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Chromosomal abnormalities in the first and second polar body

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Abstract

Aneuploidy free oocytes may be pre-selected by testing the first and second polar bodies removed from oocytes following their maturation and fertilization. We present here our experience on the application of the method in IVF cycles from patients of advanced maternal age. Overall, 5590 oocytes were obtained from 917 cycles and tested by polar body sampling and fluorescent in situ hybridization (FISH) analysis using specific probes for chromosomes 13, 16, 18, 21 and 22. FISH results were available in 4599 (82.2%) of 5590 oocytes studied, from which 2077(45.2%) were with aneuploidies. Thirty six point one percent of aneuploidies were of the first meiotic origin, and 29.3% of the second meiotic origin. Most errors in the first meiotic division were represented by chromatid errors. The transfer of embryos deriving from 2014 of 2520 aneuploidy free oocytes in 821 treatment cycles resulted in 182 (22.2%) clinical pregnancies and 140 healthy children born after confirmation of the polar body diagnosis. Polar body testing of oocytes provides an approach for pre-selection of aneuploidy free embryos, improving pregnancy rate in IVF patents of advanced maternal age.

Keywords: Preimplantation diagnosis; Chromosome 13, 16, 18, 21 and 22 aneuploidies; FISH; First and second polar body; IVF patients of advanced maternal age

1. Introduction

We previously reported the possibility of genotyping oocytes by testing the first (I) and second (II) polar body (PB) (Verlinsky et al., 1997). The method appeared to be of particular relevance for IVF patients of advanced maternal age, whose reduced pregnancy rates were shown to be due to the increased incidence of age-related aneuploidies (Verlinsky et al., 1996, 1998b). This has been confirmed by previous reports on the application of PB testing of oocytes in this group of patients, which also revealed a high frequency of aneuploidy deriving from the fist and second meiotic errors (Verlinsky et al., 1998a). Further data are presently being collected to investigate the clinical significance and the impact of oocyte aneuploidy testing on improving IVF efficiency in the patients of advanced maternal age. The present experience of the application of PB testing in IVF patients of advanced maternal age is described below demonstrating a practical value of pre-selection of aneuploidy free oocytes in IVF.

2. Material and methods

The material includes 917 treatment cycles performed in 585 IVF patients 35 and older. This material was partially reported earlier (Verlinsky et al., 1996, 1998a,b). The protocol and informed consent of the study were approved by the Institutional Review Board at Illinois Masonic Medical Center. Both IPB and IIPB were removed following fertilization and studied by fluorescent specific probes for chromosomes 13, 16, 18, 21 and 22 (Vysis, Downers Grove, IL) (Verlinsky et al., 1996, 1998a,b). The fluorescent signals were scored by a Nikon Microphot-MFA microscope (Nicon, Nelvile, NY) and Optical Image Analysis System (Vysis, Downers Grove, IL).

3. Results and discussion

Of 5590 oocytes obtained from 917 treatment cycles and subjected to PB sampling and FISH analysis, re-

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Table 1
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
585	917	5590	4596	2520 (54.8%)	2077 (45.2%)

sults were available in 4596 (82.2%) oocytes. As shown in Table 1, 2077 (45.2%) of oocytes with FISH results were predicted to be aneuploid, leaving 2520 for transfer. As many as 72.6% of the oocytes with FISH results had data for both IPB and IIPB, 15% with only IPB and 12.4% with only IIPB. As seen from the summary of FISH results in the IPB and IIPB presented in Table 2, 1449 (36.1%) of IPB demonstrated abnormalities, compared to 1143 (29.3%) abnormalities in IIPB. The types of abnormalities in IPB were represented by missing chromatids in 743 (51.3%), extra chromatids in 236 (16.3%), missing chromosomes in 119 (8.2%), extra chromosomes in 10 (0.7%) and complex abnormalities, involving different types of abnormalities, in 341 (23.5%) (Table 3). The proportion of abnormal oocytes with missing and extra chromatids in their second polar bodies was 44.1 and 39.1%, respectively. The second polar bodies of the rest of oocytes were with complex abnormalities (16.8%), involving missing and extra chromatids of different chromosomes (Table 4). Of 2077 abnormal oocytes, 933 (44.9%) were with meiosis I errors, 627 (30.2%) with meiosis II errors, and 517 (24.9%) with both meiotic errors (Table 5). The same chromosome was involved in 517 of these oocytes, resulting in a balance status in 187 (36.2%) of them. As many as 250 (41.8%) of the abnormal oocytes in this group were with different chromosomes involved, representing the majority of the oocytes with complex errors discussed below.

As many as 827 (39.8%) of abnormal oocytes had complex errors, involving the same chromosome in both meiotic divisions in 243 (29.4%), and more than one chromosome in 584 (70.6%) (Table 6), of which 496 (84.9%) were with abnormalities of two and 88 (15.1%) with abnormalities of all three chromosomes studied. This corroborates with earlier reports on a possible increase of mitotic spindle formation errors with age (Battaglia et al., 1996). The other data in support of spindle formation errors may be obtained from the aneuploidy rates with the application of additional chromosome specific probe for PGD of age-related aneuploidies (Table 7). As seen from these data, the aneuploidy rate of 39.8% was detected by the application of two chromosomes specific probes (chromosomes 18 and 21) for analysis of 2839 oocytes in 484 clinical cycles (Verlinsky et al., 1998b). However, with addition of the third chromosome specific probe (chromosome 13) for testing of age-related aneuploidies in additional 226 clinical cycles, involving the analysis of further 1095 oocytes, the overall aneuploidy incidence has only increased from 39.8 to 42.6.1% (Verlinsky et al., 1998a). The further addition of two more chromosome specific probes (chromosomes 16 and 22) for testing 207 additional cycles, involving the analysis of 1125 oocytes, led to 45% aneuploidy rate. Thus, it can be expected that

Table 2
Summary of FISH analysis in the first (IPB) and second (IIPB) polar bodies

FISH data	IPB		IIPB	
	Number	%	Number	%
Normal	2567	63.9	2752	70.7
Abnormal	1449	36.1	1143	29.3
Total	4016	100	3895	100

Table 3
Types of errors in the first meiotic division segregation patterns

Types	Number	%
Extra chromatid	236	16.3
Missing chromatid	743	51.3
Extra chromosome	10	0.7
Missing chromosome	119	8.2
Complex	341	23.5
Total	1449	100

Table 4
Types of chromosomal abnormalities in the second polar bodies (IIPB)

Type of FISH pattern	Number	%
Extra signal	504	44.1
Missing signal	447	39.1
Complex	192	16.8
Total	1143	100

Table 5
Types of abnormal oocytes based on first (IPB)- and second (IIPB)-polar body FISH analysis

Types of abnormal oocytes	Number	%
IPB + IIPB	517	24.9
IPB	933	44.9
IIPB	627	8.2
Total abnormal	2077	100

Table 6
Complex aneuploidies

Number of abnormal oocytes	Complex errors	Involving the same chromosome in each PB	Involving >one chromosome		
			Two chromosomes	>Two chromosomes	Total
2077	827 (39.8%)	243 (29.4%)	496	88	584 (70.6%)

Table 7
Aneuploidy rates with application of additional chromosome specific probe for PB FISH analysis of oocytes

Years of analysis	Patients/cycles	Oocytes studied/results	Abnormal oocytes (%)
1994–1997 Chromosome 18 and 13/21 specific probes	337/484	2839/2376	39.8
1997–1988 Chromosome 13, 18 and 21 specific probes	456/710	4255/3471	42.6
1999–Present Chromosome 13, 16, 18, 21 and 22 specific probes	585/917	5590/4596	45.2

Table 8
Clinical outcome of transfers of selected aneuploidy free embryos

Cycles	Normal oocytes	Total oocytes transferred	Transfers	Pregnancies	Children born
917	2520	2014	821	182[a]	140

[a] 13 pregnancies ongoing and 49 that resulted in spontaneous abortions.

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 7, showing higher proportion of abnormal oocytes with two or more chromosomes involved, compared to similar data described earlier, in which only 207 of 917 clinical cycles were performed using additional chromosome 16 and 22 probes.

Of 4596 oocytes with FISH results, 2520 were predicted to be aneuploidy-free, from which 2014 were transferred in 821 treatment cycles, resulting in 182 (22.2%) clinical pregnancies and 140 healthy children born after confirmation of the polar body diagnosis (Table 8). The exclusion from transfer of 2077 aneuploid oocytes should have contributed to 22.2% pregnancy rate in our group of patients whose average maternal age was 38.6 years. The ongoing randomized trial on the subject will allow investigating if the impact of pre-selection on aneuploidy free oocytes on the efficiency of IVF is statistically significant. It may also be suggested that pre-selection of aneuploidy free oocytes may be even of a higher value for younger IVF patients, because much higher number of oocytes are available for testing. This will allow improving the standards of assisted reproduction practices, substituting the present used blind selection of oocytes by genetic testing and selection of oocytes with the highest possible potential to result in pregnancy.

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