

Outcome of preimplantation genetic diagnosis of translocations

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Objective: To review 35 cases of preimplantation genetic diagnosis (PGD) of translocations with several methods, including telomeric probes.

Design: Retrospective study.

Setting: Clinical IVF laboratory.

Patient(s): Thirty-five couples with one partner carrying a chromosomal translocation.

Intervention(s): PGD of translocation after polar-body or embryo biopsy.

Main Outcome Measure(s): Pregnancy outcome.

Result(s): Several trends were observed. First, PGD can achieve a statistically significant reduction in spontaneous abortion, from 95% to 13%. Second, the chances of achieving pregnancy are correlated with 50% or more of the embryos being chromosomally normal. Third, patients with robertsonian translocations produced fewer abnormal gametes and more pregnancies than did patients with reciprocal translocations. Fourth, a new fluorescence in situ hybridization protocol for PGD of translocations, which involves applying telomeric probes, has proved adequately reliable with a 6% average error rate.

Conclusion(s): PGD of translocations achieves a statistically significant reduction in spontaneous abortion, both for polar-body and blastomere biopsy cases. Pregnancy outcome depended on the number of normal embryos available for transfer, with patients having <50% abnormal embryos achieving the most pregnancies. Because robertsonian translocations caused fewer abnormal embryos than reciprocal translocations, they also resulted in higher rates of implantation. (Fertil Steril® 2000;73:1209–18. ©2000 by American Society for Reproductive Medicine.)

Key Words: Robertsonian translocation, reciprocal translocation, mosaicism, PGD

Balanced translocations occur in 0.2% of the neonatal population but at a higher rate among infertile couples and patients with recurrent abortions. In a recent report, balanced translocations were found in 0.6% of infertile couples, 3.2% of couples who failed >10 IVF cycles, and 9.2% of fertile couples who experienced ≥3 consecutive first-trimester abortions (1). It was also found in 2%–3.2% of men requiring intracytoplasmic sperm injection (2–4).

Preimplantation genetic diagnosis (PGD) can be offered to carriers of balanced translocations as an alternative to prenatal diagnosis and pregnancy termination of unbalanced fetuses. In recent years, PGD for structural chromosome abnormalities has been attempted by a variety of approaches.

Analysis by fluorescence in situ hybridization chromosome painting of first polar bodies so far has been the most successful method; it uses two chromosome-painting probes applied to the translocated metaphase chromosomes in the polar body shortly after egg retrieval (5, 6). With this approach, the frequency of spontaneous abortions in pregnant patients was significantly reduced ($P < .001$), from 95% lost fetuses in natural cycles to 12.5% after PGD (6, 7).

Verlinsky and Evsikov (8) recently obtained chromosomes from human second polar bodies by injecting the second polar body into an enucleated M-II oocyte. The oocyte is then activated to produce a pronucleus of the second polar body and cultured in Okadaic acid to

produce metaphase chromosomes. A similar method applied to blastomeres was described recently by Willadsen et al. (9, 10) and is based on the observation that after blastomere fusion to a freshly matured, enucleated cow oocyte, the transferred nucleus becomes arrested in a configuration resembling M-II. This technique has already been applied clinically, resulting in chromosomally normal offspring (9).

The methods used hitherto either are useful only for female carriers of translocation or involve the use of sophisticated micromanipulation techniques that require a high level of skill to fix the metaphase chromosomes for proper analysis. An alternative to the analysis of metaphase chromosomes is the use of fluorescence in situ hybridization on interphase blastomeres. This method can be applied to translocations of any parental origin or for inversions.

Specific spanning probes can be developed that expand the breakpoints of each translocation (11–13) or inversion (14). Probes can be used that are distal to the breakpoints or telomeric probes, either for translocations (11, 15, 16) or pericentric inversions (17). For robertsonian translocations, enumerator α -satellite or locus-specific probes can be used to detect aneuploid embryos (18).

Only spanning probes can differentiate between balanced and normal embryos. Unfortunately, the production of case-specific breakpoint-spanning probes is expensive and time consuming. Using commercially available telomere probes instead would considerably simplify PGD of translocations and inversions. In the present study, we report the use of telomeric probes in five clinical cases.

Applying a variety of the above methods, we have performed 35 cases of PGD for translocations. Some characteristics of these cases, such as type of translocation, number of abnormal embryos, and biopsy and PGD methods, are correlated with pregnancy outcome.

MATERIALS AND METHODS

PGD With Previously Described Methods

Thirty-five cases of PGD for translocations were performed using various previously published methods, including chromosome painting of first polar bodies (5–7), analysis of interphase blastomeres with breakpoint-spanning probes (11, 13), use of enumerator probes for interphase analysis of male robertsonian translocations (11, 18, 19), and fusion of blastomeres with cow eggs (9). Most of these cases have been reported previously. The purpose of this summary is to find trends that could explain the failure to achieve pregnancy. Pregnancy outcomes for all 35 PGD cases were correlated with position of the breakpoints, the percentage of abnormal embryos, sex of the carrier, general methodology, and type of translocation. Pregnancy losses were compared in natural and PGD cycles.

Subjects for PGD With Telomere Probes

Five couples in which one of the partners carried a balanced translocation underwent PGD with a new fluorescence in situ hybridization protocol that involves the use of telomeric probes.

Case A

A male, 32 years old, was the carrier of a balanced translocation with karyotype 46,XY,t(10;13)(q22.3;q14). His wife was found to be karyotypically normal. The couple had experienced six previous spontaneous abortions from natural cycles. The male partner was normospermic.

Case B

A female, 28 years old, was the carrier of a balanced translocation with karyotype 46,XX,t(6;10)(q23;q26). Her husband was found to be karyotypically normal. The couple had experienced no spontaneous abortions but had chosen to terminate two unbalanced pregnancies, both after natural conception. The karyotypes of these two induced abortions were both 46,XY,der(10)t(6;10)(q23;q26). The couple underwent a single cycle of IVF followed by PGD.

Case C

A female, 30 years old, was the carrier of a balanced translocation with karyotype 46,XX,t(10;14)(q26.1;q22.1). Her husband was found to be karyotypically normal. The couple had experienced two previous spontaneous abortions from natural conception, with no genetic evaluation of the aborted fetuses. The male partner had mild oligoasthenospermia requiring intracytoplasmic sperm injection for the PGD cycle.

Case D

A 35-year-old male was the carrier of a balanced translocation with karyotype 46,XY,t(10;18)(q24.1;p11.2). His wife was found to be karyotypically normal. The couple had experienced 15 spontaneous abortions from natural conception, of which 5 could be genetically analyzed, and all were unbalanced. The couple had also undergone one previous unsuccessful cycle of IVF. The male partner was normospermic.

Case E

A male, 42 years old, was the carrier of a balanced translocation with karyotype 46,XY,t(11;22)(q25;q13.1). His wife was found to be karyotypically normal. The couple had experienced one previous spontaneous abortion from natural conception. The male partner was normospermic.

Technique of PGD With Telomere Probes

Blastomeres used in pilot studies and as controls were obtained from embryos donated for research; all exhibited arrested development or were otherwise unsuitable for embryo replacement. They were obtained from the IVF program of The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center, in accordance with

guidelines approved by the Internal Review Board of Saint Barnabas Medical Center, including individual written informed consent.

PGD was performed on embryos of consenting couples in which one or the other partner was a carrier of a translocation. A separate Internal Review Board protocol and individual consent forms were used for these patients. Embryo biopsy was performed on day 3 of development, and replacement was done the next day. Embryo biopsy and blastomere fixation were performed as described previously (20, 21).

The fluorescence in situ hybridization procedure recommended by Cytocell (Adderbury, UK) was followed with some modifications. Ten microliters of hybridization solution (Cytocell) was mixed and supplemented with 1 μ L of a centromeric probe, labeled in Spectrum Aqua (Vysis), for one of the chromosomes involved in the translocation. In some instances, two centromeric probes were added, one for each chromosome involved in the translocation. In these instances, 1 μ L of each probe was added, with one probe labeled in Spectrum Aqua and the other in a 1:1 proportion of Spectrum Green and Spectrum Orange (Vysis).

The final mixture was added on a coverslip supplied by Cytocell, which had telomere probes for the two arms involved in each respective translocation, one labeled with Cy3 and the other with fluorescein isothiocyanate conjugate. The coverslip, with the centromere-supplemented hybridization solution, was flipped over the slide containing the fixed cells to be analyzed so that the hybridization solution and the coverslip-attached telomere probe faced the cells. The slides were placed for 5 minutes at 37°C, denatured at 75°C for 5 minutes, and left overnight to hybridize. The next morning, the slides were washed in $0.4 \times$ SSC for 2 minutes at 72°C, and the signals were detected with the antibodies supplied by Cytocell as specified in their protocol, without modification.

The scoring criteria to differentiate false positives and false negatives from mosaicism have been described previously (22). To classify chromosomal abnormality types by fluorescence in situ hybridization, we used the criteria described by Munné et al. (23), but with an exception. Mosaics with <25% abnormal cells were considered normal, and the abnormal cells were considered errors of fluorescence in situ hybridization because it was difficult to differentiate mosaics from errors in embryos with six or fewer cells.

RESULTS

PGD With Telomere Probes

In each of the translocation carriers, the probes were tested first on the fixed lymphocytes in combination with a centromeric probe to make sure that the telomere probes properly characterized the translocation. In all translocations, the lymphocytes in interphase showed one signal for each telomere probe associated with the chromosome labeled with the centromeric probe, whereas the other signal

for each telomere probe was not, thus demonstrating that these probes properly characterized the translocation (Fig. 1a). In addition, ≥ 50 lymphocytes in interphase were analyzed for each patient and used as controls. The error rates were 3.3% for case A, 4.1% for case B, 11.3% for case C, 11.6% for case D, and 10% for case E. These errors consisted of either one missing or one extra telomere signal. On average, the error rate was 7.8% (24/306).

Embryos donated for research were used as blastomere controls. All the cells of these embryos were fixed to differentiate between errors and mosaicism, and ≥ 20 blastomeres per case were analyzed. Table 1 shows the results for the five sets of probes used. The error rates ranged from 0% (0/21, case B) to 10% (3/30, case C), with an average error rate of 6% (8/131).

The number of normal embryos detected in these translocation cases was low (Table 2). Overall, only 5 of 32 embryos were considered normal or balanced. The rest were classified as unbalanced ($n = 25$), balanced tetraploid (embryo 6, case A), or without results for lack of a nucleus in the biopsied cell (embryo 2, case E). Figure 1b–d shows several abnormal blastomeres from cases in which telomeric probes were used.

All 5 normal embryos were transferred. Most of the nontransferred embryos were biopsied again, and all or most of the cells were analyzed. Of the nontransferred embryos, 6 were confirmed to have the same abnormality as the one initially diagnosed by PGD; 11 were reclassified as mosaics, but all still contained only abnormal cells; and 7 could not be reanalyzed, either because the patient did not consent (case C) or for other reasons. Of the remaining 3 embryos, the 1 without diagnosis showed arrested development and was found to be normal after reanalysis (embryo 2, case E), and the other 2 had been misdiagnosed (10% misdiagnosis, or 2/20 reanalyzed). One of the misdiagnoses concerned embryo 3 from case D, in which the PGD diagnosis of abnormal was not confirmed, although the embryo was still unbalanced. The other misdiagnosis concerned embryo 6 from case A, which was a mosaic 2N/4N, balanced or normal for the translocation, in which the biopsied cell was tetraploid.

In total, from five cycles only 7 of 32 embryos (22%) were found to be normal or mostly normal (embryo 6, case A), whereas at least 11 of the 25 abnormal ones were mosaics. The 3 embryos of case A diagnosed as normal were transferred, but the patient did not become pregnant. Case B had only abnormal embryos, so none were replaced. According to the PGD results, case C produced 2 normal embryos, which were transferred but did not result in clinical pregnancy. Out of 7 embryos, case D had only 1 normal embryo, which developed poorly and did not implant after transfer. Finally, case E produced 8 embryos, of which 1 could not be diagnosed by PGD for lack of a nucleus in the biopsied cell; all of the rest were unbalanced. None of the embryos were transferred.

Fluorescence in situ hybridization on lymphocytes (a), blastomeres (b–d), and polar bodies (e, f) with the use of probes for detection of translocations. Centromeric probes labeled in gold (a, d) or aqua (b, c) were used in combination with telomeric probes labeled in cy5 and fluorescein isothiocyanate conjugate (a–d). Painting probes labeled in green and orange were used in metaphase-stage polar bodies (e, f). Figure 1a shows a normal lymphocyte belonging to the carrier of translocation 46,XY,t(11;22)(q25;q13.1) (case E). The marker probes were yellow for chromosome 11, green for telomere 11q region, and red for telomere 22q region. Figure 1b represents an unbalanced blastomere from embryo 7 of case E, containing one green signal for tel11q, three red signals for tel22q, and two yellow signals for a marker on chromosome 22. Figure 1c shows an unbalanced blastomere from embryo 4, case B, carrier of a 46,XX,t(6;10)(q23;q26) translocation, showing two green signals for tel14q, one red signal for tel10q, and one blue signal for the centromere region of chromosome 10. Figure 1d depicts an unbalanced metaphase-stage blastomere from embryo 1 of case A, showing three green signals for tel10q, two red signals for tel13q, and three yellow signals for centromere 10. Figure 1e shows an unbalanced metaphase-stage polar body containing a green signal for chromosome 2 and red with a green spot for derivative chromosome 14 from a case in which the female was the carrier of a 46,XX,t(2;14)(q23;q24) translocation. Figure 1f shows an unbalanced metaphase-stage polar body with a red signal for chromosome 21 and green with two red spots for a derivative robertsonian (14;21)(10q;10q) translocation.

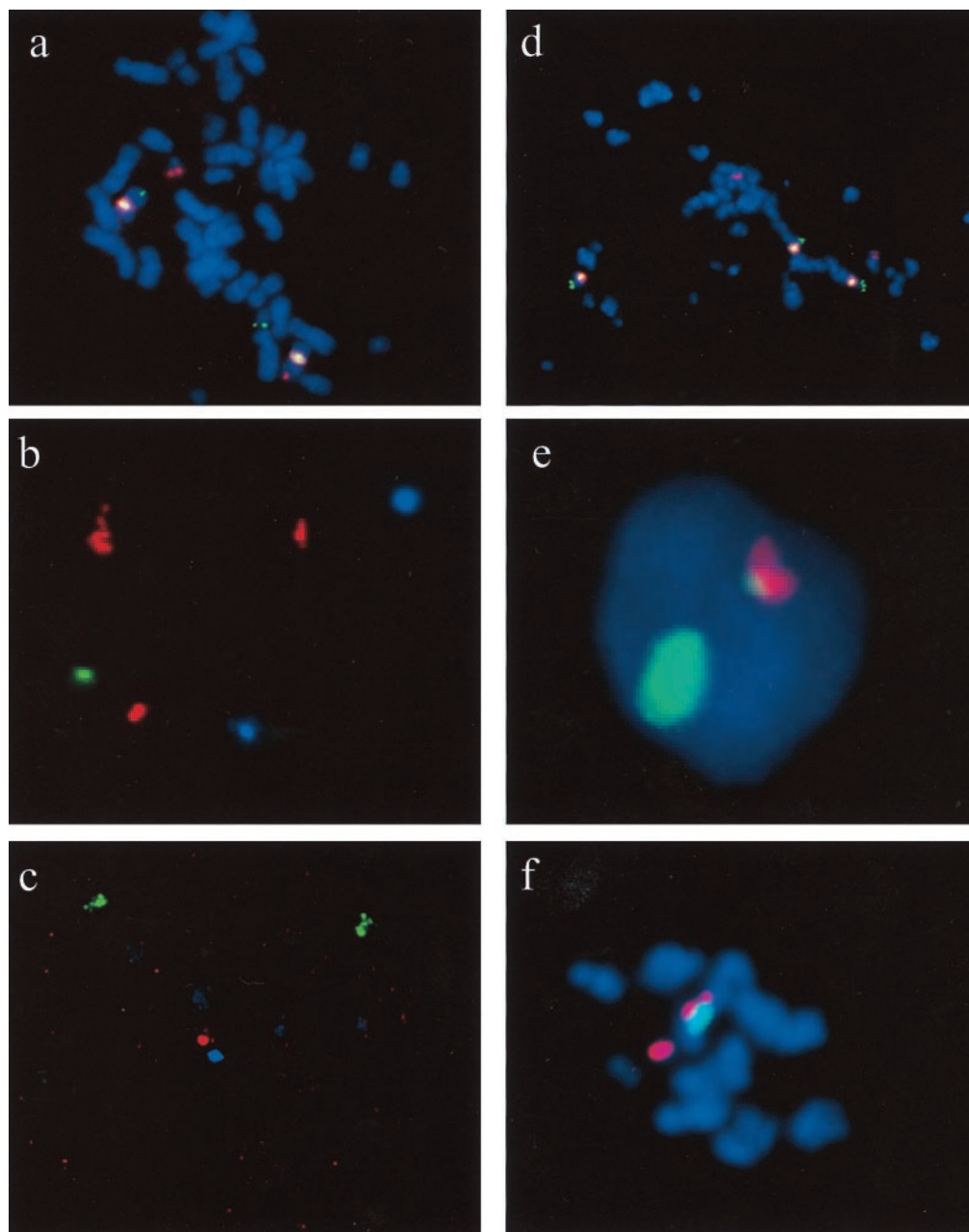


TABLE 1

Control blastomeres.

Fluorescence in situ hybridization result	No. of cells	Embryo diagnosis
Case A: 46,XY,t(10;13)(q22.3;q14)		
Embryo 1: 2(tel10q)2(tel13q)2(cen10)	4	Normal
Embryo 2: 2(tel10q)2(tel13q)2(cen10)	4	Normal
2(tel10q)2(tel13q)1(cen10)	1 (error)	
2(tel10q)1(tel13q)2(cen10)	1 (error)	
Embryo 3: 2(tel10q)2(tel13q)2(cen10)	10	Normal
Embryo 4: 6(tel10q)3(tel13q)6(cen10)	1	Mosaic
3(tel10q)2(tel13q)3(cen10)	1	
2(tel10q)3(tel13q)2(cen10)	1	
1(tel10q)2(tel13q)1(cen10)	3	
Case B: 46,XX,t(6;10)(q23;q26)		
Embryo 1: 2(tel6q)2(tel10q)2(cen10)	2	Mosaic 2n/3n
3(tel6q)3(tel10q)3(cen10)	1	
Embryo 2: 2(tel6q)2(tel10q)2(cen10)	7	Normal
Embryo 3: 1(tel6q)1(tel10q)2(cen10)	2	Mosaic aneuploid
3(tel6q)3(tel10q)2(cen10)	1	
Embryo 4: 2(tel6q)2(tel10q)2(cen10)	7	Mosaic aneuploid
3(tel6q)3(tel10q)2(cen10)	1	
Case C: 46,XX,t(10;14)(q26.1;q22.1)		
Embryo 1: 2(tel10q)2(tel14q)2(cen10)	6	Normal
Embryo 2: 2(tel10q)2(tel14q)2(cen10)	3	Mosaic for chromosome 14
2(tel10q)1(tel14q)2(cen10)	1	
2(tel10q)3(tel14q)2(cen10)	2	
1(tel10q)1(tel14q)2(cen10)	1 (error)	
Embryo 3: 2(tel10q)2(tel14q)2(cen10)	3	Normal
1(tel10q)2(tel14q)2(cen10)	1 (error)	
Embryo 4: 2(tel10q)2(tel14q)2(cen10)	5	Normal
Embryo 5: 2(tel10q)2(tel14q)2(cen10)	7	Normal
1(tel10q)2(tel14q)2(cen10)	1 (error)	
Case D: 46,XY,t(10;18)(q24.1;p11.2)		
Embryo 1: 2(tel10q)2(tel18p)2(cen10)	5	Mosaic 2n/n/3n
1(tel10q)1(tel18p)1(cen10)	2	
1(tel10q)2(tel18p)1(cen10)	1 (error)	
3(tel10q)3(tel18p)3(cen10)	1	
Embryo 2: 2(tel10q)2(tel18p)2(cen10)	6	Normal
Embryo 3: 2(tel10q)2(tel18p)2(cen10)	6	Mosaic 2n/4n
4(tel10q)4(tel18p)4(cen10)	2	
Case E: 46,XY,t(11;22)(q25;q13.1)		
Embryo 1: 8(tel11q)8(tel22q)8(cen22)	2	Octaploid
Embryo 2: 2(tel11q)2(tel22q)2(cen22)	3	Normal
Embryo 3: 2(tel11q)2(tel22q)2(cen22)	6	Mosaic
2(tel11q)1(tel22q)2(cen22)	2 (errors)	
1(tel11q)1(tel22q)1(cen22)	1	
4(tel11q)4(tel22q)4(cen22)	2	
Embryo 4: 2(tel11q)2(tel22q)2(cen22)	6	Normal
Embryo 5: 2(tel11q)2(tel22q)2(cen22)	6	Normal
Embryo 6: 2(tel11q)2(tel22q)2(cen22)	3	Mosaic
2(tel11q)0(tel22q)2(cen22)	1	
3(tel11q)4(tel22q)3(cen22)	1	

Munné. PGD of translocations. Fertil Steril 2000.

Pregnancy Outcome in 35 Translocation Cases

We have performed a total of 47 cycles for PGD of translocations in 35 patients. Of these, 16 cases were analyzed with painting probes on first polar bodies, 8 with

enumeration probes for robertsonian male translocations, 5 with spanning probes, 5 with telomere probes (described earlier), and 1 with fusion of blastomere and cow eggs to produce metaphase chromosomes. For each method, 22, 12, 7, 5, and 1 (total 47) cycles were performed, respectively. These cases are summarized in Table 3.

Most pregnancies were obtained by first-polar-body painting (45% of cycles), followed by enumerator probes; the use of telomeric or spanning probes resulted in no pregnancies. These differences may be attributable to biopsy method, type of translocation, sex of the carrier, or a combination of all these factors.

Table 4 shows the pregnancy rates (PRs) and percentages of abnormal oocytes and embryos in cases with similar characteristics. In patients with robertsonian translocations, significantly more pregnancies were produced per cycle (50%) than in cases involving reciprocal translocations (21%) ($P = .03$, F test). Similarly, when there was a terminal breakpoint in the translocation, fewer pregnancies per cycle were obtained (17%) than when none was present (41%) ($P = .026$, F test). In embryo-biopsy cases, the difference was even greater, with 0% pregnancies in cases involving at least one terminal breakpoint compared with 40% in those with no terminal breakpoints ($P = .037$, F test).

Sex and biopsy method also influenced the outcome, but these were found not to be statistically significant, probably because of the small sample size. A multifactorial statistical analysis was not applied because the numbers in each category (type of translocation, biopsy type, sex, and position of breakpoints) were too small.

Chromosomal Abnormalities in the Embryos of 35 PGD Translocation Cases

Another way to measure PGD outcomes was to evaluate the numbers of abnormal oocytes and embryos resulting from the remaining factors (see also Table 4). There was a good correlation between the percentage of chromosomal abnormalities and PR. For instance, patients with >50% normal eggs or embryos achieved significantly fewer pregnancies per cycle (8/33, 24%) than patients with <50% abnormal eggs or embryos (8/14, 57%) ($P = .028$, F test). Figure 1b–f shows abnormal blastomeres and polar bodies analyzed using different methods.

Overall, in embryo-biopsy cases we found an average of 75% abnormal embryos (Table 4), regardless of sex of the carrier, terminal breakpoints, or type of translocation (robertsonian or reciprocal), with few differences between subgroups of cases; hence the overall lower number of pregnant cycles (25%).

Cases of polar-body biopsy in which mosaicism and other abnormalities were not detected showed significantly fewer chromosome abnormalities in robertsonian translocation cases (42%) than in those with reciprocal translocations (71%) ($P < .001$, χ^2 test).

TABLE 2

PGD and reanalysis results.

PGD diagnosis	Reanalysis	Final diagnosis	Segregation
Case A: 46,XY,t(10;13)(q22.3;q14)			
1 Unbalanced	Unbalanced	Unbalanced	3:1
2 Normal	Not reanalyzed	Normal	Alternate
3 Unbalanced	Not reanalyzed	Unbalanced	3:1
4 Normal	Not reanalyzed	Normal	Alternate
5 Unbalanced	Unbalanced	Unbalanced	3:1
6 Tetraploid	Mosaic 2n/4n	Mosaic 2n/4n	Alternate
7 Normal	Not reanalyzed	Normal	Alternate
Case B: 46,XX,t(6;10)(q23;q26)			
1 Unbalanced	Unbalanced	Unbalanced	3:1
2 Unbalanced	Mosaic chaotic	Mosaic chaotic	Unknown
3 Unbalanced	Mosaic chaotic	Mosaic chaotic	Unknown
4 Unbalanced	Mosaic chaotic	Mosaic chaotic	Unknown
5 Unbalanced	Mosaic chaotic	Mosaic chaotic	Unknown
Case C: 46,XX,t(10;14)(q26.1;q22.1)			
1 Unbalanced	Not reanalyzed	Unbalanced	Adjacent I
2 Normal	Not reanalyzed	Normal	Alternate
3 Unbalanced	Not reanalyzed	Unbalanced	3:1
4 Normal	Not reanalyzed	Normal	Alternate
5 Haploid (1PN)	Not reanalyzed	Haploid	Unknown
6 Unbalanced	Not reanalyzed	Unbalanced	Unknown
Case D: 46,XY,t(10;18)(q24.1;p11.2)			
1 Unbalanced	Unbalanced	Unbalanced	Adjacent II
2 Unbalanced	Unbalanced and mosaic	Unbalanced and mosaic	Adjacent I
3 Unbalanced	Unbalanced	Unbalanced	Adjacent II
4 Normal	Not reanalyzed	Normal	Alternate
5 Unbalanced	Not reanalyzed	Unbalanced	Unknown
6 Unbalanced	Unbalanced	Unbalanced	Adjacent II
7 Unbalanced	Unbalanced and mosaic	Unbalanced and mosaic	Unknown
Case E: 46,XY,t(11;22)(q25;q13.1)			
1 Unbalanced	Unbalanced and mosaic	Unbalanced and mosaic	Unknown
2 No nucleus	Normal	Normal	Alternate
3 Unbalanced	Unbalanced	Unbalanced	3:1
4 Unbalanced	Not reanalyzed	Unbalanced	3:1
5 Unbalanced	Unbalanced and mosaic	Unbalanced and mosaic	Unknown
6 Unbalanced	Unbalanced and mosaic	Unbalanced and mosaic	Unknown
7 Unbalanced	Unbalanced and mosaic	Unbalanced and mosaic	Unknown
8 Unbalanced	Unbalanced and mosaic	Unbalanced and mosaic	Unknown

Munné. PGD of translocations. *Fertil Steril* 2000.

Reduction in Spontaneous Abortions in 35 PGD Cases

The 15 patients (16 cycles of PGD) who became pregnant after PGD had 38 previous pregnancies, of which 35 ended in spontaneous abortions. Compared with 2 spontaneous abortions among 16 pregnancies when these same patients underwent PGD, there was a statistically significant reduction in pregnancy losses after PGD ($P < .001$).

DISCUSSION

Taking into account factors that could affect PRs, such as biopsy type, sex, presence of terminal breakpoints, robertsonian or reciprocal translocation, and number of chromo-

somally normal embryos available for transfer, we found some interesting correlations.

Pregnancy Rate Is Correlated With 50% or More Chromosomally Normal Embryos

We found that when the number of abnormal embryos or eggs was $>50\%$, few pregnancies were achieved; but when the number was $<50\%$, the PR was noticeably higher. This result is found because most female carriers are fertile, and, with normal embryos, they achieve pregnancy easily. In agreement with our observations, other studies about PGD of translocations showed high rates of abnormal embryos, which did not result in pregnancy in any reported cases (16, 18).

TABLE 3

Summary of PGD cases performed by different methods and factors involved in their outcomes.

Translocation	Cycles	Method	Robertsonian	Terminal breakpoint	Sex	No. analyzed	Percent abnormal	Pregnancy outcome
t(13;14)(q10;q10)	1	Enumeration	Y	N	M	12	67	1 ongoing
t(14;15)(q10;q10)	2	Enumeration	Y	N	M	22	64	1 infant
t(9;11)(p24;q12)	1	Fusion	N	N	F	3	0	1 infant
t(13;14)(q10;q10)	2	Enumeration	Y	N	M	12	67	1st cycle: 1 spontaneous abortion (unknown) 2nd cycle: 1 infant
t(13;14)(q10;q10)	1	Enumeration	Y	N	M	2	50	1 ongoing
t(13;14)(q10;q10)	1	Enumeration	Y	N	M	2	100	No transfer
t(10;14)(q26;q22)	1	Telomeric	N	Y	F	6	100	No transfer
t(11;22)(q26;q13)	1	Telomeric	N	Y	M	7	100	No transfer
t(13;14)(q10;q10)	3	Enumeration	Y	N	M	13	92	No transfer*
t(6;11)(p22;p15)	2	Spanning	N	Y	M	11	78	No transfer*
t(1;4)(p36;q31)	2	Spanning	N	Y	F	12	83	No transfer*
t(13;14)(q10;q10)	1	Enumeration	Y	N	M	4	50	Not pregnant
t(13;14)(q10;q10)	1	Enumeration	Y	N	M	4	75	Not pregnant
t(4;10)(q33;p12)	1	Spanning	N	Y	M	8	88	Not pregnant
t(3;4)(p25;p26)	1	Spanning	N	N	M	9	89	Not pregnant
t(10;13)(q22;q14)	1	Telomeric	N	N	M	7	57	Not pregnant
t(6;10)(q23;q26)	1	Telomeric	N	Y	F	6	66	Not pregnant
t(12;20)(p13;q13)	1	Spanning	N	Y	F	2	50	Not pregnant
t(10;18)(q24;p11)	1	Telomeric	N	Y	M	10	80	Not pregnant
t(5;10)(p15;q24)	1	PB painting	N	Y	F	6	83	1 ongoing
t(2;14)(q23;q24)	1	PB painting	N	N	F	9	89	1 ongoing
t(4;14)(p15;q24)	2	PB painting	N	Y	F	11	72	1 spontaneous abortion (balanced)
t(7;20)(q22;q11)	1	PB painting	N	N	F	6	50	2 infants
t(8;21)(q13;q21)	1	PB painting	N	N	F	2	100	No transfer
t(11;16)(q21;q22)	2	PB painting	N	N	F	18	56	No transfer*
t(14;18)(q22;q11)	1	PB painting	N	N	F	6	66	No transfer*
t(13;17)(q14;q12)	1	PB painting	N	N	F	8	62	Not pregnant
t(2;14)(q31;q24)	1	PB painting	N	N	F	3	66	Not pregnant
t(6;13)(q23;q21)	1	PB painting	N	N	F	33	76	Not pregnant
t(13;14)(q10;q10)	2	PB painting	Y	N	F	13	38	1 ongoing
t(14;21)(q10;q10)	3	PB painting	Y	N	F	25	44	1 ongoing
t(14;21)(q10;q10)	2	PB painting	Y	N	F	25	44	1 ongoing
t(13;14)(q10;q10)	1	PB painting	Y	N	F	9	46	1 ongoing
t(13;14)(q10;q10)	1	PB painting	Y	N	F	5	0	2 infants
t(13;14)(q10;q10)	1	PB painting	Y	N	F	13	55	3 infants

Note: Y = yes; N = no; M = male; F = female; PB = polar body.

* No transfer because normal eggs did not fertilize or normal embryos were arrested.

Munné. PGD of translocations. Fertil Steril 2000.

It is interesting that a sizable part of the abnormalities detected in this and other studies (16, 18) were chaotic mosaics, with all or many of their cells abnormal, and therefore presumably unbalanced. High rates of mosaicism have been detected by fluorescence in situ hybridization (24–27), and even higher rates have been found in some translocation cases (11, 18). The reason for high mosaicism rates in translocation cases is unknown.

Because the risks of preimplantation and periimplantation loss are mostly unknown, it is difficult to counsel translocation-carrier couples about their chances of successful pregnancy. Segregation types found on spontaneous abortions and live births do not coincide with those found in sperm chromosome analysis (28) because many segregations are

lethal before or shortly after implantation. Further cases of translocation PGD and chromosome analyses of sperm and oocytes are needed to provide more accurate risk assessment of embryo loss.

Reduction in Spontaneous Abortions

As reported previously (6), PGD of translocations substantially increases the couple's chances of sustaining a pregnancy to full term. In the previous study (6), only female carriers were evaluated, but this study reviews both male and female carriers. In these 35 cases of PGD of translocations, we have demonstrated a statistically significant decrease in spontaneous abortions ($P < .001$): from 92% of the pregnancies in natural cycles to 12.5% in PGD cycles.

TABLE 4

Summary of factors that may affect PRs and chromosome abnormalities.

Factors	Pregnancies per cycle	Abnormal
A: Embryo biopsy–female carrier–reciprocal–terminal breakpoints	0 (0/4)	81 (21/26)
B: Embryo biopsy–female carrier–reciprocal–no terminal breakpoints	100 (1/1)	0 (0/3)
C: Embryo biopsy–male carrier–reciprocal–terminal breakpoints	0 (0/5)	86 (31/36)
D: Embryo biopsy–male carrier–reciprocal–no terminal breakpoints	0 (0/2)	73 (12/16)
E: Embryo biopsy–male carrier–robertsonian	42 (5/12)	70 (50/71)
F: Embryo biopsy–total reciprocal (F=A+B+C+D)	8 (1/12)	79 (64/81)
G: Embryo biopsy–total robertsonian (G=E)	42 (5/12)	70 (50/71)
H: Embryo biopsy–total male carrier (H=C+D+E)	26 (5/19)	76 (93/123)
I: Embryo biopsy–total female carrier (I=F)	20 (1/5)	72 (21/29)
J: Embryo biopsy–total terminal breakpoints (J=A+C)	0 (0/9) ^a	84 (52/62)
K: Embryo biopsy–total no terminal breakpoints (K=B+D+E)	40 (6/15) ^a	69 (62/90)
L: Polar body biopsy–female carrier–reciprocal–terminal breakpoints	67 (2/3)	78 (13/17)
N: Polar body biopsy–female carrier–reciprocal–no terminal breakpoints	22 (2/9)	70 (59/85)
O: Polar body biopsy–female carrier–total reciprocal (O=L+N)	33 (4/12) ^b	71 (72/102) ^c
P: Polar body biopsy–female carrier–robertsonian	60 (6/10) ^b	42 (38/90) ^c
Q: Total embryo biopsy (Q=A+B+C+D+E)	25 (6/24)	75 (114/152)
R: Total polar body biopsy (R=O+P)	45 (10/22)	57 (110/192)
S: Total female carriers (S=I+R)	41 (11/27)	NA ^d
T: Total male carriers (T=H)	26 (5/19)	NA ^d
U: Total reciprocal (U=F+P)	21 (5/24) ^e	NA ^d
V: Total robertsonian (V=G+P)	50 (11/22) ^e	NA ^d
W: Total no terminal breakpoints (K+N+P)	41 (14/34) ^f	NA ^d
X: Total terminal breakpoints (J+N)	17 (2/12) ^f	NA ^d

Note: Values are percentages (n/n). NA = not applicable.

^a $P = .037$, F test.

^b $P = .040$, F test.

^c $P < .001$, χ^2 test.

^d Because more chromosome abnormalities can be found by doing embryo biopsy than by polar-body biopsy, the percentage of abnormalities in the combination of cycles performed by both biopsy methods is meaningless.

^e $P = .030$, F test.

^f $P = .026$, F test.

Munné. PGD of translocations. *Fertil Steril* 2000.

For most translocation patients, consecutive pregnancy loss is their major incentive in enrolling in a PGD program. The unbalanced products of the translocation are usually lethal, and therefore the true risk is that of pregnancy loss. We believe that growing embryos to the blastocyst stage therefore cannot select against unbalanced embryos (29) because many unbalanced embryos implant and then abort spontaneously. Translocation carriers wish to prevent where possible the disturbing recurrent miscarriages and to sustain pregnancy to term.

Other Correlations

Couples with robertsonian translocations achieved significantly higher PRs than couples with reciprocal translocations. It could be argued that more cases of robertsonian translocations were examined by polar-body biopsy, thereby making the biopsy method the overriding factor. But when the cases were grouped according to biopsy methods (Table 4), there was a tendency in both groups for higher PRs in robertsonian than in reciprocal translocation cases.

In patients with reciprocal translocations, the production

of unbalanced gametes is likely to occur as a consequence of two mechanisms (30): one from meiotic crossing over, involving the critical region between the centromere and the breakpoint (31), and the other from abnormal meiotic segregation (32). By contrast, robertsonian translocation results in unbalanced gametes only as a consequence of abnormal meiotic segregation because there is no critical region. For instance, sperm chromosome analyses in patients with robertsonian translocations have shown only between 0% and 14% abnormal gametes (33–38), whereas in reciprocal translocation, this proportion ranges between 18.4% and 72.1% (33, 39).

We are not aware of any comprehensive study on oocyte segregation of translocations, but the present results suggest trends similar to those found in sperm. However, our embryo results do not show differences between robertsonian and reciprocal translocations. The fact that many embryos were mosaics, regardless of the type of translocation, may be the reason that these differences were not observed at the embryo stage.

Another trend that we observed was a lower PR in cases with at least one terminal breakpoint. The probable cause was the high rate of abnormalities found in these embryos (84%); however, the source of these abnormalities is not always unbalanced gametes. For instance, a wide range of unbalanced gametes (18%–77%) was found among patients with at least one terminal breakpoint in which the sperm were analyzed (33, 39).

One could compare the damage produced by embryo biopsy vs. polar-body biopsy, assuming that there is a similar population of translocation types and other factors in both groups (19). Unfortunately, even if we had a population of such cases, the cases of polar-body biopsy were analyzed at the oocyte stage, whereas embryo-biopsy cases were analyzed at the third or fourth day of development. The abnormalities found in the two groups are therefore not really comparable.

The Telomere Approach

A variety of techniques may be used for PGD of translocations. Here we have described a new approach consisting in the use of telomeric probes. Until the recent availability of commercial telomeric probes, it was necessary to find specific probes distal to the breakpoints (11, 15–17), a laborious, expensive, and time-consuming task. Using a telomeric probe simultaneously for each arm involved in the translocation, one can detect all abnormal segregations for any translocation. Even better, the use of two telomeric probes in combination with one or two centromeric ones, as we did in this series of cases, produces a more robust test because it differentiates between unbalanced and nonsense events resulting from errors of fluorescence in situ hybridization.

In the present series of cases, the error rate was between 6% and 10%. It was calculated on the basis of results obtained with control lymphocytes and control blastomeres. On the basis of reanalysis of embryos deemed abnormal after PGD, this compares well with other PGD tests (21, 40). The errors detected in control cells were mostly caused by overlaps, signal splitting, and poor probe penetration.

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