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human reproduction LETTERS TO THE EDITOR

Which blastocysts should be considered for genetic screening?

Given the exciting rapid evolution of genetic technology we, along with many others, are contemplating the idea of preimplantation genetic screening of all blastocysts. In this context we were interested in the recent paper by Fiorentino *et al.* (2014). They reported on the application of both array-comparative genomic hybridization (CGH) and next generation sequencing (NGS) using instrumentation from Illumina, Inc. They showed 99.5% concordance between the two technologies and 38.5% of embryos having trophectoderm biopsy proved euploid. Following the transfer of 50 screened embryos in 47 women, they had 32 clinical implantations (64.0%) with all those cases proceeding to live births.

Before expending the rather large financial outlay in setting up similar technology in our own facility, we would like to initiate a debate by presenting data showing that morphological assessment of blastocysts can provide similar high implantation rates. Our data, which is supplemental to a larger study (Yovich *et al.*, 2015), question the relevance of applying the advanced genetics in facilities that already have high implantation rates.

Table I shows the implantation rates from 529 single embryo transfers in a hormone controlled cycle where vitrified embryos were warmed utilizing the Cryotop method (Kuwayama *et al.*, 2005). It can be seen that those embryos graded 4AA or 5AA on morphological criteria according to Gardner and Schoolcraft (1999) implant at 63–65% level; i.e. equivalent to the genetically screened embryos reported by Fiorentino *et al.* Figure 1 shows the regression line for blastocysts of all gradings, indicating that there is a reliable predictive value in these gradings ($R^2 = 0.9715$).

Table I Clinical pregnancies and live births according to blastocyst grading categorized from lowest to highest pregnancy rate following single embryo transfer.

Blastocyst scores	6BB	6BA	6AB	4BB	5BB	3BB	6AA	5BA	3BA	3AA	5AB	4AB	4BA	3AB	5AA	4AA	Total
Blastocyst groups	**Low group <30%**			**Modest group 30–39%**			**Medium group 40–49%**					**High group 50–59%**			**Top group 60–69%**		
# CP	0	0	0	13	4	8	2	10	6	13	12	46	24	41	37	55	271
# Transfers	2	2	2	41	12	22	5	23	13	28	25	89	45	76	59	85	529
PR	0%	0%	0%	32%	33%	36%	40%	43%	46%	46%	48%	52%	53%	54%	63%	65%	51%
# LB	0	0	0	10	1	5	1	8	2	6	7	36	19	32	31	47	205
LB rate	0%	0%	0%	24.4%	8.3%	22.7%	20.0%	34.8%	15.4%	21.4%	28.0%	40.4%	42.2%	42.1%	52.5%	55.3%	39%

The blastocyst groups are categorized according to implantation rates. Data derived from Yovich *et al.* (2015).
CP, clinical pregnancy; PR, pregnancy rate; LB, live birth.

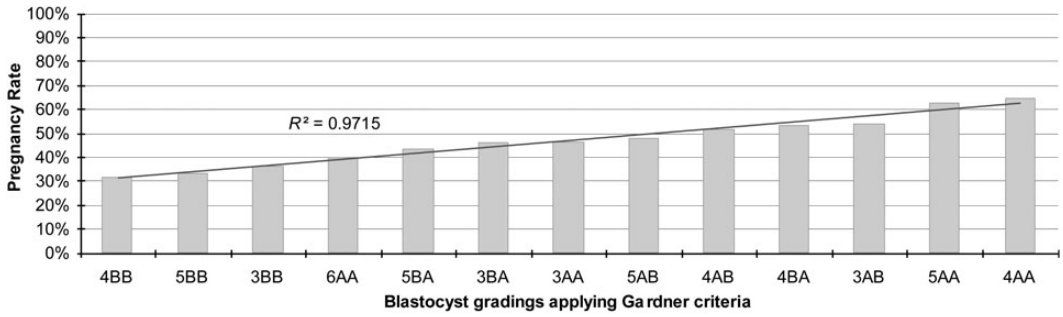

Figure 1 Pregnancy rate from single vitrified blastocyst transfer according to post-warm blastocyst grading at time of transfer, categorized from lowest to highest implantation ratings. Three groups excluded with no pregnancies from six transfers—hatched blastocysts 6BB, 6BA and 6AB. Data derived from Yovich *et al.* (2015).

Perhaps only those embryos graded in the Modest to Medium groupings should be considered for genetic screening. Blastocysts categorized in the High group and Top groups will not benefit from screening as the chance of a healthy live birth is not improved.

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Reply: Which blastocysts should be considered for genetic screening?

Sir,

We thank the authors for their interest in our paper (Fiorentino *et al.*, 2014a). We welcome the opportunity to discuss the advantages of Preimplantation Genetic Screening (PGS) versus morphological assessment of blastocysts and further stimulate the discussion on this important topic.

The authors claim that the transfer of embryos graded 4AA/5AA based on morphological criteria can provide an implantation rate equivalent to that achieved with the transfer of chromosomally screened embryos. However, they did not provide data on the cohort of patients involved in their study. As a consequence the results may be biased from the comparison of different groups of patients.

Our study included poor prognosis patients, for which embryo transfer has often involved the unique euploid blastocyst available, regardless of its morphological score. Despite so, the live birth rate obtained with blastocysts graded 4AA/5AA reported by the authors (54%) is lower than the overall live birth rate achieved in our study (62%). This value increases to 64% if considering embryos graded 4AA/5AA only (data not shown). Therefore, the concern raised by Yovich and colleagues appears unsupported.

Our study provided evidence that morphological and developmental embryo characteristics are weakly correlated with their viability. In fact, a high rate (55%) of aneuploid embryos has been detected, including those graded AA. Similarly, other studies involving comprehensive chromosome screening (CCS) and well-established criteria for the assessment of embryo morphology demonstrated that chromosome aneuploidies are common among embryos of optimal morphological score (40%), while overrepresented in embryos considered to be of poor morphology (60%) (Alfarawati *et al.*, 2011; Fragouli *et al.*, 2013, 2014). Furthermore, in our center we performed a study evaluating the correlation between standard morphology and ploidy status of 1036 blastocysts; 378 of them (36.5%) were classified as top quality blastocysts (4AA, 5AA or 6AA). In this group with high potential of implantation, 217 (57.4%) were found to be aneuploid blastocysts (unpublished data). These data demonstrate that morphologic analysis cannot be relied on to ensure transfer of chromosomally normal embryos.

Recently, several randomized controlled trials (RCTs) have also demonstrated the clinical efficacy of PGS technology versus morphological assessment (Yang *et al.*, 2012; Forman *et al.*, 2013; Scott *et al.*, 2013). For example, Yang *et al.* (2012) investigated the usefulness of PGS in young and good prognosis patients, demonstrating beneficial effect of PGS in terms of enhanced implantation and delivery rates in this group of patients. The ongoing pregnancy rate was significantly higher in the PGS group compared with the morphologically selected embryos group (69.1 versus 41.7%, respectively). Scott *et al.* (2013) investigated PGS usefulness in patients younger than 43 years old, demonstrating significantly higher sustained implantation rates per transfer (66.4 versus 47.9%) and higher delivery rates per cycle (84.7 versus 67.5%) in the PGS group compared with the control group.

A point to note is that, inevitably, aneuploid embryos fail to implant, and those that do implant will generally result in pregnancy loss or in live birth of children with chromosomal aberrations. As a consequence, PGS not only has the potential to improve the clinical outcome of IVF techniques, but the enhanced selection empowered by PGS may also provide a practical way to substantially lower the risk of adverse reproductive outcomes related with the transfer of chromosomally abnormal embryos without compromising clinical outcomes.

The benefit of PGS in terms of cost effectiveness may be more difficult to assess, as this question inevitably involves a subjective decision taken by patients after counseling with the clinicians. Although an additional cost is associated with CCS, it would be lower compared with the cost of repeated ART cycles. Moreover, the emerging CCS technologies, such as Next Generation Sequencing (NGS), allow simultaneous evaluation of multiple samples from different patients in the same sequencing run (Fiorentino *et al.*, 2014a,b). This feature holds the potential to substantially lower the costs associated with PGS.

To conclude, in view of current knowledge, it is our opinion that CCS may be beneficial even if performed by testing all blastocysts, and not only those with the lower morphological scores. This approach may provide (i) improved IVF clinical outcome, (ii) no impact on developmental potential of the embryos and (iii) a cost-effective approach for the patients.