Possible interchromosomal effect in embryos generated by gametes from translocation carriers

L. Gianaroli1,3, M. C. Magli1, A. P. Ferraretti1, S. Munne2, B. Balichia1, T. Escudero2 and A. Crippa1

1S.I.S.ME.R., Reproductive Medicine Unit, Via Mazzini 12, 40138 Bologna, Italy and 2St. Barnabas Medical Center, West Orange, NJ, USA
3To whom correspondence should be addressed. E-mail: sismer@sismer.it

BACKGROUND: The incidence of abnormal pregnancies in carriers of balanced translocations depends strictly on the chromosomes involved in the translocations. The aim of this study was to verify whether conventional aneuploidy screening could be advantageously combined with preimplantation genetic diagnosis (PGD) for translocations. METHODS: Twenty-eight carriers of Robertsonian and reciprocal translocations underwent 43 PGD cycles; specific probes were used to screen the translocation in 172 embryos generated by 35 cycles; most of these embryos were also screened for chromosomes 13, 16, 18, 21, 22 (n = 166), XY (n = 107), 1 (n = 17) and 15 (n = 88). For the remaining eight cycles (carriers of reciprocal translocations) only the chromosomes involved in common aneuploidy screening were investigated on the 40 embryos generated in vitro. RESULTS: In Robertsonian translocations, the proportion of embryos with abnormalities due to the translocation was 21%, common aneuploidies contributed 31% of total abnormalities, whereas the remaining 36% of embryos had abnormalities due to both types of chromosome. For reciprocal translocations, the chromosomes involved in the translocation were responsible for 65% of total abnormalities; only 6% of the embryos were abnormal for common aneuploidies and 16% carried abnormalities due to both the chromosomes involved in the translocation and those not related to the translocation. CONCLUSIONS: An interchromosomal effect seems to play a role in the case of Robertsonian translocations, where the relevant contribution of aneuploidy exposes the couple to an additional risk of abnormal pregnancy.

Key words: aneuploidy/interchromosomal effect/preimplantation genetic diagnosis/translocations

Introduction

Chromosomal translocations originate from chromosome partition followed by reunion in a different configuration. Clinical effects are very severe in the case of unbalanced rearrangements due to the altered amount of chromosomal material. Conversely, no loss or gain of genetic material occurs in balanced rearrangements and no consequences for the phenotype occur unless the breakpoints affect a functional gene. However, the production of a high proportion of gametes with an unbalanced genetic complement is strictly related to translocations and causes an increased risk of spontaneous abortions and abnormal offspring.

A linkage has been reported between infertility and chromosomal translocations which occur at a rate of 0.6% among infertile couples compared with an incidence of 0.2% in the general population (Hook and Hamerton, 1977). This figure is significantly higher in couples with multiple IVF failures or recurrent miscarriages (3.2 and 9.2% respectively) (Stern et al., 1999).

In recent years, preimplantation genetic diagnosis (PGD) has been offered to carriers of balanced translocations with the aim of improving the clinical outcome by selecting for transfer those embryos with a normal or balanced chromosomal complement. Different approaches have been proposed. Chromosome painting probes applied to first polar body and second polar body after metaphase conversion have been used for the diagnosis of translocations of maternal origin (Munne et al., 1998a; Verlinsky and Evsikov, 1999). For the analysis of interphase chromosomes in blastomeres, specific spanning probes can be developed for both Robertsonian (Rob.T) and reciprocal translocations (Rec.T); however, their preparation is expensive and time consuming (Munne et al., 1998b; Pierce et al., 1998). More recently, the simultaneous use of commercially available telomeric probes in combination with centromeric probes has provided an alternative approach for the detection of the abnormal segregation in Rec.T (Scriven et al., 1998; Munne et al., 2000). Similarly, enumerator α-satellite or locus-specific probes enable the detection of aneuploid embryos in the case of Rob.T (Conn et al., 1998).

The aim of the present study was to verify whether conventional aneuploidy screening, as normally performed to increase the take-home baby rate after IVF (Gianaroli et al., 1999a; Munne et al., 1999), could be combined with PGD for
translocations. The possibility that a chromosomal rearrangement could affect
the behaviour at meiosis of other chromosomes has always been
a controversial issue in human genetics. The use of
several probes specific for different positions along the chromo-
somes has shown pairing defects in translocation heterozygotes
not only in the chromosomes affected by the translocation but
in other chromosomes as well. The resulting interchromosomal
effect could be a consequence of small breaks in the formation
of homologue synapses or could depend on defects in the
alignment or chromosome condensation (McKim and
Hawley, 1995).

The final goal was the development of a system, based on
multiple round hybridization, that could maximize the chances
of pregnancy in carriers of translocations by avoiding the
transfer of aneuploid embryos.

Materials and methods

Patients

Twenty-eight patients, carriers of a balanced translocation, attended
the S.I.S.M.E.R. Reproductive Medicine Unit to undergo IVF treatment
cycles for infertility in combination with the screening of aneuploidy
on the in-vitro-generated embryos. Indication for PGD was the
presence of a Robertsonian or a reciprocal translocation in one of the
partners. Fifteen couples entered the Rob.T group and 13 the Rec.T
group. Nine of the 28 couples had a history of repeated abortions:
three with Rob.T and six with Rec.T which accounted for a total of
23 abortion events (Table I).

Five couples with a Rec.T did not have their embryos screened
for the translocation due to the impossibility of having the correct
spanning probes available in a short time; therefore they decide to perform
eight conventional PGD cycles with the screening for the
chromosomes XY, 1, 13, 15, 16, 18, 21 and 22.

Between September 1996 and May 2001, 43 PGD cycles for
translocation were performed. Induction of multiple follicular growth
was accomplished by the administration of exogenous gonadotrophins
after a long desensitization protocol with long-acting GnRH analogues
(Ferraretti et al., 1996). Ovulation was induced by hCG administration;
34–36 h later, oocytes were transvaginally collected via ultrasound
guidance, and cultured in Earle’s balanced salt solution (EBSS)
supplemented with 10% heat-inactivated maternal serum (MS), in a
5% CO2 moist gas atmosphere at 37°C. Oocyte insemination was
performed by ICSI or conventional IVF depending on semen
sample indices.

Assessment of fertilization and embryo development

At 14–18 h after insemination, oocytes were checked for the presence
of pronuclei and polar bodies. Regularly fertilized oocytes were
cultured individually in EBSS 15% MS and scored at 40, 62 and
88 h post-insemination. Number and morphology of nuclei and
blastomers, and percentage of fragmentation were recorded. After
embryo biopsy, embryos were transferred to blastocyst growing
medium (Vitrolife Sweden AB, Gothenburg, Sweden). Transfers
were performed into the uterine cavity; only embryos diagnosed
as chromosomally normal or balanced were transferred. Clinical
pregnancies were assessed by ultrasound analysis as the presence of
a gestational sac with fetal heartbeat. The implantation rate (IR)
defined the ratio between the number of gestational sacs with
fetal heartbeat and the total number of embryos transferred. The
implantation rate per pregnant patient (IRPP) was calculated as the
number of gestational sacs with fetal heartbeat divided by the total
number of embryos transferred in the pregnant patients.

Embryo biopsy

Day 3 embryos with ≥4 regular blastomers and a percentage of
fragmentation ≤50% were selected to undergo embryo biopsy for
fluorescent in-situ hybridization (FISH) analysis. Embryos were
manipulated individually in HEPES-buffered medium overlaid with
36 h pre-equilibrated mineral oil. One blastomer was aspirated by
using a polished glass needle, which was introduced into the perivitel-
line space after opening a breach of ~20 μm in the zona pellucida
with acidic Tyrode’s solution. The biopsied cell was transferred to
hypotonic solution, fixed in methanol:acetic acid on a glass slide, and
dehydrated in increasing ethanol series.

Fluorescent probes and FISH

Table II represents the scheme of the study, and Table III the
translocations and corresponding probes. In all, 212 embryos were
analysed by two-, three- or four-round FISH. In the first round (or
first two rounds in the case of Rec.T), the chromosomes involved in
the translocation were tested; in the following round, chromosomes
13, 16, 18, 21 and 22 were tested, followed by the last round involving
the probes for chromosomes XY, 1 and 15. A total of 111 embryos
was generated by the 23 cycles of the Rob.T carriers and screened
for the chromosomes involved in the translocations as well as, in 105
of them, for chromosomes 13, 16, 18, 21 and 22; 95 were also
screened for chromosomes XY, 76 for chromosome 15 and 10 for
chromosome 1. A total of 101 embryos was analysed in the case of
Rec.T; five of the patients had 40 embryos, generated by eight cycles,
analysed only for chromosomes 13, 16, 18, 21, 22 in the first round
FISH, and chromosomes XY, 1 and 15 in the second round; whereas
61 embryos from the remaining 12 cycles were diagnosed for the
chromosomes involved in the translocation (first round FISH) as well
as for the chromosomes 13, 16, 18, 21 and 22; in addition, chromo-
somes XY, 1 and 15 were investigated in 12 embryos and chromosome
1 in seven embryos.

For Robertsonian translocations, enumerator probes for interphase
nuclei were used: in cases of t(13;14) and t(14;21) the MultiVysion
PB panel from Vysis (Vysis Inc., Downers Grove, IL, USA) was used

<table>
<thead>
<tr>
<th>Table I. Patients’ history</th>
<th>Rob.T carriers</th>
<th>Rec.T carriers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>15</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>No. patients with previous abortions (%)</td>
<td>3</td>
<td>6</td>
<td>9 (32)</td>
</tr>
<tr>
<td>No. abortions (mean ± SD)</td>
<td>6 (2.0 ± 1.5)</td>
<td>17 (2.8 ± 1.7)</td>
<td>23 (2.5 ± 1.7)</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>4</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td>After ICSI</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Rob.T = Robertsonian translocation; Rec.T = reciprocal translocation.
in the first round for the detection of chromosomes 13, 16, 18, 21 and 22, followed by a second round with a probe specific for chromosome 14 (binding to the 14q11.2 region) labelled with Spectrum Green™ (Vysis). The screening for t(13;21) was achieved by a single round hybridization with the Multivision PB panel from Vysis.

For reciprocal translocations, telomeric and centromeric probes were used in a combination that properly characterized the translocation (Munne et al., 2000). Depending on the availability of fluorochromes, one- or two-round FISH were used. In the preparatory phase to PGD, the probes were tested on metaphases obtained from the carrier’s lymphocytes where one of the telomeric probes was located in the same chromosome labelled with the centromeric probe. The probe mixture was evaluated on lymphocytes in interphase as well to estimate the efficiency of the procedure.

The hybridizing solution was added to the fixed nuclei and co-denaturation of nuclear DNA and probes was achieved by heating at 73°C for 5 min; hybridization followed for at least 3 h at 37°C.

The conventional aneuploidy screening used multicolour FISH in a two step protocol for the simultaneous detection of the chromosomes 13, 16, 18, 21 and 22 (Multivision PB; Vysis) in the first round followed by an additional round including the probes for the chromosomes XY, 1 and 15. The scoring criteria adopted to classify the FISH signals have been previously described (Munne et al., 1998c).

The whole procedure required 8–16 h depending on the number of FISH rounds required to complete the analysis programmed for each case; consequently embryo transfer was scheduled on day 4 (Gianaroli et al., 1999b).

Embryo spreading
The cells from 84 non-transferable embryos were spread after a short incubation with acidic Tyrode’s solution to digest the zona pellucida. The nuclei obtained (475) were fixed and hybridized with the same probes used for PGD according to the same protocol already described. The results obtained were compared with the 1-cell diagnosis in order to verify the efficiency of the technique (Gianaroli et al., 2001).

Statistical analysis
Data were analysed by Student’s t-test and χ²-analysis applying the Yates correction, 2×2 contingency tables.

Results
The overall FISH and clinical results of Rob.T are shown in Table IV. A total of 131 embryos was generated from 23 treatment cycles in carriers of Rob.T whose mean maternal age was 35.5 ± 3.6 years; 111 of them showed regular development and were selected for FISH analysis. Following FISH, 26 were diagnosed as normal or balanced (23%) whereas
conventional prenatal diagnosis: PGD was followed by conventional aneuploidy screening (FISH) and clinical results after conventional aneuploidy screening (group 1) and after the analysis of the chromosomes involved in the translocation followed by conventional aneuploidy screening (group 2).

The results of the Rec.T cycles are reported in Table VI; they were divided into two groups depending on the type of analysis: in the first group, conventional aneuploidy screening was performed (five patients which underwent eight cycles, mean maternal age 34.6 ± 5.1 years), and in the second group, PGD of translocation was followed by conventional aneuploidy screening (11 patients and 12 cycles, mean maternal age 34.1 ± 4.9 years).

In the first group, 17 of the 40 embryos analysed were euploid for the eight chromosomes tested (43%); embryo transfer was performed in seven cycles by replacing 2.4 ± 0.5 chromosomally normal embryos which generated one clinical pregnancy and an implantation rate of 5.9%. Thirteen of the detected 23 abnormalities (56%) involved one of the chromosomes related to the translocation which were diagnosed as part of the conventional aneuploidy screening (see Table III), seven were independent from the chromosomes involved in the translocation (30%) and three were haploid or polyploid (13%).

In the second group, 61 embryos were screened for the chromosomes involved in the translocation and conventional aneuploidy. The overall results demonstrated only seven embryos with a normal or balanced chromosomal condition (11%), whereas the remaining 54 were diagnosed as abnormal (89%). Consequently, only four cycles were transferred (33% of the oocyte retrievals) with a single pregnancy that miscarried at 7 weeks gestation (no information on the fetal karyotype was available).

The observed chromosomal abnormalities were: unbalanced arrangement of the chromosomes involved in the translocation in 44 embryos (81% over total abnormalities); in 35 of them they were the only abnormality detected whereas in the remaining nine aneuploidy for another chromosome was also found; three embryos carried aneuploidy for chromosomes different from those involved in the translocation (6%) and seven were haploid or polyploid (13%).

Finally, Figure 1 represents the cellular stage of the embryos at 62 h post insemination before embryos biopsy was per-
formed. The percentage of embryos at the 4-cell stage was significantly higher in the case of Rec.T (35 versus 17%, \( P < 0.025 \)), while embryos with \( \geq 7 \) cells were more frequent in Robertsonian translocations (\( a \cdot b \cdot P < 0.025 \)).

The main differences between Rob.T and Rec.T are summarized in Table VI. The data of Rec.T only refer to group 2 where both the chromosomes involved in the translocation and those independent from the translocation were screened. Although the percentage of chromosomally abnormal embryos did not vary significantly between the two categories, the distribution of the abnormalities detected was totally different. Most of the defects were due to the chromosomes related to the translocation with the highest incidence in Rec.T (65%) compared with Rob.T (21%; \( P < 0.001 \)). Conversely, a more relevant contribution to aneuploidy from chromosomes not related to the translocation was detected in the case of Rob.T (31%), compared with Rec.T (6%, \( P < 0.001 \)); this difference did not seem to be related to maternal age, which was similar in both Rob.T and Rec.T couples (\( P = 0.35 \), not significant).

The simultaneous presence of numerical variations derived from chromosomes related to the translocation and from other chromosomes contributed 36% of total abnormalities in Rob.T versus 16% in Rec.T (\( P < 0.025 \)). Similar results occurred in the proportion of aneuploid embryos not related to the translocation when group 1 was also taken into consideration: 67% in Rob.T (31 + 26/85) and 40% in Rec.T (9 + 3 + 20/77; \( P < 0.005 \)). The results obtained from the FISH analysis of 84 non-transferable embryos after spreading of their blastomeres revealed 93% overall confirmation of PGD results (78/84). Four of the six non-confirmed embryos had a different abnormality (5%): one was monosomy 16 instead of monosomy 18 and belonged to a patient with 13;14 translocation; and three were chaotic mosaic embryos that had been diagnosed as monosomic by PGD from three patients with translocations 13;14, 18;20 and 1;22 respectively. Finally, two embryos classified as monosomy 13 generated by two patients with a 13;14 and a 13;21 translocation each, turned out to be chromosomally normal giving a misdiagnosis rate of 2%.

**Discussion**

As already reported, carriers of Rob.T derive a notable benefit from FISH analysis (Munne et al., 1998d, 2000; Evsikov et al., 2000; Scriven et al., 2001). According to the current results, the high percentage of chromosomally abnormal embryos detected in these couples, (77%; Table IV) made the selection by PGD a useful tool to achieve pregnancy in 35% of the cycles undergoing oocyte retrieval. This outcome is especially relevant when considering that even in the case of embryos at the 7–8-cell stage on the morning of day 3, chromosomal abnormalities were detected in 65% of them, implying that in these categories of patients normal morphology and development, or blastocyst growth are not sufficient criteria for euploidy (Evsikov et al., 2000). As repeated pregnancy losses are a frequent consequence of unbalanced rearrangements, it is hardly sustainable, despite some reports (Ménézo et al., 1997), that transferring at the blastocyst stage prevents abnormal implantation.

The analysis of chromosomal abnormalities revealed that 58% (82/139) of them were due to the chromosomes directly involved in the translocation, either alone or in combination with other chromosomes (Table V). The concomitant study of other chromosomes was essentially aimed at maximizing the chances of pregnancy in these patients by avoiding the transfer of aneuploid embryos. Actually, 26 of the 111 embryos diagnosed by FISH carried abnormalities due to chromosomes different from those involved in the translocation: nine were monosomic, 10 trisomic, one showed simultaneous monosomy and trisomy and six carried complex abnormalities. Interestingly, five of these trisomies involved chromosome 21 and were detected in embryos generated by carriers of the translocation 13;14. In other words, almost a fourth of the screened embryos were removed from the possibility of being transferred by the screening for conventional aneuploidy. Unfortunately, one misdiagnosis occurred and a trisomic 21 embryo was transferred to a patient 37 years whose husband carried a 13;14 translocation; this was due to partial overlapping of two signals which, according to the scoring criteria adopted, were diagnosed as one. Since then, scoring criteria has been revised, and, in the presence of a split signal, a specific telomeric probe is used to confirm the diagnosis (Magli et al., 2001). In consideration of the clinical implications related to trisomy 21, a new strategy using two different probes for this chromosome has been designed: one probe is locus specific whereas the other, used in a second-round hybridization, targets the telomere. Under these conditions, the risk of missing a trisomy 21 is close to zero (Magli et al., 2001).

Patients with Rec.T entered this study following two different approaches depending upon the type of probes used. The results from group 1 were included since the FISH results

![](image.png)
were different when the chromosomes related to the translocation were not fully characterized with specific probes, confirming that carriers of Rec.T are highly exposed to unbalanced products (Table VI).

In Table V, data from group 2 only were considered in order to distinguish the contribution to total abnormalities from the chromosomes related to the translocation from those not involved in the translocation. As reported, 81% of the abnormalities in Rec.T were due to the chromosomes involved in the translocations (either alone or in combination with others) and this was significantly higher compared with the situation found in Rob.T (58%; \( P < 0.01 \)). This could be explained by the differences in the meiotic behaviour of these two types of translocations (Scriven et al., 1998). During meiosis, the chromosomes involved in the Rob.T pair their homologous segments forming a trivalent figure, which only can segregate in an alternate way (producing balanced or normal gametes) or in an adjacent way (producing unbalanced gametes). If the chromosomes involved in the translocation are of the same size (D:D or G:G), the translocation is more prone to segregate in an alternate way than if the chromosomes are of different size (D:G). Rec.T pair their homologous segments forming a tetravalent figure, which can segregate in five different ways: alternate (producing balanced or normal gametes), adjacent I and II (producing unbalanced gametes), 3:1 or 4:0 (also producing unbalanced gametes). Depending on the position of the breakpoints in the chromosomes involved in the translocation and the size of these chromosomes, the translocation would be more prone to segregate in a 2:2 way (alternate and adjacent segregations) or a 3:1 or 4:0 way (Boue et al., 1984; Pellestor et al., 1987; Martin and Hultén, 1993).

Although only a few cases were studied, it is very clear that the two categories of balanced translocations have a completely different molecular basis, and this leads to a diverging clinical outcome after PGD. This diversity is already perceived at the morphological analysis of in-vitro-generated embryos, whose development is significantly retarded in the case of Rec.T (Figure 1), although unbalanced embryos can develop to blastocysts (Evsikov et al., 2000). The reason for this behaviour is not clear, especially when considering that the majority of the translocation cases presented here were paternal in origin, and that until the 4–8-cell stage the embryo relies upon the oocyte machinery for the first cleavage divisions (Braude et al., 1988). Similarly, the high rates of mosaicism detected in translocation cases do not have a clear explanation (Conn et al., 1998; Iwarsson et al., 2000).

The FISH results obtained in this study basically suggest two considerations. First, the high proportion of abnormalities due to the chromosomes involved in the translocations is significantly higher in carriers of Rec.T. This reduces markedly the number of embryos available for transfer and makes the quality of the response to hormonal stimulation critical for these patients’ clinical outcome. Second, an interchromosomal effect could be postulated in the case of Rob.T due to the relevant contribution of aneuploidy involving chromosomes unrelated to the translocation. The analysis of the aneuploid events associated with chromosomes different from those involved in the translocation revealed that, as already mentioned, five of the detected trisomies resulted from chromosome 21; in total, six of the monosomies and eight of the trisomies were due to one chromosome from the D group (13, 14, 15) or the G group (21, 22) which are the chromosomes entering Rob.T. In order to evaluate a putative interchromosomal effect, the contribution of aneuploidy in age-matched patients with a normal karyotype was estimated; 17% of 235 embryos diagnosed in 46 ICSI cycles, which were treated during the same period, were monosomic or trisomic. This value is similar to the aneuploidy rate found in embryos generated by Rec.T carriers, but is significantly lower when compared with Rob.T (\( P < 0.025 \)).

Contradictory data have been reported on the analysis of sperm obtained from carriers of translocations, with some reports in favour of an interchromosomal effect both in Robertsonian and reciprocal translocations (Rousseaux et al., 1995; Mercier et al., 1998; Blanco et al., 2000; Estop et al., 2000; Morel et al., 2001; Oliver-Bonet et al., 2001; Shi and Martin, 2001) and others demonstrating no evidence of this phenomenon (Martin, 1988; Pellestor, 1990; Martin et al., 1992; Syme and Martin, 1992; Blanco et al., 1998). According to these reports, the occurrence of an interchromosomal effect seems to depend both on the type of chromosomes and the chromosomal regions involved, resulting in a particular meiotic configuration that could determine an increased sperm disomy (Estop et al., 2000). In addition, a direct correlation between poor quality sperm and alteration in the recombination frequency of other chromosomes has been reported, suggesting that infertile carriers are at higher risk for interchromosomal effects (Pellestor et al., 2001). Furthermore, a follow-up study of the genetics and epidemiology of Down’s syndrome in >2×10^5 births postulated an interchromosomal effect in seven infants out of the 391 Down’s infants which were karyotyped (Stoll et al., 1998).

Taken altogether, these observations suggest that the high incidence of aneuploidy detected by PGD in this study for chromosomes unrelated to the translocation, could be due to the fact that most of these embryos were generated by infertile patients. The use of ICSI, which was necessary in 80% of the cases, could have contributed an additional element since the possibility of a natural selection against aneuploid sperm following conventional insemination (or natural conception) was completely bypassed.

Additional data are certainly required to support the hypothesis of an interchromosomal effect associated with carriers of translocations; particularly important would be to promote the inclusion in the aneuploidy screening of other chromosomes, reassured by the finding that multiple-round hybridization (at least four, as used in this study) does not seem to affect FISH efficiency.

References
Interchromosomal effect and translocation carriers


Estop, A.M., Cieply, K., Munne


