

A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection

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Objective: To compare the karyotype of babies conceived through ICSI with that of naturally conceived babies.

Design: Prospective controlled study.

Setting: The Egyptian IVF-ET Center, Cairo, Egypt.

Patient(s): Four hundred and thirty babies conceived through ICSI and 430 babies conceived naturally.

Intervention(s): ICSI and karyotyping.

Main Outcome Measure(s): Abnormal karyotype.

Result(s): Four hundred and thirty consecutive babies conceived through ICSI who were delivered in one hospital had 15 abnormal karyotypes (3.5%). Of the 15 babies, 7 were of female phenotype and 8 of male phenotype. Six babies had sex chromosome anomalies, 8 had autosomal anomalies, and 1 had combined sex chromosome and autosomal anomalies. A control group of 430 consecutive babies conceived naturally who were delivered in one hospital had no abnormal karyotype. The difference between the two groups was significant ($P < .001$).

Conclusion(s): ICSI carries a small but significant increased risk of abnormal karyotyping to the offspring. This risk appears to be equally distributed between autosomal and sex chromosome anomalies. (Fertil Steril® 2001;76:249–53. ©2001 by American Society for Reproductive Medicine.)

Key Words: Karyotype, prospective study, ICSI

The introduction of ICSI brought great concern about the safety of the procedure (1). Since the mid-1990s, infertility centers worldwide have studied the outcome of ICSI pregnancies. These studies revealed no increase in the incidence of congenital anomalies (2–6). The obstetric outcome of ICSI was found to be similar to that of conventional IVF and other assisted reproduction techniques (7, 8). Mental and physical development have also been studied (9–11).

The incidence of chromosomal aberrations in ICSI babies compared with the general incidence in national registries has been investigated (6, 12–15). A tendency toward an increased rate of chromosomal anomalies in ICSI babies compared with rates in national regis-

tries has been observed, yet the rates reported by various investigators differ widely. Bonduelle et al. (6) reported the rate of chromosomal anomalies to be 2.6%, whereas in't Velt et al. (12) found it to be 33% in a small series of ICSI babies. In a larger study, van Opstal et al. (13) found a rate of 12%, and Loft et al. (14) reported a rate of 3.4%. Although thousands of children conceived through ICSI have been born, the number of infants in reported outcome studies is still small (15). All investigators have stressed the need for more information and further study. We therefore sought to clarify the incidence of chromosomal anomalies after birth in babies conceived through ICSI and compare it with the incidence in a control group of naturally conceived babies.

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MATERIALS AND METHODS

We performed a prospective controlled study on the chromosomal makeup of babies conceived through ICSI. The study protocol was approved by our institutional review board. Our study was designed to have adequate statistical power to detect a 2.5% difference in chromosomal anomalies between the ICSI group and the control group. The planned sample size of 430 patients per group provided 80% power and a two-sided significance level of 0.05 to detect such a difference. Informed consent was obtained from parents of all babies included in this study.

Babies Conceived Through ICSI

From January 1997 to December 1999, we enrolled women who became pregnant after ICSI in our center and who were observed by the obstetricians of our center during pregnancy and delivery at one hospital. All ICSI pregnancies from our center, which were observed by other obstetricians inside or outside the country, were excluded. The ICSI group included 430 babies born from 320 consecutive deliveries. In these 320 couples, the procedure was performed by using ejaculate sperm in 275 couples with male factor infertility and surgically retrieved sperm in 45 couples, including 36 with obstructive azoospermia and 9 with nonobstructive azoospermia. Karyotyping was performed for all babies. Cordocentesis was performed by using a heparinized hypodermic syringe to draw 5 mL of blood from the fetal end of the cord at birth, or a peripheral blood sample was taken by performing venipuncture with a heparinized hypodermic syringe.

Controls

Controls were 430 babies born from 418 consecutive deliveries at Cairo University Hospital, Cairo, Egypt. All pregnancies resulted from natural conception. Karyotyping was performed for all babies by using the same methods as those for babies conceived through ICSI. Before the start of ICSI and after counseling, karyotyping for parents was suggested but was not done routinely. Karyotyping was done for 6 parents in whom an abnormal karyotype was detected in offspring. The remainder of the parents of babies with abnormal karyotypes declined karyotyping. Our standard stimulation protocol (16) and ICSI technique (17) were performed as described elsewhere. Karyotyping was done at our associated cytogenetics laboratory by using the method of Verma and Babu (18).

RESULTS

The ICSI group included 430 babies, of whom 220 were singletons, 198 were from twin pregnancies, and 12 were from triplet pregnancies. Two hundred and six were male and 224 were females. The mean maternal age was 30 ± 5.2 years (range, 17–41 years). Eighty-nine (30.6%) mothers were older than 35 years of age. The mean paternal age was

TABLE 1

Abnormal karyotypes in babies conceived through ICSI.

| |
|-----------------------------|
| 45,XO |
| 47,XY+21 |
| 47,XXY |
| 47,XX+21 |
| 47,XYY |
| 47,XXX(15)/46,XX(5) |
| 46,XY,t(3;15)(p13;p11.2) |
| 46,XY,t(7;18)(q31.3;q23) |
| 46,XX,inv(3)(p24.2,p25.3) |
| 45,XX,der(13;14)(q10;q10) |
| Chimerism 46XY,46XX |
| 47,XXY |
| 47,XXY,inv(3),t(4,8) |
| 46,XY,t(20;21)(p12.2;q11.2) |
| 47,XX+mar |

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38.9 ± 9.2 years (range, 26–55 years) overall and 37.8 ± 8.7 years in the subgroup of ICSI babies with abnormal karyotype; this difference was not significant.

Karyotyping was performed for all babies. Karyotype was normal in 415 babies (96.5%) and abnormal in 15 babies (3.5%). Table 1 provides more information on the abnormal karyotypes. Eleven babies from singleton pregnancies and 4 from multiple pregnancies had abnormal karyotypes. Of the 15 babies with abnormal karyotypes, 7 were of female phenotype and 8 of male phenotype. Six babies had sex chromosome anomalies, 8 had autosomal anomalies, and 1 had a combined sex chromosomal and autosomal anomalies (Table 1). Fourteen (3.6%) of 388 babies conceived through ICSI using ejaculated sperm and 1 (2.4%) of 42 of those conceived by using surgically retrieved sperm in nonobstructive azoospermia had abnormal karyotype.

Results of karyotyping of 6 parents of babies with abnormal karyotypes is shown in Table 2. The control group included 430 babies, of whom were 406 singletons and 12 twins; 236 were females and 194 were males. The mean maternal age was 28.5 ± 4.1 years (range, 18–39 years); 120 (29.1%) were older than 35 years of age. The mean paternal age was 37.2 ± 8.2 years (range, 24–52 years). The mean maternal and paternal ages and the percentages of patients older than 35 years of age did not differ significantly between the ICSI and control groups. Positive consanguinity was found in 31 of 320 couples (9.7%) in the ICSI group and 46 of 418 couples (11%) in the control group; this difference was not significant. All 430 control babies had normal karyotype. In this regard, the ICSI and control groups differed significantly (chi-square, 15.66; $P < .001$; relative risk, 31.0 [95% CI, 1.86–516.45]). Thirty-one of 320 couples (9.7%) in the ICSI group and 46 of 418 (11%) in the control group had positive consanguinity; this difference was not significant.

TABLE 2

Paternal and maternal karyotypes in 6 couples whose babies had abnormal karyotypes.

| Karyotype of baby | Paternal karyotype | Maternal karyotype |
|---------------------|---|--------------------|
| 47,XY+21 | 46,XY | 46,XX |
| 47,XXY | 46,XY | 46,XX |
| 47,XX+21 | 46,XY | 46,XX |
| 47,XX+mar | 45,XY,der (13;14)(q10;q10); balanced Robertsonian translocation | 46,XX |
| 46,XY,t(3,15) | 46,XY,t(3;21)(p13;p10); a rare case of jumping translocation | 46,XX |
| 47,XXX(15)/46,XX(5) | 46,XY | 46,XX |

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DISCUSSION

Several reports have shown that the incidence of congenital malformations is not increased after ICSI (3, 5). However, an increased incidence of chromosomal anomalies in babies conceived through ICSI has been reported (6, 12, 14, 19) (Table 3). The natural selection process of the spermatozoon does not occur in ICSI, and risk for injection of abnormal sperm may be increased, possibly causing a 10-fold increase in the incidence of gross chromosomal anomalies (20).

Apart from the invasiveness of ICSI, infertile men are known to have a higher incidence of chromosomal aberrations and thus produce spermatozoa with a higher rate of chromosomal abnormalities (21, 22). When azoospermia is present, peripheral blood chromosome analyses have traditionally yielded an 8% to 15% prevalence of chromosome abnormalities; this prevalence is even higher in patients with nonobstructive azoospermia (23). Studies of men with oligospermia have shown a smaller but significant increased rate of chromosome abnormalities consisting almost exclusively of autosomal translocations rather than numeric sex chromosome aneuploidies (24).

We performed postnatal study of the chromosomal makeup of ICSI babies to add further information. It has been reported that conventional IVF does produce an increase in chromosome anomalies compared with naturally conceived babies (25). For this reason and for practical purposes, we selected a control group of naturally conceived babies. In all published chromosomal studies of babies conceived through ICSI, data were compared with those from a national registry data or those from a retrospective matched group of babies conceived through IVF. Because the characteristics of the general population may differ from those of study patients, we used a control group that was as similar as possible to the study group (26) to try to reduce the effect of possible confounders.

We found that the rate of chromosomal abnormalities is significantly higher in babies conceived through ICSI than in those conceived naturally. The percentage of women older than 35 years did not differ between groups and thus does not account for differences in results of karyotyping, nor did the percentage of couples with consanguinity differ significantly between groups. Fourteen of 388 babies (3.6%) conceived through ICSI using ejaculated sperm and 1 of 42 babies (2.4%) conceived by using surgically retrieved sperm in nonobstructive azoospermia had abnormal karyotype. However, we could not compare these results because of the small number in the latter group. Our 3.5% rate of abnormal karyotype is slightly higher than the 2.6% rate reported by Bonduelle et al. (6) and is similar to the 3.4% rate reported by Loft et al. (14) (Table 3).

In 1995, in't Veld et al. (12) published an alarming report that cited a 33.3% rate of de novo chromosomal abnormalities among 15 babies born after ICSI. In a later publication (13), the same group reported a lower incidence of 12.8% among 71 babies. The investigators noted that their series consisted of a selected population referred for prenatal diagnosis and involved five different ICSI centers. It is clear that a reliable estimate of the frequency

TABLE 3

Rate of chromosomal anomalies among ICSI conceived babies reported in the literature.

| Study (reference) | No. of ICSI conceived babies | Abnormal karyotypes (%) |
|------------------------------|------------------------------|-------------------------|
| Bonduelle et al., 1999 (6) | 1,082 | 2.6 |
| Loft et al., 1999 (14) | 206 | 3.4 |
| in't Veld, et al., 1995 (12) | 15 | 33.3 |
| van Opstal et al., 1997 (13) | 71 | 12.8 |
| The present study | 430 | 3.5 |

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of chromosomal anomalies can only be derived from large prospective studies.

In 1999, Bonduelle et al. (6) found 28 babies with abnormal karyotype, 10 (0.9%) of whom inherited it and 18 (1.66%) of whom had de novo sex chromosome aberrations. This rate is significantly higher than that observed in the general population. They also reported a higher incidence of autosomal aberrations (0.36%) compared with the general population (0.07%). The father was the carrier in all but 1 case of inherited chromosomal anomaly, indicating that the high risk was not due to the ICSI procedure itself.

In our study, paternal and maternal karyotyping was done for only 6 of 15 couples who ICSI-conceived babies had chromosomal anomalies. The lack of karyotyping in the remaining 9 couples is a limitation of our study, as it precluded calculation of the percentage of de novo sex chromosome anomalies. The 9 couples declined karyotyping even though they gave consent for us to perform karyotyping their babies; perhaps the couples feared detection of anomalies in their karyotypes after abnormal karyotypes were found in their babies. The couples were also worried that finding abnormal karyotypes might adversely affect their future management of their infertility problem. It is therefore recommended that all couples undergoing ICSI be counseled for karyotyping before the procedure; the patient will more likely consent to karyotyping at this stage. Results of karyotyping will also be helpful in assessing risk of abnormal karyotyping in offspring.

Loft et al. (14) reported results of karyotyping in 206 of 730 infants born after ICSI in a Danish national study (Table 3). They reported six major chromosomal abnormalities (2.95%) and one inherited structural anomaly (0.5%). They reported no sex chromosomal aberrations and suggested that this might be due to the relatively few instances of karyotyping done. In the two large published studies (6, 14), antenatal diagnosis was performed; in contrast, we performed postnatal karyotyping. Even though all parents in these published series were counseled and prenatal karyotyping was suggested, 1,082 of 1,987 couples with an ICSI-induced pregnancies (54.5%) (6) and 209 of 730 couples (28.6%) (14) had karyotyping.

All pregnant women who had ICSI at our center but were observed and delivered in other hospitals were advised to have their babies karyotyped. They also received letters from their physicians with recommendations and a request to send data from physical examination and karyotypes of babies. However, we excluded these data because physicians and patients tend not to report normal findings but report anomalies, which might falsely increase the incidence of abnormal karyotype.

In conclusion, ICSI has a small but significant increased risk of producing abnormal karyotype in offspring. This risk appears to be equally distributed between

autosomal and sex chromosomal anomalies. Couples should undergo karyotyping before attempting ICSI and should be counseled according to the current data and assured that the increased risk for chromosomal abnormalities in offspring is small.

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