Comparison of pregnancy outcome after intracytoplasmic sperm injection and in-vitro fertilization*

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The aim of this study was to compare pregnancy characteristics and perinatal outcome of intracytoplasmic sperm injection (ICSI) pregnancies with pregnancies obtained after in-vitro fertilization (IVF). Retrospectively, 145 ICSI pregnancies were matched with 145 IVF pregnancies using the last menstruation data. The main outcome measures were preclinical and clinical abortions, ectopic pregnancies, multiple gestations, prenatal morbidity, prematurity, Caesarean section, birthweight, perinatal mortality and malformations for singletons, twins and triplets. Although patients were significantly younger (P < 0.001) in ICSI (31 years) than in IVF (33 years), their infertility duration (5 years) was similar. The mean number of transferred embryos (2.7 embryos per transfer) was similar in IVF and ICSI. The rates of preclinical (15%) and clinical abortions (11% in ICSI versus 15% in IVF) were not different. Four ectopic pregnancies were observed in the IVF group and none in the ICSI group. In ICSI, two minor malformations were detected and two therapeutic abortions were performed respectively for polyamorphinations and suspicion of cystic fibrosis. The rate of congenital malformation was 2.8% in ICSI and 2.2% in IVF. In this last group, one therapeutic abortion for malformation of neural tube was performed and two minor malformations were detected. The rate of aborted embryonic sacs before 16 weeks of gestation was not significantly lower in ICSI compared with IVF (13.7% versus 20%). The rate of multiple gestations was similar in both groups (31% in IVF and 35% in ICSI). The number of Caesarean sections was similar in IVF and in ICSI and was twice as frequent for twins versus singletons. The number of singletons born by Caesarean section was 21% after ICSI and 17% after IVF. Mean birthweights and gestational ages at birth for twins were significantly higher (P < 0.05) in ICSI than in IVF (2488 versus 2281 g and 36.5 versus 35.5 weeks). This difference was not observed for singletons. In conclusion, pregnancy characteristics and perinatal outcome after ICSI showed no increase in the number of pathologies in comparison with IVF.

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Materials and methods

Between September 1993 and January 1996, 145 ICSI and 145 IVF pregnancies, which had resulted from assisted reproduction techniques in our fertility clinic, were matched using the last menstruation data. Among the ICSI pregnancies, one after micro-epididymal sperm aspiration (MESA) and four after testicular sperm extraction (TESE) were included. Pregnancies obtained after frozen and thawed embryo transfers (either with IVF or ICSI) were excluded from the analysis. Clinical IVF indications were tubal disease, endometriosis, endocrinopathy, polycystic ovarian disease, immunological and cervical infertility, male subfertility, idiopathic infertility, combined pathologies, and long-standing infertility with failure of other medical and surgical treatments. Male subfertility was defined by a spermogram showing one or more abnormal parameters including number, motility and morphology of spermatozoa. ICSI indications included fertilization failure or severe oligoasthenoteratozoospermia (total motile spermatozoa count after preparation <40 000). Screening before treatment included systematic detection of the nine most frequent mutations of cystic fibrosis and fragile X mutation for women. In the

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The ovarian stimulation protocol was performed with gonadotrophin-releasing hormone analogue (buserelin acetate, Suprefact spray; Hoechst Inc., Frankfurt am Main, Germany), human menopausal gonadotrophin (HMG; Humegen, Organon, Oss, The Netherlands; Pergonal, Serono, Aubonne, Switzerland) and human chorionic gonadotrophin (HCG; Pregnyl, Organon; Profasi, Serono). The ovarian stimulation and oocyte retrieval procedure through vaginal puncture under ultrasound guidance, as well as embryo culture in modified Earle’s balanced salt solution (EBSS) and transcervical replacement, have been described elsewhere (Englert et al., 1992). The ICSI procedure used was based on the technique of Palermo and has also been described previously (Van den Bergh et al., 1994).

Embryos of the highest quality were selected for transfer and no more than three embryos were replaced 48 h after oocyte retrieval (Devreker et al., 1996). All patients were given luteal phase supplementation by the daily administration of 100 mg of natural progesterone (Sterop Laboratories, Brussels, Belgium) up to 8 weeks gestation.

Pregnancies were diagnosed by at least two positive HCG measurements. Preclinical pregnancy was considered if β-HCG 11 days after oocyte retrieval was positive and if the second value of β-HCG 14 days after oocyte retrieval was higher than the first. Echographic screenings with 5 MHz transvaginal ultrasound were performed on days 25, 31, 38, 45 and 52 after oocyte retrieval. Clinical pregnancies were confirmed by the observation of a gestational sac at ultrasound. The duration of ICSI or IVF pregnancies was calculated from the day of oocyte retrieval to the day of termination of pregnancy or delivery, with an addition of 14 days. Early abortion was defined as clinical pregnancy loss that occurred before 16 weeks. More advanced pregnancy loss up to 26 weeks gestation was considered as late abortion. A stillbirth was defined as intrauterine fetal death that occurred after week 26. Premature delivery was defined as live birth before week 37 of gestation. Major malformation was defined as a condition requiring surgical correction or causing functional impairment (Bondeulle et al., 1996). To assess the congenital abnormalities, paediatric reports (examination of the infant in the maternity or in the neonatal intensive care unit) were used, there being a systematic examination of the baby at birth by a paediatrician in the hospital where the mother delivered.

Ethical aspects and follow-up of pregnancy

Patient counselling included information about the novelty of this procedure of assisted fertilization. Patients gave written informed consent which included an agreement to prenatal diagnosis by chorionic villus sampling (CVS) or amniocentesis, as well as a clinical examination of children born after ICSI. The protocol was reviewed and approved by the local ethical committee of the medical campus of Brussels French-speaking Free University. Follow-up of pregnancies was reported by the gynaecologists to our unit by means of a questionnaire.

Statistical analysis

Statistical analysis included Student’s t-test and χ² test when required. A difference at the 5% level of significance was considered the threshold of probability. Computations were performed using the Statistics Package for Social Sciences software for Windows 95.

Results

Although the mean age of patients in ICSI (31 years) was significantly lower than in IVF (33 years) (P < 0.001), the mean infertility duration (5 years) was similar in both groups. The characteristics (infertility factors, age, parity) of the women and their partners in both groups are listed in Table I. The mean number of transferred embryos was similar in both groups (2.7 embryos per transfer). Numbers of preclinical abortions, miscarriages, late abortions, ectopic pregnancies and deliveries are summarized in Table II.

Rates of abortions of preclinical and clinical pregnancies were similar in ICSI and in IVF. This study did not demonstrate an increasing risk of anembryonic pregnancy (i.e. the development of a gestational sac without any signs of embryonic development) or of first-trimester abortions. There were four late abortions in ICSI and two in IVF. In both groups the causes of late abortions were cervical incompetence or premature rupture of the membranes. Four ectopic pregnancies occurred in IVF and none in ICSI (P = NS).

The probability of developmental arrest of each intrauterine sac (represented by the ratio between the number of miscarriages and vanishing sacs divided by the total number of sacs) was not different in ICSI and in IVF (14.3 and 20% respectively).

The rate of multifetal pregnancies was similar in ICSI and in IVF (Table II). Embryo reduction was performed for two triplet pregnancies in both groups and triplets with embryo reduction were counted as twins. The outcome of singleton, twin and triplet pregnancies after ICSI and IVF were compared separately. For singletons, there was no difference in prematur-

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<th>Table I. Clinical characteristics of patients and their partners</th>
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<td>Women with infertility factors (%)</td>
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<td>Age of women (years)</td>
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<td>Nulliparous women (%)</td>
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| Values in parentheses are percentages. |

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<tr>
<th>Table II. The outcome of intracytoplasmic sperm injection (ICSI) and in-vitro fertilization (IVF) pregnancies</th>
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<td>Therapeutic abortion</td>
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*One or more abnormalities at spermiogram.

fertilization failure in previous IVF trial.

ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilization.

| Values in parentheses are unknown. |

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<th>ICSI IVF</th>
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<td>Preclinical abortions</td>
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<td>Born infants</td>
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*One outcome of triplet was unknown. |

Values in parentheses are percentages.
Twin IVF 1 (1.6) 3 (4.8) 59 (95) 3092
ICSI twins were significantly higher than in IVF twins (*P* triplet) grouped according to gestational age at birth

*Significance at *P* < 0.05.

The gestational ages of one ICSI and one IVF singleton were unknown.

**Table V. Complications during pregnancy for ICSI and IVF singletons and twins**

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<th>Singleton</th>
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<tr>
<td></td>
<td>ICSI (%)</td>
<td>IVF (%)</td>
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<tr>
<td>Hospitalization</td>
<td>8 (11.9)</td>
<td>8 (12.1)</td>
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<tr>
<td>Hypertension</td>
<td>6 (9.0)</td>
<td>5 (7.6)</td>
</tr>
<tr>
<td>Placenta praeviaa</td>
<td>1 (1.5)</td>
<td>1 (1.5)</td>
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<tr>
<td>Placental abruptiona</td>
<td>1 (1.5)</td>
<td>1 (1.5)</td>
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<tr>
<td>PROM</td>
<td>2 (3.0)</td>
<td>3 (4.5)</td>
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<tr>
<td>SGA</td>
<td>1 (1.5)</td>
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*Haemorrhage was present and for one of these pathology was diagnosed. PROM, premature rupture of membranes; SGA, small for gestational age (<5th percentile).*

For twins, the ICSI group was delivered one week later than IVF (*P* < 0.05) (Table IV). Thus, the birthweights in ICSI twins were significantly higher than in IVF twins (*P* < 0.05) (Table III). The outcome of the two triplets was similar in both groups (Table IV). The mean birthweights of triplet babies were in accordance with gestational age: 1604 ± 126 g for IVF and 1775 ± 214 g for ICSI (*P* = NS).

Complications such as preterm labour requiring hospitalization, bleeding after placenta praevia or placental abruption, hypertension associated with pre-eclampsia as well as premature rupture of membranes and small for gestational age are summarized in Table V. There were no pathological differences between ICSI and IVF singleton pregnancies. More obstetric complications occurred in twins compared with singletons in ICSI and in IVF. In twins, some differences that did not reach statistical significance appeared. There were more prenatal hospitalizations for risk of preterm labour and premature rupture of membranes in IVF twin pregnancies compared with those after ICSI. These data were in accordance with the lower gestational age at delivery in the IVF twin group (35.5 weeks).

Delivery by Caesarean section was more frequent in twin pregnancies (triplets were not included as all were delivered by Caesarean section) than in singletons. The number of Caesarean sections was similar in ICSI and in IVF for twins (41 and 33% respectively) and for singletons (21 and 17% respectively).

In the ICSI group, one baby died intrapartum. This was a twin pregnancy, the baby was small for gestational age, and an emergency Caesarean section was performed for fetal distress. In IVF, one infant died at 4 months, the diagnosis being sudden death. Two therapeutic abortions (2/141; 1.4%) were performed in the ICSI group; one had renal malformation with fetal hydrops and oligohydramnios and the other was aborted for high suspicion of cystic fibrosis after echographic and biochemical analysis. The mother carried the DF508 mutation; the father had no detected mutation and the indication of ICSI was severe oligoasthenotatozoospermia. Further analysis revealed that the fetus carried the mutant gene of his mother. In the IVF group, one therapeutic abortion (1/126; 0.9%) was performed for a complex neural tube malformation supposedly of recessive origin (one affected child was born 12 years earlier). In both groups, all the karyotypes were normal. Among the 141 infants born after ICSI, only two minor malformations were observed. One infant who was a twin had an interauricular communication discovered at birth, though the malformation was not detected at ultrasound during pregnancy. The other baby presented perineal hypospadias. Among the 126 infants of the IVF group, only two minor malformations were also observed (1.6%). One infant was small for gestational age and had an auricular appendix. There were abnormalities in the brain evoked auditory response, but a control was needed as the condition may have been due to a dysmaturity linked to the infant’s small for gestational age. The other infant presented an umbilical hernia. The four karyotypes of these ICSI and IVF children were normal. The congenital malformation rate represented by the ratio between the number of malformations at birth and the number of therapeutic abortions divided by the total number of live births and stillbirths was similar in ICSI (2.8%) and in IVF (2.2%).

Among the 141 ICSI infants, 101 karyotypes (71.6%) were known while for the other 40 infants parents refused amniocentesis or CVS because of the risk of miscarriage. Physical examinations of these infants at birth were normal. Of the 101 karyotypes, four (4%) were abnormal. In one triplet...
pregnancy embryo reduction of the smaller embryo was performed. Cytogenetic analysis of this embryo revealed triploidy, though this was the only de-novo abnormality. The other three abnormalities were inherited from the father (carrier of the robertsonian translocation). Of these, one singleton had the same balanced translocation as his father, and one case was twins, one of whom had an unbalanced translocation while his brother had a balanced translocation. A selective embryo reduction of the abnormal fetus was performed. In the last case, also concerning twins, CVS was performed and showed that one fetus carried the balanced translocation. Unfortunately, the patient aborted both twins at 13 weeks (one month after CVS). In IVF, only eight karyotypes were available (amniocentesis for maternal age) and were normal. For one ICSI singleton a prenatal diagnosis for fragile X was performed as the mother was found to be a carrier of a premutation at the fragile X locus (one normal locus and one normal). The fetus was male and a carrier of his mother’s normal locus.

Discussion

Although first introduced in the early 1990s, ICSI is currently performed in hundreds of IVF centres world-wide, and an estimated 5000 ICSI children have now been born following this procedure. However, ICSI bypasses all natural sperm selection processes and the long-term implications for the life expectancy and fertility of the children are unknown (Cummins et al., 1995). It is important to compare the outcome of ICSI pregnancies, the rates of major and minor congenital malformations, and the karyotypes of children with those resulting from IVF. Wisanto et al. (1995) considered that the comparison of pregnancy outcome after ICSI and IVF was not straightforward. ICSI patients represent an almost homogenous group in which the majority of female partners are perfectly fertile. On the other hand, IVF patients are more heterogeneous, with both male and female factors represented. In this study 70% (101/145) of the IVF men presented one or more sperm abnormalities with or without abnormal sperm-mucus penetration. Only 42% of women in ICSI were perfectly fertile in this study. The ICSI group was heterogeneous versus the IVF group. The frequency of nulliparity observed in ICSI was as high as that reported by Wennerholm et al. (1996). As described in the literature (Wisanto et al., 1995), the incidence of ectopic pregnancy was low (0.9% and none in this study). In contrast, some authors observed 5% ectopic pregnancy after IVF, a finding which supports the idea of tubal damage as the underlying cause in the pathogenesis of ectopic pregnancy after IVF (Wisanto et al., 1995). As described in other studies (Wennerholm et al., 1996), the rate of abortion was similar in ICSI and in IVF and also to that seen in the general population. In evaluating the safety of the procedure, the number of vanishing sacs over the total number of sacs is a good parameter (Govaerts et al., 1996). Wisanto et al. (1995) reported 8.5% vanishing twins and triplets and 11.3% miscarriages. This rate of 20% is similar to those reported in the present study for ICSI (14%) and IVF (20%). Complications reported by Wennerholm et al. (1996) and Wisanto et al. (1995) in ICSI pregnancies (prematurity, low birthweight, small for gestational age and hypertension) and in spontaneous pregnancies are summarized in Table VI. The rate of these complications among singletons was similar in ICSI pregnancies and in the general population. Nevertheless, many reports show a high incidence of preterm delivery, low birthweight and small for gestational age infants among IVF singletons (Rizk et al., 1991; Tan et al., 1992). Risk factors such as increased maternal age, lower previous parity and the underlying cause of infertility have been suggested, though these data were not observed in our study. Previous studies of obstetric outcomes in IVF (Doyle et al., 1992; Tan et al., 1992) have reported a higher incidence of multiple pregnancy with their complications (preterm delivery, low birthweights and high rate of Caesarean sections). In this study, 35% and 31% of ICSI and IVF deliveries respectively were multiple. Mean birthweights and mean gestational ages were significantly lower in twins than in singletons. Nevertheless, mothers of ICSI twins delivered later (mean 36.5 weeks gestation) than IVF ones (mean 35.5 weeks) and birthweights were significantly higher (2488 g in ICSI and 2281 g in IVF; P < 0.05). These data were not found in singletons and need to be confirmed in a larger group of subjects. Rates of Caesarean section were higher in ICSI and IVF than in the general population, even if multiple gestations were excluded, though this phenomenon could be linked to the stress of the obstetrician.

An important question debated about ICSI infants is the risk of congenital malformations, with several studies having
reported similar rates of congenital malformations for both IVF pregnancies and the general population (2.2% versus 3.3%) (Alsalili et al., 1995). The rates of congenital malformations in ICSI infants reported by several authors are summarized in Table VII and are similar to those of most national registries and assisted reproduction surveys.

Minor malformations were found in 87 of the 423 children born after ICSI (Bonduelle et al., 1996). These authors reported 19 minor heart problems which were detected at routine heart ultrasonography performed on ICSI babies born intramuros. As with one case detected in this study, all were transient or not expected to need surgical intervention. Leppig et al. (1987) found 39.9% of children with one or more minor anomalies in a survey of 4305 newborns from a population of fertile women. Two minor malformations in the ICSI (2/141) and IVF (2/126) groups were found in this study (4/267; 0.07%). The hypospadias of the ICSI baby did not require surgical intervention. For the newborn IVF twin who had inguinal hernia and alteration of the brain evoked auditory response, these anomalies were considered by the paediatricians as complications of being small for gestational age (delay in the normal development).

In’t Veld et al. (1995) reported five chromosomal anomalies in 15 pregnancies tested because of advanced maternal age, though this rate of 33% seems to be the result of a small series and was not confirmed by other authors (Table VII). Bonduelle et al. (1996) found one abnormal karyotype among 293 fetuses, and four cases (1.3%) with benign familial structural aberrations, all of which were inherited from their infertile fathers and thus certainly not induced by the microinjection technique. In this study, the three structural anomalies inherited from the fathers were reciprocal translocations. Robertsonian or reciprocal translocations are found >10-fold more in men presenting with hypofertility than in the general population (Fraccaro, 1983). Aberrations occurring in the paternal karyotypes are expected at 0.5% in the general population (Moosani et al., 1995). These structural aberrations involve high risks for the fetuses and it is imperative to perform paternal pretreatment genetic screening and antenatal diagnosis in these cases. The large series (585 karyotypes) of Liebaers et al. (1995) reported 1% chromosomal anomalies of which five out of six were sex chromosome anomalies after ICSI. In this study, no sex chromosome anomalies were detected but a larger study is necessary to verify this. Recently, several studies (e.g. Moosani et al., 1995) have been devoted to defining key regions on the Y chromosome that are suspected to contain genes which have important roles in spermatogenesis. The frequency of Y microdeletions is higher in populations of 32 infertile ICSI father/son pairs (9.4%) than in the general population (Moosani et al., 1995). However, time will tell if the process of assisting infertile couples to achieve conception also assists in producing males who, having inherited microdeletions from their fathers, will also be faced with infertility when they are adults.

In conclusion, the pregnancy characteristics and perinatal outcome of the ICSI and IVF babies were similar. These data are reassuring with regard to the safety of the ICSI procedure, and in this study no more congenital malformations and karyotype abnormalities were observed in ICSI. However, while some studies reported a slightly higher incidence of sex chromosome abnormalities after ICSI, others (e.g. Moosani et al., 1995) suggested that male infertility is partly due to genetic origin, even when the karyotype is normal. However, karyotypes of men and ICSI babies should always be made available. In the future, other techniques such as screening the DNA of infertile men to detect some anomalies could be available.

References

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