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DEBATE—CONTINUED

What next for preimplantation genetic screening (PGS)? Experience with blastocyst biopsy and testing for aneuploidy

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Blastocysts more commonly have a normal karyotype than cleavage-stage embryos do. Moreover, blastocysts have also made a metabolic transition from catabolism and recycling of the oocyte's reserves and resources, processes that fuel the first 3 days of cleavage. Although not all blastocysts are karyotypically equal, it is still to be determined to what extent a mosaic karyotype might be a normal feature among embryos, both at the cleavage stage and the blastocyst stage—and when looking for karyotypic abnormalities by embryo biopsy might help the chance of implantation rather than harm it. It is also still impractical to look at all the chromosomes that can, through their aneuploidy, stand in the way of successful embryonic and fetal development. We report a randomized clinical trial of blastocyst biopsy followed by preimplantation genetic screening (PGS) for aneuploidy using 5-colour FISH. The trial was suspended and then terminated early when we were unable to show an advantage for PGS. If we are correct in assuming that mitotic non-disjunction is common by the stage of the blastocyst (and that it is much less ominous than meiotic non-disjunction), then further studies of effective PGS of blastocysts for aneuploidy require methods of analysis that cover all the chromosomes and can differentiate the triallelic and monoallelic states of meiotically derived aneuploidies from the biallelic state of mitotic aneuploidies.

Blastocysts have a normal karyotype more commonly than cleavage-stage embryos do (Sandalinas *et al.*, 2001; Clouston *et al.*, 2002). Blastocysts have also made a metabolic transition from catabolism and recycling of the oocyte's reserves and resources to a state of dependence on the new embryonic genome and activation of a more anabolic metabolic state, both of which are required for successful blastulation and further subsequent development (Jansen and Burton, 2004). Not all normally developing blastocysts are karyotypically equal, but it is still to be determined to what extent a mosaic karyotype could be a normal feature among embryos and to what extent looking for karyotypic abnormalities by embryo biopsy can help the chance of implantation rather than harm it.

Mastenbroek *et al.* (2007) reported that biopsy of Day 3 (cleavage-stage) embryos for limited PGS for aneuploidy of chromosomes 1, 13, 16, 17, 18, 21, X and Y reduced the chance of an ongoing pregnancy in women aged 35–41 having *in vitro* fertilization (IVF). The detrimental result could have more than one cause, including the inadvertent exclusion from transfer of mosaic embryos in which there happened to be just one trisomic cell following a mitotic non-disjunction event (Kuliev and Verlinsky, 2004) (the remaining complimentarily monosomic cell usually being non-viable), embryos that therefore might have 'self-corrected' if given the opportunity to implant, but also including more prosaic practical difficulties such as the time the embryo spends being manipulated in potentially imperfect culture conditions and the loss of up to 12.5–25% of the biomass of such an early embryo. We agree with Harper *et al.* (2008) that further randomized clinical trials are indicated but suspect that other advances will be needed before PGS can reach its full potential.

We have shown over the last several years that culture for 5–6 days and biopsy of trophectoderm after blastulation (de Boer *et al.*, 2004; McArthur *et al.*, 2005) is accompanied by substantially higher implantation and live birth rates when performed for genetic diagnosis (gene mutations or chromosomal translocations) than when biopsy is performed for these indications on Day 3 (McArthur *et al.*, 2008). Here, we report our experience in conducting a randomized controlled clinical trial of

blastocyst biopsy versus no biopsy for the purpose of PGS using FISH for limited detection of aneuploidy.

Between August 2004 and November 2006, we studied the impact of screening IVF embryos for aneuploidy in younger infertile women (<38 years, median 33.5 years), employing biopsies of trophectoderm performed on Day 5 or 6 of *in vitro* development, which is after blastulation, when the embryo typically has >100 cells, and in which the inner cell mass, destined to form the fetus, is not directly disturbed (de Boer *et al.*, 2004; McArthur *et al.*, 2005). Agreement to have only one embryo transferred, known as elective single embryo transfer or eSET, was a precondition for entry and all women were in their first or second attempt at IVF. No women had cycles cancelled because of poor response. Patients were withdrawn from the study before randomization if there were fewer than eight ovarian follicles over 1 cm diameter at 8–10 days of stimulation, fewer than four embryos with seven or more cells on Day 3 of culture, or fewer than two blastocysts for biopsy on Day 5 or 6. (During an initial period, randomization was on Day 3 after retrieval, but two women randomized to the control group and who had embryos prepared for later biopsy are included on the basis of intention to treat, although there were no suitable blastocysts; after this experience, randomization was changed to the morning of planned biopsy, 5–6 days after oocyte retrieval.) Allocation at randomization was on the basis of an instruction in a sequence of sealed envelopes in which random numbers had been used to direct the allocation (this method had not yet resulted in equal sample sizes by the time of analysis and suspension of the trial). Biopsies consisted of 2–9 trophectoderm cells, were carried out after laser-assisted opening of the zona late on Day 3 or on Day 4, and were tested by 5-color fluorescent *in situ* hybridization for chromosomes 13, 18, 21, X and Y (each step has been described in detail elsewhere) (Henman *et al.*, 2005; McArthur *et al.*, 2005).

We compared outcomes between the screened group (Group A, normal 5-color pattern in all the removed trophectoderm cells for the transferred embryo) and the principal control group (Group B, with embryo micromanipulation and zona opening but no biopsy); we also made comparisons with the women who were withdrawn from the study before randomization because of suboptimal responses to stimulation (Group C) and with women who were eligible but elected not to take part in the study (Group D). We considered that a 15% increment in primary outcome (intended to be live births) would be clinically important and estimated that 300 women would need to enter the study to demonstrate such an improvement with an 80% likelihood of detecting a difference with less than a 1:20 chance of false acceptance of the null hypothesis that PGS makes no difference. Group A includes one patient who after biopsy had no suitable embryo to transfer.

The results up to the time the trial was stopped in December 2006 are given in Table I, together with a subsequent further analysis of the four groups utilizing the end-point of live births with a baby taken home.

Unexpectedly, the embryos subjected to zona opening by near infra-red laser opening of the zona, 1–2 days before transfer at the stage of blastocyst (Group B), produced the highest pregnancy rate of the groups (60.9%), which while not statistically significantly different to either the biopsied embryos (Group A, $P = 0.16$, the primary comparison) or the eligible but non-participating women's embryos (Group D), the trend was opposite to that required to disprove the null hypothesis. Furthermore, it was clear that (non-biopsied) Groups B and D were accumulating many more cryostored blastocysts for future attempts at pregnancy than was the case for (biopsied) Group A. For these reasons, the trial was suspended at that point. Subsequent comparison of the live birth rates has seen this difference between the groups increase and, in a number of comparisons, reach statistical significance.

The pregnancy rate after blastocyst stage culture and eSET in the 554 women not participating in the study over the course of the trial was quite high (58.7% of oocyte retrieval procedures overall) and is consistent with results we (Jansen, 2005; Henman *et al.*, 2005; McArthur *et al.*, 2005) and others (e.g. Milki *et al.*, 2004) have reported previously for predominantly single-blastocyst-based IVF transfers. Our 45% pregnancy rate following blastocyst culture and biopsy compares favourably with the 25% clinical pregnancy rate reported after Day 3 biopsy by Mastenbroek *et al.* (2007).

The basis for the strong performance of the embryos in Group B (the principal control group) requires further investigation to ascertain if it is due to a reason other than chance alone. Assisted hatching by opening of the zona, although advocated from

Table I. Live birth rates per oocyte retrieval from fresh embryo transfers among groups eligible for PGS from biopsy of blastocysts on Day 5 or 6 of development

Consented *n* =	Entered *n* =	Pregnant *n* =	FH +ve *n* =	no LB *n* =	M/C *n* =	LB *n* =
Group A. Biopsy *(Including one patient in whom all biopsied were abnormal and not transferred)*						
55	55	25 45.5 (%)*†	22 39.3 (%)*†	35	5*†	20 35.7 (%)*†
Group B. No Biopsy Control *(intention at time of randomization)*						
46	46	28 60.9 (%)*	27 58.7 (%)*	17	1*	27 58.7 (%)*
Group C. Poor Response, Withdrawn *(women with no oocyte retrieval not entered)*						
111	106	37 34.9 (%)	30 28.3 (%)	79	7	27 25.4 (%)
Group D. Eligible, Non-Participant *(elective single blastocyst transfer)*						
n.a.	554	325 58.7 (%)† *$P = 0.16$ †$P = 0.06$	288 52.0 (%)† *$P = 0.07$ †$P = 0.12$	283	54† *†n.s.	271 48.9 (%)† *$P = 0.03$ †$P = 0.09$

Groups A, B and D are elective single-blastocyst transfers. Group C includes poor responders with up to two blastocysts transferred. LB, live birth; M/C, spontaneous abortion; n.a., not applicable; n.s., not significant.

time to time for the embryos of older women to facilitate implantation, has not been shown to be benefical among women <40 or with good blastocyst development. Or it could be that too strict a set of criteria for assumed meiotic non-disjunction led to over-interpretation and rejection of otherwise optimally developing blastocysts that would have developed normally if left unscreened. In this respect, there was a substantial and highly significant difference in the mean embryo utilization rate (the proportion of embryos transferred or cryostored), which fell from 44% in Group B (the controls) to 25% in Group A (the PGS group) ($\chi^2 = 6.2$, $P < 0.02$). On the other hand, a linear decline in IVF fecundity as measured by live births has been observed from age 33 to 45 years (Jansen, 2003) and on subsequent analysis of the trial results that had led to its suspension an inadvertent but significant difference in randomization outcome with respect to average female age in favour of the controls was revealed (Group A, 34.5 years versus Group B, 32.1 years, $t = 2.84$; $P < 0.01$); correcting for this difference in age and standardizing for age 33.3 years partly removes the apparent difference between Groups A and B (adjusted live birth rates of 41.1% and 55.2%, respectively) but does not eliminate it.

It remains to be shown, therefore, that routine screening for aneuploidy can benefit young infertile women undergoing IVF. It is notable that PGS is hypothesized to decrease implantation failure: our study showed no improvement in the spontaneous abortion rate afforded by biopsy, with a trend in the other direction (Table I). We believe that a meaningful advance in the field of PGS requires more than a limited screening for trisomies such as 13, 16, 18, 21, 22, X and Y, given that aneuploidies of these chromosomes do not necessarily prevent implantation. We need, instead, a molecular method that can recognize the mono- and tri-allelic states of meiotic non-disjunction in any of the 24 chromosomes.

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