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SYMPOSIUM: QUALITY MANAGEMENT IN ASSISTED REPRODUCTIVE TECHNOLOGY

Quality control standards in PGD and PGS

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Abstract Preimplantation genetic diagnosis (PGD) aims to test the embryo for specific conditions before implantation in couples at risk of transmitting genetic abnormality to their offspring. The couple must undergo IVF procedures to generate embryos *in vitro*. The embryos can be biopsied at either the zygote, cleavage or blastocyst stage. Preimplantation genetic screening uses the same technology to screen for chromosome abnormalities in embryos from patients undergoing IVF procedures as a method of embryo selection. Fluorescence *in-situ* hybridization was originally used for chromosome analysis, but has now been replaced by array comparative genomic hybridization or next generation sequencing. For the diagnosis of single gene defects, polymerase chain reaction is used and has become highly developed; however, single nucleotide polymorphism arrays for karyomapping have recently been introduced. A partnership between IVF laboratories and diagnostic centres is required to carry out PGD and preimplantation genetic screening. Accreditation of PGD diagnostic laboratories is important. Accreditation gives IVF centres an assurance that the diagnostic tests conform to specified standards. ISO 15189 is an international laboratory standard specific for medical laboratories. A requirement for accreditation is to participate in external quality assessment schemes.

KEYWORDS: chromosomal abnormality, embryo, preimplantation genetic diagnosis, preimplantation genetic screening, quality control, single gene disorder

The current status of PGD and PGS

Initial clinical application of PGD

Preimplantation genetic diagnosis (PGD) was first introduced in 1989; since then, genetic testing has seen major advances. It was developed as an alternative to prenatal diagnosis, for couples at risk of transmitting a genetic abnormality to their children. Couples must undergo IVF procedures to generate embryos *in vitro*, even though many of the couples who undergo PGD are fertile. The embryos can be biopsied by the embryologists at the zygote stage (removal of the first and second polar body), cleavage stage (removal of one to two blastomeres from the six- to eight-cell embryo)

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and blastocyst stage (removal of some trophectoderm cells) (Harton et al., 2011a).

Up until recently, almost all PGD cycles were carried out on blastomeres after cleavage-stage biopsy (Harper et al., 2012; Moutou et al., 2014), Numerous studies, however, have found that cleavage-stage embryos have high levels of chromosomal mosaicism, which means that biopsied cells may not be representative of the rest of the embryo (Fragouli et al., 2011; Harper et al., 1995; Munné et al., 1995; Taylor et al., 2014a). This is especially important when trying to conduct PGD for a chromosome abnormality. Polar body biopsy is rarely used as it only gives genetic information on the maternal genome. In recent years, the IVF community has seen an increase in the use of blastocyst transfer (Glujovsky et al., 2012), and this has been reflected in the increased use of blastocyst biopsy for PGD (Moutou et al., 2014).

Genetic testing should be carried out by a specialized genetic testing laboratory. The first cases of PGD used polymerase chain reaction (PCR) to detect a Y chromosome sequence for sexing for X-linked disease (Handyside et al., 1990).

Testing by FISH

Fluorescence in-situ hybridization (FISH) replaced PCR as the method of choice for embryo sexing (Griffin et al., 1994; Munné et al., 1995) and for chromosome analysis for patients carrying a Robertsonian or reciprocal translocation (Conn et al., 1998; Fridstrom et al., 2001; Mackie Ogilvie and Scriven, 2002). Individual tests had to be validated for each couple as separate probe combinations were needed for every translocation. Also, FISH is not an efficient technique to use at the single cell level (Ruangvutilert et al., 2000). At this time, some groups decided that PGD technology using FISH to analyse as many chromosomes as possible might be useful as an embryo selection method for patients of advanced maternal age, repeated implantation failure or repeated spontaneous abortion (when the chromosomes in the parents were normal) (Munné et al., 1995; Verlinsky et al., 1995). This technique is usually referred to as preimplantation genetic screening (PGS) and should be differentiated from PGD, as it is for a different group of patients and for a different reason.

Testing by PCR

For couples at risk of a single gene disorder, PGD is usually carried out using PCR (Harper and SenGupta, 2012). This technique has become highly sophisticated over the years, with one of the most important developments being multiplex PCR, which allows the analysis of the mutation and also a contamination check (Harton et al., 2011b). The causes of contamination are numerous, including cumulus cell contamination or from people handling the cells (SenGupta and Delhanty, 2012). Molecular-based analysis for PGD can either be carried out by direct PCR amplification of the biopsied embryonic sample or following whole genome amplification (WGA). For direct PCR analysis, inclusion of two informative short tandem repeat (STR) linked markers (within 1cM/1MB), flanking each side of the mutation site, minimizes the risk of misdiagnosis owing to allele dropout at any one locus or owing to contamination. Flanking markers allow the detection of crossover events in the region and assessment of the reliability of linkage analysis in these circumstances. The haplotype of the STR markers in phase with the mutation can be determined by identification of the shared haplotype between family members of known disease status. The limitation of direct PCR analysis is that an individual test has to be developed for each couple, which is time consuming and expensive. Each test has to be validated before being applied clinically.

The mutation site can be included for amplification in the multiplex reaction. Minisequencing is a commonly used method for mutation detection (Fiorentino et al., 2006). For a *de novo* mutation in a male partner, the haplotype in phase with the germline mutation can be determined by analysis of a single sperm. Similarly, for a *de novo* mutation in a female partner, polar bodies can be used but these must be biopsied sequentially and analyzed separately. Alternatively, phasing of alleles can be carried out from the analysis of embryos during the PGD treatment cycle; however, problems can arise when only a few embryos are available for analysis. If all the embryos do not show the mutation and have the same haplotype, it is difficult to be certain that the mutation was not present or if allele drop out had occurred at the mutation site in all the embryos. In such cases, rebiopsy may be an option or cryopreservation of embryos that are blastocysts with analysis of whole embryos that arrest to confirm the STR phasing with mutational analysis.

Whole genome approaches

The introduction of whole genome amplification (WGA) methods have enabled high throughput technologies to be used, which have increased the amount and type of information that can be obtained from an embryo biopsy sample (Hughes et al., 2005). Coupled with this is a reduction in work-up time and the need for patient-specific protocols. Techniques using WGA products are being applied clinically, such as preimplantation haplotyping (PGH), which allows genotyping of multiple STR markers by PCR, or karyomapping (Single nucleotide polymorphism genotyping using an array) to carry out PGD by linkage analysis (Handyside et al., 2010; Renwick et al., 2010; Thornhill et al., 2015). The haplotypes obtained using these methods can also identify monsomies and trisomies of meiotic origin, and can potentially be used to identify imbalances in embryos from translocation carriers and also distinguish between normal and balanced chromosome complements. Array comparative genome hybridization (CGH) identifies chromosomal imbalance in a WGA product, and has been used in both PGD for chromosomal rearrangements and for PGS. Next-generation sequencing (NGS) has also been applied for PGS (Tan et al., 2014; Wells et al., 2014). It is expected that NGS will become the method that is primarily used for detecting chromosomal imbalance and mutation analysis either as separate tests or combined together in one analysis (Tan et al., 2014; Treff et al., 2013). Currently, these whole-genome approaches rely on whole-genome amplification. The type of amplification used determines the artefacts that may be introduced into the sample and thereby affect the accuracy of the diagnostic test. Therefore, extensive validation of WGA in the context of the method of analysis (PGH, array comparative genomic hybridization, karyomapping or NGS) with the indication for testing