

# Preimplantation genetic diagnosis increases the implantation rate in human in vitro fertilization by avoiding the transfer of chromosomally abnormal embryos

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**Objective:** To verify the percentage of chromosomally abnormal preimplantation embryos in patients with a poor prognosis and possibly to increase the chance of implantation by selecting chromosomally normal embryos.

**Design:** A prospective, randomized, controlled study.

**Setting:** In vitro fertilization program at the Reproductive Medicine Unit of the Società Italiana Studi Medicina della Riproduzione, Bologna, Italy.

**Patient(s):** In a total of 28 stimulated cycles, the maternal age was  $\geq 38$  years and/or the patient had  $\geq 3$  previous IVF failures, factors that indicated a poor prognosis. After consent, 11 patients underwent preimplantation genetic diagnosis for aneuploidy, whereas 17 controls underwent assisted zona hatching.

**Intervention(s):** Simultaneous analysis of chromosomes X, Y, 13, 18, and 21 in a blastomere biopsied from day-3 embryos. Chromosomal analysis was performed with fluorescence in situ hybridization. Assisted zona hatching was performed on day-3 embryos from the control-group patients.

**Main Outcome Measure(s):** Embryo morphology, results of fluorescence in situ hybridization, clinical pregnancies, and implantation.

**Result(s):** In the study group, a total of 61 embryos were analyzed by fluorescence in situ hybridization, and 55% were chromosomally abnormal. Embryo transfer with at least one normal embryo was performed in 10 cycles. Four clinical pregnancies resulted, with a 28.0% implantation rate. In the control group, 41 embryos were transferred in 17 cycles after the assisted zona hatching procedure, yielding four clinical pregnancies and an 11.9% implantation rate.

**Conclusion(s):** Infertile patients classified as having a poor prognosis have a high percentage of chromosomally abnormal embryos. The advantage of selecting and transferring embryos with normal fluorescence in situ hybridization results has an immediate impact on implantation. (Fertil Steril® 1997;68:1128-31. © 1997 by American Society for Reproductive Medicine.)

**Key Words:** Aneuploidy, assisted zona hatching, unexplained IVF failures, in vitro fertilization, multicolor FISH, poor-prognosis patients, preimplantation diagnosis

The introduction of IVF techniques in reproductive medicine has revolutionized the approach to human infertility. The generally good results obtained now offer infertile couples the true possibility of taking home a baby. It is well known that an increase

in maternal age is correlated with a diminished implantation efficiency and a higher rate of abortion. However, unexplained multiple IVF failures also have been reported in young patients.

It is conceivable that chromosomal aberrations in the transferred embryos may represent the major cause of failed implantation, given the high rates of chromosomal abnormalities commonly found in embryos from stimulated cycles (1). Indeed, data from spontaneous abortions indicate that aneu-

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ploidy is more frequent than expected, which then corresponds with the lower implantation rate observed in patients who were  $\geq 38$  years of age (2). Therefore, preimplantation genetic diagnosis of the chromosomal complement was performed in in-vitro generated embryos from patients with a poor prognosis with use of the multicolor fluorescence in situ hybridization technique (1, 2). Although the technique consists of simultaneous screening of only five chromosomes per single cell, valuable information on the most frequent aneuploidies, polyploidies, and haploidies can be obtained.

Patients included in the study were classified as having a poor prognosis if they were  $\geq 38$  years and/or if they had undergone  $\geq 3$  previous unsuccessful IVF attempts. The study was carried out with the aim of verifying whether chromosomal abnormalities in preimplantation embryos could represent the cause of the low success rate in these patient categories. Consequently, the ultimate goal was the possibility of increasing the implantation and ongoing pregnancy rate through the selection of chromosomally normal embryos.

## MATERIALS AND METHODS

### Patients

From September to December 1996, a total of 28 infertile patients classified as having a poor prognosis were seen at the Società Italiana Studi Medicina della Riproduzione clinic in Bologna. They were invited to participate in a study aimed at determining the chromosomal status of preimplantation in vitro generated embryos. This work was approved by the local Institutional Review Board.

After signing informed consent, the patients were divided into two groups: in the study group, preimplantation genetic diagnosis of aneuploidy was performed in 11 patients (mean  $\pm$  SD maternal age,  $34.8 \pm 4.2$  years), whereas in the control group, embryos from 17 patients (mean  $\pm$  SD maternal age,  $34.4 \pm 4.1$  years) underwent the standard assisted zona hatching procedure (3). Each couple was able to select one of the two groups. The criteria for inclusion in one of the two groups were age  $\geq 38$  years ( $n = 3$ ; control,  $n = 5$ ) and/or  $\geq 3$  previous IVF failures ( $n = 8$ ; control,  $n = 12$ ). Only one patient (included in the age category of the study group) presented with both inclusion criteria.

Patients underwent induction of multiple follicular growth consisting of a long desensitization protocol with long-acting GnRH analogue and exogenous gonadotropins, as described previously (4). Sperm samples classified as severely pathologic required implementation of the intracytoplasmic sperm injection

(ICSI) technique for oocyte insemination ( $n = 5$ ; control,  $n = 7$ ).

### Assessment of Fertilization and Embryo Evaluation

Approximately 16 hours after insemination, the oocytes were checked for pronuclei and polar bodies. Monospermic zygotes were left in culture, and embryo evaluation was performed at 40 and at 60–62 hours after insemination. The number and morphology of blastomeres and the percentage of fragmentation were recorded. Day 3 embryos from the study group underwent embryo biopsy, whereas assisted zona hatching was performed in the control group.

Only clinical pregnancies confirmed by ultrasonography were considered in this study. The implantation rate was calculated as the number of gestational sacs with fetal heartbeat divided by the total number of embryos transferred.

### Embryo Biopsy and Fluorescence In Situ Hybridization Analysis

Embryo biopsy and blastomere fixation were performed on day 3 monospermic embryos, as described previously (1). For the fluorescence in situ hybridization analysis, five DNA probes were used for the simultaneous detection of chromosomes X, Y, 13, 18, and 21 (5). Briefly, the probes were labeled as follows: chromosome Y with Spectrum Aqua (Vysis, Naperville, IL), chromosome 18 in Spectrum Orange and Spectrum Aqua (Vysis; 1:1 mixture of probes), chromosome X with Spectrum Orange and Spectrum Green (Vysis; 1:1 mixture of probes), chromosome 13 with Spectrum Orange (Vysis), and chromosome 21 with biotin (Heinz Ulrich G. Weier, PhD, Lawrence Berkeley National Laboratory, Berkeley, CA), which was detected by fluorescein isothiocyanate conjugate-labeled avidin.

The hybridization solution was made by adding 1  $\mu$ L of each probe, previously concentrated to 3  $\mu$ L, to 7  $\mu$ L of whole-chromosome-paint-hybridization buffer (Vysis). The resulting 10  $\mu$ L of hybrid solution was added to the fixed blastomeres on a glass slide, covered with an 18  $\times$  18-mm coverslip, and denatured at 78°C for 3 minutes. The slide was left to hybridize for 4 hours at 37°C in a dark, moist chamber.

After washing in 0.4  $\times$  SSC at 72°C for 2 minutes, the fluorescence-labeled avidin was added, followed by anti-avidin. Finally, the slide was counterstained in 4'-diamidino-2-phenylindole in antifade solution (Vysis) and observed on a fluorescence microscope (Olympus BX60; Olympus, Tokyo, Japan) equipped with a triple-band pass filter set

**Table 1** Characteristics of embryos generated from the study and control groups.

Patient*	Age (y)	No. of oocytes retrieved	No. of embryos obtained	No. of embryos analyzed	No. of embryos with normal FISH complement	No. with aneuploidy	No. with other abnormalities	No results with FISH†	No. of embryos transferred	Pregnancy
A	39	12	12	7	2	2	3	0	2	Yes
B	39	5	4	4	1		3	0	1	
C	41	13	10	10	5	3	2	0	4	Yes
D	37	24	9	6	2	1	3	0	2	Yes
E	33	5	5	5	2		3	0	2	
F	37	4	4	4	0	2		2‡	0	
G	30	13	5	5	4			1‡	4	
H	28	11	5	4	2		2	0	2	
I	32	5	3	3	1	1	1	0	1	
J	31	18	15	10	4	2	3	1§	4	Yes
K	35	3	3	3	3			0	3	
1	40	18	12						4	
2	36	4	4						4	
3	32	19	7						5	
4	36	20	8						5	Yes
5	39	16	9						4	
6	30	27	10						4	
7	41	8	7						3	
8	40	9	5						4	Yes
9	32	18	7						4	Yes
10	31	7	5						4	
11	34	10	5						4	
12	35	7	5						3	
13	29	7	4						3	
14	30	12	8						4	
15	31	18	7						4	
16	30	11	5						4	
17	39	9	5						4	Yes

Note: FISH = fluorescence in situ hybridization.

\* Patients A–K belong to the study group; patients 1–17 represent the control group.

† No. of embryos for which FISH did not give information regarding the chromosomal status of the cells analyzed.

‡ Lost during fixation.

§ Nucleus hypercondensed.

for simultaneous observation of Spectrum Orange/Green/Aqua.

## RESULTS

As depicted in Table 1, a total of 75 embryos were generated from the study group. Of them, 61 embryos (81%) were considered morphologically suitable for replacement and therefore were biopsied for fluorescence in situ hybridization analysis. In 11 blastomeres, no result was obtained because of the absence of a nucleus ( $n = 7$ ) or loss of the cell during the procedure ( $n = 4$ ). Of the remaining 50 embryos, 26 had a normal fluorescence in situ hybridization complement, whereas 24 had chromosomal abnormalities: 6 were monosomic and 5 were trisomic, 7 were haploid or triploid, and 6 were multinucleated with an overall abnormal chromosome complement.

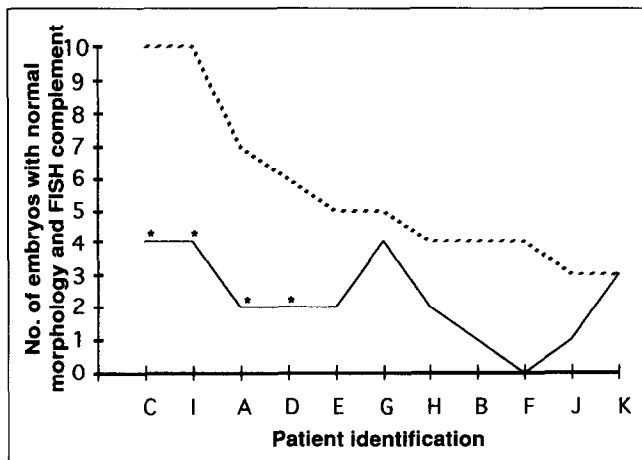
There was only one case in the cohort in which no normal embryos were detected. In the remaining 10 cases, embryos were replaced by transferring a total of 25 embryos, which were all determined to be normal by fluorescence in situ hybridization (mean

$\pm$  SD,  $2.5 \pm 1.2$  embryos per patient). Four clinical pregnancies were generated (40%), with an overall implantation rate of 28.0%. Two of the pregnancies originated from the group of patients with advanced maternal age, and two originated from the group with repeated IVF failures. All pregnancies were ongoing at the 26th week of gestation.

The percentage of embryos that were normal by fluorescence in situ hybridization did not seem to vary significantly with regard to maternal age (38%) or repeated IVF failures (46%). Similarly, the percentage of normal embryos by fluorescence in situ hybridization was comparable between patients treated with ICSI (50%) versus conventional IVF (41%).

Figure 1 shows the total number of embryos analyzed for each patient and those with a normal fluorescence in situ hybridization complement. Pregnancies occurred in patients who presented with a larger cohort of transferrable embryos (left side of the graph).

In the control group, a total of 89 embryos of the 113 obtained (79%) were considered morphologically



**Figure 1** Number of embryos with normal morphology and fluorescence in situ hybridization (FISH) complement for each patient. An asterisk indicates patients who had clinical pregnancies. ---- = embryos analyzed with FISH; — = normal embryos by FISH analysis.

suitable for transfer. The assisted zona hatching procedure was performed on 67 embryos, which subsequently were transferred with a mean ( $\pm$ SD) of  $3.9 \pm 0.6$  embryos per patient, resulting in four clinical pregnancies (23%) with an implantation rate of 11.9%. As in the study group, two of the pregnancies occurred in the group of patients who were  $\geq 38$  years old, and two occurred in the group with previous IVF failures. Cryopreservation was performed on spare morphologically suitable embryos.

## DISCUSSION

Data from this study suggest that infertile patients with a poor prognosis generate a high percentage of chromosomally abnormal embryos, which may represent the cause of failed implantation. The results from these patients (see Figure 1) illustrate that the advantages of fluorescence in situ hybridization analysis are maximized only when there is a possibility of transferring 2–4 normal embryos from a large cohort.

Without preimplantation genetic diagnosis, the chance of selecting and transferring abnormal embryos is high because even embryos presenting with normal morphology have a notable percentage of abnormalities. Indeed, in a group of patients who had the same factors indicating a poor prognosis and who underwent assisted zona hatching without preim-

plantation genetic diagnosis during the same study period (control group), the pregnancy rate was not statistically different, but the implantation rate was significantly lower than in the preimplantation genetic diagnosis group (11.9% versus 28.0%;  $P < 0.05$ ). This finding suggests that the selection of chromosomally normal embryos is associated with an increased embryo viability. Therefore, a substantial reduction in the number of embryos transferred would avoid multiple pregnancies.

After a preimplantation genetic diagnosis of aneuploidy is made, there is an immediate clinical impact derived from the application of this technique, which consists in the dramatic reduction or even exclusion of embryo transfer (mean number of embryos replaced, 2.5; a mean of 3.9 replaced in the control group). However, the identification of abnormalities in a cohort of morphologically good-quality embryos prevents the transfer of those embryos that are destined not to implant or to abort spontaneously.

Thus, especially when considering the unexplained negative outcomes of the previous IVF cycles performed in 8 of the 11 patients in the study group, it is plausible that in patients with a poor prognosis, implantation is increased by preimplantation genetic diagnosis in association with a minimized risk of delivering a trisomic fetus. Further studies are in progress to verify this hypothesis which, if confirmed, would lead to a substantial decrease in the number of embryos replaced because of their high potential to develop to term.

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