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Effect of chromosomal translocations on the development of preimplantation human embryos in vitro

S. Evsikov, Ph.D., J. Cieslak, M.L.T., and Y. Verlinsky, Ph.D.

Reproductive Genetics Institute, Chicago, Illinois

Objective: To determine the reliability of a new technique for single human blastomere karyotyping during clinical cases for preimplantation genetic diagnosis of translocations.

Design: Controlled clinical study.

Setting: Preimplantation genetic diagnosis and IVF program

Patient(s): Nineteen preimplantation genetic diagnosis cases with 11 types of translocations (10 reciprocal and one Robertsonian) involving chromosomes 1, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18, 20, 21, and 22.

Intervention(s): Blastomere biopsy followed by blastomere nucleus conversion into metaphase chromosomes. Fluorescent in situ hybridization (whole chromosome painting) was used for the detection of chromosomally unbalanced preimplantation human embryos.

Main Outcome Measure(s): Percentage of informative metaphase plates and effect of unbalanced translocations on preimplantation embryo development.

Result(s): Informative metaphases were obtained for 84% of the blastomeres. Analysis of preimplantation development of the resulting embryos showed that an unbalanced chromosomal complement does not affect embryo ability to reach the blastocyst stage in vitro.

Conclusion(s): For the translocations tested, there is no evident selection against chromosomally unbalanced embryos at the preimplantation stage of embryo development. (Fertil Steril® 2000;74:672–7. ©2000 by American Society for Reproductive Medicine.)

Key Words: Blastocyst development, blastomere biopsy, chromosome translocation, FISH, metaphase chromosomes, preimplantation genetic diagnosis

Chromosomal translocations are among the most common genetic abnormalities in humans: one in 500 newborns is a carrier of a reciprocal translocation (1). Considering that a germinal cell with a balanced reciprocal translocation can produce 32 types of gametes, only two of which would result in a chromosomally normal child (2), it is not surprising that abnormal offspring and pregnancy loss is a common cause of infertility among the carriers of translocations. For the carrier of a balanced reciprocal translocation the chances of conceiving a chromosomally abnormal embryo, depending on the translocation, is in the range of 20%–80% (3).

Very little is known about the extent and timing of selection against the embryos with chromosomal translocations. Live birth of physiologically abnormal children indicates that unbalanced translocations do not necessarily affect prenatal embryo viability. Recent studies revealed that more than 50% of chromosomally unbalanced embryos from the patients with translocations display chaotic cleavage (4) and thus are subject to elimination at the very first stages of development. Our previous results suggested that the start of cavitation initiates a selection against aneuploid cells and mosaic embryos (5). If the same is true for the embryos with chromosomal translocations, then the embryos with a normal (and perhaps also balanced) chromosomal complement could have a better chance of reaching the blastocyst stage in vitro.

Studies with animals have revealed the selective elimination of embryos with unbalanced chromosomal translocations at the cleavage stage of development (6). Moreover, it was

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Reprint requests: Yury Verlinsky, Ph.D., Reproductive Genetics Institute, 836 W. Wellington Ave., Chicago, Illinois 60657 (FAX: 773-871-5221; E-mail: rgi@flash.net).

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TABLE 1

Efficiency of the blastomere biopsy–chromosome conversion technique for the detection of unbalanced chromosomal translocations in human embryos.

Type of translocation	No. of embryos	No. of blastomeres	No. of blebs	No. of metaphases	No. of PCCs	Metaphases with chromosome loss	Analyzable metaphases (%)	Embryos analyzed (%)
Reciprocal	130	140	6	112	22	9	117 (84)	112 (86)
Robertsonian	23	24	0	20	4	1	20 (83)	19 (83)
Total	153	164	6	132	26	10	137 (84)	131 (86)

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suggested that chromosomally balanced embryos may be selected by their ability to reach the blastocyst stage in vitro (7). This implies that standard IVF followed by the transfer of only morphologically normal blastocysts might be used as a method of selecting against chromosomally unbalanced embryos. Nevertheless, selection against chromosomally unbalanced embryos using the techniques of preimplantation genetic diagnosis (PGD) has been attempted. Use of translocation-specific fluorescence in situ hybridization (FISH) probes on individual blastomeres was consistently successful for the detection of both Robertsonian and reciprocal translocations (4, 8–10). However, the development of such probes for each specific translocation case is rather expensive and time consuming. This justifies the attempts to use the whole chromosome painting (WCP) probes on the metaphase chromosomes obtained from first polar bodies or blastomere nuclei (11–13).

As reported previously, blastomere biopsy followed by blastomere fusion with mouse zygotes is an effective method for obtaining metaphase chromosomes from individual human blastomeres (14). We used this technique in conjunction with the WCP for the detection of chromosomally unbalanced embryos. Since the biopsied embryos were cultured for 5 days, anticipating transfer at the blastocyst stage, we had an opportunity to investigate in vitro development of human embryos with unbalanced chromosomal complements. The results indicate that the presence of unbalanced chromosomal translocations does not affect the embryo's ability to reach the blastocyst stage in vitro.

MATERIALS AND METHODS

The results presented in this paper are based on 19 clinical PGD cases for the patients who are carriers of reciprocal (17 cases) or Robertsonian (2 cases) translocations. The translocations involved were 46, XX, t(1;8)(q42;p11.2); 46, XX, t(5;21)(q31.1;q22.1); 46, XX, t(7;18)(q32;q23); 46, XX, t(8;22)(q24.1;q11.2); 46, XX, t(9;13)(q22;q14); 46, XX, t(11;22)(q23;q11.2); 46, XX, t(12;18)(p13.31;q21.32); 46, XY, t(1;8)(p13;q23); 46, XY, t(1;21)(p13;q11.2); 46, XY, t(13;20)(q22;q11.2); 46, XY, t(15;16)(q13;q13); and 45, XY, der(13;14)(q10;q10). The Internal Review Board of the Reproductive Genetics Institute approved this work.

Blastomere biopsy was performed on day 3 of embryo development, at the 4- to 10-cell stage. Five to 10 minutes before micromanipulation, embryos were transferred, two at a time, in Hanks' balanced salt solution (Sigma, St. Louis, MO) with 5% Protenate (Baxter Healthcare Corp., Glendale, CA) and 0.05 M of sucrose (Sigma). A single blastomere with a distinctive nucleus was removed. In some cases, cytoplasm graininess did not allow nucleus visualization. In these cases, a morphologically normal blastomere was selected.

Immediately after the biopsy, the embryos were transferred back into culture, and individual blastomeres were placed into HTF medium (human tubal fluid medium, prepared in house using reagents from Sigma) supplemented with 10% Protenate. Human blastomere fusion with mouse zygotes, embryo fixation, and slide treatment for the WCP were performed according to the previously described protocol (14). Chromosomally normal or balanced preembryos were transferred on day 5 at the blastocyst stage. The preembryos not selected for transfer were cultured in 0.1 μg/mL of Demecolcine (Sigma; blastocysts for 1–3 hours, cleavage-stage embryos overnight) and then fixed.

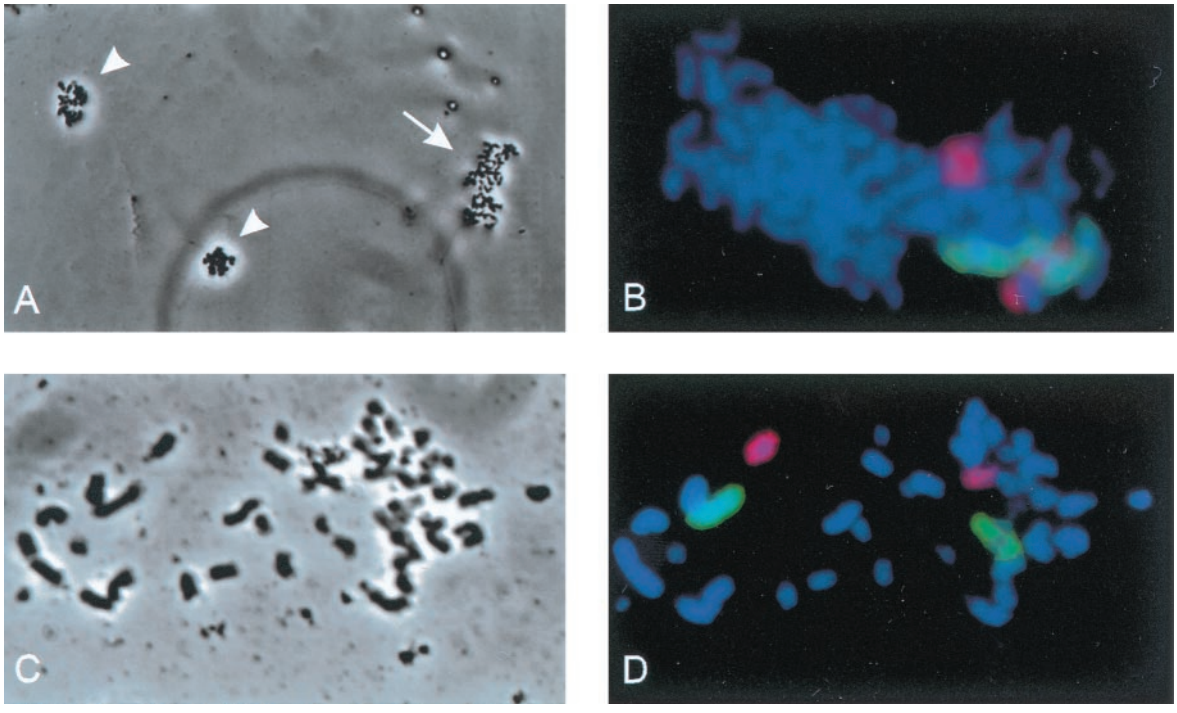
RESULTS

The effectiveness of blastomere-zygote fusion was 100%: all 164 human blastomeres were successfully fused with mouse zygotes. Six blastomeres were found to be anucleated cytoplasmic blebs; one of these embryos was rebiopsied on day 4 and found to be normal. In total, 132 metaphases and 26 prematurely condensed chromosomes (PCCs) were obtained. Ten heterokaryons could not be analyzed because of extensive chromosome loss during fixation. The results are summarized in Table 1.

Unlike slides prepared for cytogenetic analysis, heterokaryon fixation provides more control over the final chromosome spread. Since chromosome overspread is more prone to chromosome loss, it was considered unacceptable for translocation cases. The drawback of this approach is that any attempts to limit metaphase spread inevitably lead to

FIGURE 1

Metaphase plate from tetraploid heterokaryon between human blastomere and mouse zygote. Translocation case t(1;8). (**A**), Phase-contrast view of a diploid human metaphase (*arrow*) and two haploid mouse metaphases (*arrowheads*) immediately after heterokaryon fixation. (**B**), Whole chromosome painting (WCP). Chromosome 1 painted green; chromosome 8, orange. No definite conclusion can be drawn from this metaphase as to the presence of a translocation: three out of four chromosomes involved overlap. (**C**), The same embryo was biopsied on day 4 of development. Phase-contrast view of the metaphase after Pepsin treatment. (**D**), WCP. Chromosome 1 painted green; chromosome 8, orange. Metaphase has no overlaps. The embryo was determined to be normal.



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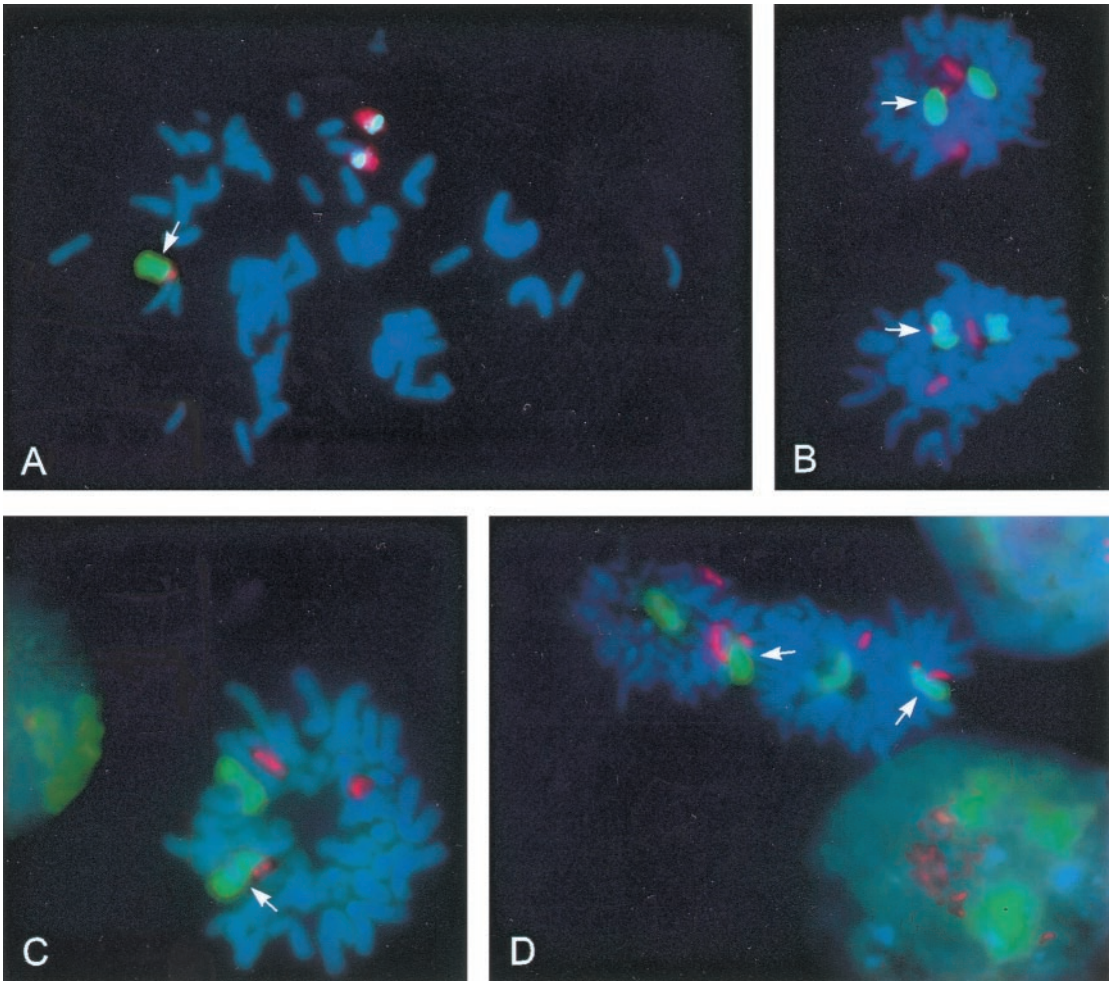
chromosome overlap, thus making some metaphases unanalyzable. One example of such a metaphase, tested for the presence of t(1;8)(p13;q23) translocation, is presented in Figure 1A. Three out of four chromosomes tested are too close to each other so that no definite conclusion as to the presence of a translocation could be made (Fig. 1B). The embryo was biopsied again, on day 4 of development at the 16-cell stage. The second metaphase analysis showed that the embryo had a normal chromosomal complement (Fig. 1D). Even after two biopsies, this embryo successfully reached blastocyst stage by day 5. In total, 16 embryos had two blastomeres removed on day 3, and three embryos were rebiopsied on day 4. From these, eight reached the blastocyst stage by day 5 of development.

There was only one case where embryo fixation did not confirm the result obtained by the blastomere biopsy. Blastomere nucleus conversion into metaphase followed by WCP showed [der(12), 18, 18] (Fig. 2A). Chromosome 12 from the unaffected parent was missing. Being chromosomally unbalanced, the respective embryo was not considered for day 4 biopsy or for transfer. It was fixed on day 6 of development, at which time it had reached the hatched blastocyst stage with more than 200 cells. Some metaphases from this blastocyst are presented in Figure 2. They were analyzable by the WCP and revealed that the actual genotype of the embryo was [12, der(12), 18, 18]. Cases like this could be explained by the presence of mosaicism at the cleavage stage, but the possibility of chromosome loss during fixation cannot be ruled out.

When used solely for the detection of unbalanced chromosomal translocations, WCP redefines the term "analyzable chromosome spread." Out of the 25 PCCs and prometaphases described here, 20 were analyzable by the WCP technique (for an example of an informative prometaphase see Fig. 2B in reference (15). Chromosome swelling due to chromatin denaturation makes some metaphases unanalyzable after WCP (see Fig. 1B). Our experience shows that photographing metaphases after fixation using an air-dry phase-contrast objective facilitates further analysis.

FIGURE 2

Translocation case t(12;18). Metaphase plates obtained from a single blastomere of a 10-cell embryo and the respective blastocyst fixed on day 6 of development (200+ cell stage). Whole chromosome painting. Arrows indicate der(12). (**A**), Blastomere nucleus conversion shows [der(12), 18, 18]: chromosome 12 painted green; chromosome 18, orange; centromeric probe for chromosome 18, aqua. (**B–D**) Metaphases obtained from the same embryo at the blastocyst stage show [12, der(12), 18, 18]. Chromosome 12 painted green; chromosome 18, orange.



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Of the 112 embryos analyzed for the presence of reciprocal translocations, 87 resulted from adjacent segregation. Twelve had trisomy, and 10 had monosomy as a result of 3-to-1 segregation; three embryos resulted from 4-to-0 segregation.

In vitro embryo development clearly showed that, at least for the translocations 46, XX, t(1;8)(q42;p11.2); 46, XX, t(7;18)(q32;q23); 46, XX, t(8;22)(q24.1;q11.2); 46, XX, t(9;13)(q22;q14); 46, XX, t(12;18)(p13.31;q21.32); 46, XY, t(1;21)(p13;q11.2); 46, XY, t(13;20)(q22;q11.2); and 45, XY, der(13;14)(q10;q10), embryos with unbalanced chromosomal translocations successfully completed preimplantation development by reaching the expanded blastocyst stage. We have not observed blastocyst development for the following translocations: 46, XX, t(5;21)(q31.1;q22.1); 46, XX, t(11;22)(q23;q11.2); 46, XY, t(1;8)(p13;q23); and 46, XY, t(15;16)(q13;q13). In all these cases, the absence of blastocysts was associated with the low number of embryos or poor overall embryo development, including normal/balanced embryos. The results are combined in Table 2.

DISCUSSION

The efficiency of PGD of translocations is presented in Table 1. Chromosomal analysis was possible for 84% and 83% of the blastomeres with reciprocal and Robertsonian

TABLE 2

In vitro developmental potential of preimplantation human embryos after preimplantation genetic diagnosis testing for the presence of unbalanced chromosomal translocations.

Genotype	No. of embryos	Morulae (%)	Blastocysts (%)
Normal	27	15 (56)	10 (37)
Balanced	17	12 (71)	4 (24)
Unbalanced	87	51 (59)	23 (26)

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translocations, respectively. Biopsy of two blastomeres is considered acceptable for PGD and would allow the analysis of all embryos. Since all embryos were transferred on day 5 of development, our strategy was based on a second, day 4 biopsy of one blastomere for the good quality embryos with questionable or no results from the first biopsy.

The results of this study show that a chromosomally unbalanced genome has no adverse effect on the viability of the preimplantation human embryo. None of the translocations investigated had a noticeable effect on the embryo's ability to reach the blastocyst stage in vitro (Table 2). This is in direct contrast to animal studies (6) and does not correspond well with the results of aneuploidy testing at the preimplantation stage of human development.

Since the advent of FISH for the study of preimplantation human development, it was noticed that morphologically abnormal cleavage-stage embryos are usually genetically abnormal (16). They are either aneuploid or have a few aneuploid blastomeres (mosaics) or are inconsistent in chromosomal complement among their blastomeres (display chaotic cleavage). Therefore, a chromosomally abnormal embryo would have a greater chance of also being morphologically abnormal at the cleavage stage of development. The developmental potential of such embryos could not be determined directly, since all of them were fixed at the cleavage stage to allow genetic analysis of all blastomeres. However, the low level of mosaicism at the blastocyst stage suggests the presence of selection for genetically normal embryos at the morula-blastocyst transition (5, 17). First-trimester spontaneous abortions mark the next stage of selection against chromosomal anomalies (18). However, the birth of physiologically and mentally abnormal children with unbalanced chromosomal complements implies that the natural selection may be delayed until the postnatal stage of embryo life.

It should be noted that the embryos with chromosomal translocations analyzed in this study should be distinguished from aneuploid embryos derived from chromosomally normal patients. The chromosomal abnormalities investigated here derive from the normal gametogenesis of a carrier of a balanced chromosomal translocation. However, aneuploidy and mosaicism, detected at the preimplantation stage, arise de novo during gametogenesis or during embryonic cleavage and reflect some errors in chromosome segregation. The causes of malsegregation may vary for each particular patient and embryo (maternal age, culture conditions, etc.) but have one thing in common: They are external to the embryonic genome, which remains transcriptionally inactive from the first meiotic division until the 4- to 8-cell stage and thus is unable to affect either chromosome segregation or embryo morphology. This leaves ooplasm deficiencies or inconsistencies in nucleocytoplasmic interactions as the primary reasons for both mosaicism and embryo fragmentation.

We suggest that aneuploidy and mosaicism, characteristic for preimplantation of human embryos, are actually the aftereffects of flaws in ooplasm maturation or nucleocytoplasmic interactions. The persistence of these deficiencies throughout preimplantation development is revealed indirectly by the presence of correlations between aneuploidy, cleavage-stage embryo morphology, and blastocyst development. It is still too early to draw any conclusions as to the extent and exact timing of the selection against chromosomally abnormal embryos. For the embryos displaying chaotic cleavage, it is already known that, although they may reach the blastocyst stage (5), none advance to postimplantation development. Chaotic embryos represent the extreme in embryo genome aberrations and are still able to develop into blastocysts; therefore it would be presumptuous to expect a complete elimination of aneuploid or mosaic embryos at the preimplantation stage of development.

Normal preimplantation development of embryos with unbalanced chromosomal translocations indicates that a chromosomally unbalanced genome does not necessarily affect the embryo's ability to reach the blastocyst stage. Further PGD cases for the carriers of chromosomal translocations will reveal the boundaries of this conclusion. It is quite evident, however, that this may be true only as long as none of the chromosomes essential for preimplantation morphogenesis are involved.

Our results show that the method of selection for normal and balanced embryos by the selection of morphologically normal blastocysts (7, 19) is not applicable for the detection of the unbalanced translocations described here.

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