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Original Article

Preimplantation genetic diagnosis by fluorescence *in situ* hybridization of reciprocal and Robertsonian translocations

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ABSTRACT

Objective: The presence of reciprocal and Robertsonian chromosomal rearrangement is often related to recurrent miscarriage. Using preimplantation genetic diagnosis, the abortion rate can be decreased. Cases treated at our center were reviewed.

Materials and methods: A retrospective analysis for either Robertsonian or reciprocal translocations was performed on all completed cycles of preimplantation genetic diagnosis at our center since the first reported case in 2004 until the end of 2010. Day 3 embryo biopsies were carried out, and the biopsied cell was checked by fluorescent *in situ* hybridization using relevant informative probes. Embryos with a normal or balanced translocation karyotype were transferred on Day 4.

Results: Thirty-eight preimplantation genetic diagnosis cycles involving 17 couples were completed. A total of 450 (82.6%) of the total oocytes were MII oocytes, and 158 (60.0%) of the two-pronuclei embryos were biopsied. In 41.4% of the fluorescent *in situ* hybridization analyses, the results were either normal or balanced. Embryos were transferred back after 21 cycles. Three babies were born from Robertsonian translocation carriers and another two from reciprocal translocation carriers. The miscarriage rate was 0%. Among the reciprocal translocation group, the live delivery rate was 8.3% per ovum pick-up cycle and 18.2% per embryo transfer cycle. Among the Robertsonian translocation group, the live delivery rate was 14.3% per ovum pick-up cycle and 20.0% per embryo transfer cycle.

Conclusion: There is a trend whereby the outcome for Robertsonian translocation group carriers is better than that for reciprocal translocation group carriers. Aneuploidy screening may possibly be added in order to improve the outcome, especially for individuals with an advanced maternal age. The emergence of an array-based technology should help improve this type of analysis.

Introduction

Chromosome translocations refer to chromosome abnormalities that are caused by the rearrangement of pieces of DNA between nonhomologous chromosomes. Chromosome translocations are mainly of two types: reciprocal and Robertsonian translocations. Reciprocal translocations often involve an exchange of material between nonhomologous chromosomes. They are found in about one in 500 to one in 625 human newborns [1,2]. These translocations are usually harmless and can be detected via prenatal diagnosis. A Robertsonian translocation involves two acrocentric chromosomes that fuse near the centromere region with the loss of the short arms. The resulting karyotype shows only 45 chromosomes in which two chromosomes are fused together. Nevertheless, there is no direct effect on the individual's phenotype, because the genes lost from the short arms of acrocentric chromosomes are nonessential genes. Robertsonian translocations can involve all combinations of acrocentric chromosomes. The most common Robertsonian translocation in humans involves chromosomes 13 and 14, with an estimated incidence of 0.97/1000 newborns [3].

Recurrent miscarriage is often related to carrying either type of translocation. In couples with two or more consecutive miscarriages, a rate of 2.3–4.5% for either Robertsonian or reciprocal translocations has been noted [4,5]. Their subsequent pregnancy outcome remains poor, with a miscarriage rate ranging from 36.4%

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to 72.4% [4,5]. Therefore, they may require genetic counseling and genetic testing prior to their next attempt to conceive.

Preimplantation genetic diagnosis (PGD) is a type of genetic testing in which a small number of cells are taken from embryos *in vitro* for testing, and only embryos unaffected by the tested condition are transferred into the woman's uterus. The main goal is to detect specific genetic diseases prior to implantation, in order to avoid the possible consequence of selective pregnancy termination; thus, this approach lowers the abortion rate.

Since our first case reported in 2004 [6], 38 cycles with PGD have been completed, involving either a Robertsonian or a reciprocal translocation. We review the results of these cycles of PGD in order to provide a reference for future improvement.

Materials and methods

A retrospective analysis was performed of all completed cycles of PGD at our center since the first reported case in 2004, in which either Robertsonian or reciprocal translocations had been detected. Couples who requested PGD but did not undergo biopsy of the embryo were not included in the review. Data on parental age, type of translocation, reproductive history, and the sex of the carrier were recorded.

Couples with either a Robertsonian or a reciprocal translocation were identified when they came for PGD assistance. After full counseling and informed consent, they underwent *in vitro* fertilization (IVF) and embryo transfer (ET). Either the long protocol with a gonadotropin-releasing hormone agonist or the gonadotropin-releasing hormone antagonist protocol was used. Ovum pick-up (OPU) using transvaginal ultrasound was carried out 34–36 hours after the administration of human chorionic gonadotrophin (Profasi; Serono, Modungo, Italy), after two or more follicles had reached a mean diameter of 18 mm.

Oocyte collection, insemination, and embryo culture

Oocytes were collected in a 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid-buffered commercial flushing medium (Cook Medical, Brisbane, Queensland, Australia). After serial washing, each oocyte recovered was maintained at 37°C in a separate drop of fertilization medium (Cook Medical) equilibrated with 6% CO_2 in air. Intracytoplasmic sperm injection was carried out using motile spermatozoa prepared by the swim-up procedure. The zygotes were checked for the presence of pronuclei and polar bodies at 18–21 hours after insemination or microinjection. Zygotes with two pronuclei were cultured at 37°C in a separate drop of cleavage culture medium (Cook Medical) equilibrated with 6% CO_2 in air until Day 3.

Embryo biopsy and blastomere fixation

Embryos that had reached seven or more cells (Grades I or II) on Day 3 were selected for biopsy [7]. A 1.48 μm (infrared) diode laser (Fertilase; MTM, Medical Technologies, Montreux SA, Switzerland) connected to an inverted microscope (Diaphot 300; Nikon, Tokyo, Japan) was used for zona drilling. The drilling consisted of four 10-millisecond pulses. A 25–30 μm opening was made to allow the entry of the embryo biopsy pipette (Cook Medical). One blastomere with a clear nucleus was aspirated from each embryo. A second blastomere was aspirated only if the first blastomere fixation had failed.

The aspirated blastomere was transferred and washed in phosphate-buffered saline at room temperature. It was then placed in a 10 μL drop of fixative (0.1% Tween 20, 0.01 M HCl in distilled water) on top of a poly-1-lysine-coated slide. The fixative was spread by continuous and gentle blowing until the cytoplasm dissolved. The final position of the nucleus was marked using a diamond pen. Dehydration was performed using ethanol of different concentrations (70%, 90%, and 100%), and then the dry slide was ready for the fluorescent *in situ* hybridization (FISH) procedure.

Fluorescent in situ hybridization

The FISH method performed in our laboratory followed the procedures described by Chen et al [8] and Harper and Delhanty [9,10]. For the two translocation types, specific commercial probes from Vysis (Abbott Laboratories, Abbott Park, Illinois, USA) were chosen, which were able to identify the translocated and non-translocated portions of the chromosomes (see Table 1). For each PGD cycle, the probes were first tested on the interphase nuclei of male human leukocytes [9–11]. FISH signals were counted using the criteria described by Hopman et al [12].

Embryo transfer and follow-up

As soon as the genotype of biopsied blastomeres were identified, only the unaffected embryos or normal diploid embryos were transferred, usually on Day 4. Pregnancies were confirmed by urinary tests or serum human chorionic gonadotropin levels at 2 weeks after ET. Clinical pregnancies were defined as the presence of one or more fetal hearts at 6–7 weeks of gestation. The implantation rate was defined as the number of fetal hearts per 100 embryos transferred. The delivery rate was defined as the percentage of pregnancies with delivery per OPU and per ET procedure. Prenatal diagnosis (2^{nd}-trimester amniocentesis) was recommended to confirm the PGD results. Postnatal physical examinations were also performed.

Statistical analysis

The fertilization rates and also the FISH results related to the normal and balanced rates were compared between different subgroups. Chi-square tests were used, and $p < 0.05$ was considered significant.

Results

Among the couples requesting IVF and PGD, because of either a reciprocal or a Robertsonian translocation, a total of 19 couples completed OPU cycle. These consisted of 12 couples with reciprocal translocations (8 male and 4 female carriers) and seven couples with Robertsonian translocations (1 male and 6 female carriers). In total, 17 couples went through at least one completed PGD cycle. The remaining two couples (11%) did not have any embryo biopsy results due to the lack of good embryos. As a consequence, 38 PGD cycles from 17 couples were completed.

The karyotype and clinical features of the 19 couples are listed in Table 1. Three out of nine male carriers suffered from a male factor. One was a Robertsonian translocation and the other two were reciprocal translocations. The most frequent Robertsonian translocation was der(13;14) (q10;q10). Among the reciprocal translocations, chromosome 4 was most frequently involved and chromosomes 1, 6, 10, 11, 13, and 15 were also common.

Segregation modes of the zygotes in female or male carriers with Robertsonian translocation were as follows: normal 2:1 alternate, balanced 2:1 alternate, and the other six unbalanced types, including 2 modes of 2:1 adjacent 1, 2 modes of 2:1 adjacent 2, and 2 modes of 3:0 nondisjunction. Therefore, only two of these modes of embryos complements could be considered transferable. The respective fluorescent signal patterns were then recorded and