

Chromosome abnormalities in embryos obtained after conventional in vitro fertilization and intracytoplasmic sperm injection

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Objective: To compare the rate of numerical chromosome abnormalities in embryos derived from bipronucleated zygotes produced by intracytoplasmic sperm injection (ICSI) and conventional IVF.

Design: Embryos were classified by maternal age and morphological and developmental characteristics to avoid bias when comparing chromosome abnormalities in ICSI and IVF embryos.

Setting: The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center, West Orange, New Jersey.

Patient(s): Seventy-nine couples undergoing IVF and 53 couples undergoing ICSI.

Intervention(s): Embryos donated for research were fully biopsied, and their cells were analyzed by fluorescence in situ hybridization with specific probes for chromosomes X, Y, 13, 18, and 21 and some with also a probe for chromosome 16.

Main Outcome Measure(s): Embryo chromosome abnormalities.

Result(s): A total of 245 embryos obtained through conventional IVF and 136 embryos obtained through ICSI were analyzed. There were no statistical differences between the rates of numerical chromosomal abnormalities detected in the IVF (61%) and ICSI (52%) embryos analyzed. Regarding gonosomal aneuploidy, the same rate was found in both ICSI (1%) and IVF groups (2%).

Conclusion(s): If the parents are chromosomally normal, the results indicate that, at the embryo level and before any embryo selection has occurred in utero, ICSI does not produce more numerical chromosomal abnormalities than conventional IVF. (Fertil Steril® 1998;69:904–8. ©1998 by American Society for Reproductive Medicine.)

Key Words: Aneuploidy, mosaicism, monosomy X, preimplantation genetic diagnosis, gonosomal trisomies

Intracytoplasmic sperm injection (ICSI) now is applied widely in the treatment of male factor infertility. Fertilization rates and pregnancy rates with this technique have improved significantly in the past 2 years. Because the source or the quality of sperm used in the injection does not appear to have a bearing on the success of the procedure (1), ICSI is performed in cases previously excluded from treatment. Therefore, concern exists over the health of the potential offspring resulting from this technique in some of the most severe cases.

There have been reports of offspring with high rates of sex chromosome abnormalities (5 of 15 cases) (2) associated with ICSI. Later studies have not confirmed these high rates in sex chromosomal abnormalities (5 of 585) (3),

although they are still higher than in the general population but are, in fact, in line with rates found in infertile men (0.8% for oligospermic and 13.9% for azoospermic) (4). Higher than expected rates of sex chromosome abnormalities have been attributed to gonosomal mosaicism (5) and/or to male meiosis impairment, leading to both oligospermia and aneuploidy (6). Fortunately, in large series of ICSI cases, the rate of chromosomal abnormalities was similar to those of the general population (1, 7).

Although studies of offspring suggest that ICSI is moderately safe, ICSI still could be a teratogen, producing an “all-or-none” response, i.e., the eggs or embryos affected would die or not produce any perceivable dam-

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age. At the oocyte and embryo level, few studies have been performed comparing chromosome abnormalities in embryos derived from ICSI and conventional IVF cycles. Plachot et al. (8), using karyotyping techniques, found that the rate of aneuploidy and chromosome breakage in unfertilized oocytes from ICSI and conventional IVF cycles was similar. However, more diploid oocytes were found in unfertilized oocytes from conventional IVF (12.5%) than from ICSI cycles (1.7%). This was attributed to the fact that most female partners of ICSI patients are fertile. On the other hand, more premature chromosome condensation was found in ICSI (57%) than in conventional IVF unfertilized oocytes (37%), indicating that oocyte activation fails more commonly after ICSI.

Embryos derived from tripronucleated and mononucleated zygotes produced by ICSI also have been studied. Although tripronucleated embryos from IVF were mostly mosaics (9, 10), tripronucleated zygotes from ICSI were mostly triploid (10, 11). This indicates that the presence of two paternally inherited centrioles profoundly disturbs the correct distribution of chromosomes between the daughter cells (12). Embryos derived from mononucleated zygotes produced by IVF were mostly fertilized and diploid (10, 12). There is evidence that these are derived from the fusion of pronuclei (13). In contrast, mononucleated zygotes produced by ICSI were mostly haploid and/or mosaics and not fertilized (10, 13).

The chromosomal integrity of embryos derived from bipronucleated ICSI zygotes is still being debated. There are no published studies considering differences in maternal age, embryo morphology, and developmental rates. When these parameters were taken into account in a large study of IVF embryos, significant differences in the frequency of chromosomal abnormalities were found (14). The present study compares chromosome abnormalities in ICSI and IVF embryos with consideration of maternal age, embryo morphology, and developmental rate.

MATERIALS AND METHODS

Classification of Embryos

The monospermic embryos developing from bipronucleated zygotes used in this study were obtained from patients undergoing IVF treatment for infertility. Patients undergoing IVF treatment at The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center donated these embryos for research. A written consent was obtained from all patients donating their supernumerary embryos for research. The study was in accordance with guidelines approved by the internal review board of Saint Barnabas Medical Center. Two groups of embryos were used: those developing from eggs fertilized through conventional insemination or through ICSI. Each group was subdivided into two maternal age groups: (up to age 39 and 40 and older).

Similarly, embryos were divided into three groups according to developmental and morphological characteristics: arrested, slow and/or dysmorphic, and nondysmorphic normally developing embryos as described previously (14). Arrested embryos were those that had not cleaved during a 24-hour period. Dysmorphic nonarrested embryos were those with >15% fragmentation and/or with multinucleated blastomeres. Slowly developing embryos were those that have five or less cells on the 3rd day of development but have cleaved in the last 24 hours. Nondysmorphic normally developing embryos were those with six or more cells on the 3rd day of development that had cleaved in the last 24 hours and had no multinucleation and $\leq 15\%$ fragments. The classification of embryos in these subgroups eliminates any bias in the data due to maternal age or embryo morphology.

Biopsy, Fixation, and Fluorescence In Situ Hybridization Analysis

Embryos were disaggregated on day 3 of development, and blastomeres were individually fixed on glass slides as described previously (15). Some of the blastomeres were analyzed by fluorescence in situ hybridization (FISH) using simultaneously X-, Y-, 13-, 18-, 21-chromosome-specific probes following Munné and Weier (16) without modification. The other blastomeres were analyzed with the same probe mixture plus a 16-chromosome-specific probe. All the probes were obtained from Vysis. Chromosome X was labeled with spectrum aqua, chromosome Y with a mixture of spectrum aqua, orange and green, chromosome 13 with a mixture of spectrum orange and green, chromosome 16 with spectrum green, chromosome 18 with a mixture of spectrum aqua and orange, and chromosome 21 with spectrum orange.

The criteria to distinguish FISH failure from mosaicism were as published previously (17). The specific FISH signals detected in a given blastomere were considered to reflect a true chromosome constitution in the following instances: [1] blastomeres with two gonosomes, 13-, 16-, 18-, and 21-chromosome-specific signals; these were considered diploid blastomeres; [2] embryos in which all the blastomeres had the same abnormality, such as aneuploid, haploid, or polyploid embryos; [3] individual blastomeres that have only one signal per chromosome pair; these were considered haploid cells; [4] individual blastomeres that had three or more signals per chromosome pair; these were considered polyploid cells; [5] individual blastomeres that had extra or missing signals compensated respectively for the missing or extra signals in sibling blastomeres. We considered that these blastomeres belonged to an embryo with mosaicism generated by mitotic nondisjunction; [6] blastomeres showing fewer signals than their sibling blastomeres and belonging to mosaic embryos resulting from the uneven cleavage of a blastomere without previous DNA synthesis. An example would be an embryo with mostly XX 13131616 1818 2121 cells, plus XO 13O 16O 1818 OO and XO 13O 16O OO 2121 cells; [7] the same criteria (1–6) were also used for

TABLE 1

Numerical chromosome abnormalities in embryos used in ICSI and IVF.

Chromosome abnormality	Age ≤39 years		Age >39 years		All ages	
	IVF	ICSI	IVF	ICSI	IVF	ICSI
No. of embryos analyzed	135	102	110	34	245	136
Percent normal	39	48	28	24	34	42
Percent aneuploid*	10	6	12	18	11	9
Percent gonosomal					2	1
Percent haploid (mosaic or not)	2	5	2	6	2	5
Percent polyploid (mosaic or not)	10	4	13	12	11	6
Percent with extensive 2N mosaicism	28	20	38	38	33	24
Percent with low 2N mosaicism	10	18	7	3	9	14

* 4 monosomies 13; 2 nullisomy 13; 6 monosomies 21; 2 monosomies 18; 6 monosomy 16; 2 trisomy 13; 3 trisomy 16; 7 trisomy 21; 1 trisomy 18; 3 monosomy X; 1 disomy Y; 1 trisomy XXY; 1 trisomy XYY.

multinucleated blastomeres; and [8] blastomeres with less or more than two gonosomes or chromosome-13, 16-, 18-, or 21-specific signals were considered, respectively, FISH false-negative or false-positive errors unless one of the prior criteria (1–7) applied.

The scoring criterion from Munné and Weier (16) to differentiate close signals from split signals was used. According to such criterion, when two signals for the same chromosome are found at a distance of two or less domains, they normally represent a split signal belonging to a single chromosome instead to two chromosomes.

Definition of Chromosomal Abnormality Types Detected by FISH

Chromosome abnormalities that could be detected by FISH are aneuploidy, haploidy, polyploidy, and mosaicism. Normal embryos were those in which each cell had two chromosomes of each kind studied. Aneuploid embryos could be monosomic or trisomic. Monosomic embryos were those in which the same chromosome was missing in each cell. Trisomic embryos were those in which each cell had three chromosomes of the same type instead of two. Embryos in which all the cells had only a single chromosome of each kind were considered haploid. However, haploid mosaics are common. Embryos were classified as haploid mosaics if, on average, each cell had one chromosome of each kind. Polyploid embryos had three or more copies of each chromosome in each cell. Embryos were classified as polyploid mosaics when, on average, the embryo had three or more copies of each kind of chromosome in each cell and no normal cells—quite a common occurrence.

An embryo was considered to be a diploid mosaic when at least one cell was normal. When all cells were abnormal and the embryo was mosaic, and the average number of chromosomes per cell was close to diploid, the embryo also was considered a diploid mosaic. Because diploid mosaicism is so common, extensive diploid mosaicism, possibly detri-

mental to embryonic development, was defined as the occurrence of more than three eighths of the cells in a diploid embryo being chromosomally abnormal. The reasons for this cutoff were explained previously (14).

Statistical Analysis

The χ^2 test was used to compare morphological groups for abnormalities detected by the same probe. The Mantel-Haenszel χ^2 test was used to compare heterogeneous percentages, i.e., those obtained after the addition of the percentage of abnormal embryos found with X, Y, 13, 18, and 21 probes and those with the X, Y, 13, 16, 18, and 21 probes.

RESULTS

A total of 245 embryos obtained through conventional IVF from 79 patient procedures and 136 embryos obtained through ICSI from 53 patient procedures were analyzed. In total, FISH results were obtained in 2,010 cells (average 5.2 cells per embryo).

Table 1 presents the incidence of numerical chromosome abnormalities for the chromosomes studied, in conventional IVF and ICSI embryos, according to maternal age. There were no statistical differences between the percentage of morphological and developmental types included in the conventional IVF and ICSI groups (Table 2). Because of this lack of difference, the two groups of embryos could be compared.

No statistical difference in total rates of chromosomal abnormalities, including or excluding low diploid mosaicism, were found in conventional IVF embryos (66% and 57%, respectively) and ICSI embryos (58% and 46%). In addition, no statistical differences were found either between the two groups of embryos regarding specific chromosome abnormalities, neither in total, nor within maternal age groups. Most important, a similar rate of gonosomal aneuploidy was found in both ICSI (1%) and IVF groups (2%).

TABLE 2

Morphological and developmental characteristics of analyzed embryos.

Type of embryo	Age \leq 39 years		Age >39 years		All ages	
	IVF	ICSI	IVF	ICSI	IVF	ICSI
No. of embryos	245	136	135	102	110	34
Percent arrested	23	20	19	19	28	26
Percent slow/dysmorphic	56	52	59	53	53	50
Percent normally developing	21	27	22	28	19	24

The most common chromosome abnormality, regardless of insemination procedure, was mosaicism, which occurred in about 40% of all embryos or about two thirds of all chromosome abnormalities, followed by aneuploidy, polyploidy, and haploidy.

On the basis of 1,459 IVF and 865 ICSI cycles, implantation rates (defined as sacs per embryo replaced) were identical (25.6%). Similarly, the percentage of multiple pregnancies (based on fetal heart beats) after ICSI (48%, 220 of 460) was similar to the percentage after IVF (50%, 350 of 699).

DISCUSSION

Congenital malformation and chromosome abnormalities in children born after the application of ICSI occur at rates similar to those in the general population (1). The ICSI procedure still could result in deleterious chromosome abnormalities not detected at birth perhaps caused by disturbance of the oocyte metaphase plate at the time of injection, among other possibilities. On the other hand, injection of a spermatozoon with an impaired centrosome (11), a structure required for spindle formation, could result in mosaicism at the first embryonic division and the demise of the embryo (17). The present study accounts for differences in maternal age and embryo morphology when comparing chromosomal integrity of ICSI and IVF embryos.

No significant differences were observed either in the overall frequency of chromosome abnormalities or in specific types of abnormalities, such as gonosomal aneuploidy or mosaicism. That means that the injection of the sperm into the cytoplasm is not disturbing the M-II metaphase spindle, probably because the injection is performed further from the spindle, which is supposed to be close to the first polar body. Our findings contribute to previous reassuring observations on ICSI offspring (1) and indicate that, at the embryo level, there is the same proportion of chromosomal abnormalities as that in conventional IVF produced embryos.

Assisted reproductive technology techniques, however, still produce an unacceptably high number of multiple pregnancies, which may result in complications for both the offspring and the mother. Multiple pregnancies may be

avoided in the future through blastocyst culture or better embryo selection based on genetic (preimplantation genetic diagnosis) or other parameters. Even though the data on embryos and offspring places ICSI at the same risk level of conventional IVF, it is probably advisable to screen the male partners in ICSI couples for chromosomal abnormalities. Conventional karyotype analysis of peripheral blood should be able to detect translocations and other chromosomal abnormalities in IVF patients requiring ICSI, which have a higher incidence than the rest of the population (8 translocations in 261 patients) (18).

When a translocation carrier is identified, we recommend preimplantation genetic diagnosis to prevent the replacement of unbalanced embryos. This genetic diagnosis recently was proved to be effective in selecting normal embryos (19, 20, 21). Similarly, screening for chromosome-Y microdeletions (22) also should be considered before ICSI because they occur in 7%–30% of the male factor population (23). Although PGD of Y microdeletions has not been attempted as yet, it is likely in the near future.

Blood tests on male candidates for ICSI will not detect, however, any abnormalities generated during spermatogenesis. According to Martin (6), disturbances in the male meiosis in chromosomally normal males may lead to oligospermia and aneuploidy, in particular of the divalent XY. The FISH on sperm cells with X and Y probes should be enough to detect ICSI candidates with unusually high rates of sex chromosome abnormalities. Cases with higher than a certain rate of sex chromosome abnormalities may be indicated for preimplantation genetic diagnosis.

The present study found no unusual increase in sex chromosome abnormalities after ICSI, although the numbers in our study are small as yet. Nevertheless, if other ICSI-induced abnormalities had been present, as postulated above by spindle disturbance, our study would certainly have detected their presence.

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