The use of Preimplantation Genetic Diagnosis to prevent transmission of Haemophilia A and B.

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For couples at risk of having a child affected with haemophilia, being able to detect and select an unaffected embryo for implantation removes much of the anxiety that would otherwise be associated with pregnancy. In this way, they can be assured from the beginning that the pregnancy is unaffected by haemophilia and avoid the difficult decision on whether or not to terminate an affected pregnancy.

First introduced in 1990[1], preimplantation genetic diagnosis (PGD) is an effective screening tool that enables couples to select embryos fertilised through in-vitro fertilisation (IVF) to be transferred on the basis of their genetic make-up. As such, it can be used to identify hereditary disorders such as haemophilia in individuals undertaking IVF as a means of conceiving.

Haemophilia A and haemophilia B are X-linked conditions with an incidence of approximately 1 in 5,000 and 1 in 30,000 male births respectively.[2] The factor VIII gene is located on Xq28 and consists of 26 exons. 45% have an inversion in intron 22. The remainder have a point mutation (majority) or a deletion/insertion (5%). Importantly, 2% do not have a detectable mutation on sequencing.[2] The factor IX gene is located on Xq27 and consists of 8 exons. The majority of mutations are point mutations; deletions/insertions make up the rest.[2]

The downside of PGD for haemophilia is the procedure may result in discarding male embryos; the benefits, however, are significant. PGD allows parents a clear diagnosis about the health of their embryos as well as the option to keep or discard them if they are found to be affected by haemophilia. If they choose to implant the affected embryo they will have been forewarned and have time to prepare for the birth and care of a baby with haemophilia.

The process of PGD

PGD can only be conducted on embryos fertilised through IVF and couples begin the process, outlined below, by visiting a fertility specialist:[3]

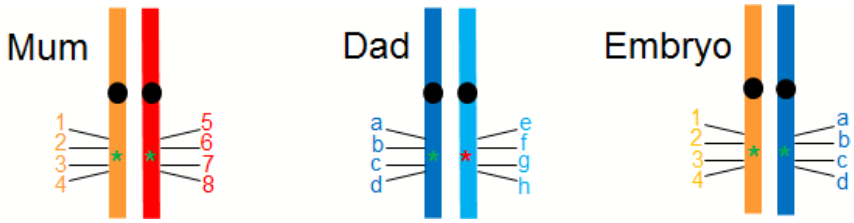
1. **Fertility specialist** – the couple are initially reviewed by a fertility specialist for a thorough work-up. If PGD is thought to be suitable, they are referred for genetic testing/counselling.

2. **Genetic testing and counselling** is performed by a multidisciplinary team consisting of a Clinical Geneticist, PGD scientist, Counsellor and Nurse. The team thoroughly assess the family, make a family-specific recommendation, and provide adequate information and support. For single gene disorders such as Haemophilia, a unique test must be established for the couple.

   The primary test uses linkage markers, which are small highly variable regions of DNA near the gene in question (Figure 1). These linkage markers, in addition to the direct mutation where possible, form a DNA fingerprint.

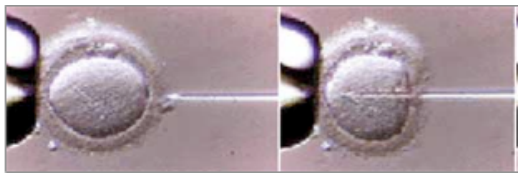
   This test can take up to 12-weeks to complete and must be finished prior to commencement of the cycle.

   Figure 1: use of linkage markers to identify a mutation (red * denotes mutation).



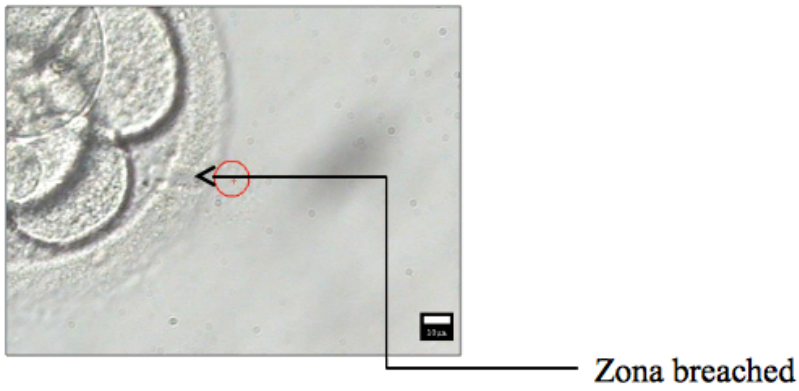
3. **Stimulated IVF cycle** – the woman undergoes a stimulated cycle of IVF to enable the creation of embryos. In brief, FSH injections are given to stimulate the growth of many follicles within the ovary. Cycle progress is tracked via blood tests and ultrasounds. When several large follicles are present, a 'trigger' injection is given to initiate the final stage of oocyte maturation. The egg collection procedure occurs 36-hours later. This procedure is usually performed via conscious sedation. Under ultrasound guidance, a needle is passed through the lateral vaginal fornices, and into the follicles. The follicles are then drained and oocytes collected. Embryos are created via IntraCytoplasmic Sperm Injection (ICSI) in order to prevent DNA contamination from other sperm/maternal cells (Figure 2).

   Figure 2: IntraCytoplasmic Sperm Injection:



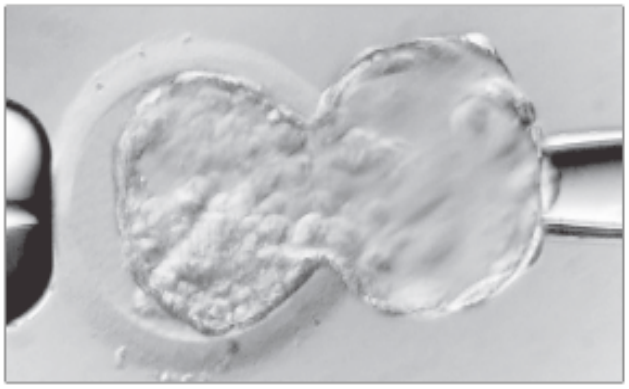
4. **Embryo biopsy** – embryos are cultured in a routine fashion until day 3. On day 3, the surrounding shell of the embryo (zona pellucida) is breached using a laser. This facilitates 'hatching' on day 5 – 6 (Figure 3).

Figure 3: Laser assisted hatching.



The embryos are then cultured to blastocysts. Blastocysts consists of a group of cells (inner cell mass) that will develop into the embryo proper surrounded by a layer of cells (trophectoderm) that will develop into the future placenta. The expanding blastocyst, 'hatches' through the weakness that was created in the zona on day 3 – it is these cells that can be safely biopsied (Figure 4).

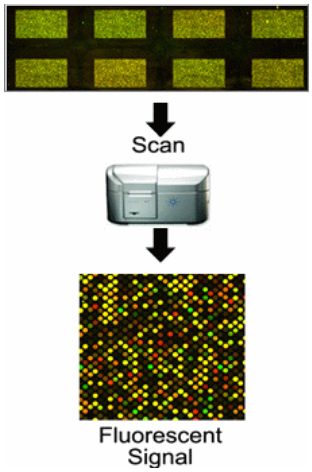
Figure 4: Blastocyst biopsy.



5. **PGD** is then performed either in conjunction with CGH or on its own. Multiple modalities can be used to analyse DNA. Whilst polymerase chain reaction (PCR) is commonly used for single gene disorders, comparative genomic hybridisation (CGH) is another modality commonly used to provide information on the entire genome. The cells biopsied from the embryos are washed, transferred into a reaction tube and their DNA extracted. PCR enzymes are used to duplicate (amplify) specific small segments of the genome into millions of copies. Analysis of the mutation and associated linkage markers are then used to sort embryos according to their genotype.

   Couples may wish to combine PCR and CGH to provide more information about their embryos. In brief, the entire genome is amplified (as described above). A mixture of 'normal' DNA is amplified at the same time to serve as a reference. A fluorescent red dye is attached to the reference DNA and green dye to the embryo DNA. The two samples are then mixed and applied to a microarray slide. The microarray contains thousands of unique DNA segments from across all chromosomes. The embryo and reference DNA 'stick' to the matching DNA on the array. A scanner reads the colour at each spot (Figure 5).

Figure 5: array Comparative Genomic Hybridisation.



Equal red and green = normal 2 copies of the chromosome.

Green means more embryo DNA = trisomy (3 copies).

Red means less embryo DNA = monosomy (1 copy).

The accuracy of PGD is 95%. Unfortunately, despite all the efforts from the PGD team, the desired outcome may not be reached. This may be due to:

- No embryo suitable for biopsy
- For technical reasons, no diagnosis could be reached
- All embryos deemed abnormal
- Misdiagnosis

6. A 'fresh' **embryo transfer** (on the same day as testing) can occur where PCR alone is used as the test result is usually available on the same day. Where CGH is used, however, the result will not be known for some time. This necessitates 'freezing' all embryos and transfer in a later cycle with no negative consequences regarding future pregnancy rates.

7. **Prenatal testing** of all pregnancies that have arisen from PGD is recommended as accuracy of the test is 95%.

PCR, polymerase chain reaction; FSH, follicle-stimulating hormone

References:

1. Handyside, A.H., Kontogianni, E.H., Hardy, K. & Winston, R.M. (1990) Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature, 344, 768–770
2. Firth HV, Hurst JA, and Hall JG. Oxford Desk Reference: Clinical Genetics. Oxford University Press, Oxford, 2005.
3. Preimplantation Genetic Diagnosis <www.genea.com.au>.