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Prevention of Age-Related Aneuploidies by Polar Body Testing of Oocytes

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Purpose: *We previously demonstrated that aneuploidy-free oocytes may be preselected by testing the first and second polar bodies removed from oocytes following their maturation and fertilization. The present paper describes the results of the application of the method in 659 in vitro fertilization cycles from patients of advanced maternal age.*

Methods: *Using micromanipulation techniques, 3943 oocytes were tested by polar body sampling and fluorescent in situ hybridization analysis using specific probes for chromosomes 13, 18, and 21.*

Results: *Fluorescent in situ hybridization results were available for 3217 (81.6%) of 3943 oocytes studied, of which 1388 (43.1%) had aneuploidies; 35.7% of the aneuploidies were of first meiotic division origin, and 26.1% of second meiotic division origin. Most errors in the first meiotic division were represented by chromatid malsegregation. The transfer of embryos deriving from 1558 of 1829 aneuploidy-free oocytes in 614 treatment cycles resulted in 131 clinical pregnancies and 88 healthy children born after confirmation of the polar body diagnosis.*

Conclusions: *Polar body testing of oocytes provides an accurate and reliable approach for prevention of age-related aneuploidies in in vitro fertilization patients of advanced maternal age.*

KEY WORDS: preimplantation diagnosis; chromosome 13, 18, and 21 aneuploidies; FISH; first and second polar bodies; IVF.

INTRODUCTION

It has been shown previously that the genotype of oocytes may be evaluated by testing the first (I) and second (II) polar bodies (PBs), extruded in the process of maturation and fertilization of the oocyte (1). Although this was initially applied for couples at high risk of having children with single-gene disorders, the method appeared to be of particular relevance for in vitro fertilization (IVF) patients of advanced maternal age, whose reduced pregnancy rates may be due to the increased incidence of age-related aneuploidies (2,3). The application of PB testing to IVF patients of advanced maternal age revealed a high frequency of aneuploid oocytes due to the first and second meiotic division errors. The major types of abnormalities were represented by chromatid errors, which were shown by follow-up studies to result in aneuploid embryos (4). Further data will be needed to investigate the clinical significance and the impact of aneuploidy testing of oocytes on prevention of age-related aneuploidies and improvement of IVF efficiency in patients of advanced maternal age.

The present paper describes experience with the application of PB testing for prevention of age-related aneuploidies in IVF patients of advanced maternal age, demonstrating the practical value of preselection of aneuploidy-free oocytes in assisted reproduction practices.

MATERIALS AND METHODS

The material included 659 treatment cycles performed in 425 IVF patients aged 35 years and older who volunteered to participate in preimplantation genetic diagnosis by PB testing of oocytes. This includes 363 patients (538 cycles) described earlier (2–4). All patients were from the Chicago area, and the protocol and informed consent for this study were approved by the Institutional Review Board at Illinois Masonic Medical Center. Both the IPB and the IIPB were removed following fertilization and studied by fluorescent specific probes for chromosomes 13, 18, and 21 (Vysis, Downers Grove, IL) (2–4). The fluores-

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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
425	659	3943	3217	1829 (56.9%)	1388 (43.1%)

cent signals were scored by a Nikon Microphot-MFA microscope (Nicon, Nelvile, NY) and an Optical Image Analysis System (Vysis).

RESULTS AND DISCUSSION

Of 3943 oocytes obtained from 659 treatment cycles and subjected to PB sampling and fluorescent in situ hybridization (FISH) analysis, results were available for 3217 (81.6%) oocytes (4.88 oocytes per cycles). As shown in Table I, 1388 (43.1%) of the oocytes with FISH results were predicted to be aneuploid, leaving 1829 (2.7 per cycle) for transfer. As many as 70.5% of the oocytes with FISH results had data for both the IPB and the IIPB, 16.3% for only the IPB, and 13.2% for only the IIPB. As seen from the summary of FISH results for the IPB and IIPB presented in Table II, 996 (35.7%) of IPBs demonstrated abnormalities, compared to 703 (26.1%) abnormalities in IIPBs. The types of IPB abnormalities were missing chromatids in 519 (52.1%), extra chromatids in 160 (16.1%), missing chromosomes in 88 (8.8%), extra chromosomes in 7 (0.7%) and complex abnormalities, involving different types of abnormalities, in 222 (22.3%) (Table III). The proportion of abnormal oocytes with missing and extra chromatids in their IIPBs was 45.9% and 41%, respectively. The IIPBs of the rest of the oocytes had complex abnormalities, involving missing and extra chromatids of different chromosomes (Table IV). Our data on the proportion of different types of abnormal oocytes analyzed by both the IPB and the IIPB remained similar to those described earlier (3), despite an additional 286 abnormal oocytes included in the analysis (Table V). Of 1388 abnormal oocytes, 685 (49.4%) had meiosis I errors, 392 (28.2%) had meiosis II errors, and 311 (22.4%) had both meiotic errors. The involvement of different chromosomes in the latter group of abnormal oocytes is shown in Table VI. The same chromosome was involved in more than half of these oocytes (181 of 311), resulting in a balanced status in 114 (36.7%) of them (an example of the resulting balanced oocytes

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

	IPB		IIPB	
FISH data	No.	%	No.	%
Normal	1795	64.3	1990	73.9
Abnormal	996	35.7	703	26.1
Total	2791	100	2693	100

Table III. Types of Errors in the First Meiotic Division Segregation Patterns

Types	No.	%
Extra chromatid	160	16.1
Missing chromatid	519	52.1
Extra chromosome	7	0.7
Missing chromosome	88	8.8
Complex	222	22.3
Total	996	100

Table IV. Types of Chromosomal Abnormalities in Second Polar Bodies

Type of FISH pattern	No.	%
Extra signal	323	46
Missing signal	288	41
Complex	92	19
Total	703	100

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

Type of abnormal oocytes	No.	%
IPB + IIPB	311	22.4
IPB	685	49.4
IIPB	392	28.2
Total	1388	100

Table VI. Oocytes with Both the IPB and the IIPB Abnormal

Both PBs abnormal for chromosome 18	29 (9.3%)
Both PBs abnormal for chromosome 21	122 (39.2%)
Both PBs abnormal for chromosome 13	17 (5.5%)[a]
Abnormal for >1 same chromosomes	13 (4.2%)
Abnormal for different chromosomes	130 (41.8%)
Total with both PBs abnormal	311 (100%)
Total balanced	114 (36.7%)

[a] Testing started from 1997.

with a missing signal for chromosome 21 in the IPB and an extra signal in the IIPB is shown in Fig. 1). Of 181 of the abnormal oocytes with the same chromosomes involved in meiosis I and meiosis II errors, 122 had chromosome 21 aneuploidy, and only 29 had chromosome 18 aneuploidy (analysis of chromosome 13 is not applicable, as the corresponding probe has been used only since 1997). As many as 130 (41.8%) of the abnormal oocytes in this group had different chromosomes involved, representing the majority of the oocytes with complex errors discussed below.

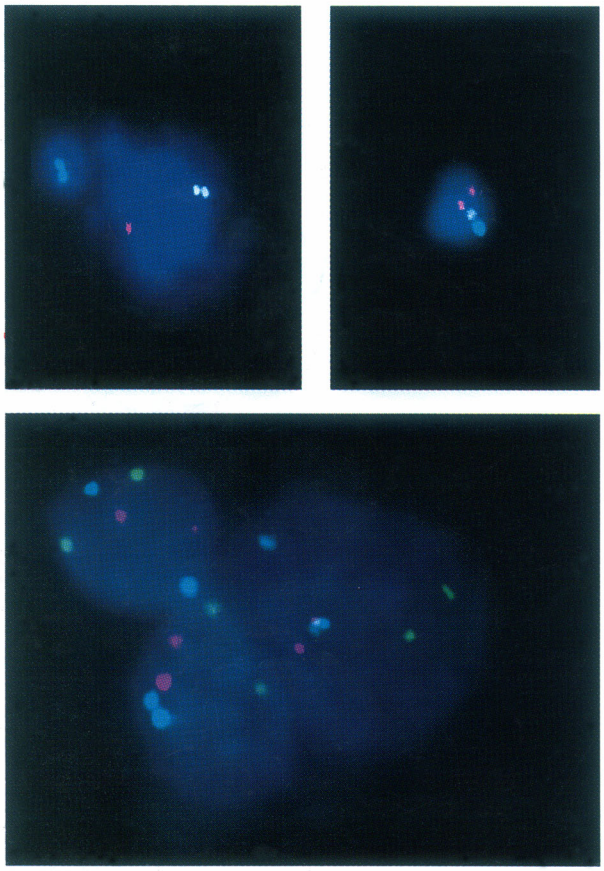

Fig. 1. Pattern of signals in the first and second polar bodies studied by FISH, using chromosome 13-, 18-, and 21-specific probes, predicting a balanced karyotype. An oocyte with both the first (upper left) and the second (upper right) polar body, the first showing one signal (orange dot) for chromosome 21 (instead of the two expected signals) and the second showing two signals (two orange dots) for chromosome 21 (instead of the one expected signal). Other signals in the first and second polar body are normal. Two signals for chromosome 13 (two yellow/green dots) and two for chromosome 18 (two aqua/blue dots) are seen in the first polar body, and one signal for chromosome 13 (one yellow/green dot) and one for chromosome 18 (one aqua/blue dot) in the second polar body. Three blastomeres (bottom) showing normal patterns of signals for chromosome 21 (two orange dots), chromosome 13 (two yellow/green dots), and chromosome 18 (two aqua/blue dots).

Study of chromosome-specific patterns of errors in the first and second meiotic divisions was possible in 1646 oocytes for chromosomes 18 and 21 and 909 oocytes for chromosome 13 (Table VII). Of 284 oocytes with chromosome 21 errors, 133 (46.8%) originated from meiosis I, 87 (30.6%) from meiosis II, and 64 (22.6%) from both meiotic divisions. A similar pattern was observed for chromosome 13 errors, with 35 (44.3%) originating from meiosis I, 27 (34.2%) from meiosis II, and 17 (21.5%) from both. Although the chromosome 18 errors also occurred predominantly in meiosis I (107 of 179), this is opposite to the data derived from postnatal cases of trisomy 18, shown to originate predominantly in meiosis II (5). Further data must be collected to investigate the biological significance of these differences.

As many as 527 (37.9%) of the abnormal oocytes had complex errors, involving the same chromosome in both meiotic divisions in 168 (31.9%) and more than one chromosome in 359 (68.1%) (Table VIII), of which 324 had abnormalities of two, and 35 had abnormalities of all three chromosomes studied. This further supports earlier reports suggesting a possible increase in mitotic spindle formation errors with age (6). The other observation supporting this hypothesis is a minor increase in the aneuploidy rate with the application of an additional chromosome-specific probe for preimplantation genetic diagnosis of age-related aneuploidies (Table IX). As shown by these data, an aneuploidy rate of 39.8% was detected by the application of two chromosome-specific probes (chromosomes 18 and 21) for analysis of 2839 oocytes in 484 clinical cycles (3). However, with the addition of a third chromosome-specific probe (chromosome 13) for testing of age-related aneuploidies in the most recent 175 clinical cycles, involving the analysis of a further 841 oocytes, the overall aneuploidy incidence has increased only from 39.8 to 43.1%. As shown by the above analysis, the application of additional chromosome-specific probes will probably affect the proportion of abnormal oocytes with complex errors, rather than the overall incidence of aneuploid oocytes. This is also in agreement with the data presented in Table VIII, showing a higher proportion of abnormal oocytes with two or more chromosomes involved, compared to similar data described earlier, in which only 54 of 538 clinical cycles were performed using an additional chromosome 13 probe (3).

Table VII. Origin of Nondisjunction in Aneuploidies by PB FISH Analysis of Chromosomes 13, 18, and 21

Chromosome	No. oocytes studied	No. abnormal	MI	MII	MI and MII
13	909	79 (8.7%)	35 (44.3%)	27 (34.2%)	17 (21.5%)
18	1646	179 (10.9%)	107 (59.8%)	49 (27.4%)	23 (12.8%)
21	1646	284 (17.3%)	133 (46.8%)	87 (30.6%)	64 (22.6%)

Table VIII. Complex Aneuploidies

No. abnormal oocytes	Complex errors	Involving the same chromosome in each PB	Involving >1 chromosome		
			2 chromosomes	>2 chromosomes	Total
1388	527 (37.9%)	168 (31.9%)	324	35	359 (68.1%)

Table IX. Aneuploidy Rates with Application of an Additional Chromosome-Specific Probe for PB FISH Analysis of Oocytes

Years of analysis	Patients/cycles	Oocytes studied/results	Abnormal oocytes (%)
1994–1997, without chromosome 13-specific probe	337/484	2839/2376	39.8
1997–present, with chromosome 13-specific probe	425/659	3943/3217	43.1

As mentioned, of 3217 oocytes with FISH results, 1829 were predicted to be aneuploidy-free, of which 1558 (2.5 per cycle) were transferred in 614 treatment cycles, resulting in 131 (22.3%) clinical pregnancies and 88 healthy children born after confirmation of the PB diagnosis (Table X). In addition to avoiding the transfer of embryos resulting from 1388 aneuploid oocytes (2. 1 per cycle), contributing to the prevention of the birth of children with common aneuploidies, PB testing of oocytes will also improve the chances of IVF patients becoming pregnant. Of 3943 oocytes obtained from 659 IVF patients of advanced maternal age (6 per cycle), only half (3 per cycle) would have been selected in routine IVF. In the absence of genetic preselection, this number may have incidentally included at least one or two aneuploid embryos, which would lead to reproductive failures. In addition, because at our center the number of embryos transferred is limited to three, selection of aneuploid embryos might have caused euploid embryos not to be transferred. The exclusion from transfer of as many as 1388 aneuploid oocytes (2.1 per cycle), therefore, should have contributed to the 22.3% pregnancy rate in our group of patients whose average maternal age was 38.6 years. However, more data will be needed to investigate the impact of preselection of aneuploidy-free oocytes on the efficiency of IVF.

Table X. Clinical Outcome of Transfers of Selected Aneuploidy-Free Embryos

Cycles	Normal oocytes	Total oocytes transferred	Transfers	Pregnancies	Children born
659	1829	1558	614	131[a]	88

[a] Eighteen pregnancies ongoing and 37 that resulted in spontaneous abortions.

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