Preimplantation genetic diagnosis

Jamie Campbell

Mary Porteous

Abstract

This article aims to inform about the current practices in Preimplantation Genetic Diagnosis (PGD) from a United Kingdom perspective. Progress in the field has been marked over the last decade and new techniques have superseded traditional analysis. The review moves from governance and data collection for PGD to the patient journey. We discuss embryo biopsy and the challenges of analysis when starting with a small amount of DNA. Segments are introduced which introduce basic principles to allow better appreciation of the tests and when they should be applied. We discuss PGD for single gene disorders by preimplantation genetic haplotyping, including for autosomal dominant de novo mutations. Translocations are also discussed and PGD by microarray analysis. We conclude by considering next generation sequencing with the advantages and challenges this may provide to the field.

Keywords diagnosis; embryo biopsy; genetic; haplotyping; microarray; preimplantation

Introduction

Preimplantation genetic diagnosis (PGD) offers couples at risk of a pregnancy affected by a disease with Mendelian inheritance, the opportunity to choose to transfer an unaffected embryo during an in-vitro fertilization cycle. PGD circumvents the issue of prenatal diagnostic testing with termination of an affected pregnancy. The process involves a cycle of IVF with similar livebirth rates per cycle. Progress in the natural sciences with concurrent development in bioinformatics have been critical to the success of PGD with techniques now available to examine the whole genome in detail, starting from a single cell. However, such developments can raise concerns in the wider society. Governance of PGD in the UK is the responsibility of the Human Fertilisation and Embryology Authority (HFEA). The HFEA is a statutory, non-governmental body whose committees license and monitor fertility clinics. Each condition to be tested by PGD must be licensed by the HFEA. For a full list of licensed conditions visit www.hfea.gov.uk. The HFEA guidance adheres to the Human Fertilisation and Embryology Act 2008 which governs the creation of human embryos outside the body and their use in treatment and research.

Outcomes

The European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium gathers international data annually. The number of PGD cycles continues to increase and success rates are also improving. The indication for PGD has a significant impact on resultant pregnancy rates however. The greatest influence on outcome remains maternal age.

Outcomes for Guy's and St Thomas' PGD Centre 1997–2015 (total number of cycles 2487)

- Cycles proceeding to Embryo transfer – 69%
- Clinical Pregnancy Rate per egg collection – 31%
- Clinical Pregnancy Rate per Embryo Transfer – 42% (>56% in the last 3 years)

Preliminary steps

When couples are referred to the clinical genetics department for pre-PGD counselling, the first appointment focusses on confirming the condition in question and any genetic analysis that has been performed in the family. A detailed family pedigree is taken which clarifies inheritance patterns and identifies appropriate individuals to include in the analysis. Informed consent is sought for the provisional lab work-up and blood samples are taken. After the initial discussion, it may be necessary to review medical records of a family member or confirm genetic reports. NHS funding is contingent on the couple meeting specific criteria which may vary between regions. The provisional genetic work-up involves clinical scientists designing and testing genetic markers for use prior to the PGD. The process generally takes between 2 and 5 months dependant on the condition and samples available (Figure 1).

Critical criteria for PGD

(adapted from National Services Division Scotland PGD Framework for decision making 2014)

- The genetic condition in the family must be known and conveys the risk of a serious condition affecting the embryo
- As a couple, they must have no living, unaffected child or untested child (adult onset disease)
- Serum AMH level ≥7.5 pmol/L or antral follicle count >8
- Female age <39 at referral with a BMI 18.5–30
- Both partners must be non-smokers (minimum of 3 months)
- No alcohol during the treatment
- No illicit or harmful drug use (including methadone)
- The couple must have lived at the same address for >2 years
- No living unaffected or untested children born to the couple

When and what to biopsy

The aim of embryo biopsy is to achieve the highest yield of DNA whilst minimizing harm to the developing embryo. There are two main methods in use in the UK.

Jamie Campbell *MBChB MRCPCH Clinical Genetics Department, Centre for Genomic and Experimental Medicine, Western General Hospital, Edinburgh, UK. Conflicts of interest: none declared.*

Mary Porteous *BSc MBChB MSc MD FRCP FRCPath Clinical Genetics Department, Centre for Genomic and Experimental Medicine, Western General Hospital, Edinburgh, UK. Conflicts of interest: none declared.*

Please cite this article in press as: Campbell J, Porteous M, Preimplantation genetic diagnosis, Obstetrics, Gynaecology and Reproductive Medicine (2017), https://doi.org/10.1016/j.ogrm.2017.11.002

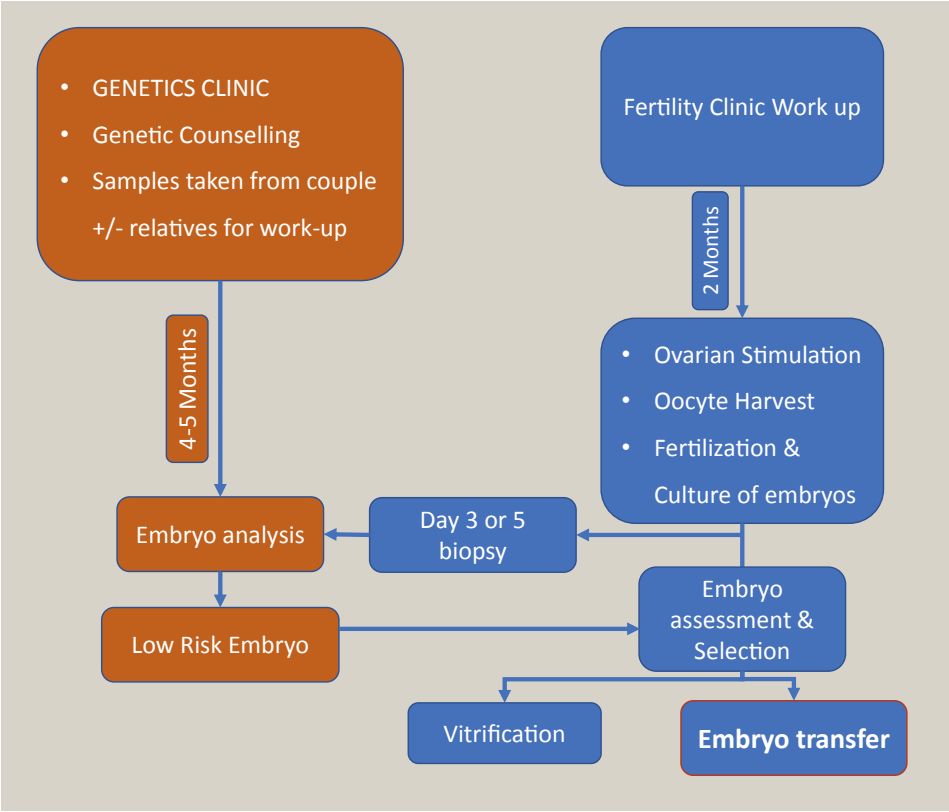

Figure 1 PGD Workflow showing the different time scales and input from genetics and the fertility clinic.

Blastomere biopsy

Cleavage stage aspiration of a single blastomere (Figure 2b) is performed on day 3 when the embryo consists of 6–8 cells. Firstly, the zona pellucida (ZP) is disrupted using an infra-red laser. A holding pipette steadies the embryo whilst a fine aspiration pipette uses a vacuum and sheering forces to extract a blastomere. This time scale allows for fresh embryo transfer even after the more time-consuming analysis such as microarray.

Trophectoderm biopsy

Blastocyst biopsy is performed on days 5–7. Blastocysts are a mass of approximately 70–100 cells differentiated into trophectoderm (TE: precursor of the placenta) on the perimeter, and the inner cell mass which will become the fetus (Figure 2c). Extraction of 8–10 trophectoderm cells leaves the fetal progenitor cells relatively undisturbed. The increased yield reduces the artefacts and bias of polymerase chain reaction and reduces the risk of mosaic results. Embryos that make it to blastocyst stage have a higher live birth rate per transfer compared to cleavage stage transfer. However, fewer embryos reach this stage of development and there is limited time for analysis when attempting fresh embryo transfer. With increased use of embryo vitrification, TE biopsy is fast becoming the most popular approach for DNA collection.

Polar bodies are the byproducts of meiosis I & II (Figure 2a). The 1st Polar body can be biopsied from an oocyte, avoiding

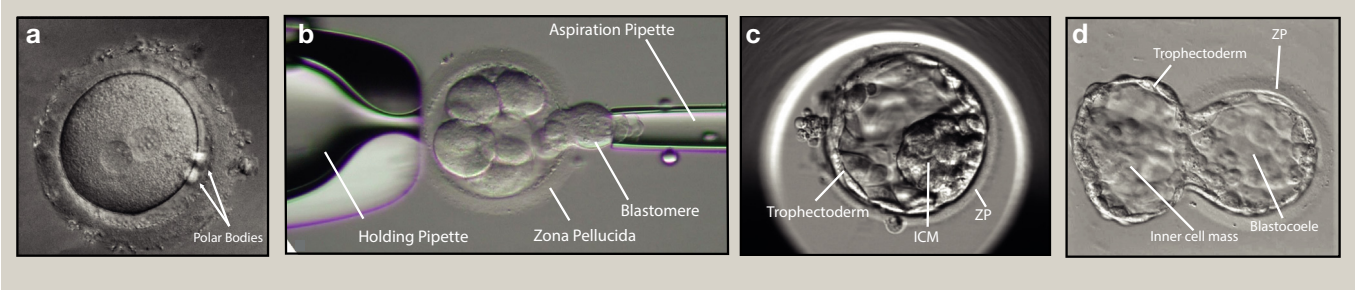

Figure 2 Fertilisation and Embryonic stages. **a**: a fertilized oocyte in the "2 pro-nucleus" stage with both polar bodies evident in the zona pellucida. **b**: Cleavage stage biopsy of a single blastomere. **c**: Blastocyst with early signs of hatching. **d**: Blastocyst at further stage of hatching. ZP – zona pellucida, ICM – inner cell mass.

Please cite this article in press as: Campbell J, Porteous M, Preimplantation genetic diagnosis, Obstetrics, Gynaecology and Reproductive Medicine (2017), https://doi.org/10.1016/j.ogrm.2017.11.002