Deep impact: sequencing embryo biopsy specimens at increasing depth

Despite major advances in assisted reproductive technologies (ARTs), it remains the case that more than half of all *in vitro* fertilisation (IVF) treatments do not ultimately result in the birth of a child. It is clear that the majority of embryos created through IVF are not capable of producing a viable pregnancy, with most failing to implant in the uterus or miscarrying soon afterwards.

In order to increase a patient's chance of a live birth, embryos are usually assessed according to morphological criteria and those considered to display optimal developmental features are prioritised for transfer. However, morphology and viability are only weakly correlated and consequently selection of embryos on this basis has only a limited capacity to improve the outcome of IVF treatment. Importantly, aneuploidy (an incorrect number of chromosomes) cannot be reliably identified via morphological evaluation. Aneuploidy is extremely common in preimplantation embryos and is believed to be the leading cause of implantation failure as well as being responsible for at least two-thirds of all miscarriages.

To aid selection of viable embryos, the analysis of chromosome copy number in cells biopsied during preimplantation development (often referred to as preimplantation genetic screening, PGS) has been proposed. The efficiency of PGS has been a matter of debate ever since its introduction into clinical practice in the mid-1990s. Although in theory, the transfer of euploid embryos should increase live birth rates per transfer, initial PGS approaches based on chromosomal analysis using fluorescent *in situ* hybridisation (FISH) failed to enhance overall IVF outcomes in randomised clinical trials.

The success of PGS is highly dependent on the accuracy of the aneuploidy detection method used. Methods utilising FISH had the obvious limitation that only a handful of chromosomes could be tested in each embryo. Additionally, published clinical trials were exclusively carried out at the cleavage stage, a

time of genetic instability during which mitotic errors lead to a high rate of mosaicism. Together, incomplete cytogenetic evaluation, combined with the possibility that the biopsied cell might not be fully representative of the embryo, due to mosaisicm, create a significant risk of diagnostic error and thus it is not surprising that early randomised PGS studies failed to yield clear benefits.

During the past two decades, PGS strategies have evolved and changed dramatically, overcoming former limitations. In an effort to offer more accurate screening of embryos, a variety of comprehensive aneuploidy detection techniques have been validated. These include microarray-based methods such as comparative genomic hybridisation (aCGH) or single-nucleotide polymorphism analysis (SNP array), as well as quantitative real-time polymerase chain reaction (qPCR). Furthermore, advances in embryology, specifically extended embryo culture and trophectoderm biopsy, have enabled a shift away from analysis at the cleavage stage towards testing of blastocysts. Trophectoderm biopsy is thought to be associated with a lower risk of damage to the embryo and, as a greater number of cells is collected, to provide a more representative and reliable indication of the chromosomal content of the embryo, reducing the risk of misdiagnosis due to mosaicism.

Several randomized controlled clinical trials have now been performed utilising comprehensive aneuploidy screening at the blastocyst stage (Forman et al., 2013; Schoolcraft et al., 2012; Scott et al., 2013; Yang et al., 2012). The cumulative data obtained from all of these trials provides strong evidence in support of the hypothesis that PGS-based embryo selection can significantly improve IVF outcomes. Importantly, beneficial effects were not only observed in patients at high risk of generating aneuploid embryos, but also in those considered to be of ‘good prognosis’ and ‘low risk’.

Given the continuous development of innovative, accurate and cost-effective screening technologies, coupled with a growing weight of clinical evidence, it can be expected that PGS will be increasingly utilised as an embryo selection tool. Indeed, it seems likely that the proportion of IVF cycles in the United States that employ PGS will reach 20% during 2015. In particular, next

generation sequencing (NGS) is emerging as a powerful technology to assess chromosome copy number in preimplantation embryos. Due to declining sequencing costs, the clinical implementation of NGS promises high-accuracy aneuploidy detection, high throughput, and a lower cost for PGS than has previously been possible (Wells et al., 2014).

It can be expected that future refinements of sequencing technology will have profound implications for the way in which embryo viability is determined. Increased resolution and genome coverage, obtained through ever deeper sequencing, has the potential allow an even more comprehensive analysis of the embryo's genetic status. Although some technical obstacles still remain, it is anticipated that accurate derivation of an embryos entire genome sequence prior to transfer to the uterus will become feasible in the coming months or years. Clearly, such possibilities raise many ethical questions. However, to date, most studies conducted on embryos have focused on the application of NGS for the purpose of PGS, detecting gross chromosomal changes (i.e. aneuploidy) (Fiorentino et al., 2014; Wells et al., 2014; Yin et al., 2013). In this issue of *RBMOnline*, Fan et al. report validation and clinical application of an NGS technique that combines comprehensive aneuploidy screening and the detection of pathogenic subchromosomal copy number variations (CNVs; microdeletions and microduplications).

Small CNVs can be associated with severe phenotypes, depending on genomic location and the genetic content of the affected chromosomal region. Data obtained during prenatal testing suggest that they occur in more than 1% of pregnancies. Unlike aneuploidy, the incidence of which is strongly associated with female age, the risk of a child affected by a pathogenic CNV is similar for all women. As a result, detrimental CNVs are actually more common than Down syndrome in children born to younger mothers.

At present, standard PGS techniques (e.g. aCGH, SNP array, qPCR) do not provide sufficient coverage of the genome to screen for small CNVs. To address this issue, Fan and colleagues set out to create a methodology that allows subchromosomal alterations (~1 Mb in size) to be detected as well as aneuploidy. After initial optimisation of the sequencing-based strategy on

genomic DNA and cell samples with known CNVs, the technique was applied to a total of 34 biopsies derived from blastocyst-stage embryos. Perhaps surprisingly, five embryos (15%) were identified as carriers of a CNV, of which three were classified as benign. Most importantly, two embryos (6%) were found to be affected by microdeletions of types known to cause severe disease syndromes.

The true incidence of clinically relevant CNVs at the preimplantation stage is not known. However, the findings of the study carried out by Fan and co-workers suggest that they may be relatively common in early human embryos, displaying a frequency much higher than observed later in pregnancy or at birth. Further studies with larger sample sizes will be needed in order to confirm the exact frequency of pathogenic CNVs during preimplantation development and to provide a better understanding of their relevance in terms of embryo viability and health. It is important to note that increasing the resolution of cytogenetic analysis to a point at which CNVs with well-defined clinical effects can be reliably detected will inevitably result in the occasional identification of alterations that are of unknown phenotypic impact. As a consequence, there will be uncertainty concerning the status of some of the embryos tested, resulting in significant challenges for patient counselling. It is not yet clear how often such incidental findings will be observed in preimplantation embryos.

In summary, rapid technological advances over recent years have enabled a comprehensive analysis of the entire chromosome set. Most recently, the introduction of NGS into clinical practice has opened up the possibility of a much deeper analysis of the embryonic genome than has previously been possible. An increase in genetic information will inevitably lead to a greater chance of incidental findings and discovery of genetic variants of uncertain clinical effect, posing new challenges for data interpretation, embryo diagnosis, and subsequent patient counselling. Nonetheless, there can be little doubt that the additional information obtained will have the potential to significantly improve the assessment of embryo viability, ultimately benefiting patients by enhancing treatment success rates.

Acknowledgements

The authors were supported by the NIHR Oxford Biomedical Research Centre.

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