Accuracy of FISH analysis in predicting chromosomal status in patients undergoing preimplantation genetic diagnosis

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Objective: The purpose of this study was to determine the positive predictive value (PPV) and negative predictive value (NPV) of FISH analysis and to determine which chromosomal abnormalities are most frequently confirmed.
Design: Prospective observational.
Setting: IVF laboratory.
Patient(s): Two hundred forty-one embryos were analyzed from 98 patients.
Intervention(s): FISH reanalysis.
Main Outcome Measure(s): Embryos that would have been discarded in patients undergoing preimplantation genetic diagnosis (PGD) were fixed and FISH reanalysis was performed. Results of reanalysis were compared with the day 3 diagnosis while PPV and NPV were calculated.
Result(s): Among the 241 embryos, 198 embryos were abnormal and 43 were normal by day 3 FISH analysis. The PPV was 83% and the NPV was 81%. PPV was also determined for specific categories of aneuploidy, and certain abnormalities such as monosomies, trisomies, tetrasomies, and polyploidies were frequently confirmed on reanalysis (PPV >80%), whereas Turner syndrome diagnosis was not (PPV = 17%).
Conclusion(s): FISH analysis offers a PPV of 83% and NPV of 81% when evaluating a single blastomere in conjunction with PGD. FISH errors and mosaicism are primarily responsible for the errors associated with FISH analysis in PGD. (Fertil Steril® 2008;90:1049–54. ©2008 by American Society for Reproductive Medicine.)

Key Words: PGD, FISH, aneuploidy, confirmation, PPV, NPV

Preimplantation genetic diagnosis (PGD) is a procedure that allows testing of embryos for specific genetic disorders before they enter the uterus and before pregnancy has begun (1). The first clinical application of PGD was described by Handyside et al. (2) in 1989. PGD was initially used for Mendelian disorders and has been more widely used since 1996, when locus-specific FISH probes became available (3).

Preimplantation genetic diagnosis is performed in patients who undergo IVF procedures and is classified by the ESHRE PGD Consortium into the following categories: [1] high-risk PGD, which is done for patients with chromosomal abnormalities and/or single gene defects; [2] low-risk PGD, which has the goal of increasing pregnancy rates in IVF patients, for example, patients with advanced maternal age, repeated miscarriages, or repeated IVF failures (4).

Embryo biopsy can be done at three different times during embryo development. The first one is polar body biopsy, in which the first and/or second polar bodies are removed and analyzed (5, 6). The second and most commonly performed time of biopsy is at the cleavage stage, often 3 days after the eggs are harvested. The last stage is the blastocyst stage, and biopsy at this stage is least frequently performed at this time (4).

When blastomere biopsy is performed, usually one blastomere is removed, and if removal of two cells is needed, the embryo should be at least at the six-cell stage (7). It is also best to select a blastomere that has one nucleus (8).

Misdiagnosis rates for PGD by single-cell blastomere vary with reporting centers. It is therefore recommended that confirmatory diagnosis is performed on embryos diagnosed as abnormal as part of quality control for each lab (4). Mosaicism can contribute to misdiagnosis by single blastomere biopsy and FISH analysis, and has been reported to be prevalent in up to 50% of embryos that are four- to eight-cell stage (9–12). In a previous smaller study by Munne et al. (13), the false negative rate and false positive rates have been reported to be 1.5% and 19%, respectively. Several other studies have reported similar error rates.

With this background in mind, the aims of this study were to [1] determine the positive predicted value (PPV) and negative predictive value (NPV) of FISH analysis of day 3 single-blastomere biopsy in low-risk PGD patients, as well as to [2] determine which chromosomal abnormalities are most frequently confirmed.

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MATERIALS AND METHODS

In between June 2004 and December 2004 at the Assisted Reproductive Technology Reproductive Center in Beverly Hills, CA, patients who were scheduled for an IVF-PGD procedure consented to the study. The study was approved by the Western institutional review board, and consent was obtained before the day of the oocyte retrieval. The patients who underwent retrieval had received gonadotropins in conjunction with a GnRH agonist or antagonist protocol, and ovulation was triggered with hCG approximately 36 hours before oocyte retrieval. Those patients desiring PGD had their embryos analyzed 3 days after oocyte retrieval when one blastomere was removed and FISH analysis was performed (Fig. 1A).

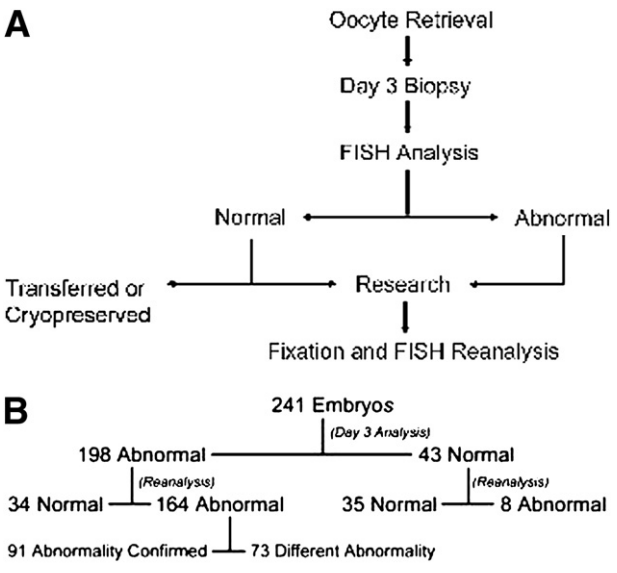
The embryos read as normal were either transferred or cryopreserved. The embryos that were diagnosed as abnormal on day 3 and would have therefore been discarded were included in the study. Those normal embryos that were not deemed suitable for cryopreservation were also included in the study. Those embryos that were diagnosed as inconclusive or those that had no signal after confirmation with FISH was performed were excluded from the study.

The embryos that were included in the study were then fixed entirely to slides on days 4–7 using Tween-20 solution and Carnoy's fixative (3:1 methanol to acetic acid solution), a modification of previously described techniques (14, 15), after which the slides were then treated in pepsin for cytoplasm removal. Following fixation, the slides were then placed in methanol, were allowed to dry, and a map of the blastomere nuclei was then established for future FISH analysis.

A five-chromosome probe (Vysis) for chromosome 13 (red), 18 (aqua), 21 (green), X (blue), and Y (yellow) was then applied, after which DNA codenaturation was performed at 73°C for 5 minutes, followed by hybridization at for 4 hours at 37°C. In a few cases, when three chromosomes were checked on day 3, a three-chromosome probe was applied on confirmation day as well (chromosomes 18, X, and Y). The slides were then washed in 0.4 × Sodium chloride/Sodium citrate with 0.3% NP-40 at 73°C for approximately 2 minutes and then in 2 × Sodium chloride/Sodium citrate with 0.1% NP-40 at room temperature for 1 minute. Antifade solution was then applied for counterstaining, and FISH analysis was done using a fluorescence microscope (Leica, St. Gallen, Switzerland) with filter sets for observation of spectrum red/aqua/green/blue and yellow (Applied Imaging Corp., San Jose, CA). All nuclei were analyzed, counted, and the results were then entered in a computerized data base. Statistical analysis involved the calculation of PPVs and NPVs, along with their corresponding standard errors for the abnormal and normal embryos as well as for specific chromosomal abnormalities. Computed specificity and sensitivity were projected based on the observed PPVs and NPVs in this study and prevalence for patients screened by the same lab.

FIGURE 1

(**A**) Diagram of methods. (**B**) Embryos evaluated for the study and the number of embryos in each category.



DeUgarte. Accuracy of FISH analysis in PGD patients. Fertil Steril 2008.

RESULTS

Overall, a total of 4,125 nuclei from 241 embryos were analyzed from 98 patients (average age 38.5) who underwent IVF-PGD, after 10 embryos were excluded secondary to lack of signal on FISH analysis. Of these, 198 embryos were abnormal and 43 were normal by day 3 FISH analysis (Fig. 1B). Confirmation with FISH was performed and the PPV, NPV, and the standard error were calculated (Table 1).

Embryos were confirmed as abnormal when >50% of the nuclei were deemed abnormal on confirmation and normal if >50% of the nuclei were determined normal on confirmation with FISH. This was derived from previous studies using this cutoff value such as that by Ziebe et al. (16). Among the embryos diagnosed as abnormal on day 3, 164 of 198 were confirmed as abnormal on confirmation (83% = PPV). Thirty-four embryos diagnosed as abnormal on day 3 were, in fact, normal, and the reasons for misdiagnosis were as follows: 10 were mosaic, 6 had signal overlap, 4 had split signals, 3 were tetraploidies, 2 were conservative diagnosis, 2 from Y failing hybridization, and the others were in the following categories: chromosomal 'self-correction,' fragmented DNA, and inaccurate diagnosis.

Among the embryos diagnosed as normal on day 3, 35 of 43 were confirmed as normal on confirmation, giving us an NPV of 81%. The projected sensitivity and specificity were calculated to be 94% and 60%, respectively, and were derived based on a presumed 39% prevalence of abnormal embryos in the same lab. All eight embryos that were abnormal in this category were misdiagnosed secondary to mosaicism.