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Incidence of congenital malformations in children born after ICSI

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The aim of this study was to determine the incidence of congenital malformations in a complete cohort of children born after intracytoplasmic sperm injection (ICSI). The medical records were retrieved for 1139 infants, 736 singletons, 200 sets of twins and one set of triplets. The total number of infants with an identified anomaly was 87 (7.6%), 40 of which were minor. The incidence of malformations in children born after ICSI was also compared with all births in Sweden using data from the Swedish Medical Birth Registry and the Registry of Congenital Malformations. For ICSI children, the odds ratio (OR) for having any major or minor malformation was 1.75 [95% confidence interval (CI) 1.19–2.58] after stratification for delivery hospital, year of birth and maternal age. If stratification for singletons/twins was also done, the OR was reduced to 1.19 (95% CI 0.79–1.81). The increased rate of congenital malformations is thus mainly a result of a 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, exact 95% CI 1.09–6.50) which may be related to paternal subfertility.

Key words: intracytoplasmic sperm injection/malformations

Introduction

Intracytoplasmic sperm injection (ICSI) has been in clinical use since 1991 (Palermo *et al*., 1992) and has revolutionized the treatment of severe male infertility. More than 20 000 children have been born worldwide with the use of this technique (de Mouzon and Lancaster, 1997). The primary question regarding ICSI is if the technique *per se* carries an increased risk of genetic aberrations and malformations in the children. This has been a special concern since spermatozoa with impaired motility and morphology are often utilized and, furthermore, immature spermatozoa derived from the epididymis and the testis are also used in combination with this technique and it may rather be the selection of patients that constitutes a risk.

Previous reports have been reassuring regarding the outcome of children born after ICSI, showing no increase in major malformations (Bonduelle *et al*., 1994, 1995, 1996a,b, 1998a; Liebaers *et al*., 1995; Palermo *et al*., 1996; Wennerholm *et al*., 1996). The incidence of major malformations detected at birth or during the perinatal period in these studies was reported to range between 0.95% and 3.6%, not significantly different from that observed after conventional IVF or from that expected in the general population. A re-evaluation of some of these results has provided a less reassuring interpretation (Kurinczuk and Bower, 1997): their re-analysis compared birth defects in the Belgian ICSI children cohort (n = 420) to 100 454 infants born in Western Australia and concluded that the odds ratio (OR) for major birth defects was higher after ICSI [OR 2.0; 95% confidence interval (CI) 1.4–2.9]. However, the authors cautioned about confounders, e.g. a lower maternal age, a lower multifetal pregnancy rate and a possible lower surveillance in the reference population. Recently, a statistically significant increase in sex chromosomal aberrations (0.83%) was reported in a total of 1082 prenatal tests in pregnancies after ICSI (Bonduelle *et al*., 1998b). Methodological shortcomings in the previous studies such as small numbers and lack of proper controls also emphasize the need to collect data from other centres and proper control groups.

We have studied the rate of congenital malformations in a cohort of children born after ICSI using data from the medical records, the Swedish Medical Birth Registry (MBR) and the Registry of Congenital Malformations (RCM). A comparison has been made with all births in Sweden and also with births after conventional IVF during corresponding time periods.

Materials and methods

The study group included all infants born in Sweden after ICSI performed at the two IVF clinics in Göteborg, Sahlgrenska University Hospital and Fertility Center Scandinavia. The Swedish definition of birth comprises all liveborn and stillborn babies delivered after 28 weeks of gestation. The study population included all children born as a result of ICSI from the first child born in 1993 to the last one born as a result of ICSI performed before January 1998. Altogether, 1139 infants were born in Sweden, 736 singletons, 200 sets of twins (400 infants) and one set of triplets. Infants delivered abroad (n = 53) were excluded from this study. Since there is no registry of therapeutic abortions in Sweden, terminated pregnancies were excluded from comparisons with conventional IVF and population data, but are discussed separately.

The number of children born after ICSI as a function of sperm origin (ejaculated, epididymal or testicular spermatozoa) and the replacement of fresh or frozen–thawed pre-embryos respectively is shown in Table I. Medical records were retrieved for all infants.

Table I. Number of children (singletons, twins and triplets) born after ICSI as a function of sperm origin and the replacement of fresh or frozen–thawed pre-embryos, respectively

	Transfer of fresh pre-embryos			Transfer of frozen–thawed pre-embryos			Total no.
	Ejaculated spermatozoa	Epididymal spermatozoa	Testicular spermatozoa	Ejaculated spermatozoa	Epididymal spermatozoa	Testicular spermatozoa	
Singletons	571	33	21	100	5	6	736
Twins	328	30	8	28	4	2	400
Triplets	3	0	0	0	0	0	3
Total no.	902	63	29	128	9	8	1139

These were also identified in the Swedish MBR and the RCM (Källen, 1987) in order to find any diagnoses which were missing in the medical record. The MBR also allowed the calculation of the expected numbers of malformed infants from all births.

The MBR has been in existence since 1973 (Cnattingius *et al*., 1990) and covers nearly all births in the country (1–2% missing). It is based on standardized medical documents used at all delivery units and at the paediatric examination of newborn infants. When using the registry in this study, stratification for year of birth, delivery hospital and maternal age was done. Using the Mantel–Haenszel technique, ICSI infants were compared with all infants born after the above-mentioned stratifications and an OR with 95% CI was determined for having a diagnosis of congenital malformation according to the International Classification of Diseases (ICD) (ICD-9, 1977; ICD-10, 1992).

A possible excess of genito-urinary defects, major cardiovascular defects and gastrointestinal defects in children conceived by ICSI was shown in the Australian study (Kurinczuk and Bower, 1997). Previous studies have shown an association between hypospadias and reduced paternal fertility, notably with paternal problems (Sweet *et al*., 1974) and between oesophageal atresia and maternal infertility (Robert *et al*., 1993).

A similar procedure was therefore performed for three specific conditions: hypospadias, congenital heart defects (excluding persistent ductus arteriosus [PDA]), and intestinal atresia. For these specific conditions, the expected number of cases was determined from the population and compared with the observed numbers using exact tests based on Poisson distributions. The observed/expected ratio represents an estimate of the risk ratio (RR).

Results

Of 1139 infants born in Sweden after 937 ICSI procedures performed at the two IVF clinics in Göteborg, 1008 infants (830 deliveries among 780 women) were identified in the MBR. Of the missing 107 deliveries, 101 occurred during 1998 when the MBR was not yet finalized. The other six deliveries had thus not been reported to the MBR or could not be identified because of errors in the identification numbers: in four of these instances the women were foreign citizens and no complete identification number had been given. Fifty-three infants delivered abroad were excluded from this study, since they were not part of the MBR. None of these infants had any registered malformation according to the medical records which were scrutinized.

Tables II (relatively serious malformations) and III (minor or variable anomalies) present the identified malformations, divided into those which had a malformation diagnosis in the MBR and those which were only identified from the medical records and/or RCM. The total number of infants with an identified anomaly was thus 87 (7.6%), 40 of which were mild or uncertain conditions. Only eight of the malformations not recorded in the MBR were severe enough to be reportable to the RCM, four of which had actually been reported. Tables II and III also specify which infants were born in twin births.

Relatively serious malformations were more common in twins than in singletons (OR 2.24, 95% CI 1.47–3.19). In order to estimate the expected number of infants with congenital malformations, only infants registered in the MBR could be used as there was no reference information based on medical record screening. A total of 57 infants with any malformation diagnosis was identified in the MBR among the 1008 infants. The OR for having any congenital malformation registered in the MBR among ICSI children versus the total population was 1.75 (95% CI 1.19–2.58) after stratification for delivery hospital, year of birth and maternal age. Of these 57 malformed infants, 10 (18%) had malformations directly related to preterm birth (PDA and undescended testicles) and twins were especially over-represented. If stratification for singletons/twins was also done, the OR was reduced to 1.19 (95% CI 0.79–1.81). In singletons, the OR was 1.39 (95% CI 0.82–2.38) and in twins 0.86 (95% CI 0.45–1.63). None of the latter OR differed significantly from 1.0. The low OR in twins may be partly due to the fact that after ICSI the majority of twin pairs are dizygotic but in the general population a substantial proportion is monozygotic and the malformation risk is slightly increased among the latter. In two sets of twins in our study group, the twins had the same malformation (hypospadias occurred in both infants in one twin pair and PDA occurred in both infants in one twin pair delivered preterm).

A few specific malformations appeared to exist in excess. There were seven infants with hypospadias but only six were identifiable in the MBR (one was mis-coded). The expected number of infants with hypospadias, stratifying for year of birth, delivery hospital, maternal age and parity, was 2.01. The relative risk (RR) was 3.0 (exact 95% CI 1.09–6.50).

Two infants with an intestinal atresia were identified in the MBR and two additional infants had intestinal atresia but were not identified in the MBR. The expected number of infants with intestinal atresia recorded in the MBR was 0.33.

There were eight infants with a cardiac diagnosis (disregarding PDA) registered in the MBR. The expected number of infants with such a diagnosis in the MBR was 9.6, stratified

Table II. Relatively serious congenital malformations in 1139 infants (400 twins) born after ICSI

Diagnosis	No. of infants with malformation in MBR	Additional infants with malformation[a]	Total no. of infants with malformation	No. of twins with malformation
Corpus callosum agenesi		1	1	1
Cataract	1		1	
Microtia	1		1	
Fallot's tetrad	1		1	
VSD	3	2	5	1
VSD + ASD		2	2	2
VSD + pulmonary stenosis		1	1	
Valvular aortic stenosis	1		1	
Aortic and mitral stenosis		1	1	
Hypoplastic left heart syndrome	1		1	1
Complex cardiac defect		1	1	1
Coarctatio aortae	1		1	
Cleft lip	1		1	1
Cleft palate	1		1	
Cleft palate + microphthalmia + foot deformity	1[b]		1	
Oesophageal atresia + absent thumb	1		1	
Small bowel atresia	1		1	
Duodenal atresia + tricuspidal malformation + lung malf.		1	1	
Anal atresia + sacrum hypoplasia		1	1	
Diaphragmatic hernia		1	1	
Omphalocele + cystic kidney + Fallot's tetrad	1		1	1
Hypospadias (1 mis-coded in the MBR)	5		5	3
Hypospadias + preauricular appendix	1		1	1
Hypospadias + valgus deformity	1		1	1
Hydronephrosis	3		3	1
Ureteral reflux	1		1	
Pes equinovarus adductus		2	2	1
Pes equinovarus + unstable hip		1	1	
Absent hand + ASD		1	1	1
Arthrogryphosis	1		1	1
Trisomy 21	1		1	
Trisomy 13	1		1	
46,XX, t (12q;?)[c]	1		1	1
Nager syndrome		1	1	1
Goldenhar syndrome		1	1	1
Total number	30	17	47	20

[a]Identified from medical records and/or the RCM.
[b]Stillborn infant, trisomy 13 discussed but not verified.
[c]Unbalanced translocation with partial monosomi of chromosome 12q and partial trisomy of another unidentified chromosome.
MBR = Medical Birth Registry; RCM = Registry of Congenital Malformations; VSD = ventricular septum defect; ASD = atrial septum defect.

for year of birth, maternal age, parity and delivery hospital. When all sources were used, 15 infants with cardiac defects were identified (the expected number was seven or eight). Among them, five were serious (expected number two or three).

There were three infants with chromosome aberrations and one with a genetic syndrome (Nager syndrome). One further infant had a possible but unverified trisomy 13.

There were two further multi-malformed infants, one of which was diagnosed with Goldenhar syndrome.

Table IV compares the presence of major malformations among the 1008 ICSI infants and the 5446 infants born after conventional IVF (Bergh *et al*., 1999). Among the latter, only infants reported to the MBR or RCM were included and a similar restriction was therefore made for the ICSI material. Some differences appeared to exist. Most notable perhaps was the complete absence of neural tube defects or hydrocephaly in the ICSI material. The difference in rates (14/5446 and 0/1008), however, may be random. The lower exact confidence limit of the OR was 0.85 (not significant). The rate of neural tube defects registered in Sweden was 5.5 per 10 000 and of hydrocephaly 1.6 per 10 000. The expected number of such defects among infants born after conventional IVF was thus

Table III. Minor or variable anomalies in 1139 infants (400 twins) born after ICSI

Diagnosis	No. of infants with malformation in MBR	Additional infants with malformation[a]	Total no. of infants with malformation	No. of twins with malformation
Preauricular appendix	3	3	6	3
Unspecified cardiac defect	1[b]		1	
PDA	5		5	5
PDA + valgus foot deform.			1	
Tooth in lower jaw	1		1	1
Undescended testicle	5	3	8	3
Unilateral kidney agenesis		1	1	1
Unstable hip	4	4	8	1
Valgus foot deformity	2		2	1
Polydactyly	2	1	3	1
Polysyndactyly	1		1	
Syndactyly	2	1	3	
Total number	27	13	40	16

[a]Identified from medical records and/or the RCM.
[b]The infant was regarded as without any cardiac defect at the age of 7 months.
MBR = Medical Birth Registry; RCM = Registry of Congenital Malformations; PDA = persistent ductus arteriosus.

Table IV. Presence in the Registry of Congenital Malformations (RCM) or the Medical Birth Registry (MBR) of some severe congenital malformation diagnoses among infants born after conventional IVF (n = 5446) and after ICSI (n = 1008)

Diagnosis	Conventional IVF		ICSI	
	n	%	*n*	%
Anencephaly	3	0.06	0	0
Spina bifida	4	0.07	0	0
Hydrocephaly	7	0.13	0	0
Cleft lip and/or palate	11	0.20	0	0.30
Oesophageal atresia	3	0.06	1	0.10
Small bowel atresia	2	0.04	1	0.10
Anal atresia	2	0.04	1	0.10
Limb reduction defect	3	0.06	0	0
Diaphragmatic hernia	1	0.02	0	0
Omphalocele	1	0.02	1	0.10
Hypospadias	13	0.24	7	0.69
Trisomy 21	7	0.13	1	0.10
Other chromosome anomaly	4	0.07	2	0.20

3.9 and after ICSI was 0.8. There thus seemed to be an increased risk of such defects after conventional IVF but this was not demonstrated after ICSI.

Hypospadias seemed to occur more frequently after ICSI than after conventional IVF but the difference (7/1008 and 13/5446) was not statistically significant: OR = 2.92 (exact 95% CI 0.98–7.90, not significant). The population rate of hypospadias was about two per 1000 and the expected number of hypospadias was therefore ~11 after conventional IVF and two after ICSI.

The only other malformation with a reasonably high frequency was oro-facial clefts — the rates were similar in ICSI and IVF and the OR was 0.73 (95% CI 0.19–4.26), thus not significant.

Prenatal diagnosis

During the study period, four pregnancies after ICSI were interrupted because of the presence of fetal abnormalities, which were detected by second trimester ultrasound scanning. These abnormalities included trisomy 18, polycystic kidney disease and acrania (two cases).

As a routine, all patients with singleton pregnancies were offered an early amniocentesis for karyotyping, but it was not compulsory. Prenatal karyotyping was performed on 149 fetuses (13.1%). Abnormal results were found in four cases (2.7%). One singleton was a trisomy 18 and the parents chose to terminate the pregnancy in this case. Two were familial structural anomalies inherited from the father. One twin had an unbalanced translocation (see Table II).

Discussion

Even though the medical records could be identified for all infants born after ICSI, they could not all be identified in the MBR. This is mainly due to the fact that the registry is not yet complete for 1998, but even for previous years some cases are missing. There are generally 1–2% of births missing in the MBR and in this study of infants born after ICSI before 1998 there are six missing births among 836 deliveries (0.7%).

The present study illustrates the problems in evaluating data obtained by screening of medical records for selected patients, e.g. infants born after IVF or ICSI. Rates of malformations obtained in this manner are difficult to compare with rates determined, for instance, from population registers (Lancaster, 1987). We found that a substantial number of congenital malformations were not included in the MBR and if data from medical record screening had been compared with rates from the MBR, the malformation risk associated with ICSI would have been exaggerated. Even after restriction to conditions identified in the registry, however, an excess risk was seen in children born after ICSI, amounting to 75% [i.e. OR 1.75 (95% CI 1.19–2.58)]. This excess risk can to a large extent be explained by conditions associated with multiple and premature birth, notably PDA and undescended testicle. It is also plausible that paediatric examination and recording of data concerning

infants born after IVF and ICSI may be more detailed than for the average baby.

There is one condition which seems clearly over-represented among infants born after ICSI, namely hypospadias. This condition has been associated with reduced parental fertility (Källen *et al*., 1991) and notably with paternal problems (Sweet *et al*., 1974). An association with ICSI is therefore plausible. Hypospadias has also been associated with progestin exposure during organogenesis (Harris, 1990). Progesterone or progestins are often given after IVF treatment as luteal phase support. This association is probably due to confounding from the major reason for progestin therapy, bleeding in early pregnancy, an association seen irrespective of whether progestins have been used or not (Källén *et al*., 1992). The apparently higher frequency of hypospadias after ICSI than after conventional IVF supports the explanation that the association between hypospadias and ICSI is due to confounding by paternal subfertility.

When specific malformations were compared between infants born after ICSI and after conventional IVF, there was an apparent lack of neural tube defects and hydrocephaly observed among the former but an over-representation among the latter, even though this difference may be random. These conditions are associated with twinning, and the rate of twin pregnancies in the conventional IVF material is higher (27%) than in the ICSI material (20%). It is, however, also possible that neural tube defects are specifically related to female infertility, as has been repeatedly suggested although never proven (cf. review by Elwood *et al*., 1992).

The crude increased rate of malformation in ICSI depends mainly on the presence of conditions related to prematurity and multiple births (e.g. PDA, undescended testicle). The only specific effect is seen on hypospadias which may be due to an association between paternal subfertility and hypospadias.

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