(REVIEW ARTICLE)

PREIMPLANTATION GENETIC SCREENING

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ABSTRACT

Preimplantation genetic diagnosis was first successfully performed in 1989 as an alternative to prenatal diagnosis for couples at risk of transmitting a genetic or chromosomal abnormality, such as cystic fibrosis, to their child. From embryos generated in vitro, biopsied cells are genetically tested. From the mid-1990s this technology was employed as an embryo selection tool for patients undergoing IVF, screening as many chromosomes as possible, in the belief that selecting chromosomally normal embryos would lead to higher implantation and decreased miscarriage rates. This procedure, preimplantation genetic screening (PGS), was initially performed using FISH, but 11 randomized controlled trials of screening using FISH showed no improvement in IVF delivery rates. Progress in genetic testing has led to the introduction of array comparative genomic hybridization, quantitative PCR, and next generation sequencing for PGS, and three small randomized controlled trials of PGS using these new techniques indicate a modest benefit. Other trials are still in progress but, regardless of their results, PGS is now being offered globally. In the near future, it is likely that sequencing will be used to screen the full genetic code of the embryo.

Key words: Preimplantation genetic diagnosis; Preimplantation genetic screening; next generation sequencing; biopsy; mosaicism

What is PGS?

Preimplantation genetic diagnosis (PGD) was developed in the late 1980s as an alternative to prenatal diagnosis for couples at risk of transmitting a genetic or chromosomal abnormality to their children (Handyside et al., 1990, Harper and Handyside, 1994, Harper, 1996). Couples go through *in vitro* fertilization procedures to create embryos *in vitro*, cells are biopsied and analysed by a number of genetic tests, such as the polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH). The majority of PGD cases are for common genetic abnormalities, such as cystic fibrosis and haemophilia or chromosome abnormalities such as translocations (De Rycke et al., 2015). PGD has been a controversial procedure as some feel it is unethical to select embryos on their genetic or chromosomal make-up and PGD is not allowed in some countries, such as Germany and Switzerland, which do allow prenatal diagnosis and termination of pregnancy.

In the mid 1990s several groups used PGD technology as an adjunct to IVF as an embryo selection tool mainly for patients with advanced maternal age (AMA) or repeated implantation failure (Munné et al, 1995a and b, Verlinsky *et al.* 1995) and this has been called preimplantation genetic screening (PGS). It is well established that the prevalence of chromosome abnormalities increases with advanced maternal age (Hassold and Hunt, 2009) so applying the technique to older women going through IVF is logical as they are going to produce a high number of chromosomally abnormal embryos. The limitations have been that women of advanced maternal age produce fewer embryos of lower quality, human embryos show a high level of chromosomal mosaicism and chromosome abnormalities in embryos may be one cause of implantation failure, but there may be other reasons.

This paper outlines the biopsy and genetic testing procedures and discusses the randomized controlled trials (RCTs) performed to date. But first, the effect of mosaicism on PGD and PGS will be discussed.

Mosaicism

It has been difficult to examine chromosomes of human embryos using traditional karyotyping methods as embryos are slowly dividing and have a small number of cells. Some early karyotyping studies managed to examine a few cells from human embryos and data suggested that embryos exhibited chromosomal mosaicism (Angell 1997; Zenzes 1992). With the advent of FISH it was possible to examine all of the cells from an embryo but only for a few chromosomes (Harper et al, 1995, Munne et al, 1995b). Because embryonic cells contain a high level of cytoplasm, normal karyotype spreading techniques for FISH and karyotyping were sub standard. Coonen et al (1994) developed a method of spreading embryos and blastomeres which allowed a whole human embryo to be examined by FISH (Harper et al., 1994). These studies showed that human embryos exhibited high levels of mosaicism and

“chaotic” embryos, where every nucleus showed a different chromosome arrangement (Delhanty et al., 1993, Harper et al, 1995, Munne et al, 1995b).

It is now known that the majority of cleavage stage embryos and blastocysts show chromosomal mosaicism (Fragouli and Wells, 2011, Greco et al., 2015). This has been an issue for PGD and PGS as the biopsied cell may not be representative of the remaining embryo and can lead to false positives and false negatives. The use of next generation sequencing (NGS) for PGS has shown that the transfer of mosaic embryos can led to healthy deliveries (Greco et al., 2015).

Biopsy

There are three stages when cells can be removed from an embryo for PGD and PGS: polar bodies from the oocyte/zygote, blastomeres from cleavage stage embryos and trophectoderm cells from the blastocyst. At all stages the embryo is surrounded by the zona pellucida which needs to be breached to allow removal of cells.

The earliest stage of biopsy is polar body biopsy. Both polar bodies are required for diagnosis but the first polar body starts to degenerate soon after forming and the second polar body is only extruded at fertilization. The polar bodies can be removed simultaneously (Montag et al, 2009) or sequentially (Strom et al, 1998), both procedures having advantages and disadvantages. In sequential biopsy, the first polar body is biopsied on day 0, the day of the egg collection and the second polar body is biopsied on day 1, after fertilization. This method ensures that both polar bodies are of good quality and also allows for identification of polar body 1 and 2 but requires two biopsy procedures. In the simultaneous method, both polar bodies are biopsied on day 1 as soon as the second polar body is extruded. Using this method it is often difficult to tell the difference between the two polar bodies and polar body 1 may have degenerated.

A limitation of polar body biopsy is that it tests for maternal errors only. This may be an advantage for PGS for advanced maternal age as most of the errors are due to maternal meiosis. Polar body biopsy cannot test for post zygotic errors, including post zygotic mosaicism which can be looked on as an advantage or a disadvantage. A disadvantage of polar body biopsy is the cost. Polar body biopsy is very time consuming to the embryology team as biopsies need to be done at unsocial hours. And the diagnosis is expensive; if 10 eggs are collected, 20 tests need to be carried out. Using array CGH this is very costly but if NGS is used, it may be cheaper if the samples are batched. Either way, it will still cost more than blastocyst biopsy as from 10 embryos, we would expect to obtain approximately 5 blastocysts – each blastocyst forming one test. Therefore PGS at the blastocyst stage is about a quarter of the cost of polar body biopsy. As a result of these issues, polar body biopsy has not been applied to many PGD or PGS cases (De Rycke et al., 2015).

The first cases of PGD performed at the Hammersmith Hospital in the UK used blastomere aspiration from day 3 cleavage stage human embryos (Handyside et al., 1990). Acid Tyrodes solution was used to breach the zona and a small pipette was used to gently aspirate 1-2 cells from the 6-8 cell embryo. Acid Tyrodes was a crude method of zona drilling and was rapidly replaced by the use of a non-contact laser. It was only in the ESHRE PGD Consortium data collection for cycles performed in 2004 that the laser was used more often that acid Tyrodes (Harper et al, 2008). The laser allows for a shorter time period to perform the drilling and more accurate size of the hole. At this stage, human embryos are starting to compact and so the procedure can be technically difficult. $Ca^{2+}Mg^{2+}$ free media is used to reduce compaction and aid aspiration (Dumoulin et al., 1998).

Cleavage embryo biopsy was the most widely applied technique for PGD and PGS until relatively recently (Harper et al., 2012). It stopped being so popular due to being limited to one cell, mosaicism and the lack of positive data from RCTs for PGS.

The majority of PGS is now done using blastocyst biopsy (De Rycke et al., 2015). In IVF, culturing embryos to the blastocyst stage is considered to be a method of embryo selection as only good quality embryos will reach the blastocyst stage (Bolton et al., 2015). For blastocyst biopsy, the zona can be breached on day 3 or day 5 (Kokkali et al, 2005, McArthur et al, 2005). The embryos are returned to culture and the trophectoderm cells start to herniate through the hole. On day 5, bout 5 trophectoderm cells are cut from the embryo.

There are several advantages of using blastocyst biopsy for PGS. Blastocysts are less mosaic, there are more cells to analyse which makes the diagnosis more accurate, there are fewer embryos to analyse which makes it cheaper and blastocyst transfer is a method of embryo selection in itself as almost half of embryos will not develop to the blastocyst stage, and since PGS is a selection method, it makes sense to test those embryos that are already selected and finally, in almost all PGS cases, embryo transfer is done at the blastocyst stage.

Vitrification of human embryos has been shown to be a very efficient technique (Vajta and Kuwayama 2006, Chang et al., 2011). Vitification allows efficient cryopreservation of embryos either before or after the biopsy. A cost effective way of performing PGS is to biopsy and then vitrify embryos which allows more time for the diagnosis and also batching of samples which makes the diagnosis much cheaper. It also allows the samples to be sent to another location, even country, for the diagnosis (Harton et al., 2010).

PGS has been separated into two versions: version 1: the use of FISH examining a few chromosomes and version 2: comprehensive chromosome screening.

PGS version 1, FISH and cleavage stage biopsy

From 1995 to 2010, PGS version 1 used fluorescent in situ hybridisation on polar bodies or cleavage stage embryos (Munne et al, 1995a and b, Verlinsky et al, 1995). Numerous papers claimed that the technique worked and thousands of cycles were conducted worldwide. From 2004-2010, eleven randomised controlled trials showed that this technique did not result in an increase in delivery rates; some studies showing a significantly reduced delivery rate (Staessen et al., 2004, 2008; Stevens et al., 2004; Mastenbroek et al., 2007; Blockeel, 2008; Hardarson et al., 2008; Mersereau et al., 2008; Debrock et al., 2008; Meyer et al., 2009; Schoolcraft et al.,2009, Jansen et al., 2008). At this time, the ESHRE PGD Consortium, the ASRM and the BFS wrote statements that PGS using FISH and cleavage stage biopsy was unproven and that RCTs should be conducted on polar bodies and trophectoderm biopsy using efficient methods that analysed all chromosomes (Harper et al, 2010, Anderson and Pickering, 2008, The Practice Committee of the Society for Assisted Reproductive Technology and the Practice Committee of the American Society of Reproductive Medicine, 2008).

Four reasons have been put forward to explain the failure of PGS version 1. First is the FISH technique, which when applied to one cell, is not a very efficient procedure and only gives about 80-95% specificity, depending on the number of probes used. Second the high level of mosaicism in cleavage stage embryos may mean that an abnormal cell is removed from an otherwise normal embryo which may have implanted. Thirdly, the biopsy of cells from an embryo may have a negative effect on implantation which may out-weigh the benefits of putting back a chromosomally normal embryo. And lastly, most studies have concentrated on poor prognosis patients (mainly advanced maternal age), who produced few embryos usually of poor quality.

PGS version 2, comprehensive chromosome screening

In recent years new technologies have emerged that are more efficient than FISH and allow analysis of all of the chromosomes from interphase nuclei. PGS version 2 involves using these methods, such as array comparative genomic hybridisation (a-CGH) (Geraedts et al, 2011, Magli et al, 2011, Mamas et al., 2012), NGS (Fiorentino et al 2014A and 2014B, Bono et al 2015, Tan et al 2014, Huang et al., 2016) and quantitative PCR (Forman et al, 2013, Scott et al, 2013). For PGS v2, as well as applying the technique to poor prognosis patients, such as advanced maternal age and repeated IVF failure, good prognosis patients have been included. Due to the problems of post zygotic mosaicism seen using cleavage stage biopsy, most, but not all, groups have concentrated on polar body and blastocyst biopsy (De Rycke et al., 2015).

After validation of a-CGH for PGS (Magli et al., 2011, Geraedts et al., 2011) ESHRE (European Society of Human Reproduction and Embryology) has embarked on a multi-centre RCT for patients with advanced maternal age using polar body biopsy. Polar body biopsy using a-CGH is an expensive technique as both polar bodies need to be analysed. But the technique will not be confused by post zygotic mosaicism which may be an advantage or disadvantage. The results should be published in 2017.

Three RCTs have been reported using trophectoderm biopsy on good prognosis patients and shown an increase in ongoing pregnancy rates (Yang et al., 2012, Forman et al., 2013 and Scott et al., 2013. The size of all three studies was small: 55, 89 and 72 patients in the treatment group respectively. The limited sample size makes it impossible to draw firm conclusions from the three trials but they indicate a modest benefit from embryo selection using trophectoderm biopsy (Dahdouh et al., 2015, Lee et al., 2014). Lee et al., (2014) concluded that "high-quality experimental studies using intention-to-treat analysis and cumulative live birth rates including the comparative outcomes from remaining cryopreserved embryos are needed to evaluate the overall role of PGS in the clinical setting". But globally PGS accounts for more than all the other indications for PGD added together, according to the USA data (Chang et al., 2016) and the ESHRE PGD Consortium Data (De Rycke et al., 2015). Kushnir et al., (2016) reanalysed the SART data and concluded that PGS decreased the chances of live birth and that the improvements reported were likely to be as a consequence of patient selection.

If poor prognosis patients are undergoing PGS, such as those of advanced maternal age who by definition produce few embryos, a trend in many IVF units is to bank embryos by vitrifying all the embryos from several cycles of IVF before warming all the embryos and performing the biopsy and diagnosis. In some USA centres it is routine for patients to go through four egg collections before PGS is applied. From this cohort – there is an increased chance that a 'normal' embryo will be found, leading to increased pregnancy rates per embryo transfer procedure but very low pregnancy rates per egg collection (Orris et al., 2010).

Next generation sequencing (NGS) is rapidly becoming the technique of choice for PGS and for the detection of chromosomal imbalances in patients with translocations (Fiorentino et al 2014A and 2014B, Li et al., 2015, Tan et al., 2014, Huang et al., 2016) with improved results compared to a-CGH (Yang, et al., (2015), Fiorentino et al., 2014B). Hopefully very soon larger, well-designed RCTs using a-CGH and/or NGS on trophectoderm cells will be published (Harper et al., 2016). Indeed these studies will be welcome, so that the controversy surrounding PGS can finally be put to rest, but until they are published, we have insufficient information to make an informed decision.

Conclusion

Medical screening is established in pregnant women, children, and adults. In screening embryos there is the same need to ensure efficiency and safety. IVF is a multi billion dollar business throughout the world, using technology that advances quickly. Because many techniques are introduced without proper validation or efficacy testing (Harper et al, 2011, Harper et al., 2017), there are instances of technology which has been shown not to work after it has been applied to thousands of IVF patients, who have generally paid for the privilege. IVF patients are emotionally vulnerable and after several failures are often willing to trial new procedures. When talking to patients about PGS, health professionals must discuss the advantages and disadvantages of PGS (Garcia-Velasco and Fauser, 2016) and practice evidence based medicine.

The refinement of sequencing will allow more genetic information to be obtained, and this technology is already in use in PGD for monogenic disorders with simultaneous aneuploidy screening (Yan et al 2015, Gui et al, 2016). It is highly probable that in the future all IVF embryos will undergo genetic testing using sequencing to determine which embryos are the ‘best’ from the cohort produced. Whatever the outcome of the PGS RCTs, patients may decide that it is beneficial to know the genetic make up of their embryos (Hens et al, 2013). Indeed, PGS is already applied to every embryo for every patient in some IVF clinics. PGS will finally fit its name, with PGD and PGS merging as the same technique, and will determine aneuploidy and genetic abnormalities.

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