

EDITORIAL: Embryo Damage by Sub-optimal Biopsy may interfere with clinical success of PGD for infertility

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Pregnancy rates after in-vitro fertilization (IVF) steadily decline with maternal age while pregnancy loss increases. It is widely accepted that reduced fertility with age is mostly egg related and 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, close to 50% of preimplantation embryos are abnormal, a figure increasing to nearly 80% for patients 40 and older (2,3).

This high incidence of chromosome abnormalities can be mitigated by preimplantation genetic diagnosis (PGD), often described as Preimplantation Genetic Screening (PGS), which consists of biopsying usually one cell from each IVF generated embryo and testing this biopsied cell with molecular techniques or fluorescence in-situ hybridization (FISH) before embryo transfer to the patient. Given the high rate of chromosomal abnormalities in IVF embryos, PGD was expected to improve IVF outcome by selecting for transfer only those embryos that are chromosomally normal (4). To date, more than 20,000 PGD procedures have been performed worldwide for that purpose (5, and personal communications). However, the positive impact of PGD on pregnancy outcome is highly technique-dependent: low error rates and minimal embryo damage are absolutely critical to the success of the procedure.

Initial comparative studies showed that PGD for infertility indeed increased implantation rates, decreased pregnancy loss, and reduced trisomic conceptions (6-10). However, none of these studies were randomized, nor had sufficient case numbers to detect a significant increase in live-birth rates. Randomized clinical trials in the US (and many other countries) have proved

difficult to perform because of the high cost associated and the self-pay nature of IVF and the lack of federal funds for human embryo research.

More recently, a few randomized studies have been performed in countries where IVF costs are covered by national health services. One well cited study demonstrated no difference in outcome between control and PGD treated cases (11). However, in this study, two cells were biopsied per embryo, a procedure that is avoided by most experienced centers. The removal of more than one cell is very likely to harm the embryo by compromising the developmental events that follow the biopsy, such as compaction.

Studies assessing biopsy damage without doing PGD selection have not been attempted. However, indirect evidence of the effect of the biopsy on embryo viability has been provided in a study that has assessed the loss of two cells from 5-8 cell embryos after freezing and thawing. In that study the implantation potential of the embryo decreased by more than 50% whereas the loss of one cell decreased it by only 10% (12). In a similar study, the loss of implantation potential proved proportional to the number of cells lost (13); a single cell loss decreased implantation rate by 10% and two by 20%. The embryo biopsy impact is validated by such cryopreservation data, since these data reflect embryo damage caused by routine loss of one or more cells – a condition similar to removal of cells for PGD.

Overall, the potential improvement in ART outcome caused by selecting against abnormal embryos through PGD should far outweigh the potential damage caused by the biopsy procedure (10%) if only one cell is removed. Thus Staessen et al. (11) may actually have shown a beneficial effect of PGD since the estimated damage caused by two cell biopsy (>50%) was similar to the potential of selecting against abnormal embryos by PGD (>50%).

Against this background comes a well-designed randomized study by Mastenbroek et al. (14). The sample size was large enough to determine ongoing pregnancy rate changes, and only one cell per embryo was removed. Yet the study results appear to indicate that PGD for advanced maternal age in their hands is not helpful but actually detrimental to pregnancy outcome.

A careful review of this otherwise elegantly designed study provides significant clues as to why PGD failed in the hands of the authors. In contrast to the experience of laboratories where large numbers of PGD cycles have been performed and low rates of failed analyses (<5% no results) have been encountered, the frequency of biopsied embryos without results in Mastenbroek et al. was 20%. Moreover, the implantation rate in their study was only 6% in cycles in which

biopsy was performed, diagnosis failed, and “undetermined” (no result) embryos were replaced. The implantation rate in a control group of patients who did not have PGD was 14.7%. Thus, based on these figures, these authors have shown an estimated 59% reduction in implantation potential due to the biopsy procedure. On the other hand, when the biopsied embryos with normal results were replaced, implantation rate was 16.8% (again, compared to 14.7% in the control group). It is hardly surprising that with 59% reduction of implantation potential the results of this Randomized Clinical Trial (RCT) were not salutary. Had these been more in line with an expected (10%) proportional reduction of implantation potential and only euploid embryos replaced, the result may have been positive.

We can only speculate what went wrong during biopsy and/or transfer procedures to produce such extensive damage to the embryos. One clue lies in Table 4 in which it is alluded that laser malfunction and other logistic problems occur at high frequency. It is well known that slight misalignment of lasers will still allow holes to be made in the zona pellucida yet this problem would seriously compromise embryo survival (15). A 2.8% frequency of biopsy failure is also mentioned, which is quite unusual in experienced centers.

Another factor which probably contributed to the poor results reported by Mastenbroek et al. is the failure to use DNA probes for two chromosomes that account for >10% of abnormalities in cleavage-stage human embryos, namely chromosomes 15 and 22. This further reduces the selection potential of the technique.

No data on the error rate of the technique, calculated by reanalyzing abnormal embryos not replaced and comparing that and the PGD result, are given. If the error rate is high, the embryos replaced may not have been properly selected.

A final caveat is that PGD for infertility is just a selection tool. The smaller the pool of embryos to be selected and the more embryos replaced, the less improvement in results can be expected. The consequence is that there must be 6 or more embryos for biopsy and potential replacement in order to detect an increase in live birth rate after PGD (8). In the study of Mastenbroek et al. the average number of embryos biopsied was 4.8; thus, many patients must have had only 2-3 embryos biopsied. Even if biopsy and diagnosis were done optimally, which is obviously not the case, there would have been little beneficial effect.

Notwithstanding our concern over the performance of the lab that conducted this RCT, the authors' ability to conduct a RCT needs to be praised. We realize that the onus is on established

centers and experienced PGD laboratories to conduct randomized studies to provide sound data on the efficacy of PGD for infertility. In the meantime, it is crucial that centers offering PGD demonstrate experience and quality control in biopsy and genetic testing. Training courses in biopsy are being provided through PGDIS and ESHRE. Further the PGD community in collaboration with ASRM, SART and Genetics and Public Policy Center in Washington DC is proceeding with development of a North American PGD registry to provide for longitudinal data on accuracy and safety. Unfortunately, restrictive U.S. governmental policies on embryo research have precluded a major funded RCT in the U.S.. We are hopeful this will change but irrespective we will redouble efforts to conduct RCT with private funds.

Mastenbroek et al. (14) have shown that if PGD methods had been optimized and a far lower damage rate than 59% been inflicted, PGD may well have improved IVF outcomes. Overall, we think that this work demonstrates the validity of PGD hypothesis, but also shows that embryo damage by biopsy procedure can be all too easily overlooked.

In summary, we believe that the conclusions of the current study about the lack of efficacy of PGD for aneuploidy for women of advanced maternal age may reflect more on the criticality of the biopsy procedure and the need for the use of additional chromosome probes. Drawing conclusions about the overall or potential benefit of PGD for aneuploidy for these patients from this study would be premature. The Centers with decades of experience in PGD stand challenged to conduct RCT to provide definitive results for this important issue.

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