FERTILITY AND STERILITY®
VOL. 79, SUPPL. 3, JUNE 2003

Published by Elsevier Inc.
Printed on acid-free paper in U.S.A.

# Predictive value of sperm fluorescence in situ hybridization analysis on the outcome of preimplantation genetic diagnosis for translocations

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**Objective:** To determine whether the proportion of abnormal sperm is predictive of the proportion of abnormal embryos from couples in which the males are translocation carriers.

**Design:** Controlled clinical study.

**Setting:** Private in vitro fertilization (IVF) center.

**Patient(s):** Eleven cases of reciprocal translocation male carriers.

**Intervention(s):** Blood sample and sperm sample collection from each male partner. Embryo biopsy of the embryos produced in each cycle.

**Main Outcome Measure(s):** Fluorescence in situ hybridization on lymphocyte slides to characterize each translocation case, then fluorescent in situ hybridization (FISH) with specific probes for each of the sperm samples. Preimplantation genetic diagnosis of the translocations in the 11 cases.

**Result(s):** A correlation was found between the percentage of abnormal gametes and the percentage of abnormal embryos, and a predictive equation is proposed for this relationship: $A = -55 + (1.9 \times B)$, where $A$ is the percentage of abnormal embryos and $B$ the percentage of abnormal sperm.

**Conclusion(s):** The predictive value of the sperm analysis was established. Patients with 65% or less chromosomally abnormal sperm have a good chance at conceiving; patients with higher rates would need to produce 10 or more good quality embryos to have reasonable chances of conceiving. (Fertil Steril® 2003; 79(Suppl 3):1528–34. ©2003 by American Society for Reproductive Medicine.)

**Key Words:** FISH, PGD, IVF, sperm, translocation carriers, miscarriage

Received April 4, 2002; revised and accepted September 12, 2002.

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0015-0282/03/$30.00
doi:10.1016/S0015-0282(03)00252-8

Translocation carriers are found in 0.2% of the neonatal population, 0.6% of infertile couples, 2% to 3.2% of males with severe infertility, and up to 9.2% of couples experiencing recurrent miscarriages. Translocation carriers have high risks of infertility, recurrent spontaneous abortions, and conception of chromosomally abnormal pregnancies (1–4). Studies of development indicate that unbalanced embryos are as likely as normal embryos to develop to blastocyst stage, thus implanting and resulting in spontaneous abortions and/or affected offspring at the same rate (5). Preimplantation genetic diagnosis (PGD) has been offered to carriers of translocations as an alternative to prenatal diagnosis and pregnancy termination of unbalanced fetuses.

A variety of fluorescent in situ hybridization (FISH) methods have been attempted for PGD for translocations (5–14). By selecting only normal and balanced embryos after PGD for replacement, these patients should have a higher chance of conceiving and a reduced probability of spontaneous abortions and chromosomally unbalanced offspring. Indeed, PGD results indicate that patients undergoing PGD of translocations obtain a pregnancy rate comparable with regular in vitro fertilization (IVF) patients and a very significant reduction in the frequency of spontaneous abortions (7, 13). However, the pregnancy rate is inversely proportional to the number of abnormal gametes (13). It is highly desirable for prospective patients to know their chances of conception in

**TABLE 1**

Probes used in each case.

| Code | Translocation | Probes used[a] |
|---|---|---|
| 1 | 46,XY,t(1;13)(q42.12;q32.2) | 1 CEP (A), 1 Tel q (O), 13 LSI (G) |
| 2 | 46,XY,t(1;18)(p36;q21) | 18 Tel q (O), 1 Tel p (G), 18 CEP (A) |
| 3 | 46,XY,t(1;6)(p22;p21.3) | 1 CEP (O), 6 CEP (A), 6 Tel p (G) |
| 4 | 46,XY,t(2;18)(q11.2;q21.1) | 18 CEP (A), 2 Tel q (O), 2 Tel p (G) |
| 5 | 46,XY,t(3;4)(q12;p15.2) | 3 CEP (A), 3 Tel q (O), 4 Tel p (G) |
| 6 | 46,XY,t(5;15)(q35;q22) | 15 CEP (A), 15 Tel q (O), 5 Tel p (G) |
| 7 | 46,XY,t(6;9)(p12;q13) | 6 CEP (A), 9 Tel q (O), 6 Tel p (G) |
| 8 | 46,XY,t(8;22)(q24.22;q11.21) | 8 CEP (A), 8 Tel q (O), 22 LSI (G) |
| 9 | 46,XY,t(11;22)(q23;q11) | 22 LSI (A), 11 Tel q (O), CEP 11 (G) |
| 10 | mos46,XY,t(3;12)(p14;q24.3)/46,XY | 3 CEP (A), 12 Tel q (O), 3 Tel p (G) |
| 11 | 46,XY,t(15;22)(q22;q13) | 15 CEP (A), 15 Tel q (O), 22 LSI (G) |

*Abbreviations:* CEP = chromosome enumerator probe; A = aqua blue; Tel = telomeric probe; q = long arm; O = orange; LSI = locus specific probe; G = green; p = short arm.

[a] Chromosome targeted followed by the type of probe and in brackets the color label.

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advance, because the procedure of IVF and PGD is not only economically daunting but also medically complex. One of the principal factors affecting their chances is the percentage of abnormal gametes.

In the present study we attempt to determine whether there is a correlation between chromosome sperm abnormalities and resulting embryo abnormalities, and if so, to determine what the level of chromosome sperm abnormalities is that would preclude chromosomally normal conception.

## MATERIALS AND METHODS

### Patient Characteristics

In each case studied, the men are the only partners affected. The women were chromosomally normal (46,XX). This study was approved by the internal committee board of Saint Barnabas Medical Center, and written consent was obtained for all patients undergoing PGD.

### Probe Preparation and Testing

Before sperm or blastomere analysis, individual probe solutions were prepared and tested in each carrier's blood. Each probe solution used a combination of three probes labeled in three different colors (Spectrum Orange, Spectrum Aqua, and Spectrum Green). All probes were obtained from Vysis Inc. (Downers Grove, IL), and were either commercially available or provided by Dr. Larry Morrison of Vysis Inc. As used previously in other studies (13), the probes were two telomeric and one centromeric, or two centromeric and one telomeric. The probes used in each case are described in Table 1.

The probe solution was prepared as previously described (13). Briefly, we mixed 1 $\mu$L each of the three probes with 7 $\mu$L of whole chromosome hybridization solution (Vysis Inc.). This probe solution was first tested in lymphocyte metaphases from the translocation carrier to verify that the translocation was well characterized; that is, we verified that the two normal chromosomes and the two derivative chromosomes were labeled with different combinations of probes (Fig. 1).

### Sperm Fixation and FISH

Semen samples were fixed in methanol/acetic acid (3:1) for FISH analyses. To prepare the slides, eight drops of fixed sperm were placed on each. Once dried, slides were then washed twice in 2× standard saline citrate (SSC, Vysis Inc.) at room temperature (approximately 22°C), for 3 minutes each, dehydrated in ethanol series (70%, 85%, 100%) for 2 minutes each, and dried at room temperature in a slanted position. When dry, slides were placed in 5-nM dithiothreitol (DDT, Sigma Chemical Co., St. Louis, MO) and 1% Triton X-100 solution (Sigma Chemical) at 37°C for 13 minutes (15).

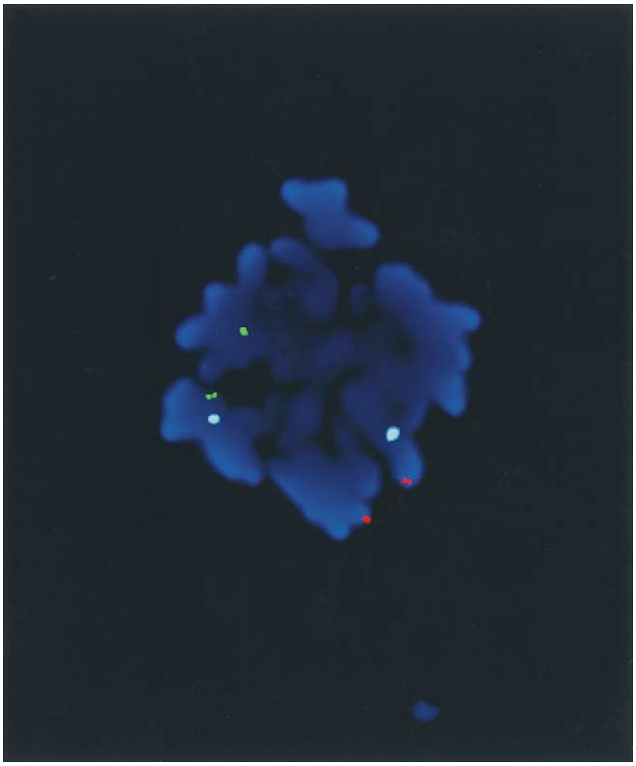
After that, the slides were denatured in 70% Formamide (Sigma Chemical) and 2× SSC solution for 5 minutes at 71°C. The slides were washed again in 2× SSC and dehydrated in ethanol series. The DNA probe solution, in an Eppendorf tube, was immersed in a water-bath for 5 minutes at 72°C. The probe was then applied to each glass slide containing the fixed sperm and was hybridized overnight at 37°C. Afterward slides were washed in 0.7× SSC at 71°C for 2 minutes, dried at room temperature, and counterstained with DAPI in antifade and covered with glass coverslips for analysis.

### Sperm Scoring

A study analyzing up to 11 different chromosomes by bicolor and tricolor FISH (16) reported ranges for sperm aneuploidy from 0.04% for chromosome 7 to 0.39% for chromosome 16; therefore, to determine differences between

FIGURE 1

Metaphase picture from patient 5 (46,XY, t(3;4)(q12;p15.2)) showing centromere of chromosome 3 labeled in aqua, q arm telomere of chromosome 3 labeled in orange, and p arm telomere of chromosome 4 labeled in green.



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patients, it is advisable to study at least 10,000 spermatozoa per male. However, translocation patients have been reported to have much higher rates of chromosomally abnormal spermatozoa, from 20% to 80% (17), so 1,000 sperm per male was considered sufficient to evaluate the overall frequency of unbalanced gametes. We analyzed a minimum of 1,000 sperm per patient. The scoring criteria of a previous study was followed without change (18), noting in particular that if two signals were separated by more than one domain (the diameter of the signal) they were counted as two different targets, and if closer, as a single target.

## Embryo Biopsy, Fixation, and FISH Analysis

One cell per embryo at the eight-cell stage was biopsied and fixed on a glass slide as previously described (8). Nine of the 11 biopsies were performed at the Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center in Livingston and one each at the other centers. All biopsied cells were fixed on slides and their analysis was done at the Institute for Reproductive Medicine and Science of Saint Barnabas. These procedures were approved by the respective institutional review boards committees and individual patient consent forms were signed in each of the participating centers.

The FISH was performed using the standard protocol provided by Vysis Inc. with slight modifications (8), employing the hybridization mixture appropriate for each translocation case. The slides were observed under a fluorescence scope (Olympus AX70; Olympus America, Inc., Melville, NY) with the DAPI, Green, Aqua, and Orange single bandpass filters.

FIGURE 2

(**A**) Closed and (**B**) open configurations at the first metaphase in meiosis.

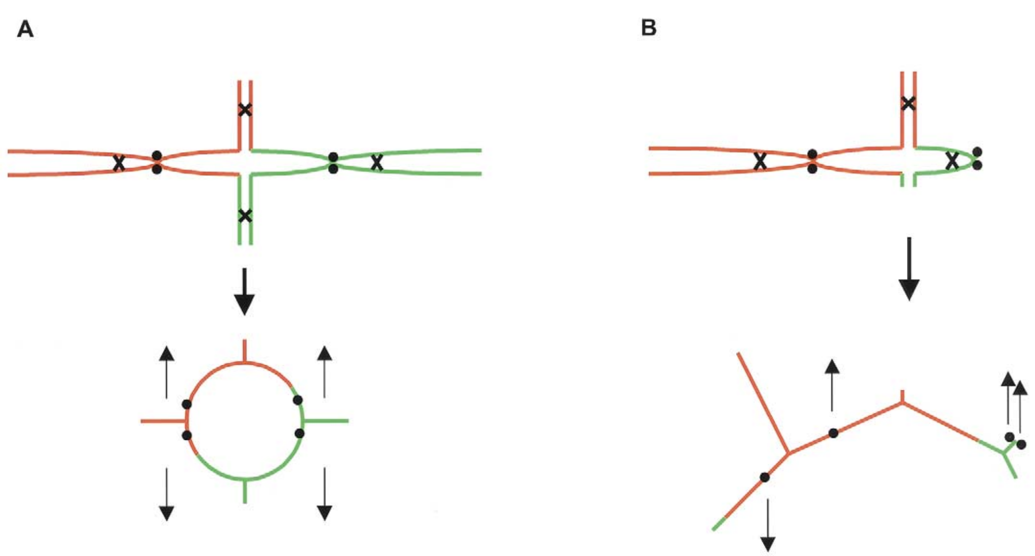


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**TABLE 2**

Results in sperm analysis.

| Code | Translocation | Alt | Adj I | Adj II | 3:1 | Others | NR | Total count |
|---|---|---|---|---|---|---|---|---|
| 1 | 46,XY,t(1;13)(q42.12;q32.2) | 37.0% | 29.0% | 8.2% | 23.3% | 1.6% | 1.0% | 1005 |
| 2 | 46,XY,t(1;18)(p36;q21) | 29.2% | 9.2% | 16.6% | 43.2% | 1.8% | 0.0% | 1000 |
| 3 | 46,XY,t(1;6)(p22;p21.3) | 37.4% | 43.1% | 7.7% | 10.7% | 0.9% | 0.2% | 1006 |
| 4 | 46,XY,t(2;18)(q11.2;q21.1) | 31.1% | 28.8% | 16.0% | 24.2% | 0.0% | 0.0% | 1079 |
| 5 | 46,XY,t(3;4)(q12;p15.2) | 23.9% | 14.7% | 24.5% | 34.2% | 2.7% | 0.0% | 1000 |
| 6 | 46,XY,t(5;15)(q35;q22) | 45.9% | 44.1% | 0.7% | 8.3% | 1.0% | 0.0% | 1032 |
| 7 | 46,XY,t(6;9)(p12;q13) | 23.6% | 14.1% | 40.1% | 21.9% | 0.3% | 0.1% | 1015 |
| 8 | 46,XY,t(8;22)(q24.22;q11.21) | 23.8% | 15.0% | 19.0% | 41.7% | 0.7% | 0.0% | 1003 |
| 9 | 46,XY,t(11;22)(q23;q11) | 21.8% | 14.4% | 31.5% | 30.3% | 1.3% | 0.7% | 1044 |
| 10 | mos46,XY,t(3;12)(p14;q24.3)/46,XY | 41.3% | 3.7% | 18.4% | 34.8% | 1.8% | 0.0% | 1000 |
| 11 | 46,XY,t(15;22)(q22;q13) | 18.6% | 15.9% | 16.0% | 43.3% | 5.8% | 0.0% | 1000 |
| Mean data | | 30.3% | 21.1% | 18.1% | 28.7% | 1.6% | 0.2% | 1016.7 |

*Abbrevations:* Alt = alternate segregation; Adj I = adjacent I segregation; Adj II = adjacent II segregation; 3:1 = 3:1 segregation; NR = no result.

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### Statistical Analysis

Analysis was done by using a weighted least squares regression, which has the effect of diminishing the impact of the patients represented by a small number of embryos.

## RESULTS

### Lymphocyte Controls for Telomeric and Centromeric Probes

The telomeric and centromeric probes were first tested on the fixed lymphocytes to make certain that every translocation was well characterized. The error rate for every probe was always lower than 5%; otherwise, a new set of probes was made until the error was found to be below 5%.

### Sperm Results

A total of 11,184 sperm were counted from the 11 patients included in the study. The proportion of balanced and normal gametes produced by alternate segregation ranged from 18.6% to 45.9%, with a mean value of 30.3% (± SD). Adjacent I segregation ranged from 3.7% to 44.1% with a mean value of 21.1% (± SD); adjacent II segregation ranged from 0.7% to 40.1% with a mean value of 18.1% (± SD); and 3:1 segregation ranged from 8.3% to 43.3% with a mean value of 28.7% (± SD). Also there were a small proportion of spermatozoa showing signal patterns that could not be explained by conventional segregation forms (ranging from 0 to 5.8%). These patterns may be caused by other phenomenon not directly related to translocations (such as polyploidy of the sperm or chromosome breakage) or technical errors (such as bad decondensation of some spermatozoa or background signals) (Table 2).

### PGD Results

Every patient underwent a single IVF cycle with PGD. A total of 99 embryos were biopsied: 96 produced nuclei and 93 gave results for the translocation. Of the 93 with results, 22 were considered normal or balanced due to alternate segregation, 42 were considered unbalanced for the translocation, and 29 were found to be abnormal by reason of aneuploidy, mosaicism, polyploidy, or haploidy. Of the 42 found to be unbalanced for the translocation, 15 had adjacent I segregation, 11 had adjacent II segregation, and 16 had 3:1 segregation (Table 3).

### Comparison Between FISH Sperm Analysis Results and PGD Results

Analysis was done by using a weighted least squares regression, which showed convincing statistical evidence ($P$=.006) of a positive association between the proportion of abnormal embryos and the proportion of abnormal sperm. The estimated equation was

$$\text{AbEmb} = -0.253 + 1.496\,(\text{AbSperm})$$

where AbEmb, and AbSperm are the proportions of abnormal embryos and abnormal sperm, respectively. The standard error of the slope parameter (1.496) was 0.419. The interpretation of this parameter is that a nominated increase (say, 0.1) in the proportion of abnormal sperm produces on average an increase one and a half times as large (0.15) in the proportion of abnormal embryos.

### Pregnancy Outcome

A total of 16 embryos were replaced in 9 of the 11 cases (cases 1, 2, 4, 5–10). Counting pregnancy as the presence of a fetal heartbeat, four of the nine transfers achieved pregnancy (cases 1, 6, 8, and 10), of which three are ongoing (cases 1, 6, and 10) and one has delivered (case 8).

In case 1, four embryos were replaced and three implanted; the couple chose to have a reduction performed, leaving two fetuses ongoing. Of these two, one is a karyo-

**TABLE 3**

Results in PGD analysis.

| Code | Translocation | Alt | Adj I | Adj II | 3:1 | Others | NR | Total embryos |
|---|---|---|---|---|---|---|---|---|
| 1 | 46,XY,t(1;13)(q42.12;q32.2) | 5 | 1 | 1 | 2 | 5 | 1 | 15 |
| 2 | 46,XY,t(1;18)(p36;q21) | 1 | 1 | 0 | 1 | 2 | 0 | 5 |
| 3 | 46,XY,t(1;6)(p22;p21.3) | 0 | 2 | 0 | 0 | 3 | 0 | 5 |
| 4 | 46,XY,t(2;18)(q11.2;q21.1) | 1 | 1 | 0 | 2 | 3 | 1 | 8 |
| 5 | 46,XY,t(3;4)(q12;p15.2) | 1 | 1 | 4 | 3 | 8 | 0 | 17 |
| 6 | 46,XY,t(5;15)(q35;q22) | 7 | 5 | 0 | 0 | 0 | 0 | 12 |
| 7 | 46,XY,t(6;9)(p12;q13) | 1 | 1 | 1 | 0 | 2 | 0 | 5 |
| 8 | 46,XY,t(8;22)(q24.22;q11.21) | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| 9 | 46,XY,t(11;22)(q23;q11) | 2 | 2 | 3 | 2 | 2 | 0 | 11 |
| 10 | mos46,XY,t(3;12)(p14;q24.3)/46,XY | 3 | 0 | 1 | 3 | 2 | 1 | 10 |
| 11 | 46,XY,t(15;22)(q22;q13) | 0 | 1 | 0 | 2 | 1 | 0 | 4 |
| Mean data | | 22 | 15 | 11 | 16 | 29 | 3 | 96 |

*Abbreviations:* Alt = alternate segregation; Adj I = adjacent I segregation; Adj II = adjacent II segregation; 3:1 = 3:1 segregation; NR = no result.

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typically normal male (46,XY) and the other is a balanced female carrier of the translocation (46,XX,t(1;13)(q42.12; q32.2)). In case 6, two embryos were replaced and one implanted. The fetus is a balanced female carrier of the inherited translocation and also has a de novo pericentric inversion in the heterochromatic region on chromosome 9 (46,XX,t(5;15)(q35, q22), inv(9qh)).

In case 8, two embryos were replaced. On of the embryos was fertilized with sperm of the translocation carrier and the other was fertilized with sperm from a donor. The mother delivered a chromosomally normal male child. The couple has purposely chosen to leave the paternity moot.

In case 10, two embryos were replaced, of which one implanted. The pregnancy is ongoing and the fetus is morphologically normal as per ultrasound, although no karyotype is as yet available (June 2001). Table 4 shows the pregnancy outcome, the embryos transferred, proportions of abnormal embryos, and proportions of abnormal sperm. There were no pregnancies in cases where only a single embryo was replaced. When there was over 60% abnormal embryos or over 63% of abnormal sperm, there were no pregnancies.

## DISCUSSION

In general, patients at our center conceive in 60% of cases on their first IVF attempt when three morphologically and developmentally normal embryos are transferred. Most translocation patients are fertile but produce a high propor-

**TABLE 4**

Pregnancy outcome.

| Code | Translocation | Pregnancy | No. of embryos replaced | Abnormal embryos (%/n) | Abnormal sperm (%) |
|---|---|---|---|---|---|
| 6 | 46,XY,t(5;15)(q35;q22) | Yes | 2 | 42 (5/12) | 54.1 |
| 10 | mos46,XY,t(3;12)(p14;q24.3)/46,XY | Yes | 2 | 60 (6/10) | 58.7 |
| 1 | 46,XY,t(1;13)(q42.12;q32.2) | Yes | 4 | 60 (9/15) | 62.1 |
| 8 | 46,XY,t(8;22)(q24.22;q11.21)[a] | Yes | 2[a] | 75 (3/4) | 76.4[a] |
| 3 | 46,XY,t(1;6)(p22;p21.3) | No | 0 | 100 (5/5) | 62.4 |
| 4 | 46,XY,t(2;18)(q11.2;q21.1) | No | 1 | 75 (6/8) | 69.0 |
| 2 | 46,XY,t(1;18)(p36;q21) | No | 1 | 80 (4/5) | 70.8 |
| 5 | 46,XY,t(3;4)(q12;p15.2) | No | 1 | 94 (16/17) | 76.1 |
| 7 | 46,XY,t(6;9)(p12;q13) | No | 1 | 80 (4/5) | 76.4 |
| 9 | 46,XY,t(11;22)(q23;q11) | No | 2 | 82 (9/11) | 77.5 |
| 11 | 46,XY,t(15;22)(q22;q13) | No | 0 | 100 (4/4) | 81.0 |

[a] Case 8 was a mixed transfer of one embryos produced with paternal sperm and one embryos produced with donor sperm. The paternal origin of the delivered baby is unknown.

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tion of unbalanced gametes that frequently result in spontaneous abortions. Thus, if enough chromosomally balanced or normal embryos are available for transfer after PGD, patients should conceive at similar rates. Actually, the rates could be even higher than for other patients, because the translocation might be their only fertility problem.

In the present series only five patients had two or more embryos available for transfer, but four of them conceived, and all of them are ongoing or have delivered. This is in line with previous observations that if enough chromosomally balanced or normal embryos are available for transfer, these patients have a good chance of conceiving and carrying the pregnancy to term (13).

It is generally understood that the meiotic behavior of reciprocal translocations depends on the chromosomes involved in the translocation, the position of breakpoints, and the crossovers in those chromosomes. Translocations between two big metacentric chromosomes with breakpoints close to the centromeres would be more prone to form a close configuration in the first metaphase of meiosis, like rings, which produces mainly 2:2 segregations (Fig. 2A). In these cases, the proportion of unbalanced gametes will be lower than in other translocations.

Other translocation cases involving small chromosomes, acrocentric chromosomes, or those with breakpoints close to telomeric regions are more prone to form open configurations like chains, which tend to produce 3:1 segregations (see Fig. 2B). These cases will have a higher proportion of unbalanced gametes than the translocations producing close configurations.

A patient's karyotype information can at best predict the most common segregation types for a specific translocation and provide the expected rate of unbalanced offspring. But it cannot foresee the chance of conceiving a normal baby in a specific pregnancy nor estimate the number of spontaneous abortions that would occur before conceiving a normal baby.

Previous studies of segregation modes have been based on post-zygotic material, and have been used to formulate rules to predict unbalanced offspring (19–21). But the specimens used in these studies came from fetuses and aborted fetuses, and because selective processes have already occurred, these specimens probably showed only the most viable segregation types. Thus, when analyzing zygotes and preimplantation embryos, is not surprising that different translocations involving the same chromosomes show very different meiotic behavior (22), and even unrelated cases with the same translocation could do the same (23).

The objective of this study was to determine if sperm chromosome abnormalities were a good predictor of pregnancy outcome, because a large number of decondensed spermatozoa can be analyzed using FISH, which gives a better statistical case-specific analysis in the unbalanced sperm forms.

With the exception of case 8, which was a mixed transfer including donor sperm embryos, pregnancy was only obtained in patients with less than 65% unbalanced sperm. In general about 20% to 25% of embryos have abnormalities attributed to oocyte factors and/or postmeiotic events (24–26), and one study reported high rates of mosaicism in some translocation patients (10). Most of these abnormalities can be detected by PGD translocation analysis. This leaves perhaps 75% of the embryos that could be affected by abnormal sperm, for which our test provides an accurate estimate. Although this was a small series, there were no differences in the total percentage of normal embryos and normal sperm. These results are in agreement with a previous study involving two Robertsonian translocation cases (27).

We conclude that translocation carriers with enough embryos of good morphology and development and with less than 65% unbalanced sperm have a reasonable chance of conceiving after IVF and PGD. But when the sperm is more than 65% abnormal, their chances at conceiving perceivably decrease. This information is very valuable for patients having to decide whether to proceed with PGD or to opt for sperm donation. We expect to confirm the present data with further PGD for translocation cases.

*Acknowledgments:* The authors thank the IVF team at SBMC, Larry Morrison, Ph.D., of Vysis, Inc., for his special attention to the individual probes used in this study, Giles Tomkin for editorial support, and Eurof Walters, Ph.D., for the statistical analysis.

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