



# The role for preimplantation genetic diagnosis in balanced translocation carriers

Jone E. Sampson, MD,\* Nadia Ouhibi, PhD, Helen Lawce, BS, Phillip E. Patton, MD, David E. Battaglia, PhD, Kenneth A. Burry, MD, Susan B. Olson, PhD

*Departments of Obstetrics and Gynecology and Molecular and Medical Genetics, Oregon Health & Science University, Portland, Ore*

## KEY WORDS

Preimplantation genetic diagnosis  
Balanced translocation  
Fluorescent in situ hybridization  
Aneuploidy

**Objective:** Preimplantation genetic diagnosis is an established technique that provides an alternative to prenatal diagnosis for patients who are at risk of transmitting a serious genetic disorder to their offspring. Preimplantation genetic diagnosis has been used for couples who have been at risk for having offspring with single gene or X-linked disorders and for screening for common age-related aneuploidy and in couples who themselves carry balanced chromosomal rearrangements. The aim of this study was to summarize our experience using preimplantation genetic diagnosis after the identification of a parental balanced translocation, specifically as it relates to the number of embryos that are suitable for transfer after preimplantation genetic diagnosis for a known translocation and aneuploidy screening.

**Study design:** This is a retrospective review of data from a single center that involved 6 couples that initiated the process of preimplantation genetic diagnosis for translocation and aneuploidy screening by fluorescent in situ hybridization.

**Results:** A total of 65 embryos were obtained, of which 56 embryos (86%) were suitable for fluorescent in situ hybridization analysis. After fluorescent in situ hybridization, 1 embryo was diagnosed as normal or balanced (1.7%). Forty-three embryos (76.8%) were unbalanced for the translocation; 8 embryos (14.3%) were aneuploid, and 4 embryos (7.1%) were uninformative. There were no clinical pregnancies.

**Conclusion:** In our experience, there are very few embryos that are available for transfer from these patients after translocation and aneuploidy screening because of multiple unbalanced segregation products and a high rate of aneuploidy. Factors that contributed to this may be related to which parent carries the translocation, methods that were used for in vitro fertilization, and advanced maternal age. Although preimplantation genetic diagnosis for translocation carriers theoretically can enhance the pregnancy rate for a couple, there are limitations. This information should be shared with couples who are contemplating preimplantation genetic diagnosis for translocation, and the options of sperm or egg donor should be considered.

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Presented at the Seventieth Annual Meeting of the Pacific Coast Obstetrical and Gynecological Society, September 16–21, 2003, Anchorage, Alaska.

\* Reprint requests: Jone E. Sampson, MD, Department of Molecular and Medical Genetics, Oregon Health & Science University, L-103, 3181 SW Sam Jackson Park Rd, Portland, OR 97239-3011.  
E-mail: sampsojo@ohsu.edu

Reciprocal translocations are a 2-way exchange of material between 2 nonhomologous chromosomes, with no net gain or loss of genetic material, merely a rearrangement. Therefore, individuals with reciprocal translocation are phenotypically normal unless the break points interrupt or modify the function of a gene or genes. In most cases, these individuals are identified after reproductive problems that involve infertility, multiple spontaneous abortions, or the delivery of a child with multiple congenital anomalies in whom an unbalanced karyotype is observed. The incidence of balanced translocations in the general population is 0.2% but increases to 9.2% in fertile couples with >3 first-trimester abortions because of the abnormal segregation of chromosomes during meiosis that resulted in abnormal gametes or an unbalanced karyotype in the embryos.<sup>1</sup>

Preimplantation genetic diagnosis (PGD) has been used for the identification of chromosome abnormalities in couples who are at risk for either aneuploidy that is based on maternal age or an unbalanced parental karyotype chromosome rearrangement (such as translocations and inversions). PGD for chromosomal abnormalities or rearrangements provides an alternative to prenatal diagnosis and termination of affected fetuses and theoretic enhancement of implantation and pregnancy rates for these couples.<sup>2</sup>

Although fluorescent in situ hybridization (FISH) probes can be designed to identify normal or balanced embryos from polar bodies or blastomeres of cleaving embryos, there are significant limitations to this technique. The process requires controlled ovarian stimulation and in vitro fertilization (IVF) and may result in too few oocytes recruited, particularly in women with advanced maternal age, which limits the number of embryos to analyze. In the analysis of translocations, unique FISH probes that flank the breakpoints of each translocation or that require the use of subtelomeric probes (specific to the chromosome ends of the translocated segments) for each affected individual must be designed and validated to detect normal and balanced products in embryonic tissue.<sup>3</sup> For reciprocal translocations, the prevalence of unbalanced gametes is estimated to range between 50% and 70%, thus potentially vastly reducing the number of embryos that are available for transfer and implantation.<sup>2,3</sup> Furthermore, an embryo with balanced or normal translocation signal may still harbor abnormalities in unrelated chromosomes.

In the past, only invasive prenatal diagnosis by chorionic villous sampling or amniocentesis was used to detect translocations. PGD, an alternative option, can now identify normal and/or balanced embryos before transfer<sup>4</sup>; we reviewed our experience with PGD in a subgroup of patients in which one or the other partner was a carrier of a balanced translocation.

## Material and methods

This is a retrospective review of couples with an identified balanced translocation who had undergone IVF at Oregon Health & Science University Fertility Consultants from March 2001 through March 2003, specifically for the purpose of using PGD to identify chromosomally normal/balanced embryos for transfer. The indication for PGD was a history of recurrent pregnancy loss, the identification of a parental translocation, or a genetic history of translocation in their family. All couples completed the informed consent process as approved by the Institutional Review Board. The clinical characteristics of the 6-translocation carrier couples that were studied are summarized in Table I.

### IVF, embryo culture and blastomere biopsy

After the construction and validation of the probes, patients were enrolled in the IVF program. The methods of ovarian stimulation, sperm processing, and techniques for fertilization and embryo culture have been described previously.<sup>5</sup> Briefly, the ovarian stimulation protocol included the use of a long-acting gonadotropin-releasing hormone agonist (0.5 mg twice daily, Lupron; Tap Pharmaceuticals, Inc, Deerfield, Ill) that was administered after a minimum of 2 weeks of oral contraceptive treatment. After biochemical evidence of pituitary suppression, (serum estradiol level, <40 pg/mL), follicle-stimulating hormone or follicle-stimulating hormone/human menopausal gonadotropin (Serono Inc, Rockland, Mass) was given twice daily (3-6 ampules per day). Follicular response was monitored with serial pelvic ultrasound examination and serum estradiol measurements. When at least 2 follicles were 17 mm, 10,000 IU of human chorionic gonadotropin (Serono, Rockland, Mass) was given intramuscularly; transvaginal ultrasound-directed oocyte retrieval was scheduled 36 hours later. After egg retrieval, conventional insemination or intracytoplasmic sperm injection (ICSI) was performed on the basis of semen criteria. All patients who underwent ICSI had subnormal semen parameters (count,  $\leq 20 \times 10^6$  sperm/mL; motility,  $\leq 50\%$ ; total motile morphologic condition,  $\leq 50\%$  or a subnormal sperm penetration assay). Fertilization was assessed 15 to 18 hours after the insemination.

Embryo biopsy was performed on 6- to 10-cell stage embryos, 3 days after insemination, in  $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free medium under oil (SAGE BioPharma) with the use of a double-needle approach. Briefly, partial zona drilling was performed mechanically to create an opening in the zona pellucida with a partial zona drilling needle. Blastomere removal was then facilitated by gentle aspiration through a biopsy micropipette. One or 2 blastomeres were removed from each embryo. After the biopsy procedure, the embryos were kept in culture in

**Table I** Patient characteristics

Patient	Carrier	Maternal age (y)	Gravity/parity	Previous IVF	Translocation	Sperm
Case 1	Male	32	2/0	Yes	46,XY,t(1;8)(p22;q13)	Normal
Case 2	Male	33	5/2	Yes	46,XY,t(7;9)(q34;q34.3)	Normal
Case 3	Male	29	2/0	No	46,XY,t(4;16)(q13;q12.1)	Abnormal
Case 4	Male	25	2/0	No	46,XY,t(4;13)(q24;q33)	Abnormal
Case 5	Female	40	1/0	Yes	46,XX,t(3;13)(q25;q31.2)	Normal
Case 6	Female	37	0/0	No	46,XX,t(15;17)(q24;p12)	Normal

Blastocyst medium (SAGE BioPharma) until the results of the genetic analysis.

### FISH and PGD

Before the initiation of the IVF cycle, commercially available probes were chosen that were appropriate for each translocation that would elucidate balanced and at least fourteen unbalanced products of each translocation. FISH was carried out according to the manufacturer's recommendations, and probes were validated according to laboratory protocol. This included hybridization to translocation carrier metaphase and interphase blood cells and to donated blastomeres.

Blastomeres were first screened with the use of the translocation probe set. After overnight hybridization, each slide was coverslipped for viewing, scoring, and capturing on a microscope (Nikon Instruments Inc, Melville, NY) with appropriate filters linked to a Cytovision imaging system (Applied Imaging, Santa Clara, Calif). Signal patterns were scored as balanced, unbalanced, or uninformative (a nonspecific scattered signal appearance on interphase FISH analysis that is uninterpretable).

Slides from balanced blastomeres were destined by immersion in 72°C distilled water for 2 minutes, dehydrated in alcohols, and air dried. They were then hybridized with the aneuploidy probes (Vysis-Abbott Laboratories, Abbott Park, Ill) for chromosomes 13, 18, 21, X and Y, according to manufacturer's recommendations and analyzed as mentioned earlier.

### Results

Four cycles of IVF and 3 cycles of IVF/ICSI were completed on 6 couples. The results of PGD are shown in Table II.

The first couple completed 2 cycles in our program. Seven embryos were available for PGD in the first cycle. Three embryos were aneuploid, and the euploid blastocyst embryo was unbalanced. One was "uninformative." The remaining embryos were not suitable for FISH, and there were no embryos that were available for transfer. In a second cycle, 12 embryos were analyzed in a total

**Table II** Clinical results

	Patient						
	1	2	3	4	5	6	
IVF/ICSI	IVF	IVF	IVF	ICSI	ICSI	ICSI	IVF
Embryos (n)	7	12	11	12	5	12	6
Unbalanced* (n)	1	5	9	9	4	10	5
Aneuploid* (n)	3	3	1	1	—	—	—
Uninformative (n)	1	1	0	1	0	1	0
No nucleus (n)	2	3	0	1	1	1	1
Transferred (n)	0	0	1	0	0	0	0
Pregnancy	No	No	No	No	No	No	No

\* Blastomeres were tested sequentially; if abnormal for the first result, the second test was not performed.

of 16 blastomere biopsies. Eight of the embryos were abnormal with respect to ploidy or unbalanced. There was 1 uninformative embryo, and 3 of the blastomere biopsy specimens were not suitable for FISH because a nucleus was not identified. Again, there were no embryos that were suitable for transfer.

There were 11 embryos for PGD from patient 2. Nine embryos were unbalanced; 1 embryo was triploid, and the 1 normal embryo was transferred but did not result in a pregnancy.

Twelve embryos were available for PGD from patient 3. Nine embryos were unbalanced. One embryo was balanced, but uninformative for aneuploidy, and then arrested before transfer. One embryo was triploid, and the last embryo was not suitable for FISH. No embryos were available for transfer.

Five embryos were available for PGD from patient 4. Four embryos were unbalanced, and 1 embryo had no nucleus. No embryos were available for transfer.

Patient 5 had 12 embryos for PGD. Ten embryos were unbalanced; 1 embryo was uninformative, and 1 embryo had no nucleus. No embryos were available for transfer.

There were 6 embryos available for PGD from patient 6. Five of the embryos were unbalanced, and the remaining blastomere was not suitable for FISH. There were no embryos that were available for transfer.

A total of 65 embryos were generated from 7 cycles. Nine embryos were unsuitable for FISH analysis

(13.8%) because of the absence of a nucleus in the biopsied cell after fixation or chromatin degradation in the nucleus. The remaining 56 embryos (86%) were tested. After FISH, 1 blastomere was identified as normal or balanced. Forty-three embryos (76.8%) were unbalanced; 8 embryos (14.3%) were aneuploid, and 4 embryos (7.1%) were uninformative. The normal embryo was transferred but did not result in a clinical pregnancy.

## Comment

Experience regarding the role of PGD in translocation carriers has been reported previously.<sup>1,4,6-8</sup> Our paper is unique in that all of our patients have balanced translocations and not Robertsonian translocations. In keeping with the results of previous studies, patients with balanced translocations produce fewer normal gametes and pregnancies than patients with Robertsonian translocations. Fridström et al<sup>9</sup> reported their experience with PGD in 7 couples with Robertsonian translocations and 8 couples with reciprocal translocations. The total number of transferable embryos in the Robertsonian group was 23 of 84 embryos (27%) versus 11 of 96 embryos (11%) in the reciprocal group. Patients with reciprocal translocations completed 17 cycles of IVF; in 9 cycles, no embryos were available for transfer. Only 1 live birth (0.06% live birth rate/cycle initiated) and 1 spontaneous abortion were reported. In contrast with our study, aneuploidy screening was not performed. In our experience, approximately 15% of the embryos were excluded from transfer after aneuploidy screening. The prevalence rate of aneuploidy is artificially low because embryos that were found to be unbalanced were not tested subsequently for aneuploidy as well. This again is in keeping with results from other centers and our own data.<sup>10</sup>

Other factors that were possibly related to the poor outcome in our small series include the preponderance of males who carried the translocation, the mode of fertilization, and the recognized impact of advancing age and aneuploidy risk. There is a theoretic disadvantage of the use of ICSI in male translocation carriers. Male translocation carriers may have  $\geq 70\%$  unbalanced spermatozoa, thus there is a statistical likelihood for the selection of a single abnormal sperm for fertilization.<sup>11</sup> Abnormal sperm count or morphologic condition in male translocation carriers may be a direct consequence of this and may affect the success rate.<sup>12,13</sup>

Although the experience in this study and others is poor, additional data are necessary to determine the efficacy of PGD in reciprocal translocation carriers. To improve accuracy of diagnosis in both maternal and paternal translocations, Verlinsky et al<sup>14</sup> have used nuclear transfer, reporting 94 cycles that resulted in 66 embryo transfers and the delivery of 15 healthy babies. How-

ever, it is uncertain whether federal regulatory agencies will allow the application of this technology in the future because this technique requires fusion of human blastomeres to mouse zygotes.

Couples who embark on this process often are motivated highly because of previous losses or infertility, but they must be counseled carefully regarding the difficulties that are inherent in these techniques. The implantation rate of unaffected embryos after PGD should be similar to that of IVF. The limited numbers of normal/balanced embryos reduce the chances of success. This information and the many pitfalls in the process should be shared with couples who contemplate these procedures and in the context of other options that include natural reproduction, freezing early-stage embryos after several cycles to increase the number of embryos that are available for screening, the use of egg or sperm donors, and consideration of adoption.

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*Editor's note:* This manuscript was revised after these discussions were presented.

## Discussion

**DR LORNA MARSHALL**, Seattle, Wash. Dr Sampson et al reported their experience with IVF and PGD for both maternal and paternal carriers of a balanced reciprocal translocation. Importantly, carriers of robertsonian translocations are not included in this report and may be expected to have different outcomes. This is a small series, in which 4 couples have a paternal balanced reciprocal translocation, and the other 2 couples have a maternal balanced reciprocal translocation. Only 1 patient had >2 unsuccessful pregnancies, and 1 patient had never conceived. These are not typical profiles of patients with recurrent pregnancy losses, which suggests that other circumstances are involved in ascertaining the diagnosis. The female partners of the male translocation carriers were all <35 years old, whereas the female translocation carriers were 37 and 40 years old. Because most reciprocal translocations arise de novo, each carrier had different chromosomes that were involved in the translocation.

The patients underwent a complex process, with ovarian hyperstimulation, ovum retrieval, and IVF being only the first step. In this study, 1 or 2 blastomeres from a 3-day old embryo were removed with micromanipulation techniques. In some other centers, polar body biopsies have been used to exclude maternal translocations. A polar body biopsy may have the advantage of being less traumatic to the embryo, but because chromosomes are only maternal in origin, it cannot be used to exclude aneuploidy of the embryo. After blastomere biopsy, the embryo is cultured to a blastocyst that is awaiting the results. Commercially available probes that are appropriate for each translocation are selected to perform the FISH. These techniques require highly trained laboratory personnel who are available 7 days per week.

The results of IVF and PGD in this series were poor. From the 7 IVF cycles in 6 couples, only 1 normal embryo was transferred back into a uterus, and no pregnancy resulted. This is discouraging, especially for a procedure that often costs \$5000 in addition to the typical cost of an IVF cycle. Pregnancy rates reflect the number of normal embryos that are available for transfer; therefore, if enough chromosomally normal embryos are available for transfer, then these patients should conceive at rates that are the same or higher than

other women their age who undergo IVF. But almost 80% of the embryos that were biopsied were unbalanced, and most of the others were aneuploid, anuclear, or uninformative, which suggests that there may be limited opportunities to find normal or balanced embryos.

Is the small sample size in this study representative of all patients with this diagnosis, or might results improve with a larger, more diverse sample? Both female translocation carriers were >35 years old. Does the age of the female partner have a significant impact on the chance of obtaining normal embryos? Because the risk of unbalanced fetuses with spontaneous conception in these patients is only 10%, does it make more sense to allow some natural selection to occur and then do prenatal diagnosis? Other reports suggest at least some success with this procedure. In the first report of successful pregnancies after PGD in 1998 for maternal robertsonian or reciprocal translocations, 1 of the 3 patients was 37 years old with a balanced reciprocal translocation. Two of 6 embryos had a balanced translocation in the polar body, were transferred, and resulted in a pregnancy and spontaneous abortion.<sup>1</sup> Since then the group at Saint Barnabas Medical Center has reported 5 of 24 pregnancies (21%) in cases of maternal and paternal reciprocal translocation carriers, contrasted with 11 of 22 pregnancies (50%) with robertsonian translocations.<sup>2</sup> The same group recently reported 4 ongoing or delivered pregnancies of 11 cases of paternal balanced reciprocal translocations.<sup>3</sup> Several other studies have reported successful pregnancies, although most of the studies have included both balanced reciprocal and robertsonian translocations. Hopefully, the publication of this series will encourage all centers to publish their results and, specifically, to separate patients with balanced reciprocal from robertsonian translocations.

I congratulate Dr Sampson et al for publishing these results. Studies that do not support the use of new technologies are at least as important as those studies that report their successes. On the basis of your series and the experience of others, can you recommend to clinicians which patients, if any, with a balanced reciprocal translocation should consider IVF and PGD?

Given your poor results with male balanced reciprocal translocation carriers, do you think there is a role for the use of FISH analysis of the sperm before the patients who are considering IVF and PGD are counseled?

Do you foresee any advances in technology or techniques that might improve the chance of conceiving a normal child after IVF and PGD when 1 partner carries a balanced reciprocal translocation?

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