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Prepregnancy Genetic Testing for Age-Related Aneuploidies by Polar Body Analysis

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ABSTRACT

Current practice for prevention of chromosomal aneuploidies involves prenatal screening and termination of pregnancy, a procedure that is not universally acceptable. We introduced prepregnancy genetic testing by sampling and fluorescence in situ hybridization (FISH) analysis of the first and second polar body (PB), to avoid fertilization and transfer of embryos resulting from aneuploid oocytes. In 395 *in vitro* fertilization (IVF) patients of advanced maternal age, the first and second PBs were removed following their extrusion from oocytes and studied by FISH, using probes specific for chromosomes 13, 18, and 21, to detect and avoid the transfer of oocytes with common aneuploidies. Overall, 3,651 oocytes obtained from 598 IVF cycles were available for FISH analysis, with 2,952 showing interpretable FISH results (80.9%). The analysis revealed 1,271 (43.1%) oocytes with aneuploidy, which were excluded from transfer and subjected to follow-up FISH analysis to confirm PB diagnosis in the cleavage or blastocyst stage embryos. Only embryos originating from 1,681 aneuploidy-free oocytes were transferred back to patients, resulting in 119 pregnancies overall, from which 78 healthy children have already been born, 35 were spontaneously aborted, and 16 are ongoing, after confirming PB diagnosis by prenatal diagnosis. The results demonstrate that PB-based preimplantation diagnosis may be used for prepregnancy screening in women with age-related risk for common aneuploidies.

INTRODUCTION

CURRENT PRACTICE for the prevention of chromosomal aneuploidies is based mainly on prenatal testing, including maternal serum screening during the first and second trimester of pregnancy, high-resolution ultrasound monitoring for congenital malformations, and prenatal diagnosis by amniocentesis or chorionic villus sampling (CVS) in women of advanced maternal age. Although these techniques make it possible to detect and avoid the birth of children with the majority of chromosomal abnormalities, the affected pregnancies detected by prenatal diagnosis have to be terminated. To avoid termination of pregnancy following prenatal diagnosis, chromosomal anomalies have to be detected in gametes or preimplantation embryos, *i.e.*, prior to the establishment of pregnancy, so that the implantation of abnormal embryos may be avoided from the outset. Because chromosomal abnormalities may also result in spontaneous abortions, the substitution of prenatal diagnosis with a prepregnancy test may also avoid pregnancy failures caused by chromosomal abnormalities.

To detect chromosomal anomalies before pregnancy, we introduced a method for genetic testing of maternal gametes by sampling the first (I) and second (II) polar bodies (PB), which are by-products of the first and second meiotic divisions, allowing a preselection of normal oocytes for fertilization and transfer (Verlinsky *et al.*, 1990, 1992, 1994, 1996a,b, 1997). Because visualization of PB chromosomes cannot be performed reliably at the present time (Dyban *et al.*, 1993; Verlinsky, *et al.*, 1994), a fluorescence *in situ* hybridization (FISH) technique, using chromosome-specific probes, was applied and demonstrated to be accurate for detecting chromosomal abnormalities in PB interphase nuclei, and for predicting the genotype of the corresponding oocytes (Verlinsky *et al.*, 1995; Munne *et al.*, 1995; Dyban *et al.*, 1996).

This paper describes our current experience in the applica-

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tion of prepregnancy genetic testing for preselection of aneuploidy-free embryos in women of advanced maternal age.

MATERIALS AND METHODS

Overall, 395 patients older than 34 years, who were referred to our IVF center for different infertility problems (598 clinical cycles), were offered prepregnancy genetic testing for common aneuploidies. Some of this material has been presented elsewhere (Verlinsky *et al.*, 1995, 1996a,b, 1998).

Using micromanipulation techniques, IPB and IIPB were removed simultaneously after maturation and fertilization of oocytes and analyzed by fluorescent probes specific for human chromosomes 13, 18, and 21 (Vysis, Downers Grove, IL), as described previously (Verlinsky *et al.*, 1995, 1996a,b, 1998). The signals were registered using a Nikon Microphot-MFA microscope (Nikon, Nelvile, NY) and Optical Image Analysis System (Vysis, Downers Grove, IL) and scored according to the previously described criteria (Verlinsky *et al.*, 1995, 1996a,b, 1998; Dyban *et al.*, 1996). In brief, the presence of paired dots (signals) for each chromosome in IPB, coupled with a single dot (signal) for IIPB, was scored as a normal pattern, *i.e.*, each signal was scored as a single chromatid of the corresponding chromosome. Accordingly, two extra or missing signals in IPB were scored as a chromosome nondisjunction in the first meiotic division, while any lack or addition of one signal in IPB or IIPB was scored as a chromatid malsegregation in the first or second meiotic divisions. Therefore, any deviation from a normal pattern of signals in IPB and IIPB was considered abnormal, and embryos resulting from the corresponding oocytes were excluded from transfer and subjected to follow up FISH analysis for confirmation of the prepregnancy diagnosis. Because this work is still at the clinical trial stage, follow-up prenatal diagnosis by amniocentesis or CVS is performed for reassurance purposes, when the transfer of the preselected embryos results in a clinical pregnancy.

RESULTS AND DISCUSSION

The number of oocytes available for prepregnancy testing in each patient varied from 1 to 20, with an average of 6 per cycle/patient. Overall, 3,651 oocytes were subjected to PB removal and FISH analysis, with conclusive FISH results available in 2,952 oocytes (80.9%). The failure of diagnosis was either due to FISH failure (14.5%) or due to fragmentation or loss of IPB or IIPB (4.7%). It may be expected that the efficiency of FISH analysis will steadily increase with further development of micromanipulation techniques for PB removal and with improvement in chromosome-specific probes and the FISH technique overall.

As seen in Table 1, the number of oocytes with abnormal FISH patterns was as high as 43.1% (1,271 of 2,952 oocytes with FISH results), based on the analysis of IPB and/or IIPB. Figure 1 demonstrates one of the observed examples of abnormal oocytes with an error in IPB. A high frequency of errors was observed both in the first and second meiotic divisions. Of 2,952 oocytes with FISH results, 912 showed errors in IPB (35.8%), compared to 641 in IIPB (26.1%) (Table 2). Although, as expected, the frequency of errors was higher in IPB, the fact that almost a quarter of IIPBs were found with aneuploidies is quite a novel observation, suggesting a comparable rate of errors in the second meiotic division, requiring further data collection. The other unexpected observation concerns the types of errors in the first meiotic division, which were predominantly of chromatid rather than of chromosome origin, as seen from the types of IPB errors presented in Table 3. The majority of these errors were represented by missing or extra chromatids: 473 (51.9%) of 912 abnormal IPBs were found to lack a chromatid, 77 (8.4%) were found to lack a chromosome, 149 (16.3%) were found with an extra chromatid; 7 (0.8%) were found with an extra chromosome; and 206 (22.6%) were found to involve different types of abnormalities. Although some of these errors may be attributable to limitations of the FISH technique, such as a lack of single or paired dots, the follow-up analysis of the resulting embryos confirmed the predicted IPB diagnosis in more than half of the cases (62.7%). The accuracy of the PB diagnosis was demonstrated both for missing and extra signals.

In contrast to the IPB data, the distribution of missing and extra signals in IIPB was relatively equal. Of 641 abnormal second PBs, 294 (45.9%) were found with extra, 267 (41.7%) with missing, and 80 (12.4%) with both missing and extra signals for the different chromosomes studied. Because no such information has previously been available, the follow-up data is of great value, because it provides data on the biological significance of the errors in the second meiotic division. As in the IPB follow-up study, the FISH analysis of embryos resulting from oocytes with IIPB errors confirmed the predicted diagnosis in 75.9% of cases, also demonstrating the accuracy of IIPB FISH analysis for preselection of aneuploidy-free oocytes. It may be argued that the IIPB errors might not matter, as they would probably not result in a viable pregnancy, but this possibility may be explored in future studies by culturing and FISH analysis of the resulting embryos at the later stages of preimplantation development.

Taking into consideration only those errors in the first and second meiotic division for which the follow up data were available (35.5% of the total sampled), the observed frequency of chromosomal abnormalities might be reduced by 34.4%, changing 43.1% (Table 1) to 28.4%, which is still high.

As we have demonstrated, aneuploidies may originate both from first and second meiotic errors. Threfore, reliable prese-

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*
395	598	3,651	2,952	1,681 (56.9%)	1,271 (43.1%)

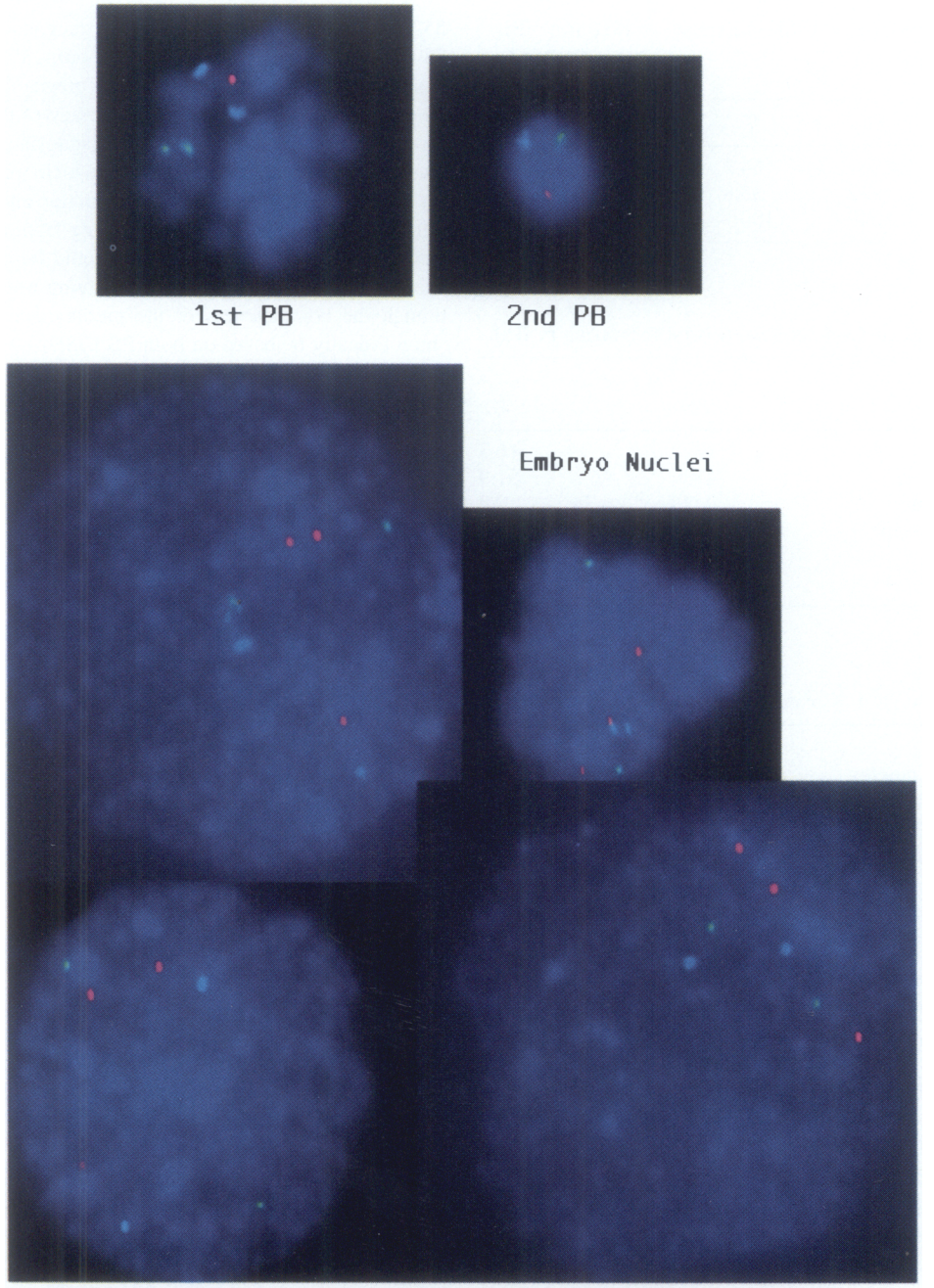

FIG. 1. Pattern of signals in IPB and IIPB studied by FISH, using chromosome 13, 18, and 21 specific probes, predicting trisomy 21. Oocyte with both IPB (upper left) and IIPB (upper right): IPB shows one signal (orange dot) for chromosome 21 (instead of two expected signals, leaving one chromatid unaccounted for chromosome 21, which was expected to be left in the corresponding oocyte). Other signals in IPB are normal: two signals for chromosome 13 (two green dots) and two for chromosome 18 (two aqua-blue dots). IIPB has a normal pattern of signals for chromosomes 13, 18, and 21: one signal for chromosome 13 (one green dot), one for chromosome 18 (one aqua-blue dot), and one for chromosome 21 (one orange dot). A follow-up FISH analysis of blastomeres (bottom), shows trisomy 21 in the embryo resulting from the corresponding oocyte: three signals for chromosome 21 (three orange dots), two for chromosome 13 (two green dots), and two for chromosome 18 (two blue dots).

lection of aneuploidy-free oocytes may be performed only if both IPB and IIPB are analyzed. Unfortunately, this was not the case in all our patients, particularly in the initial stage of study. Of 2,952 oocytes with FISH results, only 2,055 (69.6%) included results for both IPB and IIPB. More than half of these oocytes (50.9%) were normal, with the rest distributed as follows: 282 (13.7%) with errors in both IPB and IIPB, 468 (22.8%) with errors only in IPB, and 259 (12.6%) with errors only in IIPB. In the 282 oocytes with errors in both PBs, different chromosomes were involved in 119 (42.2%), with the rest involving the same chromosome errors in both IPB and IIPB. There was an apparently balanced pattern in 101 of the

TABLE 2. SUMMARY OF FISH ANALYSIS IN IPB AND IIPB

	IPB		IIPB	
FISH data	*No.*	*%*	*No.*	*%*
Normal	1,637	64.2	1,817	73.9
Abnormal	912	35.8	641	26.1
Total	2,549	100	2,458	100

TABLE 3. TYPES OF CHROMOSOMAL ABNORMALITIES IN IPB

Types of FISH patterns	*No.*	*%*
Extra chromatid	149	16.3
Missing chromatid	473	51.9
Extra chromosome	7	0.8
Missing chromosome	77	8.4
Complex	206	22.6
Total	912	100

TABLE 4. COMPLEX ANEUPLOIDIES

Number of oocytes Abnormal	*Complex errors*	*Involving 1 chromosome*	*Involving >1 chromosome*
1,271	*476*	*209*	*267*

282 oocytes (35.8%). It is of interest that 476 of 1,271 aneuploid oocytes (37.5%) were of a complex nature (Table 4). Of the 476 complex errors, 209 (43.9%) involved a single chromosome in both IPB and IIPB, with the chromosome being the same in both PBs in 150 oocytes; and 267 (56.1%) involved more than one chromosome, which were the same chromosomes in both PBs in 13 cases. This group of errors is of special interest, because these errors might suggest a mechanism for the errors observed. For example, the chromatid/chromosome nondisjunction may be due to errors in spindle formation in female meiosis. The latter have been shown to increase with advancing maternal age (Battaglia *et al.*, 1996).

Overall, 1,681 oocytes were predicted to be error-free for the chromosomes studied, and 1,433 of them resulted in the embryos acceptable for transfer in 557 treatment cycles. The number of preselected embryos available for transfer ranged from 1 to 13, with average of 2.6 per cycle, which is slightly below the number used in routine assisted reproduction practices. The 557 treatment cycles resulted in 119 pregnancies, from which 78 healthy children have already been born; 35 resulted in spontaneous abortions and 16 are ongoing pregnancies (Table 5). In all the ongoing pregnancies, the accuracy of the prepregnancy testing was confirmed by prenatal diagnosis (except for those resulting in spontaneous abortions, which were not available for karyotyping). Preimplantation detection and exclusion from transfer of the 1,271 embryos resulting from oocytes with the first and/or second meiotic errors would be expected to contribute to the patients' chances of having a normal child. Although the completeness of the prepregnancy testing, which should ideally be based on both PB FISH results, is an important prerequisite in improving the accuracy of the diagnosis, even partial information could contribute to an improved pregnancy outcome.

Because our data includes the analysis of the most common aneuploidies, involving chromosomes 13, 18, and 21, the cumulative frequency will probably represent the majority of the meiotic errors. Even if additional chromosome-specific probes are included in the PB analysis in the future, the overall frequency will probably not change considerably (although probes for additional chromosomes might increase the proportion of complex errors due to the involvement of more than one chromosome in the errors in the first and second meiotic divisions). The other important factor, which will definitely contribute to the estimated frequencies, is the relationship between errors in the first and second meiotic divisions. As mentioned, a considerable proportion of oocytes in which both IPB and IIPB were analyzed appeared to have errors in both the first and second meiotic divisions. This suggests that nondisjunction in the second meiotic division may be due to the errors in the first meiotic division, with both the consequence of errors in the spindle formation process.

The above data demonstrate the clinical significance of prepregnancy testing for age-related aneuploidies. The preselection of aneuploidy-free embryos for uterine transfer is one option to avoid prenatal diagnosis and termination of chromosomally abnormal pregnancies in women with an age-related risk for aneuploidies. Although it is too early to make conclusions about any improvement in pregnancy rates following prepregnancy diagnosis based on the PB FISH analysis, out of 557 cycles in a group of women in which the mean age was 38.5 years, 119 pregnancies were established (21.4%), and these have already resulted in the birth of 78 chromosomally normal children. Prenatal diagnosis in the resulting pregnancies, and the follow-up analysis of PB diagnosis in preselected abnormal embryos demonstrates the accuracy and reliability of PB FISH analysis as a means of avoiding the age-related risk of common aneuploidies in IVF patients of advanced maternal age. This study also provides original data on the frequency of the first and second meiotic errors in stimulated cycles, and their pre-

TABLE 5. CLINICAL OUTCOME OF TRANSFERS OF SELECTED TRISOMY-FREE EMBRYOS

Number of cycles	*Normal of oocytes*	*Total oocytes transferred*	*Number of transfers*	*Number of pregnancies*	*Number of children born*
598	1,681	1,433	557	119	78[a]

[a]60 singletons, 6 twins, and 2 triplets (16 pregnancies ongoing and 35 resulted in spontaneous abortions).

dictive value for preimplantation diagnosis of chromosomal aneuploidies.

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