Human Reproduction, Vol.25, No.3 pp. 575–577, 2010
Advanced Access publication on December 23, 2009 doi:10.1093/humrep/dep446

human reproduction DEBATE

What next for preimplantation genetic screening? A polar body approach!

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Screening of human preimplantation embryos for numerical chromosome abnormalities has been conducted mostly at the preimplantation stage using fluorescence *in situ* hybridization. However, it is clear that preimplantation genetic screening (PGS) as it is currently practiced does not improve live birth rates. Therefore the ESHRE PGS Task Force has decided to start a proof of principle study with the aim of determining whether biopsy of the first and second polar body followed by subsequent analysis of the complete chromosome complement of these polar bodies using an array based technique enables a timely identification of the chromosomal status of an oocyte. If the principle of this approach can be proven, it is obvious that a multicentre randomized controlled trial should then be started to determine the clinical value of this technique. In this way the ESHRE PGS Task Force hopes to redirect preimplantation screening from the blind alley to the main road of assisted reproduction.

One of the presumed factors causing low pregnancy rates in medically assisted reproduction is the presence of chromosomal abnormalities in preimplantation embryos. Therefore many centres have tried to improve their results by screening human preimplantation embryos for numerical chromosome abnormalities at the cleavage stage using fluorescence *in situ* hybridization (FISH) (Goossens *et al.*, 2009). Initially many low-level evidence studies (Gianaroli *et al.*, 1999; Munne *et al.*, 1999) suggested a favourable outcome of preimplantation genetic screening (PGS) of aneuploidy on implantation and pregnancy rates, but more recent high-level evidence from randomized control trials has not confirmed these initially promising findings. There are now 10 RCTs applied to both good (Jansen *et al.*, 2008; Mersereau *et al.*, 2008; Staessen *et al.*, 2008; Meyer *et al.*, 2009) and poor prognosis patients (Staessen *et al.*, 2004; Stevens *et al.*, 2004; Debrock *et al.*, 2007; Mastenbroek *et al.*, 2007; Hardarson *et al.*, 2008;Schoolcraft *et al.*, 2009). These studies have all shown that PGS has not improved the delivery rate compared with a control group, and some of these studies have shown harm or had to be terminated prematurely. Meta-analysis of these trials shows a statistically significant reduction of ongoing pregnancies after PGS [ongoing pregnancy rate per cycle of 13% (92 out of 696) after PGS versus 21% (132 out of 638) in the control group; odds ratio 0.56, 95% CI 0.42–0.76; Mastenbroek *et al.*, 2008]. Both the American Society of Reproductive Medicine and the British Fertility Society have concluded that PGS, as it is currently practiced, does not improve the live birth rates in patients with advanced maternal age, recurrent implantation failure or recurrent pregnancy loss (Anderson and Pickering, 2008; ASRM, 2008). For the observed discrepancy between the theory and the practice a number of reasons have been put forward, such as inexperience in embryo testing, the insufficient number of chromosomes tested and the harm caused by the biopsy procedure (Cohen *et al.*, 2007; Handyside and Thornhill, 2007). However, most probably chromosomal mosaicism in cleavage-stage embryos is the major factor (Coonen *et al.*, 1994; Harper *et al.*, 1995; Delhanty *et al.*, 1997; Coonen *et al.*, 2004; Baart *et al.*, 2006; Mastenbroek *et al.*, 2007; Vanneste *et al.*, 2009). Thus, in many cases, the biopsied and analysed blastomere is not representative for the rest of the embryo. As an alternative, trophectoderm biopsy

has been suggested as this would reduce the damage of the biopsy and increase the possibility of an accurate result as more cells can be analysed from the same embryo. A recent trial by the Sydney group on trophoblast PGS was terminated early since an interim analysis indicated it was not possible to show an advantage for PGS (Jansen *et al*., 2008).

Since mosaicism is not present at the zygote stage it seems to be preferable to opt for polar body biopsy. The disadvantage of polar body biopsy is, of course, that only the maternal aneuploidies can be studied (Verlinsky *et al*., 1995). However, the vast majority of human aneuploidies (more than 90%) are maternal (Nicolaidis and Petersen, 1998). Moving towards the polar body biopsy has the additional advantage that more time is available to study all the chromosomes using novel molecular techniques (microarray CGH, MLPA, etc.) after whole genome amplification (Handyside *et al*., 2004; Spits *et al*., 2006). This might circumvent the problem that, by the application of FISH, the full chromosomal complement never can be studied in an efficient and reliable way.

Comprehensive chromosomal analysis of single cells from human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization was introduced about 10 years ago (Voullaire *et al*., 2000; Wells and Delhanty, 2000). Until very recently, the technology was time consuming, and biopsied Day 3embryos needed to be frozen pending the results of the CGH, with associated compromise in embryo quality (Wilton *et al*., 2001; Munne and Wells, 2003). However, microarray-based methods will yield similar results and may be sufficiently rapid to permit comprehensive screening without the need for embryo cryopreservation. The Cambridge based company BlueGnome has elaborated an array-based CGH protocol which allows for analysis of all chromosomes from biopsied polar bodies within 11–13 h. Therefore the ESHRE PGS Task Force has decided to start a proof of principle study using their methods with the aim of determining whether biopsy of the first and second polar body, followed by subsequent analysis of the complete chromosome complement of these polar bodies using an array-based technique, enables a timely identification of the chromosomal status of an oocyte.

The specific objectives of this study are:

(i) To show that the analysis of both polar bodies can be completed within a time period that allows for fresh transfer;
(ii) To ensure the reliable identification of the chromosomal status of an oocyte in at least 90% of polar body biopsy attempts;
(iii) To test the feasibility of a multicentre randomized trial based on the technology used in the pilot study.

The polar bodies will be biopsied by the centres in Bonn (Germany) and Bologna (Italy), since they have a documented and proven history in the clinical application of this method and the experienced and trained personnel required for a proof of principle. Both centres have obtained ethical approval. The study aims to include 500 oocytes, independent from the number of patients. Assuming that 300 oocytes will be fertilized after ICSI, 600 polar bodies will be processed for chromosomal diagnosis.

Expecting a 50% aneuploidy rate, another 150 aneuploid oocytes will be processed for array analysis to verify the data obtained from the polar bodies. In addition, undiagnosed oocytes will also be analysed but only if the resulting embryos are not transferred. There will be no limitation regarding the number of patients recruited per study site and no restriction on the maternal age of the patients which will be included. First and second polar body will be separately processed for chromosomal analysis. Chromosomal analysis of first and second polar bodies will be done at each study site using the SurePlex amplification protocol and the 24sure analysis provided by BlueGnome (see http://www.bluegnome.co.uk/). The images will be scored by two independent observers in each centre. In case of discordance between the two observers the result will be categorized as 'unknown'. To estimate the concordance of the data from the polar bodies and the corresponding oocytes, there will be a blind analysis of those oocytes which are presumed to be aneuploid based on the result of the chromosomal analysis of their polar bodies.

All data (patient and cycle data as well as array data) from both study sites (polar bodies and oocytes) will be evaluated by an independent data analysis team at the University of Amsterdam. The concordance analysis will be made on the basis of the three categories euploid/aneuploid/unknown (the aim is to have at least 90% concordance).

The study will start in October 2009 and the acquisition of the data from the intended number of biopsied oocytes should be reached by the beginning of 2010. Hopefully the first data can be presented at the 26th Annual Meeting of ESHRE in Rome 2010. If the principle of this approach can be proven, it is obvious that a multicentre randomized controlled trial should be started along the same lines. With this approach the ESHRE PGS Task Force hopes to redirect preimplantation screening from the blind alley back to the main road of assisted reproduction.

Funding

Funded by ESHRE, the European Society of Human Reproduction and Embryology, Grimbergen, Belgium.

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