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Follow-up of children born after ICSI

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The comparison of outcome of assisted reproductive technology (ART) children and naturally conceived children may be hampered by the difference in characteristics of the infertile patients such as age and genetic risks. Follow-up studies are further hampered by the type of neonatal surveillance protocol, the number of individuals lost to follow-up, the size of the cohort study, and the lack of standardization, for example to define major anomalies. The limited available data on ICSI fetal karyotypes reveal that, in comparison with a general neonatal population, there is: (i) a slight but significant increase in de-novo sex chromosomal aneuploidy (0.6% instead of 0.2%) and structural autosomal abnormalities (0.4% instead of 0.07%); and (ii) an increased number of inherited (mostly from the infertile father) structural aberrations. Available data indicate that in 8319 liveborn ICSI children, the mean percentage who do not originate from singleton pregnancies was 40% (range 32.6–60.8% according to centre). Most multiples are twins, but there are also 4.4% triplets (in one survey 13.2%). This substantial increase in multiple pregnancies must be considered the most important complication of ART. The different percentages of major and minor congenital malformations cannot be compared, but overall the data in large and reliable surveys does not indicate a higher rate of malformations in ICSI children than in naturally conceived children. To date, only three studies have examined the medical and developmental outcome of ICSI children at 1 and 2 years. These do not reveal obvious problems, but in future further comparison of matched cohorts of children and case–control studies are needed before final conclusions can be drawn.

Keywords: development/fetal karyotypes/intracytoplasmic sperm injection/malformations

TABLE OF CONTENTS

Introduction
Limitations of outcome studies
Prenatal diagnosis in ICSI pregnancies
Congenital malformations in ICSI children
Further medical and developmental outcome of ICSI children
Suggestions for further studies
Acknowledgements
References

Introduction

Shortly after its introduction, it became clear that IVF was an efficient assisted reproductive technology (ART) procedure by which to alleviate female-factor or idiopathic infertility. However, the results of conventional IVF in male-factor infertility were disappointing: in a large proportion of couples there were no embryos for replacement, and couples were not accepted for IVF if the semen parameters were too impaired. The outlook for couples with severe male-factor subfertility has improved dramatically since the first pregnancies and births after ICSI were reported (Palermo *et al*., 1992; Van Steirteghem *et al*., 1993). Results of ICSI in couples with severe male-factor infertility have been similar to results from conventional IVF in female-factor infertility.

Since the introduction of ICSI there has been major concern about its safety. ICSI is indeed a more invasive procedure than conventional IVF, since when one spermatozoon is injected into the cytoplasm of the oocyte using micromanipulation, a small amount of culture medium is also injected into the oocyte and fertilization and embryo development can ensue from a spermatozoon that could not have been used successfully in other fertility treatments. More questions arose—and concern was expressed—when ICSI with non-ejaculated sperm, either epididymal or testicular, was introduced. Concern about the safety of ICSI relates to the ICSI technique itself, to the chromosomal and genetic constitution of the sperm used, and to the possibility of incomplete genomic imprinting at the time of fertilization in cases where testicular sperm are used. It is possible that incomplete imprinting does not impair fertilization or early embryonic

development, but that anomalies might become manifest later in post-natal life. As yet, there is no information available on the mechanisms and the timing of imprinting in the human embryo.

This review will provide comment on the limitations of follow-up studies of children born after IVF or ICSI, and review existing data on the outcome of ICSI pregnancies. The outcome data comprise information on prenatal karyotypes and major and minor malformations obtained prenatally or after birth, as well as on the further development of the children.

Limitations of outcome studies

It is generally accepted that the incidence of major malformations observed in the general population is 2–3%. The question to be answered is whether the anomaly rate in ART children (in this review ICSI children) is similar to that in the general population (Stevenson *et al*., 1993). The ART population may be different from the general population in terms of: (i) factors related to the infertility of one or both partners; (ii) the age of women undergoing ART (mean age 33–34 years versus 27 or 28 years in the general population); (iii) other confounding variables such as maternal toxin exposure and nutrition; and (iv) a greater likelihood that ART offspring have a genetic disorder as a result of parental transmission, for example congenital absence of vas deferens and cystic fibrosis, premature ovarian failure in patients with fragile X premutation and structural chromosomal aberrations.

The issue of an independent confounding effect linked to ovulation induction regimes is unresolved, and data should be collected to answer this question.

Factors related to the neonatal surveillance protocol may influence the anomaly rate. More vigorous surveillance in ART pregnancies than in the data collection of birth defect registries will influence the anomaly rate. The time period for the surveillance is usually 1–2 days after birth in registries, though this time period may be longer (e.g. 1 week) in ART registries.

A major pitfall exists whenever a significant proportion of a given sample is lost to follow-up. It is invalid to assume that cases lost to follow-up are comparable with cases included within the analysis. The number of cases lost to follow-up is especially important since the frequency of abnormal outcome is expected to be low.

Conclusions about ART outcome can, furthermore, be valid only if the sample size is large enough. Anomalies in liveborn children and abortuses cannot be pooled in ART registries since the latter are usually not present in birth defect registries. Further confusion may arise if data are recorded as total anomalies per total infants rather than numbers of infants with anomalies per number of infants evaluated.

The best way to assess pregnancy losses and anomalies after ART is a cohort approach recording data in a standard fashion. The survey should begin as soon as possible after pregnancy is recognized, and should further include surveillance during pregnancy at the end of the first trimester, fetal loss when it occurs during pregnancy as well as neonatal assessment at a defined time (e.g. 1 week) after delivery. Ideally, an expressly designed instrument should be used for infant evaluation. Agreeing on precise definitions and achieving consensus among examiners is a daunting task. Standardization of major anomalies can be achieved more easily, but a grey zone still exists. A pragmatic approach is to define a major anomaly as one resulting in death, causing serious handicap, or necessitating surgery. Another option is to record all malformations major and minor, per organ system, according to ICD 10 codes which is a standardized coding list (Wennerholm *et al*., 2000a,b). The skills and expertise of those carrying out the survey may also influence the anomaly rate. If examinations are conducted by geneticists or dysmorphologists, a spurious increase in ART anomaly rates will ensue in comparison with a group (control) not similarly scrutinized. Of course, examinations of both subjects and controls by geneticists would be ideal. Internal anomalies that are detectable only by ultrasound should be recorded separately. Finally, anomaly assessment should be restricted to a specified time interval.

The protocol for children follow-up used at the Dutch-speaking Brussels Free University (as a collaborative study of the Centres for Reproductive Medicine and Medical Genetics) is an example of an exhaustive follow-up study of ART (conventional IVF as well as ICSI) pregnancies and children. Before starting ICSI, the couples were asked to participate in this prospective follow-up study, which included karyotyping both partners, genetic counselling, prenatal karyotype analysis—in the first 2 years for all couples, but now optional after careful information of the different risks—and participation in a prospective clinical follow-up study of the children. This included completing a standardized questionnaire and returning it to the research nurses, and where possible visiting the Centre for Medical Genetics with the child after birth. All couples referred for assisted conception were evaluated for possible genetic risks (history, pedigree, medication, alcohol abuse, smoking, socio-economic status and possible environmental or occupational risk factors). A karyotype was routinely performed for the couple. At birth, written data concerning the pregnancy outcome were obtained from the obstetrician or paediatrician in charge. A detailed physical examination was carried out on babies born at our University Hospital. Whenever possible, babies born elsewhere were examined at 2 months in our centre by a paediatrician-geneticist. Further follow-up examinations were carried out at 12 months and 2 years to assess physical, neurological and psychomotor development. At approximately 2 years, Bayley tests were performed in order to score the psychomotor development of the children. Further psychomotor evaluation and social functioning were scheduled at a later age (Bonduelle *et al*., 1999).

In this review, several protocols for assessing malformations have been used. A widely accepted definition of major malformation consists of those malformations causing death, functional impairment or requiring surgical correction. The remaining malformations are considered minor malformations. A minor malformation, which is coded using a standardized checklist, was distinguished from normal variation by the fact that it occurs in $\leqslant 4\%$ of the infants of the same ethnic group. Malformations or anomalies were considered synonymous with structural abnormalities (Smith, 1975; Holmes, 1976). In Sweden, congenital malformations after ICSI were studied using data from medical records, the Swedish Medical Birth Registry and the Registry of Congenital Malformations; diagnosis of congenital malformations was made according to the International

Classification of Diseases (ICD-9 or ICD-10) (Wennerholm *et al*., 2000a,b).

Sometimes the European Registration of Congenital Anomalies (EUROCAT) is used; this registry includes all diagnosed congenital anomalies: (i) at birth (liveborn or stillborn); (ii) in children up to the age of 1 year; (iii) in fetuses after 20 weeks of gestation; and (iv) when termination of pregnancy is carried out because of an abnormality (Lechat and Dolk, 1993).

Prenatal diagnosis in ICSI pregnancies

The results of karyotype analyses in prenatal diagnoses after ICSI are summarized in Table I. A total of 2139 fetal karyotypes has been reported in seven studies. There were 73 abnormal fetal karyotypes: 42 de-novo chromosomal aberrations (16 sex chromosomal and 26 autosomal aneuploidies or structural aberrations) and 31 inherited aberrations. In the largest single-centre series (Bonduelle *et al*., 2002), it was concluded that there was a statistically significant increase in sex chromosomal aberrations and structural de-novo aberrations as compared with a control neonatal population.

These results are used to inform the patients during genetic counselling about the risk of chromosomal aberrations in ICSI fetuses. In our own centres, about half of the pregnant patients currently choose to have amniocentesis (AC) or chorionic villous sampling (CVS). In general, no reliable data are available on the percentages of patients pregnant after ICSI who undergo prenatal diagnosis. In a survey of counselling 107 women pregnant as a result of ICSI about prenatal diagnosis, there was a strong preference (82%) for non-invasive prenatal diagnosis (ultrasound, serum screening), while only 17% made use of AC or fetal blood sampling (Meschede *et al*., 1998). The developmental prognosis of individuals carrying a sex chromosomal anomaly should be discussed in detail. Major malformations do occur in Turner patients, but not necessarily in Klinefelter, triple XXX and XYY syndrome. Infertility is an integral finding in Klinefelter patients, but ICSI may overcome this problem for some of them (Staessen *et al*., 1996). Mental retardation does not occur more often in patients with a sex chromosomal anomaly than in normal controls. Academic achievement, however, may be somewhat impaired when compared with peers (Meschede and Horst, 1997). The question has been raised as to whether invasive prenatal testing (AC or CVS) involves additional risks for the patients undergoing ICSI. The pregnancy outcome was compared in 576 pregnancies after prenatal diagnosis with that of 540 pregnancies without prenatal diagnosis. AC was recommended for singleton pregnancies, and CVS for twin pregnancies. Prenatal testing did not increase the preterm delivery rate, the low-birthweight rate, or the very low-birthweight rate, as compared with controls. The fetal loss rate in the prenatal diagnosis group was comparable with that of the control group (Aytoz *et al*., 1998).

Congenital malformations in ICSI children

As for conventional IVF there are no data in the literature allowing a full picture of the outcome of ICSI pregnancies. This became evident in the surveys carried out by the ESHRE Task Force on ICSI (ESHRE Task Force on Intracytoplasmic Sperm Injection, 1998). Data on clinical experience from about 14 000 cycles were received from 94 centres in 24 countries, while only 24 centres completed the follow-up survey for the children, and only two centres reported major congenital malformations. It is common in all voluntary surveys on IVF outcome that data on such parameters as complications during pregnancy and perinatal outcome will be incomplete. A reliable comparison between different surveys is not possible, since the methodology used to collect the data varies from one survey to another, including such points as the way in which major or minor congenital malformations are categorized. Table II summarizes relevant items from reports of groups in the USA (Palermo *et al*., 1996), Belgium (Govaerts *et al*., 1998; Bonduelle *et al*., 2002), Spain (Van Golde *et al*., 1999) and from national cohort studies from Denmark (Loft *et al*., 1999), Australia and New Zealand (Lancaster *et al*., 2000) and Sweden (Wennerholm *et al*., 2000a). When the numbers of children surveyed are categorized in terms of originating from singleton, twin, triplet or higher-order pregnancies, it becomes evident that the overall percentage of children born who do not originate from singleton pregnancies ranges from 32.6 to 60.8%. Most of the children from multiple pregnancies are from twin pregnancies. The mean percentage of

Table I. Karyotype analyses in prenatal diagnoses after ICSI

Reference	Fetuses[a]	De-novo chromosomal aberrations		Inherited structural aberrations
		Sex chromosomal	Autosomal	
Testart *et al*. (1996)	115/NA	–	–	5
Van Opstal *et al*. (1997)	71/NA	6	3	–
Govaerts *et al*. (1998)	101/141	–	1	3
Loft *et al*. (1999)	209/720	–	6	1
Van Golde *et al*. (1999)	57/NA	1	–	1
Wennerholm *et al*. (2000b)	149/1192	–	2	2
Bonduelle *et al*. (2002)	1437/2889	9	14	19
Total abnormal karyotypes	–	16	26	31

[a]Number of fetal karyotypes/total number of fetuses eligible for prenatal karyotyping.
NA = information not available.

Table II. Liveborn children and congenital malformation rate in ICSI children

Reference	Liveborn children				Children from multiple pregnancies (%)	Prenatal mortality	Major and minor congenital malformations
	Total	Singleton	Twins	Triplets[a]			
Palermo *et al*. (2000)	2059	708	980	271	60.8	16	22 major/11 minor
Govaerts *et al*. (1998)	141	67	68	6	52.5	1	2 minor
Loft *et al*. (1999)	721	473	230	18	34.4	1.4%	2.2% major/1.2% minor
Van Golde *et al*. (1999)	119	–	–	–	–	0.8%	2 major
Wennerholm *et al*. (2000a)	1139	736	400	3	35.4	NA	47 (4.1%) 'serious' malformations
Lancaster *et al*. (2000)	2762	1861	782	119	32.6	3.0%	2.5% major
Bonduelle *et al*. (2002)	2840	1499	1228	113	47.2	1.7%	3.4% major/6.3% minor

[a]Triplets or higher-order liveborn.

children from triplet or higher-order pregnancies is 4.4% (361/8181 children). However, this percentage varies greatly per centre: in one centre the value was 13.2% (Palermo *et al*., 2000), whilst in the other five centres it ranged from 2.5 to 4.3%. The difficulties caused by multiple births arising from the practice of replacing multiple embryos has been well documented (Doyle, 1996; ESHRE Capri Workshop Group, 2000).

As indicated in Table II, the perinatal mortality varies (according to the report cited) between 1% and 3%. The number or percentages of 'major' and 'minor' congenital malformations is reported (Table II). A comparison of major and minor congenital malformations between different surveys is invalid because of the variation in methodology used.

A Swedish study (Wennerholm *et al*., 2000a) reported the rate of malformations in 1139 infants born after ICSI in two IVF clinics in Gothenburg; data were obtained from the medical records, the Swedish Medical Birth Registry and the Registry of Congenital Malformations, and compared with data for all births in Sweden and also for births after conventional IVF. There were 87 (7.6%) children with an identified anomaly, and this was minor in 40 cases. The odds ratio for ICSI children for having any major or minor congenital malformation was 1.75 (95% CI 1.19–2.58) after stratification for delivery hospital, year of birth and maternal age. The odds ratio was reduced to 1.19 (95% CI 0.79–1.81) when stratification for singletons/twins was also performed, which indicates that the increased rate of malformation is mainly a result of the high rate of multiple births. The only specific malformation which was found to occur in excess in children born after ICSI was hypospadias (relative risk 3.0, 95% CI 1.09–6.50), but this may be related to paternal subfertility.

The details of the major malformations in our own survey can be summarized as follows (Bonduelle *et al*., 2002): major malformations were found in 18 terminated pregnancies and in eight stillbirths among a total of 49 stillbirths after 20 weeks. There was one additional malformation detected prenatally, i.e. a child who had a holoprosencephaly detected at 15 weeks of pregnancy and died at birth. The major malformation rate was 3.4% (96/2840 liveborn children); the malformation rate was 3.1% in 1499 children from singleton pregnancies, and 3.7% in 1341 children from multiple pregnancies. Defining the total malformation rate as (affected livebirths + affected fetal deaths + induced abortions for malformations) divided by (livebirths + stillbirths), the figures are $(96+8+18)/(2840+49)$ or 4.2% (Bonduelle *et al*., 2002). The major congenital malformation rate was also similar between 2477 children born after ICSI with ejaculated sperm (3.4%) and with non-ejaculated [epididymal ($n=105$) or testicular ($n=206$)] sperm (3.2%) (Bonduelle *et al*., 2002). In a study reporting the outcome of ICSI in 308 cases according to the aetiology of azoospermia, the occurrence of malformations was reported in three out of 180 new-born children (Palermo *et al*., 1999). A case report (Zech *et al*., 2000) reported that in four pregnancies obtained after ICSI with elongated spermatids, two cases of major malformations resulted.

In 1997, a different and less reassuring interpretation of our data was reported (Kurinczuk and Bower, 1997). Using the classification scheme from the Birth Defects Registry in Western Australia, the authors noted that several (mostly cardiac) major defects in our Belgian series had been incorrectly classified as minor. In our commentary on this article (Bonduelle *et al*., 1997), we pointed out that most of the minor heart defects were found by routine heart ultrasonography and had all resolved spontaneously by 1 year of age—and were thus minor malformations. The disproportionately high number of cardiac malformations therefore represented an ascertainment bias due to over-reporting (as minor malformations), and were not attributable to the ICSI technique itself.

Further medical and developmental outcome of ICSI children

In May 1998, two reports appeared in *The Lancet* regarding the further development of ICSI children. One study, performed in Australia (Bowen *et al*., 1998), compared the medical and developmental outcome at 1 year of 89 children conceived by ICSI with that of 84 children conceived by routine IVF, and 80 children conceived naturally. Developmental assessment was assessed using the Bayley Scales of Infant Development from which a mental development index was derived. The incidence of major congenital or major health problems during the first year of life was similar in the three groups. The mental development index was lower for ICSI children (especially boys) at 1 year of age than for IVF or naturally conceived children; more ICSI children showed mildly or significantly delayed development in this test, which assesses memory, problem-solving and language

skills (generally in the predominant language of the country). The second study (Bonduelle *et al*., 1998) reported that at 2 years of age the mental development (as tested by the Bayley Mental Developmental Index) of 201 ICSI and 131 non-ICSI IVF children was similar between ICSI and IVF, and also comparable with that for the general population. Our conclusion was that there was no indication at this point that ICSI children have slower mental development than the general population. In a recent abstract from the Australian group (Leslie *et al*., 2001), sperm quality was reported as being a predictor of child health and development in children conceived using ICSI. These authors concluded that the degree of abnormality of sperm used in the procedure was not a significant predictor of long-term cognitive development. In a later case–control study from the UK (Sutcliffe *et al*., 1999) 123 ICSI children were compared with 123 children conceived naturally when aged between 12 and 24 months. Only singleton children were recruited; children were matched for social class, maternal educational level, region, sex and race, but not maternal age. The mean mental age and the mean Griffiths quotients were comparable; only eye–hand coordination was lower in the study group. There was no difference between the study group and control group in terms of the numbers of major and minor malformations.

Final conclusions on the developmental outcome of ICSI children may require comparisons of matched cohorts of children or case–control studies taking into account parental background and other confounding variables, such as language spoken at home (te Velde *et al*., 1998).

Suggestions for further studies

It is clear from this review that further studies are needed to address several issues in relation to the outcomes of ICSI:

1. The role of prenatal testing during ICSI pregnancies.
2. The significance of malformations among terminated pregnancies and stillbirths.
3. The specific outcome of ICSI pregnancies in cases where non-ejaculated sperm are used.
4. The incidence of abnormalities in children after replacement of frozen–thawed ICSI embryos.
5. The long-term follow-up of ICSI children.

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