Preimplantation Diagnosis by FISH: The Rambam Experience

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Purpose: Our purpose was to summarize our experience gained using fluorescence in situ hybridization for preimplantation diagnosis at the Rambam Medical Center.

Methods: Seventy-three embryos (29 cycles) were analyzed for preimplantation diagnosis for the following indications: advanced maternal age (>39 years), X-linked diseases, poor-quality embryos, repeated failure in vitro fertilization cycles and fast-dividing embryos. An additional 38 embryos with unequal pronuclei size were examined for ploidy. Biopsy of embryonic blastomeres was performed at the six- to eight-cell stage. Five fluorescence probes, for chromosomes X, Y, 13, 18, and 21, were applied for ploidy detection.

Results: Eighty-four (87%) blastomeres of the 73 embryos analyzed showed clear signals. Six of the blastomeres were lost during spreading. Two of the embryos were destroyed following biopsy. No nucleus was found in five of the blastomeres, while in nine, more than one nucleus was verified. Transfer was performed in 10 patients (32 embryos). Two pregnancies were achieved. Two healthy babies were born. Fifty-seven percent of the fast-dividing embryos demonstrated normal signals. In two groups of embryos of unequal pronuclei size, following conventional insemination and intracytoplasmic sperm injection, 50 and 11.4% demonstrated normal signals.

Conclusions: The efficiency of fluorescence in situ hybridization for aneuploidy detection is 87 and 97% for autosomes and gonosomes, respectively. The preimplantation genetic diagnosis is suitable for selected in vitro fertilization cases including fast-dividing embryos and embryos with unequal pronuclei size following regular in vitro fertilization.

KEY WORDS: fluorescence in situ hybridization; preimplantation genetic diagnosis.

INTRODUCTION

Preimplantation genetic diagnosis (PGD) is a recently introduced technique for patients undergoing in vitro

fertilization (IVF). Following genetic analysis of one or a few cells obtained by biopsy from a six- to eight-cell embryo, only "normal" embryos are transferred back to the woman's uterus (1–3). Two genetic analysis procedures are in use today: the polymerase chain reaction (PCR) (4) and fluorescence in situ hybridization (FISH) (5). The former is used in the diagnosis of single-gene disorders with defined mutations, whereas the latter is applied mostly for sex selection (when the mother is the carrier of an X-linked disease) and for aneuploidy detection. The possibility of detecting five chromosomes on one interphase nucleus (6) made this procedure applicable for the screening of most potentially viable trisomies (chromosomes 13, 18, 21, X, and Y). The aim of this article is to summarize our experience using the FISH procedure.

MATERIALS AND METHODS

Seventy-three embryos obtained from 29 IVF cycles in 24 patients were included in this study. Indications for preimplantation genetic diagnosis (PGD) included the following: maternal age (>39 years), 4 patients; X-linked hemophilia, 1 patient; paternal XO mosaicism, 1 patient; fast-dividing embryos (0 PN 18–20 hr postinsemination), 12 patients; multiple IVF cycles failure, 2 patients; multiple fragments in embryos on day 2, 1 patient; 1 PN embryos obtained following testicular biopsy and intracytoplasmic sperm injection (ICSI) procedure, 1 patient; and a combination of the above, 2 patients. In addition, 38 whole embryos with unequal pronuclear size were analyzed: 12 after conventional IVF insemination and 26 after ICSI.

Biopsy of the embryos and FISH analysis of the blastomeres were performed when the embryos arrived at the six- to eight-cell stage as described previously (7). Briefly, the zona pellucida of the embryos was dissolved using acid Tyrode's (pH 2.4; Sigma Chemical Co., St. Louis, MO) using a narrow pipette, 4 μ m in diameter. With a larger pipette, 30–40 μ m in diameter, one or two blastomeres were biopsied. Blastomeres were held in

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a hypotonic solution of 0.5% sodium citrate in water (Sigma) for 1–2 min, transferred onto a glass slide with a minimal volume of hypotonic solution, and finally, fixed by dripping a mixture of methanol:acetic acid (3:1) until complete dissolution of the cytoplasm. In the group of embryos analyzed for unequal size of pronuclei, the whole embryo was spread on a glass slide without separating the blastomeres. FISH was performed utilizing direct chromosome enumerator probes (CEP; Vysis, Stuttgart, Fasanenhof, Germany) for chromosomes X, Y, and 18 and indirect alpha satellite probes (Oncor, Gaithersburg, MD) for chromosomes 13 and 21. Whenever an abnormality was verified for chromosomes X, Y, and 18, chromosomes 13 and 21 were not analyzed. Slides were hybridized for 150 min at 37°C, followed by a series of washings with formamide and SSC solutions (Oncor). The long protocol was applied for the unequal pronuclei, with hybridization of slides overnight at 37°C. FISH signals were analyzed under fluorescence microscopy (Olympus BX 70, Japan).

RESULTS

One hundred three blastomeres from 73 embryos were analyzed. Six blastomeres (6.7%) were lost during the spreading procedure. In five blastomeres (5.8%) no nucleus was found, while in nine (8.7%) more than one nucleus was verified. In two cases (2.7%) the embryos were destroyed following the biopsy. Eighty-four blastomeres (87.5%) showed clear signals. Thirty-two embryos were transferred to 10 patients. Two normal pregnancies were achieved, and two healthy babies were recently born. Indications for PGD in these cases were advanced maternal age (40 years) and fast-dividing embryos.

Fifty-seven percent of the undocumented fast-dividing embryos showed normal signals. Of the embryos analyzed for unequal size of pronuclei, 50% of the embryos derived from conventional IVF/embryo transfer were normal, while in the ICSI group, only 11.4% embryos demonstrated normal signals for the five chromosomes checked. In the ICSI group, 10 embryos showed one set of chromosomes, suggesting activation of oocytes and not true fertilization.

DISCUSSION

The use of FISH-PGD for aneuploidy diagnosis

is promising, although the efficiency of detection of autosomal aberrations is only 87%, compared to 97% for sex determination. It is obvious that structural chromosome anomalies or aneuploidy affecting other chromosomes will not be detected by the probes used in this study. However, in the latter case most of these abnormal conceptions will be miscarried by nature. With the use of probes for additional chromosomes and newer techniques applied in molecular genetics (e.g., chromosome painting), this method may offer an alternative to the current practice in which fetuses at increased risk for chromosome anomalies, when diagnosed to be affected by chorionic villous sampling or amniocentesis, are aborted. It is obviously a valid solution for patients who are carriers of a genetic trait that is diagnosable by molecular probes and for whom the option of termination of pregnancy is not acceptable for of religious or ethical reasons. Undocumented embryos (0 PN) and embryos following conventional IVF insemination with unequal-sized pronuclei can also benefit from FISH analysis prior to transfer, as 57 and 50% of them, respectively, are normal and should not be discarded. Zygotes with unequal pronuclear size after ICSI carry, in the vast majority of cases (almost 90%), an abnormal chromosomal complement and should probably not be used for embryo transfer.

REFERENCES

1. Handyside AH, Kontogianni EH, Hardy K, Winston RML: Pregnancies from biopsied human preimplantation embryos sexed by Y specific DNA amplification. *Nature* 1990;244:768–770
2. Tarin JJ, Handyside AH: Embryo biopsy strategies for preimplantation diagnosis. *Fertil Steril* 1993;59:943–956
3. Munné S, Lee A, Rosenwaks Z, *et al.* Diagnosis of major chromosome aneuploidies in human preimplantation embryos. *Hum Reprod* 1993;8:2185–2191
4. Antonarakis SF: Chromosome mapping using DNA probes: A case study (chromosome 11) and future possibilities. In VA McKusick, TH Roderick, J Mori, NW Paul (eds). *Medical and Experimental Mammalian Genetics: A Perspective*. New York, Alan R. Liss, 1987
5. Griffin DK, Handyside AH, Harper JC, *et al.*: Clinical experience with preimplantation diagnosis of sex by dual fluorescent in situ hybridization. *J Assist Reprod Genet* 1994;11:132–143
6. Munné S, Alikani M, Tomkin G, *et al.*: Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995;64:382–391
7. Manor D, Kol S, Lewit N, Lightman A, Stein D, Pillar M, Itskovitz-Eldor J: Undocumented embryos: Do not trash them, FISH them. *Hum Reprod* 1996;11:2502–2506