ORIGINAL ARTICLE: GENETICS

Preimplantation genetic diagnosis for chromosomal rearrangements with the use of array comparative genomic hybridization at the blastocyst stage

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Objective: To establish the value of array comparative genomic hybridization (CGH) for preimplantation genetic diagnosis (PGD) in embryos of translocation carriers in combination with vitrification and frozen embryo transfer in nonstimulated cycles.
Design: Retrospective data analysis study.
Setting: Academic centers for reproductive medicine and genetics.
Patient(s): Thirty-four couples undergoing PGD for chromosomal rearrangements from October 2013 to December 2015.
Intervention(s): Trophectoderm biopsy at day 5 or day 6 of embryo development and subsequently whole genome amplification and array CGH were performed.
Main Outcome Measure(s): This approach revealed a high occurrence of aneuploidies and structural rearrangements unrelated to the parental rearrangement. Nevertheless, we observed a benefit in pregnancy rates of these couples.
Result(s): We detected chromosomal abnormalities in 133/207 embryos (64.2% of successfully amplified), and 74 showed a normal microarray profile (35.7%). In 48 of the 133 abnormal embryos (36.1%), an unbalanced rearrangement originating from the parental translocation was identified. Interestingly, 34.6% of the abnormal embryos (46/133) harbored chromosome rearrangements that were not directly linked to the parental translocation in question. We also detected a combination of unbalanced parental-derived rearrangements and aneuploidies in 27 of the 133 abnormal embryos (20.3%).
Conclusion(s): The use of trophectoderm biopsy at the blastocyst stage is less detrimental to the survival of the embryo and leads to a more reliable estimate of the genomic content of the embryo than cleavage-stage biopsy. In this small cohort PGD study, we describe the successful implementation of array CGH analysis of blastocysts in patients with a chromosomal rearrangement to identify euploid embryos for transfer. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Preimplantation genetic diagnosis (PGD), chromosomal rearrangements, array CGH, trophectoderm biopsy, blastocysts

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Carriers of a reciprocal or robertsonian translocation usually have a normal phenotype, but through the generation of unbalanced gametes through impaired chromosome segregation they have an increased risk of fertility problems, recurrent miscarriages, or offspring with an unbalanced chromosomal rearrangement. Approximately 0.5%–5% of couples with reproductive problems carry a balanced rearrangement (1–3). Preimplantation genetic diagnosis (PGD) following an in vitro

fertilization (IVF) procedure or a spontaneous pregnancy in combination with prenatal genetic testing can be offered to these couples at risk (4–7).

Preimplantation genetic diagnosis of embryos for chromosomal rearrangements is applied to select balanced or unaffected embryos and thus can yield higher pregnancy rates and prevent the transmission of unbalanced rearrangements to the offspring. To detect chromosomal rearrangements, several molecular cytogenetic techniques have been implemented (8–11).

For many years, fluorescence in situ hybridization (FISH)–based techniques have been applied on single cells, such as polar bodies or blastomeres that were retrieved by means of biopsy from oocytes/zygotes or cleavage-stage embryos, respectively. Chromosome-specific probes are hybridized to the interphase nuclei of the cells to detect chromosomal (segmental) imbalances. FISH has, despite the extensive use by many clinics around the world, drawbacks that impair the accuracy and consistency of the PGD test (12, 13). Moreover, it has been shown that embryos at these early stages are often mosaic for aneuploidies and therefore that results may not be representative of their genuine genetic status (14–21).

Through recent advances in whole genome amplification (WGA) methods, more reliable and efficient molecular techniques can be used for PGD, such as array comparative genomic hybridization (CGH), single-nucleotide polymorphism arrays, karyomapping, and massive parallel sequencing (22–34). In contrast to FISH, with the use of these techniques it is possible to perform a comprehensive chromosome screening (CCS) (33, 35). This implies that a general off-the-shelf PGD protocol can be used for most types of translocations, making specific FISH probe testing for individual couples redundant.

Another important evolution in PGD is the time point for embryo biopsy. Recent observations supporting the idea of selecting trophectoderm (TE) biopsy over blastomere biopsy are based on the use of extended culture and the fact that the embryos that become blastocysts have a greater chance of implanting by the time of transfer (36). In addition, TE biopsy is less detrimental to the embryo than blastomere biopsy and enables the investigation of more cells, which leads to a more accurate genetic diagnosis (37–39). Furthermore, progress in cryopreservation protocols has enabled the transfer of embryos in a nonstimulated natural menstrual cycle, leading to higher implantation rates (40, 41).

Being able to detect chromosomal abnormalities unrelated to the parental translocation under investigation is recognized as a benefit, especially when a technique such as array CGH is not technically demanding to apply (42). The use of array CGH for PGD in carriers of a chromosomal rearrangement on embryo biopsies has been validated technically and clinically in several studies (26, 33, 35, 42, 43). These studies show the added value of CCS in PGD and report high success rates. They also report the detection of numeric and structural abnormalities that have no direct link with the parental-derived structural chromosomal rearrangement in up to 36% of blastocysts. Whereas embryos at the cleavage stage show a high rate of mosaicism, arguing against aneuploidy detection, embryos at the blastocyst stage suffer less from this problem (43–45). A 2012 randomized controlled trial exploring the use of CCS with the use of array CGH on blastocysts concluded that, despite testing only good-prognosis IVF patients, a successful IVF outcome could be achieved if array CGH testing and elective single-embryo transfer are integrated in a clinical IVF program (46).

The present study confirms the value of array CGH on TE biopsies for PGD in carriers of a chromosomal rearrangement and highlights the increased pregnancy rates when normal embryos are transferred in nonstimulated cycles. We report the presence of new numeric and structural aberrations that were observed in embryos balanced or unaffected for the parental rearrangement. Two interesting PGD cases are discussed, clearly showing the benefit of the used strategy.

MATERIALS AND METHODS

Patients

Preimplantation genetic diagnosis of blastocysts from couples carrying a balanced chromosomal rearrangement was performed at the IVF clinic of Ghent University Hospital in collaboration with the University Hospital's Center for Medical Genetics from October 2013 to December 2015. Our Institutional Review Board approved this study (EC/UZG/2016/0354), and informed consents were obtained from all of the included patients.

A total of 224 blastocysts originating from 50 oocyte retrieval cycles (ORCs) from 34 couples were investigated with the use of array CGH. Maternal age ranged from 26 to 40 years with a mean of 32.5 years. In nine couples, one of the parents was a carrier of a robertsonian translocation, in 21 a carrier of a reciprocal translocation, in 2 of inversions, in 1 of an insertional translocation, and in 1 a double two-way reciprocal translocation. In total, 28 different rearrangements were included (see Supplemental Table 1 for more details [available online at www.fertstert.org]).

Ovarian Hyperstimulation Protocol

Controlled ovarian hyperstimulation of this study's patients was performed according to age, antimüllerian hormone levels, and previous response; the gonadotropins used were either a recombinant FSH (Gonal F; Merck Serono) or a urinary FSH (Menopur, Ferring Pharmaceuticals) at daily doses of 150–300 U. When an agonist protocol was used, 0.1 mg triptorelin (Decapeptyl) was administered subcutaneously for 7 days starting on cycle day 1, and gonadotropins were started on cycle day 3. In cases where an antagonist protocol was necessary, gonadotropins were started on cycle day 3, and 0.25 mg cetrorelix (Cetrotide) was injected subcutaneously as a daily dose from the 6th day of stimulation until the day of oocyte maturation triggering. Ultrasound monitoring was done to check the course of stimulation. As soon as 50% of the follicles >10 mm reached a diameter of ≥ 18 mm, oocyte maturation and retrieval was performed according to Vandekerckhove et al. (47).

Fertilization, Culture, Blastocyst Biopsy, and Vitrification

After intracytoplasmic sperm injection, oocytes were cultured in 25-μL drops of IVF cleavage medium (Cook) in the