of the treatment procedure as well as the strict indication for a prenatal test should take place.

A number of arguments exist to support the idea of an additional risk due to 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 disease visible at birth or later in life.

The 3.3% major malformation rate in our study is similar to that of most of the national registries and assisted reproduction surveys. We consider here the livebirth malformation rate as this is the one most commonly used, rather than a more precise figure including fetal deaths and interruptions of affected fetuses, as 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 follow-up was continued for up to 2 years. Moreover, risk figures in the national statistics are probably underestimated as it is unlikely that the malformation rates are obtained as carefully as in this survey. The reported 14 major malformations in this study, however, were noticed at birth, except for the diaphragmatical hernia that was detected only at the age of 2 weeks (this would reduce our figure to 3.0% in a national register).

Assisted reproduction surveys also have their limitations: data are obtained through standard data collection forms, most often filled in at birth. The children born after assisted procreation are not examined in a systematic manner and no follow-up is provided to detect congenital malformations or developmental problems which only become manifest 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 in comparison to this detailed prospective follow-up study of children born after ICSI.

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

no more malformations in these children than in the general population.

In our first article on 55 children born after SUZI and ICSI (Bonduelle *et al*, 1994), we reported one child with multiple congenital anomalies. Subsequently, we were unable to find any difference in malformation rate in a larger but still limited group of 130 children born after ICSI, compared to a group of 130 children born after IVF (Bonduelle *et al.*, 1995). In the current study we found 3.3% major malformations and this is comparable to the figures found in national registers (Office of Population Censuses and Surveys, 1982–88a and 1982–88b) and in IVF surveys.

We found one or more minor anomalies in 87 of the 423 children born. Of the 19 minor heart problems, most were detected at routine heart ultrasonography done for ICSI babies born *intra muros*. All were transient or were not expected to need surgical intervention. They would therefore not have been mentioned in a national register. A figure of 20.5% (87/423) for minor anomalies is in the same range as those 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). Leppig *et al.* (1987) found 39.9% of children with one or more minor anomalies in a survey of 4305 newborns (28.4% had one anomaly, 8.4% had two and 3.1% had three or more anomalies). It is important to screen for minor anomalies, as these may be a marker for major anomalies. If three or more minor anomalies are present, the risk of a major anomaly is between 19.6 and 90% according to the literature data (Marden *et al.*, 1964; Leppig *et al.*, 1987).

Surgery was needed for 21 children, 10 of whom did not have a major malformation but had an inguinal hernia (n = 7), pyloric stenosis (n = 2) or periventricular drain (n = 1). We considered inguinal hernias a delay in normal development rather than a major malformation, although surgical treatment was necessary; this rule is also the case in national registers like the Congenital Malformation Statistics of England and Wales and the Liverpool Congenital Malformation Statistics. Nor do we consider pyloric stenosis to be a major malformation, although surgical treatment was necessary, as it represents the commonest gastrointestinal condition and has a genetic basis. Moreover, several national registers [such as the Office of Population Censuses and Surveys (1982–88) report on Congenital Malformation Statistics of England and Wales and the Liverpool Congenital Malformation Statistics] exclude pyloric stenosis from their congenital malformations. The periventricular drain was needed following a neonatal complication rather than a malformation

In conclusion, this follow-up study of 423 children born after ICSI showed no increase in chromosomal abnormality, on the basis of 293 prenatal karyotypes. A slight increase in benign structural aberrations was due to transmitted aberrations from the father. Major malformations were found in an expected range of 3.3%, when compared to the figures of other studies after assisted reproductive treatment or in population registries. Before drawing any firm conclusion, further careful evaluations are necessary. These observations have to be followed through by us and by other teams using the same assisted reproductive techniques

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