

Substandard application of preimplantation genetic screening may interfere with its clinical success

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The intent of this study was to evaluate a recent randomized clinical trial evaluating the effect of preimplantation genetic screening (PGS) that reports a negative effect on pregnancy outcome. This article reviews appropriate PGS techniques and how they differ from the trial in question. A closer look at the clinical trial in question reveals significant lack of expertise in biopsy, cell fixation, genetic analysis, and patient selection. At most, this trial demonstrates that in inexperienced hands, PGS can be detrimental. No other conclusions concerning the effect of PGS on pregnancy results can be drawn from the trial. (*Fertil Steril*® 2007;88:781–4. ©2007 by American Society for Reproductive Medicine.)

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Pregnancy rates after IVF steadily decline with maternal age, and pregnancy loss increases. It is widely accepted that reduced fertility with age is mostly egg related and is not caused by reduced uterine receptivity (1). Chromosome abnormalities arising from spindle disorders in aging eggs are one of the major reasons for the reduced egg quality. Even in young patients after follicular stimulation, close to 50% of preimplantation embryos are abnormal; the figure increases to nearly 80% for patients ≥ 40 years of age (2, 3). It should be possible to mitigate this high incidence of chromosome abnormalities by aneuploidy testing through preimplantation genetic diagnosis, often described as preimplantation genetic screening (PGS) and hereafter referred to as PGS. This consists of biopsying usually one cell from each IVF-generated embryo and testing this biopsied cell with molecular techniques or fluorescence in situ hybridization before embryo transfer to the patient.

Given this unassailable rationale, PGS was expected to improve IVF outcome by selecting for transfer only embryos that are chromosomally normal (4). To date, >20,000 PGS procedures have been performed worldwide for that purpose (personal communication) (5). However, controversy still exists regarding the procedure's efficacy. The likely reason for

this is that realizing a benefit from PGS requires optimal application. Low error rate and minimal embryo damage are absolutely critical to the success of the procedure.

Without a low error rate and without expert biopsy, benefits are not necessarily realized, and the effort could even be counterproductive. Thus, PGS is no different than any surgical procedure.

Initial and subsequent comparative studies from certain large centers showed that PGS increased implantation rates, decreased pregnancy loss, and reduced trisomic conceptions (6–11). However, whereas safety has been demonstrated in experienced hands, none of these studies were randomized, nor did they have sufficient case numbers to detect a significant increase in live-birth rates. Attempts to entice patients into randomized clinical trials (RCTs) in the United States (and many other countries) proved difficult because of the high cost associated and the self-pay nature of IVF. The lack of federal funds for human embryo research has hindered all sound applications of preimplantation genetic diagnosis and PGS in the United States.

Several randomized, controlled trials (RCTs) recently have been performed in countries in which IVF costs are covered by national health services. One well-cited study from a respected center demonstrated no difference in outcome between control and PGS-treated cases (12). In that study, two cells were biopsied per embryo, a procedure that is avoided by most other experienced centers because of its possible negative impact on the embryos. Nevertheless, even with two-cell biopsies, the mean implantation rate of the PGS embryos was 17.1%, compared with 11.5% of the non-PGS group. These results demonstrated again that in experienced hands, biopsy for preimplantation genetic diagnosis is safe for embryos' viability. Another well-designed,

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randomized study recently was published by Mastenbroek et al. (13). Their sample size was large enough to determine ongoing pregnancy rate changes, and only one cell per embryo was removed. The results appear to indicate that PGS for advanced maternal age in those investigators' hands was not helpful and actually was detrimental to pregnancy outcome. Thus, results of two RCTs, especially Mastenbroek et al. (13), are at odds with those of comparative studies. This contribution explores potential explanations for these discrepancies.

BIOPSY TECHNIQUES

Removal of a blastomere is not logically salutary to embryo development. Even if performed impeccably, there are fewer cells at a stage when numbers of cells must increase. In addition, the means used to breach the zona pellucida could be deleterious to the embryo, and the environmental changes occurring while the embryo is being micromanipulated outside the incubator also can pose a risk. Thus, an inevitable effect of the biopsy must be compensated for by improvement as a result of better selection, that is, by the transfer of a euploid embryo after PGS.

Studies assessing the effects of biopsy and its repercussion on the implantation potential of the embryo have not been attempted without including PGS selection. The true effect of biopsy therefore is still unknown. However, in experienced centers, polar body, blastomere, or a combination of polar body and biopsy does not affect pregnancy rates negatively (14–16). In addition, indirect evidence concerning the effect of biopsy on embryo viability was obtained in studies assessing the loss of cells from 5- to 10-cell embryos after freezing and thawing. With loss of two cells, the implantation potential of the embryo decreased by >50%; the loss of one cell decreased its potential by 10% (17). In a similar study, the loss of implantation potential proved proportional to the number of cells lost (18); a single cell loss decreased implantation rate by approximately 10%, and that of two, by 20%. The impact of embryo biopsy after PGS can be modeled on the cryopreservation data, because the condition is similar to the case of removal of cells for PGS. The loss of development potential helps explain the well-known decrease in success rate after transfer of previously cryopreserved embryos. What is unknown is the additional effect of the zona-breaching methods on the implantation potential of the embryo, and the extent to which differences in technique and experience play roles. These effects may limit the potential for development further but also may improve development.

It follows that in PGS, pregnancy rates after removal of a single blastomere must be improved by some measure of implantation potential to overcome the initial loss of the cell and to return to baseline (unbiopsied embryos). There must then be additional benefit if the procedure is to be justified. Thus, biopsy must be done with consummate skill. Lacking this, PGS should not be performed. If more than one cell is removed, it becomes very difficult to show a benefit of

PGS; an increase over baseline of $\geq 30\%$ would be needed. If a center's optimal rate is lower, a positive result for PGS becomes nearly impossible. Thus, Staessen et al. (12) may actually have shown a scientifically beneficial effect of PGS, because the estimated damage caused by two-cell biopsy (>50%) was similar to the potential of selecting against abnormal embryos by using PGS (>50%). That is, the pregnancy rate in the biopsied group would have been much lower if it were not compensated for by the benefit of embryo selection.

Even if only one blastomere is removed, difficulties with embryo biopsy represent an explanation for why Mastenbroek et al. (13) failed to show a benefit of PGS in their hands.

Experienced programs with positive outcomes after PGS for any indication have several characteristics in common. Biopsies are done rapidly (<2 min per embryo) by experienced embryologists, the method for breaching the zona (acid, laser, mechanical) does not significantly change the pH or the temperature in the vicinity of the opening, and very few embryos or cells are damaged during biopsy. Other important factors include the use of Ca- and Mg-free media containing amino acids to prevent culture shocks and the use of sucrose to diminish cell volume. In experienced laboratories in which large numbers of PGS cycles have been performed, low rates of failed analyses (no results in <5%) have been encountered (7, 8).

By contrast, the frequency of biopsied embryos without results in Mastenbroek et al. (13) was 20%. Moreover, the implantation rate in their study was only 6% in cycles in which biopsy was performed, diagnosis failed, and so-called undetermined (no-result) embryos were replaced. The implantation rate in a control group of patients who did not have PGS was 14.7%. Simple calculation reveals that in the Mastenbroek et al. (13) experience, there was an estimated 59% reduction in implantation potential as a result of the biopsy procedure. When the biopsied embryos with normal results were replaced, the implantation rate was 16.8% (again, compared with 14.7% in the control group). Overall, it is hardly surprising that with a 59% reduction in implantation potential, the results of this RCT were not salutary. Had there been only the expected (10%) proportional reduction of implantation potential and had only euploid embryos been replaced, the result might have been positive, with a larger sample size.

One can only speculate what problems led to the untoward losses during biopsy and/or transfer procedures. One clue lies in table 4 of Mastenbroek et al. (13), in which it is alluded that laser malfunction and other logistic problems occurred. It is well known that slight misalignment of lasers will still allow holes to be made in the zona pellucida, yet this problem will seriously compromise embryo survival (19). A 2.8% frequency of biopsy failure is also mentioned, which is quite unusual in experienced centers.

GENETIC ANALYSIS

Once the cell is biopsied, fixation is a critical step to obtaining good genetic analysis and to minimizing cases in which there are no results or are errors. As noted frequently in the literature, some fixation methods are better than others (20), and failure to use the best method could also render diagnosis less accurate. For instance, if fixative is added after the cytoplasm already has been breached, DNA fibers and micronuclei can become lost, producing false-positive results. Once a nucleus has been appropriately fixed, several technical requirements regarding PGS analysis should be taken into account. First, key chromosomes that disproportionately impact the rate of aneuploidy in cleavage-stage embryos and spontaneous abortions (chromosomes 22, 16, 21, and 15, respectively) should be included (21, 22). These four, combined with other chromosomes at risk for producing liveborn trisomies (chromosomes XY, 13, and 18), are the standard cocktail of probes that is used in most PGS laboratories. To this end, the Mastenbroek et al. (13) study failed to use DNA probes for chromosomes 15 and 22, which alone account for >10% of abnormalities in cleavage-stage human embryos. This further reduces the selection potential of the technique.

Personnel analyzing the cell slides need to be highly experienced. The proper way to validate the prowess of a PGS lab is through their published error rate, which is calculated by reanalyzing abnormal embryos that were not replaced and then comparing this and the PGS result. Error rates range from as low as 4% (3) to as high as 50% (23). The higher the error rate, the less selection potential. A 50% error rate is akin to selecting embryos at random. The error rate for any important study should thus be known. Unfortunately, this was not reported by Mastenbroek et al. (13). The authors appear to have no prior publications on the topic, and hence, accuracy and quality-control measures of their laboratories are unclear.

Other standard procedures involve the use of two observers to analyze results. This minimizes error rate and number of cells with no results. Using this approach, Colls et al. (11), showed a significant improvement in pregnancy outcome.

PATIENT SELECTION

Preimplantation genetic screening for infertility is merely a selection tool. The underlying principle is that the smaller the pool of embryos available, the less improvement in results can be expected. It follows that there must exist a certain minimum number of embryos for biopsy and potential replacement to allow detection of an increase in live birth rate after PGS. This number is generally taken to be six or eight, given that 50%–80% will be abnormal (8). In Mastenbroek et al. (13), the average number of embryos biopsied was 4.8; thus, many patients must have had only 2 or 3 embryos biopsied. Even if biopsy and diagnosis had been done optimally, there would have been little beneficial effect on pregnancy rates, although a decreased risk of miscarriage and trisomic conceptions should have resulted.

RESPONSIBILITIES OF THE PGS COMMUNITY

Notwithstanding our concern over the technical performance or technique used in certain RCTs, the authors' ability to conduct an RCT needs to be praised. We realize that the onus is on established centers and experienced PGS laboratories to conduct randomized studies to provide sound data on the efficacy of PGS for infertility. The PGS community, in collaboration with the American Society for Reproductive Medicine, the Society for Assisted Reproductive Technology, and the Genetics and Public Policy Center, is proceeding with development of a North American PGS registry to provide for longitudinal data on accuracy and safety. Restrictive US governmental policies on embryo research have precluded a major, funded RCT in the United States. We are hopeful that this will change, but regardless, we will redouble efforts to conduct RCTs with private funds. In the meantime, it is crucial that centers offering PGS demonstrate experience and quality control in biopsy and genetic testing. Training courses in biopsy are provided through the Preimplantation Genetic Diagnosis International Society and the European Society of Human Reproduction and Embryology.

In summary, conclusions, even of RCTs, concerning efficacy of PGS for aneuploidy in women of advanced maternal age should not necessarily lead to the conclusion that the procedure is without benefit. Such a conclusion is appropriate only if all biologic and technical requisites are met. It is critical to properly perform the biopsy procedure, to use appropriate chromosome probes, and to identify the patients that may benefit from it. Published RCTs do not appear to have met these requirements.

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