

# First pregnancies after preconception diagnosis of translocations of maternal origin

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**Objective:** To develop an approach to preimplantation diagnosis of translocations that would not require the use of specific DNA probes for each translocation type.

**Design:** Retrospective analysis.

**Setting:** Clinic.

**Patient(s):** Two patients with 45XX,der(13;14)(q10;q10) karyotypes and one patient with a 46XX,t(4;14)(p15;q24) karyotype.

**Intervention(s):** Based on the observation that first polar body chromosomes remain at the metaphase stage for a few hours after oocyte retrieval, fluorescence in situ hybridization with chromosome-painting probes was used to perform preconception genetic diagnosis of translocation of maternal origin.

**Main Outcome Measure(s):** Oocyte FISH analysis.

**Result(s):** A total of 31 mature oocytes were produced, and results were obtained in 23 first polar bodies. After IVF-ET, all three patients became pregnant, preconception diagnoses being confirmed by prenatal diagnosis or birth.

**Conclusion(s):** Preconception diagnosis of translocations may reduce significantly the risk of chromosomally unbalanced offspring and pregnancy loss. This method is simpler than previous approaches because the need to develop specific DNA probes for each translocation type is avoided. (Fertil Steril® 1998;69:675–81. ©1998 by American Society for Reproductive Medicine.)

**Key Words:** Polar body, robertsonian translocation, trisomy 13, preimplantation genetic diagnosis

Reciprocal and robertsonian translocation carriers have a greater chance of being infertile, producing chromosomally abnormal offspring, or having multiple spontaneous abortions. Reproductive problems associated with translocations might be alleviated by performing preimplantation genetic diagnosis before ET in patients undergoing IVF-ET. Healthy infants have been born after preimplantation genetic diagnosis that resulted in embryos with X-linked genetic diseases, single gene defects, and aneuploidy being excluded from transfer (1). However, preimplantation genetic diagnosis of translocations has been rarely attempted because of the lack of a convenient method.

Chromosome analysis by conventional karyotyping cannot be used because an aver-

age of only 25% of blastomere nuclei show metaphase chromosomes after antimitotic treatment, and even fewer nuclei show banding-quality chromosomes (2). Similarly, G-banding of polar body or oocyte chromosomes cannot be achieved consistently because of the poor metaphase chromosomes obtained (3).

The only reliable method for preimplantation diagnosis of translocation is fluorescence in situ hybridization on interphase blastomeres, which requires that specific probes be developed for each type of translocation either flanking or expanding the break points (Munné S, Fung J, Cassel MJ, Márquez C, Weier HUG, unpublished data). The exception is robertsonian translocation, for which enumerator  $\alpha$ -satellite or locus-specific probes could be used to

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detect aneuploid embryos. Even in cases of robertsonian transfer, locus-specific probes cannot differentiate between balanced and normal embryos. When enough normal embryos are available, balanced embryos should not be transferred so that perpetuation of the genetic disease in the family is prevented.

In this study, we present a simple approach to preimplantation genetic diagnosis of translocations of maternal origin. It involves the use of painting probes on metaphase chromosomes from first polar bodies biopsied shortly after oocyte retrieval to differentiate between normal, balanced, and unbalanced oocytes. The analysis of first polar bodies was pioneered by Verlinsky and co-workers (4) for use in preconception diagnosis of monogenic diseases. Here we demonstrate its application to translocations of maternal origin.

## MATERIALS AND METHODS

### Source of Oocytes for Control Studies

Oocytes were obtained from the IVF program of the Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center in accordance with guidelines set by the internal review board of that center, and written consent was obtained from each patient.

Three types of oocytes were used: oocytes that failed to become fertilized after IVF, unfertilized mature oocytes from patients undergoing preimplantation diagnosis of aneuploidy, and matured in vitro oocytes at the germinal vesicle stage. Oocytes that failed to become fertilized after IVF (aged mature oocytes) were used to assess nuclear phase in aged polar bodies. To assess nuclear phase in aged polar bodies, we used unfertilized mature oocytes from which the first polar bodies were removed within 6 hours of retrieval from patients undergoing preimplantation genetic diagnosis of aneuploidy. After the nuclear phase was noted, the fixed polar bodies were used for preimplantation genetic diagnosis of aneuploidy in patients who consented to the procedure. For fluorescence in situ hybridization controls, fresh polar bodies from matured in vitro immature oocytes at the germinal vesicle stage of metaphase I were used.

Immature oocytes were cultured with inactivated follicular fluid from mature oocytes supplemented with 20% heat-inactivated human serum. They were checked for the presence of a first polar body early on the next day and every 4 hours thereafter. These polar bodies were fixed shortly after extrusion.

### Study of Metaphase Degeneration in First Polar Bodies

In a previous study, polar body chromosomes were found to degenerate shortly after retrieval or to be in interphase (5). Preliminary observations suggested that polar body chromosomes from fresh oocytes were still at the metaphase stage. To determine the best time to visu-

alize these metaphase chromosomes, polar bodies were obtained from fresh oocytes as well as oocytes aged between 24 and 72 hours.

### Clinical Cases

Three carriers of translocations agreed to undergo preimplantation genetic diagnosis.

Patient 1 was a 27-year-old female carrier who had a balanced robertsonian 45XX,der(13;14)(q10;q10) translocation. The couple was fertile, and the mother's condition as a carrier was ascertained after she miscarried a female fetus with trisomy 13. Karyotype analysis of both parents and the miscarried fetus was done elsewhere.

Patient 2 was a 33-year-old female carrier who had a balanced robertsonian transfer 45XX,der(13;14)(q10;q10). The couple was fertile, but she experienced four spontaneous miscarriages; a fifth pregnancy was terminated after detection of trisomy 13. Karyotype analysis of both parents and terminated fetus was done elsewhere.

Patient 3 was a 37-year-old female carrier who had a balanced reciprocal translocation with a 46XX,t(4;14)(p15.3;q24) karyotype, which was ascertained after repeated pregnancy loss. One of the spontaneous abortuses was karyotyped as being balanced for the translocation. Karyotype analysis of both parents and products of conception was done elsewhere.

### Oocyte Retrieval, Polar Body Biopsy, and IVF-ET

Patients underwent gonadotropin stimulation with GnRH-agonist suppression followed by administration of exogenous gonadotropins. Patients received leuprolide acetate beginning in the midluteal phase of the menstrual cycle before stimulation. This was followed by administration of four to six ampules of gonadotropins, beginning on cycle day 3. Dosages were adjusted based on individual responses using established criteria but generally were decreased when the maximum diameter of the lead follicles was >14 mm. Criteria for hCG administration included two or more follicles with a maximum diameter of between 18 and 20 mm or a single follicle of  $\geq 20$  mm in diameter. Oocyte retrievals were done transvaginally under ultrasound guidance. All visible follicles were aspirated.

At  $\leq 6$  hours after oocyte retrieval, first polar bodies from fresh metaphase-II oocytes were biopsied by partial zona dissection (6) in an area immediately in front of the polar body. A microneedle with a 15- $\mu$ m inner diameter and a tip beveled at 45° was used to penetrate the opening in the zona and draw out the polar body by suction. Polar body fixation was performed as described previously, without modification (5).

Embryos then were cultured for 3 days in human tubal fluid supplemented with maternal serum protein under min-

TABLE 1

Metaphase degeneration of first polar bodies in relation to time after oocyte retrieval.

No. of hours after retrieval	No. of polar bodies	Percentage of polar bodies at metaphase
0-6	32	90.6
10-48	58	31.0
≥72	83	14.5
Total	173	

eral oil at 37°C and 5% CO<sub>2</sub> in air. Embryo transfers were done in the afternoon of the 3rd day after retrieval.

### Fluorescence In Situ Hybridization Chromosome Painting on First Polar Bodies

For patients 1 and 2, fluorescence in situ hybridization chromosome painting (7) with whole chromosome probes specific for chromosomes 13 and 14 labeled with Spectrum-Green (Vysis, Downers Grove, IL) and Spectrum-Orange (Vysis), respectively, was used for the analysis of first polar body chromosomes. A 1.5 μL of each probe was added to 7 μL of whole chromosome probe hybridization buffer (Vysis).

For patient 3, a whole chromosome probe for chromosome 14 labeled with digoxigenin (Oncor), a whole chromosome probe for chromosome 4 labeled with Spectrum-Green (Vysis), and a probe for the 4p telomere region (D4F26; Oncor) were mixed in a 0.3:0.7:5 ratio, and 3 μL of the mixture was added to 7.0 μL of whole chromosome probe hybridization buffer (Vysis). The use of D4F26 was necessary because the whole chromosome probe for chromosome 4 did not cover the 4p telomeric region.

These hybridization solutions were codenatured with the corresponding slides by placing the mounted slides on a hot plate at 78°C for 3 minutes. The slides then were sealed with rubber cement and placed in a moist chamber at 37°C for overnight hybridization. Afterward, all slides were washed in 0.4× standard sodium citrate for 2 minutes, at 72°C for lymphocytes and 71°C for polar bodies. Slides with specimens from patients 1 and 2 were counterstained with 6-diamino-2-phenylindole in antifade. Slides with specimens from patient 3 were treated with a 1:1 mixture of rhodamine-labeled sheep anti-digoxigenin antibody (Oncor) and fluorescein-labeled avidin (Oncor), incubated for 30 minutes at 37°C, washed twice for 2 minutes in 1× phosphate-buffered detergent solution (Oncor), and counterstained with DAPI in antifade.

A fluorescence scope (BX60; Olympus, Lake Success, NY) with a triple-band pass filter was used to visualize simultaneously Spectrum-Green and fluorescein isothiocyanate (FITC), Spectrum-Orange or rhodamine, and Spectrum-

FIGURE 1

First polar body metaphase observed at phase contrast before fluorescence in situ hybridization analysis.



Aqua and the image recorded with an image analysis system (Metasystems, Belmont, MA).

## RESULTS

### Nuclear Phase of Polar Bodies

As shown in Table 1, first polar body chromosomes fixed ≤6 hours after oocyte retrieval were found to be at metaphase in 91% of the cases. This incidence decreased to 31% at ≥10 hours. One of these metaphases can be observed in Figure 1.

### Controls for Painting Probes: Results From Carrier Lymphocytes

All complete lymphocyte metaphases in specimens from patient 1 and 90% (n = 20) in specimens from patient 2 showed a chromosome 13, a chromosome 14, and a der(13;14)(q10;q10) chromosome. The other 10% of the metaphases from patient 2 showed two 13 chromosomes and two 14 chromosomes. All complete lymphocyte metaphases (n = 50) of patient 3 showed a 46XX,t(4;14)(p15.3;q24).ish t(4;14)(wcp4+,wcp14+; wcp14+,D4F26+) complement.

### Control for Painting Probes: Results From Fresh Polar Bodies From In Vitro Matured Oocytes

Forty germinal vesicle-stage oocytes that reached metaphase II after incubation were fixed, and intact polar bodies were obtained from 38 of these oocytes. In 31 cases, the polar body and its corresponding oocytes could be fixed and

TABLE 2

## Preimplantation genetic diagnosis results.

Polar body number	Fluorescence in situ hybridization*	Oocyte diagnosis
Patient 1		
1	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)*	Normal (F)
2	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (T)
3	22,X,-13.ish 13(wcp13-),14(wcp14+)	Disomy 13
6	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (T)
7	23X.ish 13(wcp13+),14(wcp14+)	Balanced
8	Doubtful	Doubtful
9	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (F)
10	Doubtful	Doubtful
11	22,X,-14.ish 13(wcp13+),14(wcp14-)	Disomy 14
12	Doubtful	Doubtful
13	22,X,-14.ish 13(wcp13+),14(wcp14-)	Disomy 14
14	23,X,-14,+der(13;14)(q10;q10).ish 13(wcp13+),14(wcp14-),der(13;14)(wcp13+,wcp14+)	Nullisomy 13
15	Doubtful	Doubtful
16	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (T)
17	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (F)
Patient 2		
1	23X.ish 13(wcp13+),14(wcp14+)	Balanced (T)
2	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (T)
3	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (A)
5	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (A)
6	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (T)
7	22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+)	Normal (no C)
Patient 3†		
1	23,X,-4,+der(4)t(4;14).ish der(4)(wcp 4+,wcp14+),(14)(wcp14+)	Unbalanced
2	Degenerated polar body	No result
3	23,X.ish (4)(wcp4+),(14)(wcp14+)	Balanced (T)
4	23,X,-4,+der(4)t(4;14).ish der(4)(wcp4+,wcp14+),(14)(wcp14+)	Unbalanced
5	23,X.ish (4)(wcp4+),(14)(wcp14+) 23,X,-4,+der(4)t(4;14).ish	Balanced (T)
6	der(4)(wcp4+,wcp14+),(14)(wcp14+)	Unbalanced

Note: A = embryo developmentally arrested; F = embryo frozen; T = embryo transferred; no C = zygote did not cleave.

\* Nomenclature according to the International System for Human Cytogenetic Nomenclature (8).

† The presence of D4F26 could not be determined because wcp4 and D4F26 were labeled both in green. D4F26 would be seen only if a der14 chromosome had been present.

analyzed. Of those, 21 were used as controls for patients 1 and 2, and only one was considered a fluorescence in situ hybridization error because the polar body showed a chromosome 13 and a chromosome 14 whereas the oocyte showed only a chromosome 14. The remaining 20 cases all showed a 23X.ish(13)(wcp13+),(14)(wcp14+) complement. Although no configuration resembling a derivative chromosome was found, four polar bodies showed chromosome 13 and chromosome 14 lying in parallel, side by side.

The other 10 eggs and corresponding polar bodies were analyzed with probe solution for patient 3. A normal 23X.ish (4)(wcp4+),(14)(wcp14+) complement was seen in each metaphase-II stage. The D4F26 probe could not be distinguished from the wcp4 because they were labeled in the same color.

### Case Results

Patient 1 produced 21 oocytes, patient 2 produced 11, and patient 3 produced 8, of which 15, 8, and 6, respectively,

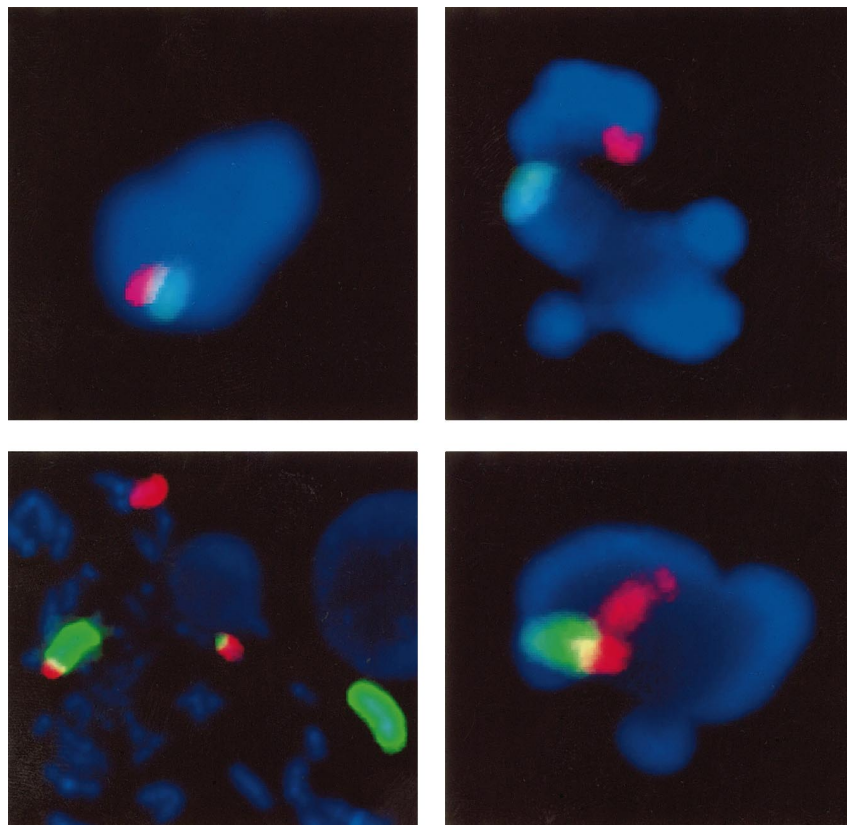
were mature metaphase-II stage. Only the mature oocytes were biopsied.

Fluorescence in situ hybridization results are shown in Table 2. Of the 17 polar bodies obtained from patient 1, 6 were found to be balanced (normal oocyte), 1 was normal (balanced oocyte), 2 were nullisomy 14 (disomy 14 in the oocyte), 1 was nullisomy 13 (disomy 13 in the oocyte), and 1 had the derivative chromosome der(13q10;14q10) plus a chromosome 13 (nullisomy 13 in the oocyte). The remaining 4 polar bodies did not produce clear results because of excessive chromosome condensation, either due to polar body damage during biopsy or to unsuitable polar body fixation. Of the normal oocytes that developed into embryos, three were transferred and three were frozen.

In the case of patient 2, no intact polar bodies were obtained in two oocytes, and of the remaining six biopsied mature metaphase II oocytes, five were balanced (normal oocyte) and one was normal (balanced oocyte). Two of

**FIGURE 2**

Chromosome painting of lymphocyte and polar body metaphases using wcp13 (green) and wcp14 (red) (*top panels*) and wcp4 (green), wcp14 (red), and D4F26 (green) (*bottom panels*). The top right panel shows a balanced polar body from patient 1 with a balanced karyotype 22,X,+der(13;14)(q10;q10).ish der(13;14)(wcp13+,wcp14+), indicating that the oocyte is normal. The top left panel shows a normal polar body from patient 1 with a karyotype 23X.ish 13(wcp13+),14(wcp14+), indicating that the oocyte had a balanced translocation. The bottom left shows a lymphocyte metaphase from patient 3 with a balanced karyotype 46,XX,t(4;14).ish der(4)(wcp4+,wcp14+),(14)(wcp14+,D4F26+). The bottom right panel shows an unbalanced polar body from patient 3, oocyte 6, with a karyotype 23,X,-4,+der(4)t(4;14).ish der(4)(wcp4+,wcp14+),(14)(wcp14+).



the chromosomally normal embryos and one of the balanced embryos were transferred because the other chromosomally normal embryos were either developmentally or morphologically unsuitable for transfer. In the case of patient 3, one polar body could not be recovered, three were unbalanced, and two were normal (egg balanced). The two balanced resulting embryos were transferred because no normal embryos were found. Examples of different configurations are shown in Figure 2. No predivision of chromatids was found on any of the analyzed polar bodies.

### Pregnancy Outcome

Patient 1 became pregnant with triplets, two of whom were identical. The couple declined prenatal diagnosis, and at birth the infants were found to be chromosomally normal (46XY); however, congenital malformation of the heart mus-

cle, a benign rhabdomyoma, was found in both monozygotic twins.

Patient 2 became pregnant with twins and delivered two healthy infants, one chromosomally normal and the other balanced for the robertsonian transfer. It is surprising that the carrier infant also was balanced for a second translocation of paternal origin, 46XXt(13p;20p), not ascertained in the father by the referring center before the preimplantation genetic diagnosis was performed. Because the polar body test can ascertain only translocations of maternal origin, this second translocation could not have been screened with this test.

Patient 3 became pregnant with a singleton but spontaneously aborted at 7 weeks. The product of conception showed a balanced karyotype, as predicted by the preconception diagnosis.

## DISCUSSION

The present study describes a simple approach to preimplantation genetic diagnosis of translocations of maternal origin. The test is based on the observation that first polar body chromosomes are mostly at metaphase shortly after oocyte retrieval, and therefore fluorescence in situ hybridization analysis with chromosome-painting probes can be performed easily for preimplantation genetic diagnosis of translocations. The test can be used for diagnosis of translocations in the general population; the incidence of translocations in the general population is relatively high (approximately 0.2%) (9).

This test has two advantages for affected couples. One advantage is that the risk of delivering trisomic offspring is reduced. For instance, 5% of newborns with trisomy 21 have their origin in robertsonian transfer carriers (10). Another advantage is that the chance of sustaining a pregnancy is increased. For instance, in the present study, five of eight embryos implanted. In addition to loss of trisomic pregnancies, translocations of maternal origin produce repeated spontaneous abortions, many of which show a balanced rather than an unbalanced translocation complement (10). By performing preimplantation genetic diagnosis and transferring only normal embryos, these patients may have a higher chance of producing a viable pregnancy.

Of these three cases, three balanced embryos were replaced on patient request because not enough normal embryos were available for transfer. Of those, two embryos implanted (but one spontaneously aborted) and the other arrived to term. It is of interest that the embryo with the balanced robertsonian transfer was the one that arrived to term.

Although this test is designed to study only the chromosomes involved in the translocation, for women of advanced maternal age it would be interesting also to screen the oocytes for common aneuploidies before ET. This can be achieved in several ways. First, after the polar bodies are analyzed for the translocations, they could be reanalyzed with chromosome X-, 13-, 16-, 18-, and 21-specific probes, following our previous protocol for cell recycling (11). Another approach could be to perform an embryo biopsy and blastomere analysis using chromosome XY-, 13-, 16-, 18-, and 21-specific probes; the disadvantage would be performing two micro-manipulation procedures on the embryo (polar body biopsy and blastomere biopsy). Finally, spectral imaging could be used to simultaneously determine translocations and aneuploidy in polar bodies, as was recently demonstrated (12).

This study is the first one to report the ratio of normal, balanced, and unbalanced oocytes in a robertsonian transfer carrier. Robertsonian transfers of paternal origin mostly produce alternate segregation with <10% of unbalanced sper-

matozoa (13). However, when the robertsonian transfer is maternal in origin, unbalanced karyotypes in amniotic fluid obtained by amniocentesis seem to be more common. For instance, amniocentesis results from carriers of 45XY, der(14;21)(q10;q10) and 45XY,der(21;22)(q10;q10) chromosomes show almost no unbalanced karyotypes when the origin is paternal but 15% when it is maternal (14). The present results support this observation because 23% of the oocytes analyzed were unbalanced. In addition, patients 1 and 2 in this study showed normal-to-balanced gamete ratios (5:1 and 6:1, respectively), which are higher than the previously reported ratio (5:4) for 45XY,der(13;14)(q10;q10) (13). In the case of patient 3, only adjacent 1 segregations were found among the unbalanced eggs. This was as expected because the exchanged segments were relatively short (14).

The present technique can be applied to any translocation of maternal origin provided that the probes that are used cover the translocated areas. Commercially available whole chromosome probes do not cover the telomere, centromere, and other regions, but as shown in the case of patient 3 in our study, telomere probes can be added to whole chromosome probes to characterize the translocation.

This test may also be difficult because it may involve predivision of chromatids, which may complicate analysis by confusing monovalent chromosomes with single chromatids (15). However, some predivision events are due to in vitro oocyte aging (5). Researchers can drastically reduce this artifact by performing the analysis shortly after oocyte retrieval or by using enumerator probes labeled in a different color than that used with whole chromosome probes (i.e., blue Spectrum-Aqua, Vysis) that can differentiate between whole chromosomes and single chromatids (two and one hybridization signals, respectively).

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