Journal of Assisted Reproduction and Genetics, Vol. 15, No. 5, 1998

Preimplantation Diagnosis of Common Aneuploidies by the First- and Second-Polar Body FISH Analysis

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Submitted: November 18, 1997
Accepted: November 18, 1997

Purpose: *A low pregnancy rate in in vitro fertilization (IVF) patients of advanced maternal age may be caused by aneuploidies originating from non disjunction in the first or second meiotic divisions. We introduced genetic testing of oocytes by sampling and fluorescent in situ hybridization (FISH) analysis of the first and second polar bodies, to avoid fertilization and transfer of aneuploid oocytes in IVF patients of advanced maternal age.*

Methods: *Three hundred and sixty-three IVF patients 34 years and older participated in the study. Using micromanipulation procedures, the first and second polar bodies were removed following their extrusion from the oocytes and studied by FISH, using probes specific for chromosomes 13, 18, and 21 to detect oocytes with common aneuploidies.*

Results: *Of a total of 538 IVF cycles, 3250 oocytes were available for FISH analysis, with conclusive FISH results in 2742 oocytes (84.3%). As many as 1102 (40%) of oocytes were predicted to be aneuploid and not transferred. Of 1640 embryos predicted to be normal, 1145 were transferred in 467 treatment cycles, resulting in 107 pregnancies (23%), from which 67 healthy children have been born, 32 pregnancies spontaneously aborted, and 15 pregnancies are ongoing after being confirmed normal by prenatal diagnosis.*

Conclusions: *Preimplantation diagnosis by first- and second-polar body FISH analysis allows us to avoid the age-related risk of common aneuploidies in IVF patients of advanced maternal age.*

KEY WORDS: preimplantation diagnosis; first polar body; second polar body; fluorescent in situ hybridization (FISH) chromosome 13-, 18-, and 21 specific probes; chromatid errors; common aneuploidies.

INTRODUCTION

Most patients referred for in vitro fertilization (IVF) are of advanced maternal age, with an elevated risk for conceiving and delivering a child with chromosome aneuploidies. Aneuploidies may also result in spontaneous abortions or nonimplantation, considerably decreasing the patient's chances of becoming pregnant. Because the risk of conceiving a trisomic fetus in women of advanced maternal age is considerably higher due to increased non disjunction occurring during maternal meiosis, the possibility of detecting trisomies before pregnancy should eliminate the need for abortion of affected fetuses. Avoiding the age-related risk of common aneuploidies may also improve the efficiency of IVF in couples of advanced maternal age.

To detect common aneuploidies before pregnancy, we introduced genetic testing of oocytes by sampling of the first and second polar bodies (PBs), allowing preselection of normal embryos for transfer (1). The application of the fluorescent in situ hybridization (FISH), using chromosome-specific probes, appeared to be accurate for detection of chromosome signals in interphase nuclei of the first and second PBs, and prediction of the genotype in the corresponding oocytes (2–5).

This paper describes the results and clinical outcome of the preselection of aneuploidy-free embryos in IVF patients of advanced maternal age.

MATERIALS AND METHODS

The present clinical trial involves 363 IVF patients older than 35 years (538 clinical cycles), including 193 patients (235 clinical cycles) described elsewhere (3–5). The informed consent form and the protocol for the study were approved by the institutional review board of Illinois Masonic Medical Center.

The first and second PBs were biopsied simultaneously after fertilization by the micromanipulation technique and analyzed by three-color fluorescent probes, including human chromosome 13-, 18- and 21-specific probes (Vysis, Downers Grove, IL) as described else-

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1058-0468/98/0500-0285$15.00/0

where (2–5). The signals were registered using a Nikon Microphot-MFA microscope (Nikon, Nelvile, NY) and an Optical Image Analysis System (Oncor, Gaithersburg, MD; currently, Vysis, Downers Grove, IL) and scored as described previously (2–5).

RESULTS

The results of the analysis of the first and second PBs in 363 IVF patients of advanced maternal age are presented in Table I. Of 3250 oocytes available for FISH analysis, conclusive FISH results were obtained in 2742 (84.3%) oocytes. Errors were observed in 1102 (40%) oocytes based on the analysis of the first and/or second PB (Fig. 1). As many as 793 (33.7%) of 2355 first PBs with FISH results and 556 (24.9%) of 2229 second PBs were aneuploid (Table II). The majority of aneuploidies were represented by missing (predominantly in the first PB) or extra chromatids (predominantly in the second PB) (Table III). Of 793 abnormal first PBs, 438 (52.2%) lacked a chromatid, 65 (8.2%), lacked a chromosome, 128 (16.1%) had an extra chromatid, 10 (1.3%) had an extra chromosome, and 152 (19.2%) involved different types of abnormalities. Of 556 abnormal second PBs, 259 (46.6%) had an extra chromatid, 232 (41.7%) had a missing chromatid, and 65 (11.7%) had both missing and extra signals for different chromosomes studied.

Results of both PBs were available in 1717 (62.6%) of 2942 oocytes with FISH data, of which 899 (52.4%) were normal, 246 (14.3%) had errors in both the first and the second PBs, 352 (20.5%) had errors only in the first PB, and 220 (14.3%) had errors only in the second PB (Table IV). Of 246 oocytes with errors in both PBs, 93 (37.8%) had different chromosomes involved and the rest involved the same chromosome errors in both the first and the second PB, resulting in an apparently balanced pattern in 52.8% of these cases (79 of 153 oocytes). Overall, 379 (34%) of 1102 aneuploid oocytes had complex errors (Table V). These included (a) errors involving more than one chromosome in either the first or the second PB; (b) errors of more than one chromosome in both PBs; (c) errors of the same chromosome in both PBs; (d) errors in both PBs, involving different single chromosomes; and (e) errors involving more than one chromosome in one and a single chromosome in the other PB. Of 379 complex errors, 169 (45.1%) involved a single chromosome, the majority (121 oocytes) being the same chromosome in the first and second PBs, and 206 (54.9%) involved more than one chromosome, which in 162 cases were the same chromosomes in both PBs.

Of 1640 oocytes predicted to be error-free for the chromosomes studied, 1298 were transferred in 467 treatment cycles, resulting in 107 pregnancies, 67 births of healthy children, 32 spontaneous abortions, and 15 ongoing pregnancies confirmed to be unaffected by prenatal diagnosis (Table VI). The pregnancy rate per transfer was 26.8% in those cycles in which oocytes were selected based on both-PB FISH analysis, compared to 20.7% when only one PB was available for analysis.

Table I. Results of PB FISH Analysis Using Probes for Chromosomes 13, 18, and 21

Couples	Cycles	Total oocytes studied	Oocytes with FISH results	Normal oocytes	Abnormal oocytes
363	538	3250	2742	1640 (60%)	1102 (40%)

DISCUSSION

As shown by the presented data, the PB testing has allowed us to detect and avoid from transfer of 1102 embryos resulting from oocytes with first and second meiotic errors, which should contribute considerably to the patients' chances of having a normal child. The observed 40% frequency of aneuploidies may be an overestimate, due to possible limitations of the FISH technique. Follow-up study of the abnormal PB results by FISH analysis of the embryos resulting from these oocytes confirmed the predicted results in 66.2% of cases, and the diagnosis was also confirmed in all resulting pregnancies, by chorionic villus sampling or amniocentesis (except for those resulting in spontaneous abortions). Although we were able to follow up only about one-third of embryos resulting from abnormal oocytes, extrapolation of these data to the whole sample suggests a possible correction of 33.8% (considering 272 of 1102 abnormal oocytes attributable to possible technical errors). This suggests the much lower frequency of 27% (730 of 2742 oocytes with FISH results). There might be different explanations for the fact that about one-third of the follow-up embryos had genotypes different from those predicted by the PB analysis. This proportion will definitely decrease with the improvement of the preparation of PB slides, as well as with the improvement of the

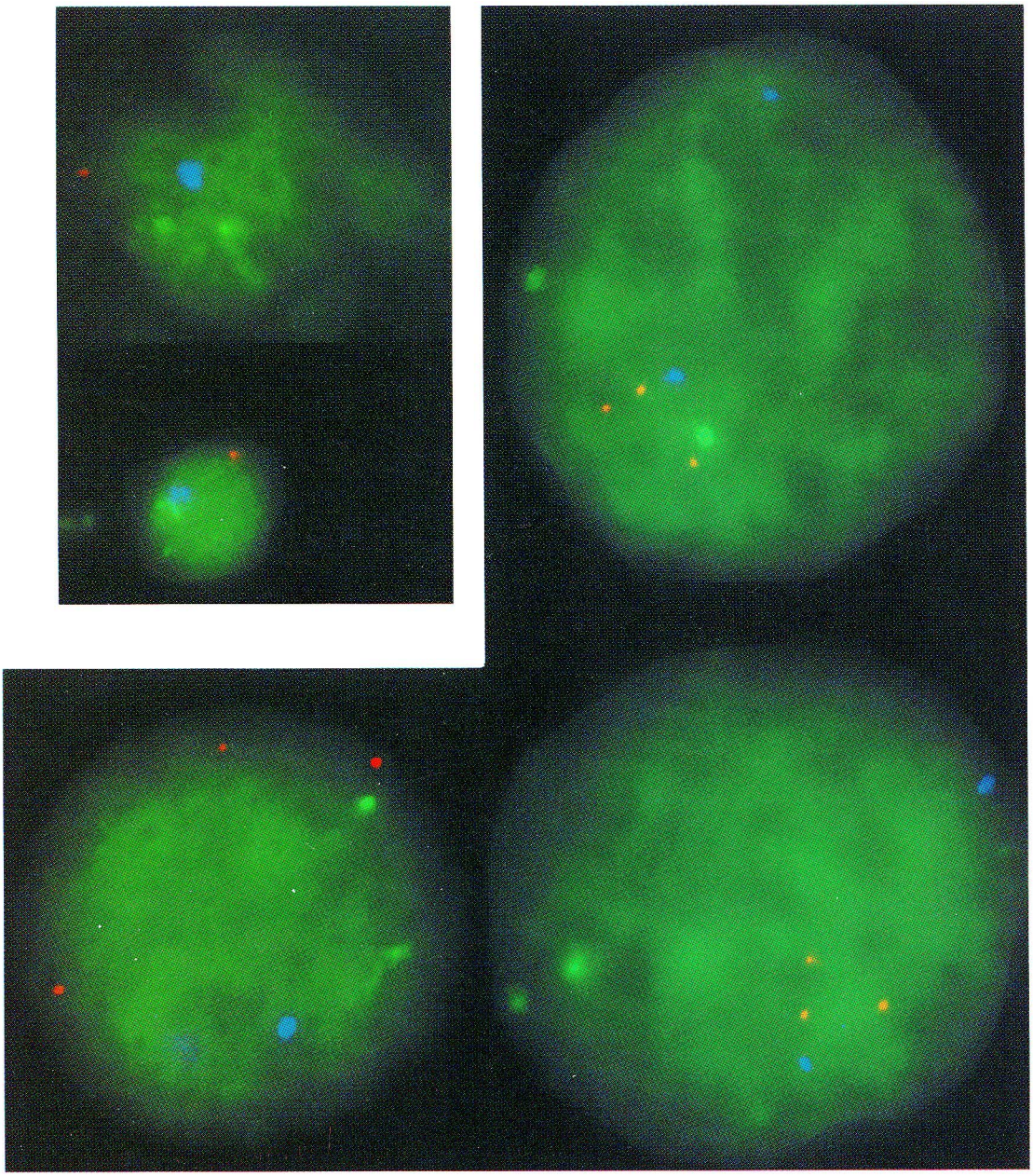

Fig. 1. Pattern of signals in the first and second polar bodies studied by FISH, using chromosome 13-, 18-, and 21-specific probes, predicting trisomy 21. An Oocyte with both the first (upper left) and the second (middle left) polar body, the first polar body showing one signal (red dot) for chromosome 21 (instead of the two expected signals). Other signals in the first polar body are normal: two signals for chromosome 13 (two green dots) and two for chromosome 18 (two blue dots). The second polar body has a normal pattern of signals: one signal for chromosome 13 (one green dot), one for chromosome 18 (one blue dot), and one for chromosome 21 (one red dot). Three blastomeres (upper right, bottom left and right) showing trisomy 21 in the embryo resulting from the corresponding oocyte: three signals for chromosome 21 (three red dots), two for chromosome 13 (two green dots), and two for chromosome 18 (two blue dots).

Table II. Summary of FISH Analysis in the First (IPB) and Second (IIPB) Polar Bodies

	IPB		IIPB	
FISH data	No.	%	No.	%
Normal	1562	66.3	1673	75.1
Abnormal	793	33.7	556	24.9
Total	2355	100	2229	100

Table III. Types of Chromosomal Abnormalities in the First (IPB) and Second (IIPB) Polar Bodies

	IPB		IIPB	
Type of FISH pattern	No.	%	No.	%
Extra signal	138	17.4	259	46.6
Missing signal	503	63.4	232	41.7
Complex	152	19.2	65	11.7
Total	793	100	556	100

FISH technique. For example, some of the missing signals might be due to artifactual loss of pieces of the first PB, resulting in the missing signals. On the other hand, hybridization failure will also result in overestimation of the missing signals, especially in the first PB. Both factors might well contribute to the approximately 3:1 ratio of missing/extra signals in the first PB. The other possible contributing factor is the completeness of the oocyte testing, which should ideally be based on both-PB FISH results, to improve the accuracy of the diagnosis. Although minor, the paternal contribution to the embryo abnormalities may also explain the possible discordance of the follow-up genotypes from those predicted.

Because our data include the analysis of most common aneuploidies, involving chromosomes 13, 18, and 21, the cumulative frequency will probably represent the majority of the meiotic errors. In addition, the fact that 34% of all aneuploidies were represented by complex errors (Table V) involving more than one chromosome, or a combination of aneuploidies in both meiotic divisions, may suggest meiotic spindle errors, which is in agreement with recent data about age-related abnormalities of meiotic spindle formation (6).

Table V. Complex Aneuploidies

No. abnormal oocytes	Complex errors	Involving 1 chromosome	Involving >1 chromosome
1102	379 (34%)	169 (45.1%)	206 (54.9%)

Finally, the other important factor that will definitely contribute to the estimated frequencies is the relationship of errors of MI and MII. As mentioned, as many as 14.3% of oocytes studied by both first and second PBs appeared to have errors in the first and second meiotic divisions, confirming the suggestions that nondisjunction in the second meiotic division may be due to abnormal first meiotic division and may also reflect errors in the spindle formation process.

The clinical significance of preimplantation diagnosis of chromosomal aneuploidies is obvious from the outcomes of the transfer of preselected embryos based on PB FISH analysis. As demonstrated, preimplantation diagnosis was confirmed by prenatal diagnosis in 107 resulting pregnancies, which have already resulted in the birth of 67 healthy children (Table VI). The overall pregnancy rate was 23.6%, reaching as high as 26.8% in those cases in which oocytes were tested by analysis of both the first and the second PBs.

In conclusion, our data show the accuracy and reliability of PB FISH analysis for avoiding the age-related risk of common aneuploidies in IVF patients of advanced maternal age. Using this method we avoided the transfer of 1102 chromosomally abnormal embryos, which would have been used in traditional IVF and could also have contributed to the pregnancy failures or spontaneous abortions. The study also provided original data about the frequency of first and

Table IV. Types of Abnormal Oocytes Based on First (IPB)- and Second (IIPB)-Polar Body FISH Analysis

Type of abnormal oocytes	No.	%
IPB + IIPB	246	22.3
IPB	547	49.6
IIPB	306	28.1
Total Abnormal	1102	100

Table VI. Clinical Outcome of Transfers of Selected Trisomy Free Embryos

Cycles	Normal oocytes	Total oocytes transfer	Transfers	Pregnancies	Children born
538	1640	1208	467	107	67[a]

[a] Eight singletons and two twins (18 pregnancies ongoing and 9 that resulted in spontaneous abortions).

second meiotic errors in stimulated cycles and their predictive value in preimplantation diagnosis of chromosomal aneuploidies.

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