

# Pre-implantation genetic diagnosis

Pre-implantation genetic diagnosis (PGD) was first pioneered at Hammersmith Hospital, London, United Kingdom, in 1989<sup>1</sup> and allows the genetic sampling and testing of the early human embryo prior to being transferred to the uterus to start a pregnancy. Although occasionally controversial, PGD is now well established in several countries and in vitro fertilisation (IVF) centres around the world. In 1993, Adelaide was the first city in the southern hemisphere to offer PGD and to produce the first baby born in Australia after PGD.

Pre-implantation genetic diagnosis tests have been developed at the single cell level to enable PGD to be performed for more than two dozen different diseases, including cystic fibrosis,  $\beta$ -thalassaemia, sickle cell anaemia, Duchenne/Becker muscular dystrophy, Huntington's disease, spinal muscular atrophy, Tay-Sachs, myotonic dystrophy, and Marfan's syndrome.<sup>2,3</sup> In addition to these, many more sex-linked disorders have been diagnosed by PGD by sexing embryos, including the first PGD cases performed.<sup>4</sup>

Genetic screening of embryos serves to reduce a couple's chance of conceiving a pregnancy affected by a serious known genetic disorder. It also increases the success rate of IVF by allowing embryos to be screened for chromosomal imbalances (aneuploidy) prior to selecting the embryos to be returned to the uterus.

Genetic screening of embryos is indicated for both fertile and infertile couples, for the following reasons:

1. when a couple know they are carriers for a genetic disorder such as cystic fibrosis or thalassaemia and wish to reduce their risk of having an affected child;
2. for those couples who wish to reduce the chance that their child will carry a particular mutation. The embryo being screened is not at risk of symptoms from the genetic disease but screening at this time will eliminate the potential for affected offspring in subsequent generations. This is most effective for sex-linked disorders where female carriers will pass the defect on to subsequent male children and where the couple wish to stop the transmission of the disorder within the family;
3. when a couple wish to choose a safe and accurate way of selecting the sex of their next child (social sex selection);
4. for carriers of translocations or chromosomal abnormalities who wish to reduce their risk of miscarriage or avoid conceiving an affected pregnancy and the subsequent use of therapeutic abortions; and
5. for infertile couples undergoing IVF to obtain a pregnancy. They may request that their embryos be screened for chromosomal disorders if they have had

recurrent implantation failures after the transfer of good quality embryos, advanced maternal age, premature ovarian failure, or recurrent miscarriage.

There are two main techniques used to analyse the genetic makeup of an embryo. The first is polymerase chain reaction (PCR) which is able to detect a single base change in the DNA code and hence determine the presence of specific DNA mutations.<sup>5</sup> The other technique is termed fluorescence in situ hybridisation (FISH). Fluorescence in situ hybridisation uses fluorescent-labelled DNA probes for each of up to five chromosomes, labelled in a different fluorescent colour. In this way up to five chromosomes may be analysed at one time when the probes bind to their respective chromosomes present in the single blastomere. The limitations of FISH include the number of colours available and the difficulties in interpretation. As highlighted in the recent article by Ng et al,<sup>6</sup> FISH is useful for detecting aneuploidy and thus eliminating the transfer of embryos that have no developmental potential or will produce offspring with an unbalanced karyotype. At least 50% of IVF embryos have been found to be chromosomally abnormal.<sup>3</sup>

An exciting new technique that has now been applied to single cells is comparative genomic hybridisation (CGH). This technique 'compares' the genome present in the unknown blastomere, labelled in a fluorescent colour such as green, with that of a known normal control cell labelled in red. If the blastomere and control cells contain the same amount of a given chromosome, that chromosome will fluoresce yellow. This indicates that the particular chromosome is neither lacking nor in excess in the blastomere compared with the known normal control cell. This technique can only detect large differences between unknown and known samples in the order of 10 megabases.<sup>7</sup> Conventional metaphase spread single-cell CGH<sup>8</sup> is limited by the long length of time required to perform this technique—approximately 3 to 5 days. In order that embryos are transferred in a fresh IVF cycle they must be returned on day 5—in other words, only 2 days are available in which to perform the analysis.

Our laboratory is developing new DNA chips that are sensitive enough to be used for single-cell CGH and require less than 2 days for analysis. This breakthrough allows embryos to be screened for aneuploidy in the fresh IVF cycle prior to transfer to the uterus. Further research will enable us to analyse every chromosome and thus screen for the presence of normal numbers of every chromosome prior to embryo transfer. A further advantage of CGH is that it is based on PCR. It will also be possible to combine a diagnosis for a specific gene such as cystic fibrosis or set of genes, as well as checking for chromosomal abnormalities prior to embryo transfer.

Most centres now use a laser to breach the zona, which is the outside shell of the egg through which the embryo must hatch and pass. No detrimental effect has been found after PGD.<sup>3</sup> Long-term follow-up of babies born as a result of embryo biopsy is required and this work may begin this year with European Union funding, as has been carried out for babies born as a result of fertilisation using single-sperm injection or intracytoplasmic sperm injection.<sup>9</sup> Most centres remove a single cell from the embryo (blastomere) or two cells in situations where increased accuracy is required. There is no evidence that removing one or two cells from an eight-cell embryo is harmful and pregnancies have still been achieved when only half of the blastomeres remain, either because of PGD or after loss of blastomeres post-thaw for the conventional IVF embryo. To avoid reducing the inner cell mass too much it is best to take just one, or at most two, cells.

Pre-implantation genetic diagnosis embryo biopsies are carried out on the morning of day 3 post-insemination. With a genetic analysis requiring 8 to 24 hours, it is possible to transfer embryos on day 3, 4, or 5. Pregnancy rates for PGD have increased with the increased use of extended culture medium and preferential transfer of embryos on day 5 at the blastocyst stage.<sup>3</sup> The European Society for Human Reproduction and Embryology PGD consortium was established to report data on PGD cycles and consists of centres from Europe, Australasia, and the US. The worldwide overall pregnancy rate for couples undergoing PGD for genetic disorders is 23% per embryo transfer procedure.<sup>3</sup>

In the future, all IVF cycles may include PGD screening to ensure healthy embryos. Ng et al's paper shows that safe and effective PGD is now available in Hong Kong.<sup>6</sup>

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