

Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed

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Objective: To verify whether advantages can derive from the implementation of preimplantation genetic diagnosis for aneuploidy in patients with a poor prognosis of full-term pregnancy, compared with conventional treatment procedures.

Design: A randomized, controlled study.

Setting: Reproductive Medicine Unit of the Società Italiana Studi Medicina della Riproduzione, Bologna, Italy.

Patient(s): In a total of 262 stimulated cycles, women presented with the following poor-prognosis indications: maternal age of ≥ 36 years ($n = 157$), ≥ 3 previous IVF failures ($n = 54$), and an altered karyotype ($n = 51$). After giving consent, 127 patients underwent preimplantation genetic diagnosis for aneuploidy, whereas 135 controls underwent assisted zona hatching.

Intervention(s): Analysis of chromosomes XY, 13, 14, 15, 16, 18, 21, and 22 was carried out with the fluorescence in situ hybridization technique in a blastomere biopsied from day 3 embryos. Assisted zona hatching was performed on day 3 embryos from the control group.

Main Outcome Measure(s): Embryo morphology and chromosomal status, number of transferred embryos, clinical pregnancies, implantation rates, and abortions.

Result(s): In the study group, 717 embryos were analyzed by fluorescence in situ hybridization, and 60% were chromosomally abnormal. A mean of 2.3 ± 0.9 euploid embryos were transferred in 99 cycles, resulting in 37 clinical pregnancies (37%) and a 22.5% ongoing implantation rate. In the control group, 126 cycles were performed with 3.2 ± 1.3 embryos transferred, yielding 34 clinical pregnancies (27%) and a 10.2% ongoing implantation rate.

Conclusion(s): The advantage of selecting embryos with a normal chromosome complement has an immediate impact on the ongoing implantation rate, especially in patients aged ≥ 38 years and carriers of an altered karyotype. (Fertil Steril® 1999;72:837–44. ©1999 by American Society for Reproductive Medicine.)

Key Words: Altered karyotype, aneuploidy, assisted zona hatching, maternal age, multicolor FISH, poor-prognosis patients, preimplantation diagnosis, unexplained IVF failures

Received March 19, 1999;
revised and accepted July
7, 1999.

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The preimplantation genetic diagnosis of aneuploidy is currently performed with the fluorescence in situ hybridization technique on blastomeres or polar bodies (1–3). The most frequently used probes are specific for the chromosomes responsible for the most common aneuploidies in the human, as detected in spontaneous abortions, although limits are imposed by the extremely reduced number of cells available for analysis. The results obtained confirm that chromosomal abnormalities can originate at three different stages: during

gametogenesis, fertilization, and embryogenesis, and the frequency of chromosomal abnormalities is higher than expected by considering the sum of aneuploidies observed in both oocytes and spermatozoa (4–7).

According to data reported in the literature, couples are especially at risk of generating chromosomally abnormal embryos when they possess one or more of the following characteristics: [1] advanced maternal age, [2] unexplained repeated IVF failures, and [3] an al-

TABLE 1

Composition of the study and control groups.

Variable	Age ≥ 36 y		≥ 3 IVF failures		Altered karyotype	
	Study group	Controls	Study group	Controls	Study group	Controls
No. of cycles	73	84	27	27	27	24
Age (y)	39.2 \pm 2.2	38.8 \pm 2.8	32.2 \pm 2.3	31.7 \pm 2.4	32.3 \pm 3.0	29.9 \pm 3.5
No. of FSH ampules	52.7 \pm 16.3	51.8 \pm 15.4	48.1 \pm 15.2	46.9 \pm 15.4	38.9 \pm 15.3	39.5 \pm 13.8
E ₂ level on day of hCG administration	1,526.7 \pm 1,003.8	1,498.6 \pm 989.3	1,524.0 \pm 896.1	1,503.7 \pm 883.6	1,530.4 \pm 639.8	1,411.0 \pm 609.2
No. of oocytes	11.0 \pm 5.5	11.1 \pm 5.4	10.3 \pm 4.7	10.1 \pm 4.5	11.3 \pm 5.4	10.9 \pm 5.3
No. of embryos	7.0 \pm 3.8	7.2 \pm 3.9	6.2 \pm 2.7	6.7 \pm 2.4	6.1 \pm 3.4	6.4 \pm 3.0

Note: Data are n or mean \pm SD.

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tered karyotype due to gonosomal mosaicism at low frequency or balanced translocations (8, 9). The chromosomal study of preimplantation embryos has provided not only information on the potential cause of embryonic loss, but also promising results for the treatment of patients with a poor prognosis of full-term pregnancy, especially in the case of advanced reproductive age. However, ethical and biologic questions are still open to debate, and there is a need to define the categories of patients who can derive a real benefit from application of the procedure (10, 11). In fact, no randomized studies of reasonable size have been performed to establish for which couples preimplantation genetic diagnosis of aneuploidy should be routinely recommended.

In the present report, patients presenting with poor-prognosis indications were included in a randomized controlled study with the aim of determining whether advantages could derive from the implementation of preimplantation genetic diagnosis of aneuploidy in preimplantation embryos in comparison with conventional treatment procedures.

MATERIALS AND METHODS

Patients

From September 1996 to July 1998, 189 patients classified as having a poor prognosis of full-term pregnancy underwent 262 IVF treatment cycles for infertility at the Società Italiana Studi Medicina della Riproduzione clinic in Bologna. They were proposed to participate in a study aimed at verifying the percentage of chromosomal abnormalities in preimplantation embryos presenting with normal morphology on day 3 of in vitro development. Inclusion criteria were: maternal age of ≥ 36 years (group 1; n = 157), ≥ 3 previous IVF failures (group 2; n = 54), and an altered karyotype detected in peripheral blood (group 3; n = 51) due to gonosomal mosaicism (n = 35) or robertsonian translocations (n = 16). The study was approved by our institutional review board.

After giving consent, the patients were divided into two

groups. In the study group (n = 127), preimplantation genetic diagnosis of aneuploidy was performed, whereas in the control group (n = 135), the embryos selected for transfer underwent the standard assisted zona-hatching procedure (12). Couples were allocated to the groups on the basis of their volunteer decision. The study and control groups were homogeneous in terms of maternal age, indication for treatment, E₂ level on the day of hCG administration, number of oocytes retrieved, and embryos generated (Table 1).

Induction of multiple follicular growth was accomplished by exogenous gonadotropins following a desensitization protocol with long-acting GnRH analogues (13). Approximately 36 hours after hCG administration, oocytes were collected transvaginally under ultrasound guidance and incubated in Earle's medium supplemented with 10% heat-inactivated maternal serum in a 5% CO₂ humidified atmosphere at 37°C. Insemination was performed with conventional IVF or intracytoplasmic sperm injection (ICSI) depending on sperm indices.

Assessment of Fertilization and Embryo Development

The numbers of pronuclei and polar bodies were evaluated at 14–18 hours after insemination. Regularly fertilized oocytes were cultured individually and examined 24 and 48 hours later. The number and morphology of nuclei and blastomeres and the percentage of fragments in the perivitelline space were assessed. Day-3 embryos from the study group with regular development were selected for embryo biopsy; in the control group, assisted hatching was performed in the embryos selected to be transferred. Transfers were performed into the uterine cavity; clinical pregnancies were confirmed by the presence of a gestational sac with a fetal heartbeat by ultrasound analysis.

The implantation rate represents the ratio between the number of gestational sacs with a fetal heartbeat and the total number of embryos transferred; when this calculation is performed for pregnant patients, the percentage obtained

defines the implantation rate per pregnant patient. The ongoing implantation rate takes into consideration only the gestational sacs of the pregnancies that are ongoing (beyond 6 months' gestation).

Assisted Zona Hatching and Blastomere Biopsy

Day-3 embryos were manipulated individually in HEPES-buffered medium overlaid with mineral oil preequilibrated for 48 hours. A breach of 20–22 μm was opened in the zona pellucida (ZP) with acidic Tyrode's solution; if fragments were present in the perivitelline space, they were gently aspirated. Embryos from the control group were then carefully washed and put in culture. In the study group, a blastomere was removed with use of a polished glass needle, which was introduced in the perivitelline space through the hole opened in the ZP. The biopsied cell was transferred to hypotonic solution (1% sodium citrate) for 1–2 minutes, and the nucleus was fixed on a glass slide using methanol–acetic acid 3:1 (3). After dehydration in ethanol (2 minutes at 70%, 85%, and 100%, respectively), the slide was incubated with the hybridization solution at 37°C in humidity for 4 hours (14).

Fluorescence In Situ Hybridization

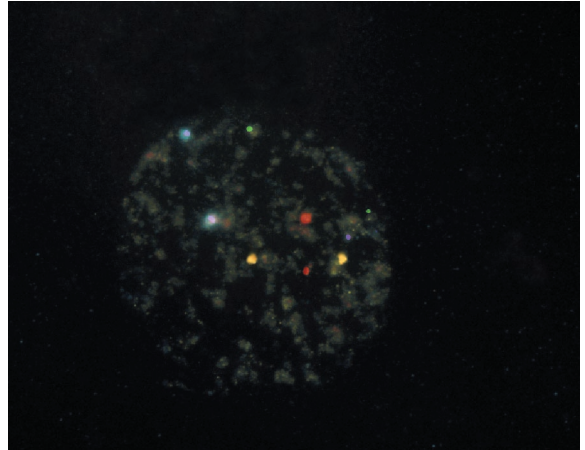
For the multicolor fluorescence in situ hybridization analysis, 6 DNA probes were used for the simultaneous detection of chromosomes X, Y, 13, 16, 18, and 21 (15). Three additional probes, specific for chromosomes 14, 15, and 22, were also implemented from December 1997; therefore, 56 cycles in the study group had embryos ($n = 293$) screened for 9 chromosomes (16).

Briefly, the probes of the first panel were labeled as follows: chromosome X with spectrum-aqua, chromosome Y with spectrum-aqua and spectrum-green, chromosome 13 with spectrum-orange and spectrum-green, chromosome 16 with spectrum-green, chromosome 18 with spectrum-aqua and spectrum-orange, and chromosome 21 with spectrum-orange. Ten microliters of the hybridization solution was applied to the glass slide containing the fixed blastomeres, and the slide was covered with an 18 \times 18-mm coverslip. The slide was then placed for 3 minutes on a hot plate preheated to 78°C, sealed with rubber cement, and placed in a dark moist chamber at 37°C for 4 hours.

After washing in 0.4 \times SSC for 2 minutes at 71°C, the slide was counterstained with DAPI in antifade solution (4',6-diamino-2-phenyl indole) and observed with a fluorescent microscope (Olympus BX40; Olympus, Tokyo, Japan) equipped with the triple-band-pass filter set for simultaneous observation of the spectrum-orange, spectrum-green, and spectrum-aqua fluorescence. When analyzed through the triple-band-pass filter set, the specific signal for the X chromosome appeared as blue, the Y as white to yellow, the 13 as orange, the 16 as green, the 18 as pink, and the 21 as red (Fig. 1).

FIGURE 1

In situ hybridization with probes specific for chromosomes XY, 13, 16, 18, and 21. The X chromosome signal appears as light blue, the Y as white to yellow, the 13 as orange, the 16 as green, the 18 as pink, and the 21 as red. The cell is from a female embryo with monosomy 18.



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For the second hybridization round, the slide was washed in 1 \times phosphate-buffered detergent and dehydrated (70%, 85%, and 100% ethanol). Ten microliters of the second hybridization solution was applied per slide. The probe for chromosome 14 was labeled with spectrum-aqua, the probe for chromosome 15 with spectrum-orange, and the probe for chromosome 22 with spectrum-green. After denaturation and hybridization (2 hours at 37°C), the slide was washed and analyzed (Fig. 2).

The error rate inherent to the fluorescence in situ hybridization procedure on one cell was defined by analyzing all the blastomeres from 148 abnormal embryos.

Statistical Analysis

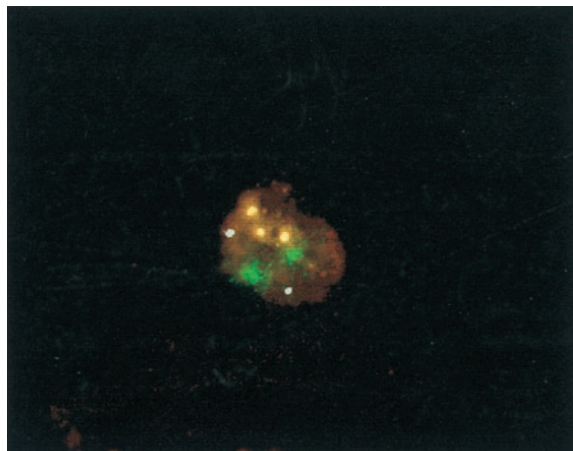
Data were analyzed by the Student's *t*-test and χ^2 analysis applying the Yates' correction and 2 \times 2 contingency tables.

RESULTS

A total of 845 embryos were obtained from the patients in the study group. Blastomere biopsy was performed on 717 of them, which were selected on the basis of regular morphology and developmental rate. After fluorescence in situ hybridization analysis, 267 were diagnosed as euploid (37%) and 428 presented with chromosomal abnormalities (60%); no result was obtained in 22 embryos because of technical problems during the procedure (3%). In 28 cycles, no chromosomally normal embryos were detected; therefore, embryo replacement was not performed. In the remaining 99 cycles, a mean number of 2.3 ± 0.9 embryos were trans-

FIGURE 2

In situ hybridization with probes specific for chromosomes 14, 15, and 22. The probe for chromosome 14 is shown labeled in light blue, the probe for chromosome 15 in orange, and the probe for chromosome 22 in green. The cell is from an embryo with trisomy 15.



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ferred, which were all assessed to be euploid by fluorescence in situ hybridization.

A total of 37 clinical pregnancies resulted (37%). Of

these, 24 women delivered 32 healthy infants, 9 pregnancies were ongoing, and 4 ended in abortion. Of the abortions, 2 were miscarriages (5%), 1 was an ectopic, and 1 aborted after amniocentesis (the fetal karyotype was normal). The overall implantation rate was 24.2%. No differences were detected between ICSI and IVF regarding either the percentage of normal embryos by fluorescence in situ hybridization or the clinical outcome.

The results obtained in the study group were analyzed on the basis of the indication for treatment and compared with the corresponding controls (Table 2). In patients aged ≥ 36 years (group 1), the study and control groups exhibited similar percentages of clinical pregnancies, whereas in the study group, the mean number of embryos transferred per patient was significantly lower and the implantation rate was higher in comparison with the control group. Concomitantly, the implantation rate per pregnant patient was superior in the study group compared with the controls (57.9% versus 38.5%; $P < .05$).

When group-1 patients were arbitrarily divided into three classes of age: 36–37 years, 38–39 years, and ≥ 40 years, the pregnancy and implantation rates characterized in the control group revealed a significant difference when comparing patients aged 36–37 years (42% and 21.0%, respectively) versus older patients (19% and 8.6%) (Table 3). Conversely, in the study group, the percentages of pregnancy and implantation did not differ among the three classes of age and were comparable to the rates observed in women aged 36

TABLE 2

Overall outcome in patients from the study and control groups.

Variable	Age ≥ 36 y			≥ 3 IVF failures		Altered karyotype		
	Study group	Control group	P value	Study group	Control group	Study group	Control group	P value
No. of cycles	73	84	—	27	27	27	24	—
Age (y)*	39.2 \pm 2.2	38.8 \pm 2.8	—	32.2 \pm 2.3	31.7 \pm 2.4	32.3 \pm 3.0	29.9 \pm 3.5	—
No. of embryos	514	610	—	167	181	164	154	—
No. of embryos tested by FISH	432	—	—	138	—	125	—	—
No. with abnormal FISH results (%)	277 (64)	—	—	74 (54)	—	77 (62)	—	—
No. of embryos transferred†	128 (2.2 \pm 0.9)	259 (3.2 \pm 0.9)	<.001	52 (2.6 \pm 0.9)	74 (3.2 \pm 1.1)	47 (2.1 \pm 0.9)	70 (3.2 \pm 1.3)	>.005
No. of cycles transferred	57	81	—	20	23	22	22	—
No. of clinical pregnancies (%)	22 (39)	25 (31)	—	5 (25)	5 (22)	10 (45)	4 (18)	—
No. of abortions (%)	1 (4)	5 (20)	—	1‡ (20)	1 (20)	2§ (20)	1 (25)	—
Implantation rate (%)	25.8	14.3	<.01	17.3	9.5	27.7	8.6	<.025
Ongoing implantation rate (%)	25.0	11.6	<.005	15.4	8.1	23.4	7.1	<.05
IRPP (%)	57.9	38.5	<.05	52.9	36.8	59.1	42.9	—

Note: FISH = fluorescence in situ hybridization; IRPP = implantation rate per pregnant patient.

* Mean \pm SD.

† Data are n (mean \pm SD).

‡ Ectopic.

§ One after amniocentesis (fetal karyotype was normal).

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TABLE 3

Pregnancy and implantation rates in patients aged ≥ 36 years: preimplantation genetic diagnosis versus controls.

Rate and group	Age group (y)			P value
	36–37	38–39	≥ 40	
Pregnancy rate (%)				
Study group	43	35	40	—
Control group	42	20	19	<.05*
Implantation rate (%)				
Study group	22.9	28.6	25.5	<.05†
Control group	21.0	11.1	6.5	<.01‡§
IRPP (%)				
Study group	57.1	80.0	46.4	<.05
Control group	43.1	38.9	25	<.05¶

Note: IRPP = implantation rate per pregnant patient.

* 36–37 years versus ≥ 38 years.

† 38–39 years: study group versus control group.

‡ ≥ 40 years: study group versus control group.

§ 36–37 years versus ≥ 38 years.

|| 38–39 years: study group versus control group.

¶ 36–37 years versus ≥ 38 years.

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and 37 years. Finally, the implantation rate observed in the oldest categories (38–39 years and ≥ 40 years) was significantly higher after preimplantation genetic diagnosis of aneuploidy than after the standard assisted-hatching procedure (28.6% versus 11.1%, $P < .05$, and 25.5% versus 6.5%; $P < .01$, respectively).

Table 2 shows the overall results in patients with three or more previous IVF failures (group 2). Despite the notable percentage of chromosomally abnormal embryos generated by these patients (54%), all indices were similar between the groups, including pregnancy and implantation rates (25% and 17.3% in the study group versus 22% and 9.5% in the controls, respectively).

Finally, group-3 patients, whose indication for treatment was an altered karyotype, had a significantly higher rate of implantation after fluorescence in situ hybridization analysis compared with the controls (27.7% versus 8.6%; $P < .025$) in association with a reduced number of embryos transferred per patient (2.1 ± 0.9 versus 3.2 ± 1.3 ; $P < .005$; Table 2). The percentage of clinical pregnancies did not differ significantly.

In Table 4, the clinical results obtained from the study group have been summarized and compared with those derived from the controls. After preimplantation genetic diagnosis of aneuploidy, the rate of cycles transferred was notably decreased compared with the control rate (78% versus 93%; $P < .001$). However, clinical pregnancies occurred with the same frequency in the two groups when calculated not only per transferred cycle, but also per started cycle. In addition, the replacement of euploid embryos was associated

TABLE 4

Clinical outcome in patients with a poor prognosis.

Variable	Study group	Control group	P value
No. of cycles	127	135	—
Age (y)*	36.3 ± 4.1	35.6 ± 3.5	—
No. of embryos transferred†	227 (2.3 ± 0.9)	403 (3.2 ± 1.3)	<.005
No. of cycles transferred (%)	99 (78)	126 (93)	<.001
No. of clinical pregnancies	37	34	—
Per started cycle (%)	29	25	—
Per transferred cycle (%)	37	27	—
Early abortions (%)	2 (5)	7 (21)	—
Implantation rate (%)	24.2	12.4	<.001
Ongoing implantation rate (%)	22.5	10.2	<.001
IRPP (%)	57.3	38.8	<.01

Note: IRPP = implantation rate per pregnant patient.

* Mean \pm SD.

† Data are n (mean \pm SD).

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with a reduced mean number of embryos transferred (2.3 ± 0.9 versus 3.2 ± 1.3 ; $P < .005$) and a higher overall implantation rate (24.2% versus 12.4%; $P < .001$) that remained significantly increased for ongoing gestational sacs (22.5% versus 10.2%; $P < .001$). Similarly, the implantation rate per pregnant patient was superior in the study group compared with the controls (57.3% versus 38.8%; $P < .01$).

The overall data obtained after fluorescence in situ hybridization analysis with 6 probes (402 embryos) and 9 probes (293 embryos) are presented in Table 5. Monosomy and trisomy involved 1 chromosome in 60 and 77 cases, respectively, and 2 or 3 chromosomes in 33 and 7 embryos, respectively. The detected frequency of aneuploidy for the tested chromosomes was as follows: 19% for chromosome 13, 27% for chromosome 16, 12% for chromosome 18, 29% for chromosome 21, and 13% for gonosomes with the first panel. When 9 probes were used, the frequency was: 7% for chromosome 13, 6% for chromosome 14, 12% for chromosome 15, 19% for chromosome 16, 7% for chromosome 18, 21% for chromosome 21, 22% for chromosome 22, and 6% for gonosomes.

A total of 148 abnormal embryos were reanalyzed with fluorescence in situ hybridization after spreading all their blastomeres. The diagnosis was confirmed in 136 embryos (92%), 4 presented with other abnormalities (3%), and 8 embryos turned out to be chromosomally normal, yielding a misdiagnosis rate of 5%. False monosomies represented the most frequent source of error, with reanalysis confirmation obtained in 83% of them (39 of 47 embryos classified as monosomic by preimplantation genetic diagnosis). Trisomies were confirmed in 94% of the reanalyzed embryos (34 of 36), haploidy and polyploidy in 93% of the cases (14 of 15 in both abnormality categories), and complex abnor-

TABLE 5

Chromosomal abnormalities detected by six and nine probes in the three study groups.

Variable	Age ≥ 36 y		≥ 3 IVF failures		Altered karyotype	
	6 probes	9 probes	6 probes	9 probes	6 probes	9 probes
No. of cycles	35	38	18	9	18	9
No. of FISH-diagnosed embryos	215	217	98	40	89	36
No. with normal FISH result	77	78	42	22	38	10
No. with abnormal FISH result (%)	138 (64)	139 (64)	56 (57)	18 (45)	51 (57)	26 (72)
No. of monosomies	30	29	12	2	15	4
No. of trisomies	29	35	3	1	10	7
No. of haploidies	10	3	4	1	5	2
No. of polyploidies	9	9	9	1	5	0
No. of mosaics	60	63	28	13	16	13

Note: FISH = fluorescence in situ hybridization.

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malities (defined as mosaics) were confirmed in all 35 reanalyzed embryos.

DISCUSSION

The data generated from this study confirm that patients with a poor prognosis develop a high percentage of chromosomally abnormal embryos despite regular morphology and developmental rate. Therefore, the low success rate in these couples may be related to the notable incidence of morphologically suitable but chromosomally abnormal embryos, indicating that the selection of embryos based on morphologic evaluation alone is not sufficient in poor-prognosis cases to guarantee the replacement of viable embryos capable of developing to full term (15, 17).

A physiologic decline in the functional and structural quality of the oocyte occurs along with maternal age (18). Accordingly, preimplantation genetic diagnosis of aneuploidy has demonstrated that the number of chromosomal abnormalities increases proportionally with the patient's age (9, 19). The expected effect of the selection of euploid embryos for transfer should be the overcoming of the age factor. The data reported here demonstrate an increased implantation rate in women aged ≥ 36 years compared with the controls. The ongoing implantation rate of embryos selected by fluorescence in situ hybridization is also significantly higher, confirming previous results showing a reduced risk of miscarriage associated with a normal chromosomal status (20).

The analysis of the error rate inherent in the technique has permitted us to define strategies to improve the reliability of fluorescence in situ hybridization. Special attention has been dedicated to decrease the incidence of false monosomies and trisomies by improving the fixation procedure and assessing the scoring criteria for the correct interpretation of fluorescent signals (16). Interestingly, mosaicism did not appear to

represent a major source of error because the implementation of several probes allowed the recognition of complex abnormalities. Although very limited information is available on the constitution of the embryos classified as euploid, the misdiagnosis rate in this study (5% of the abnormal embryos were found to be chromosomally normal) is in agreement with other published data. In view of these findings, the probability of a trisomic pregnancy occurring in older women after preimplantation genetic diagnosis of aneuploidy is reduced to the levels detected in patients aged < 34 years (9, 16).

At this point, the question arises about the age above which preimplantation genetic diagnosis of aneuploidy should be recommended to the patient, with the aim of increasing her chances of taking home a healthy infant. Indeed, most women who are > 40 years of age have only a limited number of embryos; in many centers, all of the embryos would be routinely transferred because no increase in multiple pregnancies has been reported (21). However, this debatable approach is absolutely unacceptable in the age range of 36–39 years, where cohorts of embryos are still large. Therefore, the clinical outcome of the patients included in group 1 was analyzed according to their age, with the aim of verifying whether a cutoff point exists between advantage and nonadvantage associated with the transfer of embryos diagnosed by fluorescence in situ hybridization.

As Table 3 illustrates, there was a statistically significant difference in terms of pregnancy and implantation rates in patients from the control group aged 36–37 years versus older women. This trend does not occur when embryo selection is based on the chromosomal analysis of normally developing embryos, leading to percentages of success that are equivalent regardless of maternal age. These findings suggest that endometrial receptivity is not the main cause of the decline of fertility with age, as also demonstrated by the

implantation rate calculated per pregnant patient. The influence of the uterine role can be minimized if the implantation potential of the transferred embryos is evaluated in patients who became pregnant.

According to the current results, embryos evaluated as normal by fluorescence in situ hybridization showed an implantation rate of 57.1% in pregnant women aged 36–37 years and 58.1% in patients >38 years. When compared with the controls, results were similar for the youngest patients (43.1% at 36–37 years), whereas differed significantly for the older age categories considered as a group (31.6% after 38 years; $P < .05$). This confirms the fundamental role of the chromosomal constitution in determining reproductive failure related to meiotic nondisjunction, as already postulated by the data from spontaneous abortions. Nevertheless, more data are needed to evaluate whether a different rate of implantation can be identified in relation to age in patients >40 years, to establish up to which point the oocyte quality is the prominent factor.

The second group of poor-prognosis patients considered in this study included patients who underwent at least three unsuccessful IVF cycles, despite the transfer of morphologically normal embryos. They were admitted to fluorescence in situ hybridization analysis because of the possibility that chromosomal abnormalities could represent the cause of nonimplantation. Although more than half of the embryos analyzed were diagnosed as aneuploid, the comparison with the clinical outcome in the control group did not show any difference, suggesting that other factors contribute to this poor-prognosis condition. It is interesting that the most frequent defects characterized in these patients are mosaicism and haploidy/polyploidy, the incidences of which increase proportionally with the number of previous IVF cycles (15).

These defects could be attributed to different causes that are related to disturbances in the mechanisms of cell division, where centriolar defects and asynchrony between karyokinesis and cytokinesis events may play a key role (22–24). However, the lack of difference in success rates obtained despite the selection of chromosomally normal embryos suggests that failed implantation occurs at later stages and may be determined by defects associated with either endometrial receptivity or chromosomes that are different from the ones that were screened. In addition, inability of the embryo to hatch intact from the ZP and the higher incidence of fragmentation and slow cleavage, which are frequent in this category of patients, could contribute to the poor implantation of their embryos (12).

Finally, a high frequency of chromosomally abnormal embryos (62%) was detected in patients with an altered karyotype; as anticipated, monosomy and trisomy were the most common defects both in patients with balanced translocations and in patients with gonosomal mosaicism (Table 5). Although no increase in the percentage of gonosomal aneuploidies has been observed, data from these patients

suggest that the mechanisms involved in cell division may have a predisposition not to function properly, creating monosomic or trisomic cell lines the distribution and frequency of which vary in the different tissues, including the gonads. Therefore, the possibility of selecting euploid embryos for transfer minimizes the risk of carrying a trisomic fetus or having a spontaneous abortion due to numerical chromosomal aberrations. As indicated in Table 2, the implantation potential of embryos selected by fluorescence in situ hybridization was significantly higher, suggesting that patients with an altered karyotype, especially when presenting with recurrent miscarriages, could consider preimplantation genetic diagnosis of aneuploidy as a tool to optimize the possibility of taking home a healthy infant.

The general conclusions yielded by this study are summarized in Table 4. The implementation of preimplantation genetic diagnosis of aneuploidy in patients with a poor prognosis leads to a statistically significant reduction in the number of cycles transferred (78% versus 93% in the controls; $P < .001$). However, the pregnancy rate per started cycles does not differ between the study and the control groups, indicating that a notable proportion of patients after fluorescence in situ hybridization analysis are spared the stress of undergoing the unnecessary expectation and therapy of a hopeless embryo replacement.

Furthermore, the mean number of embryos transferred per patient is significantly lower when chromosomal analysis is performed, but resulting in a higher potential of implantation. This increase is independent of maternal age (after preimplantation genetic diagnosis, 56.4% in patients aged ≤ 35 years and 57.9% in older patients; for the controls, 39.3% and 38.5%, respectively). This result confirms that in these patients, the reproductive failure may be determined by the high incidence of chromosomally abnormal embryos. This finding could have an immediate impact on the incidence of multiple pregnancies, and careful reconsideration of the number of embryos transferred is now mandatory.

The current results show that some patients can take real advantage from the chromosomal analysis of IVF-generated embryos: [1] women aged ≥ 38 years, [2] carriers of an altered karyotype, and [3] patients diagnosed with pathologies such as fibromas, myomas, diabetes, or hypertension, in which a multiple pregnancy could be extremely dangerous for the patient's health. The benefits reside not only in the reduced risk of trisomic fetuses, but also in an improved efficiency of IVF procedures and an improvement in overcoming the poor-prognosis condition.

Acknowledgments: The authors thank all the colleagues of the scientific, clinical, nursing, administrative, and technical staff of the Reproductive

Medicine Unit at S.I.S.M.E.R., whose unconditioned dedication and contributions made this study possible.

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