

Preimplantation genetic diagnosis for advanced maternal age and other indications

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Maternal aging is not only characterized by a discernible decrease in ovarian reserve, but also by aberrations in the oocyte that can lead to trisomy and reduced implantation.

Chromosomal abnormalities occur more frequently in preimplantation embryos (Table 1) than in concepti that have implanted. A sizable fraction of chromosomally abnormal embryos are obviously eliminated before prenatal diagnosis. Such loss may partly account for the decline in implantation in older women.

It has been hypothesized that selection of chromosomally normal embryos could elevate implantation in women of advanced reproductive age (1). Current technology can only assess chromosomal errors after fixation of cells, hence elimination of aneuploid embryos can only be done after polar body or blastomere analysis through preimplantation genetic diagnosis (PGD). Fluorescence in situ hybridization (FISH) allows chromosome enumeration on interphase cell nuclei, i.e., without the need for culturing cells or preparing metaphase spreads. FISH has been used in PGD of common aneuploidies (at least XY 13, 18, and 21), and more recently it has been expanded to include other chromosomes (16, 15, and 22) involved in spontaneous abortions. Although the technology has been criticized for its inability to diagnose all chromosome errors, efficiency and accuracy rates have been surprisingly high (1–4).

Goals and Results

One of the main objectives of PGD for aneuploidy is to increase the chance of implantation. This was only achieved when at least eight chromosomes were analyzed, a feat that took some years to accomplish (4). Another important objective of PGD was to reduce the incidence of spontaneous abortion. This was achieved with considerably less effort as many affected embryos were selected appropriately even when few chromosome probes were available during the early years of PGD (3). It is natural that the emphasis has been on achieving pregnancy, but many pregnancies in women 40 years of age and older spontaneously abort. In our clinic, we found a significant decrease in spontaneous abortions from 23% to 11% after PGD (3).

Avoidance of chromosomally abnormal births (trisomy) is of special concern for women of advanced maternal age. In the population of case patients that underwent PGD at Saint Barnabas, we would currently expect 3.2% chromosomally abnormal conceptions according to their specific maternal age, but we found only 0.8% (2/262) chromosomally abnormal conceptions. Another beneficial effect of PGD has been a reduction in multiple offspring. Chromosome testing not only yields smaller embryo cohorts in many patients, but there has also been the tendency to transfer fewer embryos even when larger cohorts are available.

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TABLE 1

Chromosomally abnormal embryos for eight chromosome pairs.

Age, years	25–34	35–37	38–39	40–41	42–44
Embryos analyzed	154	87	96	180	74
% Normal	61	60	47	43	39
% Aneuploid	8	10	18	26	30
% Other abnormal	31	30	35	31	31

Note: The data are from Saint Barnabas Medical Center.

Munné. PGD of aneuploidy. Fertil Steril 2002.

Disadvantages Associated With PGD of Aneuploidy

Embryo Biopsy

All of the cells of an embryo remain totipotent until about the fourth day of development. Removal of one cell may delay development for a short time; however, cell loss seems to be proportionate up until the blastocyst stage and apparently normal development continues. So far, there is no clear evidence that biopsied embryos implant less frequently than untouched ones, although more work remains to be done. It is possible, at least theoretically, that embryo biopsy may lower implantation by a few points, while the PGD selection procedure increases it. The overall balance between biopsy damage, if any, and PGD selection seems to be positive but depends highly on maternal age, particularly since the beneficial effect of PGD increases with maternal age (Table 2). Only 0.9% embryos ($n=4,248$) have been damaged during the embryo biopsy process at Saint Barnabas Medical Center. The current use of Ca/Mg-free media in selected embryos and the general improvement of the technology are expected to reduce this rate even more.

Misdiagnosis

Amniocentesis and chorionic villi sampling both use hundreds of cells per sample and leave little margin for error

TABLE 2

Maternal age and implantation after PGD in 163 controls and 163 PGD cases.

Age, years	Implantation in controls, %	Implantation after PGD, %	Implantation improvement, %
Total >35	15.2 (591)	20.9 (358)	+5.7
35.0–37	26.4 (87)	30.8 (52)	+4.4
37.1–39	19.0 (158)	21.8 (110)	+2.8
39.1–42	12.9 (271)	19.7 (152)	+6.8
42.1–45	2.7 (75)	11.4 (44)	+8.7
39.1–45 ^a	10.7 (346)	17.8 (196)	+7.1

Note: The data are from Saint Barnabas Medical Center. The numbers in parentheses are the number of embryos transferred.

^a $P<.05$.

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(less than 1%). PGD analysis normally depends on one single cell from each embryo. By reanalyzing embryos that were not replaced, we have found our error rate to be 7%. That includes a 2% false normal diagnosis rate, which could give rise to trisomic conceptions, and a 5% false abnormal diagnosis rate, which would result in normal embryos not being transferred. Given these figures, we strongly recommend that pregnant patients undergo prenatal diagnosis.

No Embryo Transfer

In our study population, 16% of patients ($n=534$) were found to have abnormalities in all embryos and therefore none were replaced. When we reanalyzed the embryos in these cases, four embryos from four patients were in fact normal. Two of these initially did not have a result and two were assessed as abnormal.

No Cryopreservation

We have found that after PGD, there are very few chromosomally normal embryos left after transfer. Of those leftover embryos, few survive freezing and thawing.

Suitable Patients for PGD

With the advent of PGD, trisomic conception can be reduced if not eliminated. The expectation of an improvement in implantation is only reasonable when one considers maternal age and number of embryos. As seen in Table 2, the improvement in conception after PGD with eight or nine probes increases in women over 37. Similarly, cohort size by itself alters the selection of normal embryos. For instance, when the number of embryos biopsied is equal to or lower than the number of embryos expected to be replaced without PGD (3–5), there can be no improvement of pregnancy rates, since any embryo available that could implant would be replaced. There are obvious advantages to an optimal follicular response.

Other Indications Besides Age

Recurrent Pregnancy Loss

Several reports have indicated an increase in chromosome abnormalities in the embryos of patients with recurrent pregnancy loss. We have demonstrated a significant reduction in spontaneous abortions if PGD is applied to carriers of translocations. The risk of miscarriage is reduced after PGD in patients with normal karyotype and recurrent embryo loss.

Repeated IVF Failure

PGD has also been used in patients with repeated IVF failure. The results so far show little direct benefit. The high rate of chromosome abnormalities in these embryos may explain the repeated IVF failure in some of these cases. However, the predictive value of future cycles based on a single PGD cycle is not always high (Table 3).

TABLE 3

Difference in chromosome abnormalities between PGD cycles in the same patient.

% Difference	Patients
<20	23
20–40	5
>40	7

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Conclusions

PGD in advanced maternal age, using present technology, can benefit women 37 years of age or older with at least six embryos of average or better quality. In these circumstances, one can expect an increase in implantation and a reduction in spontaneous abortion and trisomic offspring. With improved technology, the spectrum of women benefiting from PGD may increase. Improvements may include better cell sam-

pling with negligible effect on embryo development and molecular advances that could enumerate all 24 chromosome types in a redundant fashion to reduce misdiagnosis. Comparative genome amplification alone or in combination with microarray technology currently offers the most promise for achieving this goal in the future, but the time for analysis should be reduced considerably to make it compatible with current IVF practice.

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