Comprehensive preimplantation genetic screening and sperm deoxyribonucleic acid fragmentation from three males carrying balanced chromosome rearrangements

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Objective: To assess whether preimplantation genetic screening can successfully identify cytogenetically normal embryos in couples carrying balanced chromosome rearrangements in addition to increased sperm DNA fragmentation.
Design: Comprehensive preimplantation genetic screening was performed on three couples carrying chromosome rearrangements. Sperm DNA fragmentation was assessed for each patient.
Setting: Academic center.
Patient(s): One couple with the male partner carrying a chromosome 2 pericentric inversion and two couples with the male partners carrying a Robertsonian translocation (13:14 and 14:21, respectively).
Intervention(s): A single blastomere from each of the 18 cleavage-stage embryos obtained was analysed by metaphase comparative genomic hybridization. Single- and double-strand sperm DNA fragmentation was determined by the alkaline and neutral Comet assays.
Main Outcome Measure(s): Single- and double-strand sperm DNA fragmentation values and incidence of chromosome imbalances in the blastomeres were analyzed.
Result(s): The obtained values of single-strand sperm DNA fragmentation were between 47% and 59%, and the double-strand sperm DNA fragmentation values were between 43% and 54%. No euploid embryos were observed in the couple showing the highest single-strand sperm DNA fragmentation. However, euploid embryos were observed in the other two couples: embryo transfer was performed, and pregnancy was achieved by the couple showing the lowest sperm DNA fragmentation values.
Conclusion(s): Preimplantation genetic screening enables the detection of euploid embryos in couples affected by balanced chromosome rearrangements and increased sperm DNA fragmentation. Even though sperm DNA fragmentation may potentially have clinical consequences on fertility, comprehensive preimplantation genetic screening allows for the identification and transfer of euploid embryos. (Fertil Steril® 2015;104:681–7. ©2015 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, pericentric inversion, preimplantation genetic diagnosis/screening, Robertsonian translocation, sperm DNA fragmentation

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Male fertility has traditionally been assessed by seminogram studies and by fluorescent in situ hybridization (FISH) analysis on spermatozoa, among others. Although these parameters are informative concerning patient fertility status, these results are not always conclusive regarding the IVF outcome (1, 2).

Recently, sperm DNA fragmentation (SDF) has been suggested as a biomarker of sperm quality that may predict IVF success (3). Different techniques, such as the TUNEL assay, in situ nick translation, sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD) test, or Comet assay have been used to determine SDF (4). Moreover, the Comet assay was developed for the differential evaluation of single-strand SDF (ssSDF) and double-strand SDF (dsSDF) in human spermatozoa (5). Using the alkaline Comet assay, 52% of the DNA in the Comet tails and 45% of spermatozoa showing ssSDF have been considered the cut-off values for achieving pregnancy, as validated for clinical analysis (2, 6). Furthermore, the alkaline Comet assay has recently been shown to provide a higher sensitivity and specificity in predicting male infertility when compared with TUNEL, SCD, and SCSA (7). When analyzing SDF on fresh and cryopreserved sperm samples by the Comet assays, the alkaline Comet assay has shown a 10% increase in ssSDF from cryopreserved samples, but the neutral Comet assay has not shown significant differences (8).

Characteristic Comet assay profiles have been described in different clinical groups: low-equivalent Comet assay profiles (with low ssSDF and low dsSDF) were associated with the best prognosis for achieving pregnancy, whereas high-equivalent Comet assay profiles (with high ssSDF and high dsSDF) correlated with the worst prognosis (patients with oligoasthenoteratozoospermia, asthenozoospermia, and asthenozoospermia with varicocele) (9). Non-equivalent Comet assay profiles, with low ssSDF and high dsSDF, would not prevent achieving pregnancy but would rather be associated with a higher male-factor miscarriage risk (6). Balanced rearrangement carriers show extremely variable ssSDF and dsSDF values, with either high-equivalent or non-equivalent Comet assay profiles (9) and also variable SDF values when measured by the TUNEL assay, SCD test, or SCSA (10, 11).

Furthermore, chromosome rearrangement carriers are at risk of producing aneuploid embryos, owing to the generation of unbalanced gametes. Robertsonian and reciprocal translocation carriers also show an increased incidence of aneuploidy in chromosomes not involved in the rearrangement due to meiotic errors caused by the trivalent or tetravalent chromosome figures. This mechanism is named the interchromosomal effect (ICE) (12, 13). In addition to structural chromosome imbalances related to rearrangements, segmental imbalances produced by breaks in chromosome fragile sites (14, 15) have also been reported in preimplantation genetic diagnosis (PGD) cycles for balanced rearrangement carriers (16, 17).

In the present work, comprehensive preimplantation genetic screening (PGS) was performed on couples at risk of obtaining aneuploid embryos due to paternal balanced chromosome rearrangements and an increased SDF characterized by the alkaline and neutral Comet assays. Previous studies have been performed to analyze the association between male infertility and IVF outcome (1, 18–20); however, no comprehensive cytogenetic results have previously been provided. This is the first performed study aiming to elucidate the effect of SDF on the embryo cytogenetics and pregnancy outcome after comprehensive PGS.

MATERIALS AND METHODS

Patients

Three PGD cycles were performed on couples with the male partner carrying a balanced chromosome rearrangement, in collaboration with two IVF centers in Barcelona, Spain: Fundació Puigvert and Institut Universitari Dexeus. Females had normal karyotypes (46,XX), and the males' karyotypes and seminogram description are shown in Table 1. Semen analysis was performed according to the World Health Organization 2010 criteria (21).

Sperm DNA Fragmentation Analysis

Semen samples were obtained by masturbation after a minimum of 3 days of abstinence. Samples were cryopreserved in a 1:1 proportion with Test-yolk buffer (14% glycerol, 30% egg yolk, 1.98% glucose, and 1.72% sodium citrate), and sperm DNA fragmentation analysis was performed after thawing. Single-strand SDF and dsSDF were measured using the alkaline and neutral Comet assays, respectively, as previously described (9). Samples were stained with DAPI SlowFade Gold antifade (Invitrogen) and were evaluated using an Olympus AX70 fluorescence microscope, analyzing 400 spermatozoa per sample.

IVF and Embryo Biopsy

The patients underwent routine superovulation procedures. Embryos were fertilized on day 0 by intracytoplasmic sperm injection. On day 3 after fertilization, one blastomere from each embryo that had reached the six-to eight-cell stage was biopsied using Tyrode's acid or laser, depending on the IVF center.

Comprehensive PGS Procedure

Cell lysis and whole genome amplification (WGA) of each blastomere was performed by the SurePlex DNA Amplification System (BlueGnome), according to the manufacturer's instructions. The correct DNA amplification was assessed in a 1.5% agarose gel (smears between 200 pb and 1,500 pb). The metaphase comparative genomic hybridization (mCGH) approach was performed as previously described (22) using either XY or XXY reference DNA, amplified by the same WGA system.

Reanalysis of the Discarded Embryos

Eleven of the embryos that had been discarded owing to the presence of cytogenetic abnormalities were reanalyzed by mCGH. When possible, isolated blastomeres were processed separately; however, some embryos were processed as a whole because they were compacted or had reached the blastocyst stage.

Cytogenetic Analysis Criteria

The thresholds used to diagnose losses and gains were fixed at 0.8 and 1.2, respectively, as previously defined (23).