



Preimplantation genetic diagnosis of structural abnormalities

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Abstract

Preimplantation genetic diagnosis (PGD) of translocations can be achieved through a variety of methods. For female carriers, a possibility is by polar body biopsy and analysis of its metaphase chromosomes using painting probes. For male carriers or female carriers with terminal breakpoints, metaphase chromosomes can also be studied by fusing blastomeres to enucleated oocytes. Otherwise, interphase analysis of the translocation can be performed using distal, subtelomeric or breakpoint spanning probes. The results obtained after PGD of translocations indicate a significant decrease in spontaneous abortions after the procedure, a good selection against unbalanced oocytes and embryos, and pregnancy rates that depend on the type of translocation involved. 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 that failed over ten IVF cycles, and 9.2% among fertile couples experiencing three or more consecutive first-trimester abortions (*Hum. Reprod.* 14 (1999) 2097). They were also found in 2–3.2% of males requiring ICSI (*Hum. Reprod.* 11 (1996) 2609; *Hum. Reprod.* 13 (1998) 576). 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. The aims of PGD for translocations are to reduce the rate of spontaneous abortions that this population suffers and to minimize the risk of conceiving an unbalanced baby. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Approaches to preimplantation genetic diagnosis of translocations

Preimplantation genetic diagnosis (PGD) of translocations has been attempted using mostly fluorescent in situ hybridization (FISH). This is because on average, only 25% of blastomere nuclei show metaphase chromosomes after anti-mitotic treatment, and even fewer nuclei show banding-quality chromosomes (Santaló et al., 1995). Similarly, G-banding of polar body or oocyte chromosomes cannot be achieved consistently due to the poor metaphase chromosomes obtained. For this reason, several approaches have been developed either involving alternative ways to obtain metaphase stage chromosomes, analysis by FISH of degenerated polar body chromosomes, or by using FISH in interphase nuclei.

1.1. Metaphase analysis

Metaphase analysis of first polar bodies was proposed after the observation that more than 90% first polar bodies fixed six or less hours after retrieval are at metaphase stage (Munné et al., 1998a). The translocation can then be identified using chromosome-painting probes for the two chromosomes involved in the translocation (Munné et al., 1998a,b).

One problem with this technique is the occurrence of crossing-over and predivision of chromatids. In both cases the outcome of the second meiotic division is unclear, and the second polar body or blastomeres should be analyzed. A second problem is the occurrence of interstitial crossover with subsequent segregation of balanced and unbalanced sets of chromosomes during the second meiotic division. So far we have detected two of these events (Munné et al., 1998c). A third problem is the shortness of polar body chromosomes which implies that terminal translocations are difficult

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or impossible to see with painting probes. These probes must then be reinforced by adding telomere probes to the mixture (Munné et al., 1998b).

Although spectral imaging has been used to identify all 23 chromosomes in polar bodies (Márquez et al., 1998) this technique requires well-spread chromosomes in order to identify each one of them, something quite difficult to achieve on a regular basis with single, small, degenerated cells.

Unlike the first polar body, the second polar body nucleus is in interphase because it inherits the oocyte cytoplasm possessing chromosome-decondensing activity (Howlett and Bolton, 1985). Recently, Verlinsky and Evsikov (1999) have developed a method to produce banding-quality metaphases from second polar bodies by injecting the second polar body into enucleated M-II oocytes, followed by oocyte activation in order to produce a pronuclei of the second polar body nucleus. Then, the zygote is cultured for 1 h in okadaic acid and fixed, which is 100% effective in producing metaphase chromosomes.

Another method of obtaining metaphase stage chromosomes, but from blastomeres, has been recently published by our team (Willadsen et al., 1999). When human blastomeres were fused with enucleated cow oocytes, the nuclei changed to metaphase stage. The addition of colcemid prevented the progression of these metaphases into pronuclear stage. This approach has been used for two clinical cases of translocation. The fused blastomeres could be either banded, analyzed with painting probes, or by spectral karyotyping. A successful chromosomally normal pregnancy has resulted from it (Willadsen et al., 1999).

1.2. Interphase fluorescent in situ hybridization on blastomeres

FISH on interphase blastomeres can be applied for translocations of any parental origin or for other structural abnormalities, such as inversions. One approach has been to develop specific probes expanding the breakpoints of each translocation (Munné et al., 1998d; Weier et al., 1999) or inversion (Cassel et al., 1997). Another approach is to use probes distal to the breakpoints or telomeric probes, either for translocations (Munné et al., 1998d; Pierce et al., 1998; Munné et al., 2000) or inversions (Iwarsson et al., 1998). The exception is for Robertsonian translocations (RTs), when chromosome enumerator probes must be used to detect aneuploid embryos (Conn et al., 1998; Munné et al., 1998d; Escudero et al., in press). Only the first approach (spanning probes) can differentiate between balanced and normal embryos. Balanced embryos, when enough normal ones are available, should not be transferred in order prevent the perpetuation of the genetic disease in the family.

Breakpoint spanning probes used in interphase nuclei can detect normal, balanced or unbalanced karyotypes resulting from any translocation, inversion, deletion or duplication. They work as follows. When two breakpoint spanning probes, one for chromosome A labeled in red and one for B labeled in green are used, for instance in a translocation case, two independent green and two independent red signals are observed in normal cells. In balanced cells, the normal A appears as an independent red signal, the normal B as an independent green signal and the derivative A and B chromosomes appear as associations of a smaller red and a smaller green signal. Any other combinations represent unbalanced nuclei. This is because when the translocation occurred, each hybridization target was split in two physically separated domains of about equal intensity. Therefore, a derivative chromosome appeared as an association of a green and a red domain. To further distinguish the derivative chromosomes, we added a blue fluorescent satellite probe for the centromeric region of one of the chromosomes involved in the translocation. This approach was first presented for PGD of inversions (Cassel et al., 1997) and later applied to PGD of translocations (Fung et al., 1998; Munné et al., 1998d). Because probe development has to be performed for each breakpoint of each translocation and the method is time consuming and expensive; so breakpoint spanning probes are seldom used.

Several groups have used probes distal to the breakpoints (Munné et al., 1998d; Pierce et al., 1998; Van Assche et al., 1999) or subtelomeric probes (Munné et al., 2000). However, in order to identify any possible unbalanced event, two distal and one proximal probe, or two proximal and two distal probes should be used. The use of only one distal and one proximal probe (Pierce et al., 1998) cannot detect for instance 1:3 unbalanced embryos. A more robust protocol design is to use two probes proximal to the breakpoint and two distal to the breakpoint probes, thus differentiating between unbalanced events and 'nonsense' events produced by FISH errors, and in addition identifying numerical abnormalities for the chromosomes being analyzed (Munné et al., 1998d). This approach is the simplest of the ones described here, thanks to the recent commercialization of telomeric probes for most q and p arms (Munné et al., 2000). However, this approach cannot differentiate between normal and balanced embryos.

Finally, PGD for RTs can be performed with chromosome enumerator probes. RTs arise through the p-arm fusion of acrocentric chromosomes, and therefore, by using any probes labeling to the chromosomes involved aneuploid embryos could be differentiated from normal or balanced embryos. These are the easiest translocations to analyze by simply using enumerator probes (Conn et al., 1998; Munné et al., 1998d; Escud-

ero et al., in press) and many PGD cases have been already performed (Munné et al., 2000). There is a problem with this approach and is the occurrence of uniparental disomy (UDP) for chromosomes 14 and 15, which have phenotypic consequences, Angelman/Prader-Willi syndrome for UDP15 and precocious puberty for UDP14 (Tomkins et al., 1996). Because enumerator probes cannot differentiate the parental origin of chromosomes, UDP cannot be detected using this approach.

2. Objectives and outcome of preimplantation genetic diagnosis of translocations

2.1. Spontaneous abortions and unbalanced offspring reduction

For most translocation patients, the consecutive pregnancy loss is their major incentive for enrolling in a PGD program. The unbalanced products of the translocation are usually lethal, therefore reducing the risk to pregnancy loss. We have demonstrated that PGD of translocations substantially increases the couple's chances of sustaining a pregnancy to full term (Munné et al., 1998b, 2000). In the last review of 35 PGD translocation patients, we observed a significant decrease in spontaneous abortions ($P < 0.001$): from 95% of the pregnancies in natural cycles to 13% in PGD cycles (Munné et al., 2000). None of the 15 patients that became pregnant produced unbalanced offspring. We also believe that growing embryos to blastocyst stage cannot select against unbalanced embryos, because many unbalanced embryos implant and then are spontaneously aborted. The suggestion that growing embryos to blastocyst stage may select against unbalanced embryos (Menezo et al., 1997) is unreliable at best.

2.2. Achieve pregnancy: prognosis depends on several factors

Taking into account several factors that could affect pregnancy rates, such as biopsy type, gender of the carrier, presence of terminal breakpoints, Robertsonian or reciprocal translocation, and number of chromosomally normal embryos available for transfer, several interesting correlations appear.

We found a very good correlation between percentage of chromosomal abnormalities and pregnancy rate. For instance, cases with more than 50% abnormal eggs or embryos achieved significantly fewer pregnancies per cycle (24%) than cases with less than 50% abnormal eggs or embryos (57%; $P = 0.028$, F test; Munné et al., 2000). When other authors reported high rates of abnormal embryos, pregnancy also failed to occur (Conn et al., 1998; Van Assche et al., 1999).

Cases involving RTs achieved significantly higher pregnancy rates (50%) than cases involving reciprocal translocations (21%, $P < 0.03$; Munné et al., 2000). At least as far as polar body biopsy cases are concerned, the reason is probably that there were significantly more chromosomally abnormal oocytes (71%) in the reciprocal cases than in the RT cases (42%, $P < 0.001$; Escudero et al., 2000; Munné et al., 2000). Also, sperm chromosome analyses in patients with RTs have shown less than 26% abnormal gametes (Martin, 1988; Scriven et al., 1998; Escudero et al., in press); whereas in reciprocal translocation patients this proportion ranges between 18 and 72% (reviewed by Estop et al. (1996)).

Fewer pregnancies per cycle were obtained (17%) when there was a terminal breakpoint in the translocation, than when none were present (41%; $P = 0.026$, F test). The embryos and oocytes of translocations with at least one terminal breakpoint had the highest rate of chromosome abnormalities (84%).

High rates of mosaicism have been detected in some translocation cases (Conn et al., 1998; Munné et al., 1998d; Van Assche et al., 1999). However, while one report on RT carriers indicated a high rate of mosaicism in these patients (Conn et al., 1998), this was not confirmed by Escudero et al. (in press) in a similar study that compared sperm and embryo chromosome abnormalities in Robertsonian carriers.

3. Uncited references

Testart et al. (1996), Meschede et al. (1998), Stern et al. (1999)

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