

Commentary

Multicentre trial of preimplantation genetic screening reported in the *New England Journal of Medicine*: an in-depth look at the findings

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Abstract

A randomized clinical trial of 406 patients with advanced maternal age by Mastenbroek and co-workers recently published in the *New England Journal of Medicine* showed a significant decrease in pregnancy outcome after preimplantation genetic screening (PGS). It is our opinion that this study suffers from a number of insurmountable inaccuracies and that these are either a direct consequence of the inexperience of the team or of a general disregard of vital guidelines reported in the literature. Most importantly, the authors show that in their hands embryo biopsy may affect as many as half the embryos. The error rate was not presented, shedding doubt on the authors' abilities to reliably diagnose the biopsied cells. An evaluation of the study indicates that poor biopsy technique, sub standard fixation and FISH methods, poor IVF outcomes and inappropriate patient selection are the cause of the discouraging results obtained by these authors rather than problems inherent to PGS.

Keywords: birth rate, ICSI, infertility, IVF, PGD, PGS

Introduction

A randomized clinical trial (RCT) is a standard method of testing the efficacy of any new medical therapy. On 6 July 2007, the *New England Journal of Medicine* (NEJM) published the results of an RCT of preimplantation genetic screening (PGS) that was recently conducted in The Netherlands by Mastenbroek and co-workers (Mastenbroek *et al.*, 2007). The study purports that PGS or preimplantation genetic diagnosis (PGD) for infertility or advanced maternal age (AMA) is not just ineffective but detrimental to pregnancy outcome. The study described a multi-centre, randomized double-blind controlled trial in 406 patients comparing three cycles of IVF or intracytoplasmic sperm injection (ICSI) with and without PGS. Women in the PGS group had a significantly lower birth rate cumulatively over three cycles than those in the control arm (24 versus 35%). Surprisingly the miscarriage rates did not differ between the two groups. Although at first glance the study seems well founded, further scrutiny reveals a number of serious flaws.

The live birth rates for the control group in the study compare poorly with clinic-specific success rates published by the CDC (Centers for Disease Control and Prevention) and SART (Society for Assisted Reproductive Technology) in the USA or the HFEA (Human Fertilisation and Embryology Authority) in the UK. Single cycle success rates extrapolated from the cumulative data are also poor in both experimental and control groups. Thus, even without considering any other problems in the study, it is questionable whether the main conclusion can be taken as definitive.

It is not clear whether the teams involved in the study had much previous experience with the multifaceted and complex PGS technology, and whether the laws in The Netherlands prevented the authors from performing the requisite pilot experiments to optimize their methods? There is no previous record from these authors regarding PGS in international proceedings. What is clear is the authors' disregard for crucial details in methodology that have been discussed by other investigators in dozens of publications over the past 10 years (reviewed by Kuliev and Verlinsky, 2002; Munné, 2002, 2003; Gianaroli *et al.*, 2005; Cohen *et al.*, 2007). These studies have demonstrated that aspects of biopsy, fixation, patient selection and fluorescence in-situ hybridization (FISH) can determine success or failure of PGS.

In a large number of cases ($n > 50$), the authors decided to transfer embryos that were biopsied but had no result or diagnosis; this is quite disconcerting not only because of the questionable medical decision to routinely transfer undiagnosed embryos, but also because the test failed in 20% of all embryos biopsied – an extraordinarily high proportion compared with the 3–4% previously published by others (Gianaroli *et al.*, 1999; Munné *et al.*, 2003). The authors point to 'biopsy failure' having occurred in 2.8% and 'incomplete nucleus' in 4.9% of embryos. Yet such categories are non-existent in experienced PGS laboratories and only reflect poor biopsy and fixation techniques used by the participating groups. Biopsy failure suggests that technicians either were unable to remove a blastomere, or the biopsied cell

lysed during or after the biopsy. Incomplete nuclei presumably refer to incomplete chromosomal spreads, extensive layering of DNA, or loss of nuclear material through over-spreading, all of which may result from poor fixation technique and technician inexperience (Velilla *et al.*, 2002).

Once undiagnosed but biopsied embryos were transferred, they showed a poor implantation rate of 6%. This figure was significantly lower than the implantation rate for biopsied embryos that were diagnosed as 'normal' (16.8%) or embryos that were in the control arm of the study and were not biopsied (14.7%). What this discrepancy demonstrates is that the biopsy procedure, as performed at the participating clinics in the trial, was detrimental to embryo viability and reduced the implantation potential of the embryos by more than 50%. The authors do not note the latter but this is an important observation in that even when only normal embryos were replaced, the selection advantage provided by PGS could not have compensated for this loss in implantation potential. It should be said that if such a tremendous reduction in viability actually were to occur in PGS for AMA, one could reconsider not only PGS for AMA, but all other applications of PGS, including those that the authors of the study consider clinically useful and necessary. But in fact, this is unprecedented in the PGD/PGS literature and is an experience unique to the centres involved in this trial. Even in cryopreservation models estimating the impact of blastomere loss, such detrimental effects on viability have never been reported (reviewed by Cohen *et al.*, 2007). It is not unreasonable to speculate that if extensive embryo damage had not occurred, the same study could have possibly shown a beneficial effect of PGS in these patients.

What could explain loss of implantation potential following micromanipulation? One contributing factor may be the imprecise use of lasers by some groups practicing PGS. The laser (Zilos; Hamilton-Thorne, USA) used by these three clinics is certainly effective in ablating the zona pellucida and seems user-friendly; it has also recently received clearance for use during blastomere biopsy from the US Food and Drug Administration (FDA). Cell biopsy techniques require larger holes in the zona pellucida than are needed for assisted hatching thus exposing the embryo to much higher levels of energy. It has been suggested recently that a precise working distance may be required for safe application of the laser and that outcome may be user-dependent (Malter *et al.*, 2001; Chatzimeletiou *et al.*, 2005). The laser biopsy application is essentially untested in randomized trials and not standardized. Outcomes must also be considered cautiously because of differences in ambient temperatures, handling media composition and protein supplementation, microscopes, and magnification during biopsy. None of these details is provided in the NEJM paper, nor is there any comment on procedural, technical, or staff differences among the three participating clinics.

Regarding the FISH procedure, no data on error rates were presented. It is not clear whether clinics in The Netherlands are allowed to analyse blastomeres of non-transferred embryos in order to obtain an error rate, but this must be an absolute requirement when reporting on a large-scale investigation of the efficacy of PGS. A large error rate would quickly nullify any finding of such a study. Therefore, it is quite unfortunate that in the case of the Dutch study, this point was overlooked during the peer-review process prior to publication.

The chromosome panel used by these authors did not include probes for chromosomes 15 and 22 while it is known that these two chromosomes account for more than 10% of abnormalities in cleavage-stage embryos (Abdelhadi *et al.*, 2003; Munné *et al.*, 2004; Baart *et al.*, 2007). The patients were selected from a population of 35–41 year old women with infertility, however, the majority of these patients had few embryos. Previously published data clearly show that the efficacy of PGS for AMA is significantly reduced in patients with few embryos (Munné *et al.*, 2003).

In summary, a careful evaluation of the study by Mastenbroek *et al.* (2007) indicates that poor biopsy technique, substandard fixation and FISH methods, poor IVF outcomes and inappropriate patient selection rather than problems inherent to PGS are to blame for the discouraging results obtained by these authors.

References

- Abdelhadi I, Colls P, Sandalinas M *et al.* 2003 Preimplantation genetic diagnosis of numerical abnormalities for 13 chromosomes. *Reproductive BioMedicine Online* **6**, 226–231.
- Baart EB, van den Berg I, Martini E *et al.* 2007 FISH analysis of 15 chromosomes in human day 4 and 5 preimplantation embryos: the added value of extended aneuploidy detection. *Prenatal Diagnosis* **27**, 55–63.
- Chatzimeletiou K, Morrison EE, Panagiotidis Y *et al.* 2005 Comparison of effects of zona drilling by non-contact infrared laser or acid Tyrode's on the development of human biopsied embryos as revealed by blastomere viability, cytoskeletal analysis and molecular cytogenetics. *Reproductive BioMedicine Online* **11**, 697–710.
- Cohen J, Wells D, Munné S 2007 Removal of 2 cells from cleavage stage embryos is likely to reduce the efficacy of chromosomal tests that are used to enhance implantation rates. *Fertility and Sterility* **87**, 496–503.
- Gianaroli L, Magli MC, Ferraretti AP *et al.* 2005 The beneficial effects of preimplantation genetic diagnosis for aneuploidy support extensive clinical application. *Reproductive BioMedicine Online* **10**, 633–640.
- Gianaroli L, Magli MC, Ferraretti AP, Munné S 1999 Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with poor prognosis: identification of the categories to which it should be proposed. *Fertility and Sterility* **72**, 837–844.
- Kuliev A, Verlinsky Y 2002 Current features of preimplantation genetic diagnosis. *Reproductive BioMedicine Online* **5**, 294–299.
- Malter HE, Schimmel T, Cohen J 2001 Zona dissection by infrared laser: developmental consequences in the mouse, technical considerations, and controlled clinical trial. *Reproductive BioMedicine Online* **3**, 117–123.
- Mastenbroek S, Twisk M, van Echten-Arends J *et al.* 2007 In vitro fertilization with preimplantation genetic screening. *New England Journal of Medicine* **357**, 9–17.
- Munné S 2003 Preimplantation genetic diagnosis and human implantation – a review. *Placenta* (Suppl. B), S70–S76.
- Munné S 2002 Preimplantation genetic diagnosis of numerical and structural chromosome abnormalities. *Reproductive BioMedicine Online* **4**, 183–196.
- Munné S, Bahce M, Sandalinas M *et al.* 2004 Differences in chromosome susceptibility to aneuploidy and survival to first trimester. *Reproductive BioMedicine Online* **8**, 81–90.
- Munné S, Sandalinas M, Escudero T *et al.* 2003 Improved implantation after preimplantation genetic diagnosis of aneuploidy. *Reproductive BioMedicine Online* **7**, 91–97.
- Velilla E, Escudero T, Munné S 2002 Blastomere fixation techniques and risk of misdiagnosis for preimplantation genetic diagnosis of aneuploidy. *Reproductive BioMedicine Online* **4**, 210–217.

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