A Retrospective Follow-Up Study on Intracytoplasmic Sperm Injection

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Purpose: *Genetic aspects of male subfertility and the novelty of intracytoplasmic sperm injection (ICSI) as a new technique can influence the development of zygotes and children born after ICSI. Therefore, we evaluated the outcome of ICSI compared to in vitro fertilization (IVF).*
Methods: *Data from medical records of 233 total pregnancies and the follow-up of 132 children born after IVF and 120 after ICSI were retrospectively analyzed.*
Results: *No differences were found between ICSI and IVF for early embryonic development and obstetric outcome. In both groups the rate of women undergoing prenatal chromosomal diagnosis was low, 30.0%. The congenital malformation rate was 3.0% after IVF and 1.7% after ICSI, which was not significantly different. Follow-up on development of children born after IVF and ICSI also showed no significant differences.*
Conclusions: *Our results indicate that at this moment ICSI is a safe procedure. However, a consistent prospective follow-up is still mandatory to exclude possible risks.*

KEY WORDS: ICSI; retrospective follow-up; pregnancy; children.

INTRODUCTION

It is important to accumulate information about the safety of intracytoplasmic sperm injection (ICSI), because of theoretical risks and the novelty of the procedure. Collecting data on preembryonic development, obstetric and perinatal outcome, and follow-up of children born after ICSI could indicate possible deleterious effects of ICSI on the normal development.

Attempts to alleviate male-factor fertility problems through in vitro fertilization (IVF) have resulted in low rates of success for men with abnormal sperm characteristics. ICSI, a technique that allows microinjection of a single spermatozoon into the oocyte cytoplasm, has been proven a successful alternative in treating male-factor subfertility (1).

Since the introduction of this technique there has been concern about the risks, although theoretically, of the ICSI procedure (2–6).

(i) Risks related to the ICSI technique: (a) the manipulative procedure itself, (b) injection of foreign material, (c) mechanical activation of the oocyte, and (d) exclusion of the prezygotic selection processes.
(ii) The use of abnormal sperm: (a) an increased frequency of constitutional chromosomal abnormalities (7) and/or (b) chromosomal abnormalities in sperm (8), and (c) an underlying "genetic" disorder (9) which causes male subfertility (e.g., cystic fibrosis and Y chromosome deletions) (10); (d) gamete maturation could be impaired in epididymal or testicular sperm (11).

Microinjection of spermatozoa that have been selected from men with combined defects of sperm motility, morphology, and concentration might lead to an increased incidence of abnormal embryos. These factors might influence the development of the zygote and increase the risk of abnormal offspring.

There have been only a limited number of studies on the follow-up of ICSI. Most studies describe the successful experiences of ICSI in treating male subfertility (12,13).

At this moment follow-up studies show no abnormalities after ICSI. However, Bonduelle *et al.* (14) report that there is a significant increase in chromosomal abnormalities after ICSI. Reclassification of

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birth defects of the prospective follow-up study from Brussels showed an increased risk of having a major birth defect (15).

Recently Bowen *et al.* 1998 (16) showed an increased risk of mild delays in mental development at 1 year in children conceived by ICSI. These results are not confirmed by the Brussels group (17).

More follow-up data on ICSI should be evaluated because of theoretical risks, the novelty of the procedure, and the limited number of controversial follow-up results. Therefore we evaluated the safety of ICSI by comparing data on ICSI versus IVF.

MATERIALS AND METHODS

Patients included in this study became pregnant following treatment with IVF (group 1) or ICSI (group 2), in the year 1995, at the Reproductive Medicine service of the Institut Dexeus, Barcelona, Spain. In total, 1136 patients started a cycle of IVF or ICSI, resulting in 1026 oocyte collections.

Technical procedures relating to methods of ovarian stimulation, oocyte collection, embryo culture and transfer, sperm preparation, and microinsemination procedures were reported earlier (12,18). ICSI was performed in cases of male-factor fertility problems with impaired semen parameters or unexplained fertilization failure. Ejaculated semen was used in 90.1% of the cases, 2.7% had a microsurgical epididymal sperm aspiration, and 7.2% had a testicular sperm extraction.

Azoospermic men were offered chromosomal analysis, and in cases of congenital absence of the vas deferens, couples were screened for cystic fibrosis mutations. These couples were informed about their possible risks of cystic fibrosis and chromosomal abnormalities and subsequently a prenatal diagnosis was offered.

Couples undergoing IVF or ICSI were also offered the possibility of prenatal chromosomal diagnosis by chorion villus biopsy or amniocentesis in all cases of ICSI and advanced maternal age (IVF and ICSI).

We collected data from medical records on (a) early embryonic development, (b) obstetric outcome, (c) perinatal outcome, and (d) follow-up of children born after IVF or ICSI. In all couples included in this study oocytes had been fertilized by either IVF or ICSI. In 11 couples oocytes had been fertilized by either IVF or ICSI. Both treatments were initiated because of mild impaired semen parameters. In this group of patients it was not possible to determine the origin of the developing embryo and they were excluded from the study.

Two hundred thirty-three women had an ongoing pregnancy defined by a positive ultrasound after 8/9 weeks of gestation. Spontaneous abortion ($n = 40$) or an ectopic pregnancy ($n = 3$) occurred in 43 of 233 pregnancies. Patients were phoned and asked for cooperation when pregnancies proceeded beyond 20 weeks gestation. They were asked for additional information about obstetric outcome, perinatal outcome, and follow-up of the children as noted by their medical doctor. In cases of any abnormality more information was collected from the gynecologist and/or the pediatrician. With the use of standardized questionnaires we were able to collect complete data on 183 (92%) of 190 pregnancies proceeding beyond 20 weeks of gestation.

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 (19). Weights, lengths, and head circumferences at birth and at the time of this study were compared to the Catalan national scale (20). Physical and mental development were also compared to this scale at the age of 1, 3, 5, 7, 9, 12, 15, and 18 months by a pediatrician. Furthermore, additional information about development was evaluated by standardized telephonic questionnaires compared to the age of the children (0.5 until 1.5 years old).

Data were statistically analyzed using Student's t test for comparison between ICSI and IVF. A P value of < 0.05 (two-tailed) was taken to represent statistical significance.

RESULTS

Overall the mean maternal age was 33.67 years (range, 25–44; SD, 3.83): for IVF, 34.3 years (range, 25–44; SD, 3.94); and for ICSI, 33.1 years (range, 25–44; SD, 3.63). The mean maternal age in couples treated with ICSI was significantly ($P < 0.05$) lower than in the IVF-treated couples. The mean paternal age was not different in IVF or ICSI and was overall 35.9 years (range, 25–69; SD, 5.38).

Preconceptional risk and risks during pregnancy such as smoking, medication, and diseases during pregnancy were equally divided in both groups.

Early Pregnancy Data

The fertilization rate was lower after ICSI (50.6%) than conventional IVF (66.4%), which is probably due

Table I. Abnormal Prenatal Karyotypes

Result	Origin	Outcome	Maternal age	Paternal age
45, XY, t(13q, 14q)	Inherited father	Healthy live born	36	39
45, XO	De novo	Selective abortion[a]	31	33

[a] Twin pregnancy; the other fetus had a normal karyotype.

to the learning process of ICSI. Embryo quality (21) was the same in IVF and ICSI cycles.

The ongoing pregnancy rate per transfer was 27% after IVF and 23.2% after ICSI.

Vanishing twins and triplets defined by the decrease in the number of gestational sacs and/or fetal heart activity per ongoing pregnancy was 9 of 120 (7.5%) in IVF and 7 of 113 (6.2%) in ICSI. The rates of spontaneous abortion before 20 weeks of gestation were 22 of 120 (18.3%) in IVF and 18 of 113 (15.9%) in ICSI.

Ectopic pregnancy, an ongoing pregnancy implanted outside the uterus, was observed after IVF in only 3 of 120 (2.5%) pregnancies.

The overall ongoing pregnancy rate after 20 weeks of gestation was 190 of 233 (81.5%).

Chromosomal Analysis

One chromosomal abnormality, 45,XY, t(13q, 14q), was found in 1 of 23 azoospermic men (4.3%). This couple at risk of inheriting a chromosomal abnormality was informed about possible risks and a prenatal chromosomal diagnosis was offered when a viable pregnancy was established.

Prenatal diagnosis was performed using amniocentesis in all cases. The rate of couples undergoing this diagnosis was 57 of 190 = 30%, the same rate in both groups. Although this screening was offered in all cases of ICSI, advanced maternal age was the main reason after IVF or ICSI. In two cases of ICSI pregnancies, amniocentesis showed an abnormal karyotype. One was inherited from the father and one was de novo. The rate of a de novo chromosomal abnormality per performed karyotype detected by prenatal diagnosis karyotype is 1 of 28 (3.6%) after ICSI (Table I).

Obstetric Outcome

The overall mean gestational age was 38 weeks (range, 25–42; SD, 2.82) and did not differ between the two groups. The rate of multiple pregnancy was the same in IVF and ICSI. Overall 120 of 183 (65.6%) were singletons, 57 of 183 (31.1%) twins, and 6 of 183 (3.3%) triplets. Prematurity, live birth before 37 weeks of gestation, occurred in 22 of 96 (22.9%) after IVF and in 15 of 87 (17.2%) after ICSI. Prematurity was associated mainly with multiple pregnancy, and subsequently there was an increase in problems occurring at birth, 16% in twins and 43% in triplets. Problems occurring at birth were the same in the groups of children born after IVF, 12 of 132 (9.1%), or ICSI, 11 of 120 (8.3%). All these children needed to spend some time in the incubator for observation due to prematurity. Additional problems at birth were of respiratory (n = 5), cerebral (n = 2), and intestinal (n = 1) origin.

The mode of delivery is summarized in Table II. The rate of deliveries without any problems (spontaneous vaginal and elective cesarean deliveries) was 66.7%. The remaining cases had instrumental vaginal or urgent cesarean deliveries. No statistical differences were discovered between the two groups.

Perinatal and Neonatal Data

The number of children born from 183 pregnancies beyond 20 weeks of gestation was 252 (132 IVF and 120 ICSI).

Stillbirth, intrauterine fetal death after 20 weeks of gestation, was 4 of 132 (3.0%) and 1 of 120 (0.8%) after IVF and ICSI, respectively. The total of live borns was 247:128 after IVF and 119 after ICSI.

The early neonatal death, death of a live-born infant during the first days after birth, was 1 of 128 = 0.8%

Table II. Mode of Delivery

Mode of delivery	IVF (n = 96)	ICSI (n = 87)	Total (n = 183)
Spontaneous vaginal	37/96 (38.5%)	27/87 (31.0%)	64/183 (34.9%)
Instrumental vaginal	6/96 (6.3%)	8/87 (9.2%)	14/183 (7.7%)
Elective cesarean section	28/96 (29.2%)	30/87 (34.5%)	58/183 (31.7%)
Cesarean section on indication	25/96 (26.0%)	22/87 (25.3%)	47/183 (25.7%)

Table III. Perinatal Mortality

Definition	IVF	ICSI	Total
Stillbirth	4/132 (3.0%)	1/120 (0.8%)	5/252 (2.0%)
Early neonatal death	1/128 (0.8%)	2/117 (1.7%)	3/245 (1.2%)
Perinatal mortality	3.8%	2.5%	3.2%

in IVF and 2 of 117 = 1.7% in ICSI. Tables III and IV show the perinatal mortality cases, the sum of stillbirth and early neonatal death (no statistical differences).

The female/male ratio was equally divided and there were no differences in children born after IVF versus ICSI.

Neonatal measurements for 252 children are listed in Table V. Birth weight and head circumference compared to the gestational age were not significantly different between the groups. Children born after ICSI were significantly (1.1 cm) less tall than children born after IVF. However, this difference was within the normal range of length compared to gestational age in the national catalan register.

Congenital malformations are listed in Table VI. The rate of major congenital malformation was 4 of 132 (3.0%) after IVF and 2 of 120 (1.7%) after ICSI, the differences being nonsignificant. Minor malformations were not observed in medical reports or in telephonic standardized questionnaires.

Follow-Up of Children Born After IVF and ICSI

All children surviving the perinatal period ($n = 244$) were between 0.5 and 1.5 years old at the time of the study. Follow-up data were compared to the Catalan national developmental scale. Measurements of weights, lengths, and head circumferences of the children at the time of the study were not significantly different between IVF and ICSI.

Table IV. Clinical History of Perinatal Mortality

Number of children	Gestational age	IVF/ICSI	Cause
1	34.5	IVF	Intrauterine growth retardation in twin pregnancy
2	25	IVF	Cervical incompetence
1	41	IVF	Fetal asphixia (occlusion of birth cord)
1	37.5	IVF	Exencephaly: died 3 days after birth
1	28.5	ICSI	Abruptio placenta
2	26	ICSI	Cerebral hemorrhage; died 2 and 4 days after birth[a]

[a] In this triple pregnancy the third child died of hydrocephalus at 4 months of age.

Table V. Neonatal Measurements

Method	Birth weight (g)	Birth length (cm)	Head circumference at birth (cm)
IVF ($n = 132$)	2939	48.9*	34.6
SD	695.7	2.7	1.9
ICSI ($n = 120$)	2857	47.8*	33.8
SD	575.3	3.6	2.0
Total ($n = 252$)	2898	48.4	34.2
SD	654.7	3.3	2.0

* $P = 0.025$ (t test).

Problems occurring during the development of children born after IVF and ICSI are divided into physical problems and mental developmental problems. We found no significant differences between IVF and ICSI for physical and/or mental development. Developmental problems, both physical and mental, were discovered in 3 of 127 (2.4%) children after IVF and in 4 of 117 (3.4%) after ICSI. The developmental delays were minor and of unknown origin.

There were two children who needed medical treatment: one child born after IVF had recurrent convulsions and mental developmental delay and one child born after ICSI needed treatment because of hypothyroidism.

DISCUSSION

It is important to accumulate information about ICSI, whether the zygotes develop normally into chil-

Table VI. Congenital Malformations

Malformation		
IVF	ICSI	Total
4/132 (3.0%)	2/120 (1.7%)	6/252 (2.4%)
Exencephaly	Pes equinovarus	
Hemivertebrae	Hydronephrosis[a]	
Hydronephrosis[a]		
Vesicoureteric reflux[a]		

[a] Clinical evaluation showed no gross anatomical genitourinary tract malformation.

dren who do not show more abnormalities at birth and during their development. This is important not only because of the risks on theoretical grounds, but also because of the novelty of this technique.

The effects of micromanipulation during ICSI are probably limited. Our results did not show any difference in the early embryonic development between the two groups. This has also been shown in animal models (22,23). This study and others (12,24) indicate a limited effect on early embryonic development. However, deleterious effects could still be discovered and there is a risk of parthenogenetically activating an oocyte. This could induce a higher incidence of maternally derived chromosomal abnormalities, especially in women of advanced age.

We did not discover any significant differences between IVF and ICSI for obstetric outcome, perinatal outcome, and follow-up of children born after ICSI. The congenital malformation rate after ICSI was not significantly different from that after IVF and seemed to be in the range of the national register, 1.6% (25).

The relatively high rate of cesarean section, prematurity, and problems at birth in our study could be caused by increased maternal age and/or multiple pregnancy due to multiple embryo transfer. The high rate of cesarean section after IVF or ICSI might also be due to the final decision of the physician and couple, choosing, in their opinion, the best method of delivery after long-term fertility problems.

At this moment follow-up studies (14,17,19,26–29) show a normal obstetric outcome, no increase in congenital malformations, and normal development of children born after ICSI. However, there are some concerns that ICSI might somehow be responsible for an increased frequency of chromosomal abnormalities and fertility problems in children born after ICSI (10,14,30). At present they do not show phenotypic abnormalities in children born after ICSI (17). Moreover, Bowen *et al.*. (16) showed a significant developmental delay in children born after ICSI. Our results show normal development of children born after ICSI, but the national Catalan developmental scale (20) may not be accurate enough to detect developmental impairment after ICSI.

Prospective studies are prone to detect a higher rate of abnormalities than retrospective studies (31). We did not discover any minor malformation, which could be due to our retrospective study design. Comparing data on an international basis can be influenced by the difference in data collection. Most centers have only limited and retrospective study on the risks of ICSI (26). Conclusions on the risks of ICSI can thus be controversial (14,16,17,19,26).

Prenatal chromosomal diagnosis in our center in the year 1995 was 30% in both groups, although couples were informed about the genetic risks of ICSI. A low uptake of prenatal diagnosis in ICSI was reported earlier (32). Because of the risk of miscarriage, the present follow-up results, and the unknown phenotypic consequence of some chromosomal abnormalities, people may decide not to have a prenatal chromosomal diagnosis. In the prospective study from Brussels (14) the uptake of prenatal diagnosis was 55%.

We discovered one case of a de novo chromosomal abnormality (1/28; 3.6%). The numbers are too small to be of statistical value. Small numbers and screening patients at high risk could be responsible for the high frequency of chromosomal abnormalities (30).

Male candidates for ICSI should have a detailed genetic workup: family history, chromosomal analysis, detection of genes involved in spermatogenesis, and genetic counseling (33). It is important to indicate the group at risk for "genetic" abnormalities, although their origin is difficult to detect (14,34). The affected couple needs to understand the risks for the offspring and what preventive measures can be taken. In some cases of genetic abnormalities the couple can be offered a prenatal diagnosis (35,36).

More investigation is needed concerning genetic causes in males selected for ICSI and the risk(s) for the offspring. Our results show that ICSI is a safe procedure, but international prospective follow-up is still mandatory.

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