REVIEW PAPER

Preimplantation genetic diagnosis: State of the ART 2011

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Abstract For the last 20 years, preimplantation genetic diagnosis (PGD) has been mostly performed on cleavage stage embryos after the biopsy of 1–2 cells and PCR and FISH have been used for the diagnosis. The main indications have been single gene disorders and inherited chromosome abnormalities. Preimplantation genetic screening (PGS) for aneuploidy is a technique that has used PGD technology to examine chromosomes in embryos from couples undergoing IVF with the aim of helping select the chromosomally 'best' embryo for transfer. It has been applied to patients of advanced maternal age, repeated implantation failure, repeated miscarriages and severe male factor infertility. Recent randomised controlled trials (RCTs) have shown that PGS performed on cleavage stage embryos for a variety of indications does not improve delivery rates. At the cleavage stage, the cells biopsied from the embryo are often not representative of the rest of the embryo due to chromosomal mosaicism. There has therefore been a move towards blastocyst and polar body biopsy, depending on the indication and regulations in specific countries (in some countries, biopsy of embryos is not allowed). Blastocyst biopsy has an added advantage as vitrification of blastocysts, even post biopsy, has been shown to be a very successful method of cryopreserving embryos. However, mosaicism is also observed in blastocysts. There have been dramatic changes in the method of diagnosing small numbers of cells for PGD. Both array-comparative genomic hybridisation and single nucleotide polymorphism arrays have been introduced clinically for PGD and PGS. For PGD, the use of SNP arrays brings with it ethical concerns as a large amount of genetic information will be available from each embryo. For PGS, RCTs need to be conducted using both array-CGH and SNP arrays to determine if either will result in an increase in delivery rates.

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Introduction

Preimplantation genetic diagnosis (PGD) is performed for couples who are at risk of a specific inherited disorder (Table 1). The reproductive options for these couples are to remain childless, have no genetic testing on any pregnancy (reproductive chance), undergo prenatal or PGD, have gamete donation, or adopt. The couples who opt for PGD have already been diagnosed with their specific disorder, either because they have had an affected child, have a known family history or been diagnosed as an adult. Most of these couples are fertile, and have often been through prenatal diagnosis and termination of an affected pregnancy. PGD is not an easy option as it takes some time to validate the specific test for each couple; they have to go through IVF, and the success rates are only comparable to routine IVF patients. The added problem is that there are cases where all of the embryos are affected as can be seen from the ESHRE PGD Consortium data where for all indications there are a number of cycles that reached PGD but do not have a transfer procedure (Harper et al. 2010a).

PGD was first performed in 1989 in a set of couples who were at risk of transmitting a sex linked disease to their children (Handyside et al. 1990). The biopsy was performed by removing one cell from cleavage stage embryos (Hardy et al. 1990). A polymerase chain reaction (PCR)

Table 1 Differences between PGD and PGS

	PGD	PGS
Aims	Identify genetically normal embryos	Achieve a pregnancy/birth
	Achieve a genetically normal pregnancy/birth	
Indication	Monogenic disorder	Advanced maternal age
	X-linked disease	Repeated implantation failure
	Known chromosome abnormality	Repeated miscarriage
		Severe male factor
Fertility	Often fertile	Infertile or subfertile
Biopsy	Usually day 3	Usually day 3
		Recently polar body and blastocyst biopsy being used
Number of cells for analysis	1–2 cells	1 cell
Diagnosis	Interphase FISH for chromosome abnormalities and sexing	FISH with as many probes as possible
	PCR for monogenic disorders	Recently arrays being used
	Recently arrays being used	
Undiagnosed or inconclusive results	Never transfer these embryos	Can transfer these embryos
Prenatal diagnosis	Indicated	Indicated for the same risk factors as natural conceptions

Adapted from Harper 2009

method was used which was able to detect a segment of the Y chromosome. An absence of a band on the gel indicated a female embryo, but this result could also be obtained if the biopsied cell had failed to be put in the tube, had failed to amplify totally or if the Y sequence had failed to amplify (allele dropout, ADO).

The biopsy technique has remained relatively unchanged for two decades, the only changes being the use of a laser for zona drilling (Veiga et al. 1997) and the use of calcium magnesium free media, which helps reduce blastomere lysis (Dulmoulin et al. 1998). The majority of clinics are still using cleavage stage biopsy for PGD but polar body biopsy and blastocyst biopsy are increasing in popularity (Harper et al. 2010a).

In the early 1990s, fluorescent in situ hybridization (FISH) was applied as a more efficient technique to perform embryo sexing rather than using PCR to amplify a Y chromosome sequence (Griffin et al. 1994; Munne et al. 1993). Since this time, PCR has developed to become a highly efficient technique of high efficacy that can rapidly perform the diagnosis of numerous single gene disorders (Harper et al. 2010a; Spits and Sermon 2009). The FISH technique has remained relatively unchanged but it has been additionally applied to inherited chromosome abnormalities (Fridstrom et al. 2001; Mackie Ogilvie and Scriven 2002). The main indications for PGD can be divided into single gene disorders, inherited chromosome abnormalities and sexing for X-linked disease (Harper et al. 2010a).

There are several disadvantages of PGD. There have been a number of cases of misdiagnosis (Harper et al. 2010a; Wilton et al. 2009) and PGD is very expensive, especially in countries where prenatal diagnosis is paid for by the state but PGD may not be (such as the UK). For the patient, the most difficult part of PGD is that fertile couples have to go through IVF, which can be costly and stressful.

The current techniques used for PGD have been relatively uncontroversial, except for social sexing (Pennings and de Wert 2003; Sharp et al. 2010), PGD for HLA (Pennings and de Wert 2003; de Wert et al. 2007) and PGD for inherited cancers where penetrance in not 100% and late onset diseases, such as Huntington disease, where the parents do not wish to know if they are carriers (Quinn et al. 2009). However, PGD analysis will very likely become more controversial as there is an increase in the types of genes and diseases that can be diagnosed.

Preimplantation genetic screening (PGS)

In 1995, two teams in the USA used PGD technology to detect chromosome abnormalities in polar bodies for patients going through in vitro fertilisation (IVF) as an additional means of embryo selection (Munné et al. 1995a; Verlinsky et al. 1995) and this was applied to embryos by Munné et al. (1995b). The technique was performed in patients with advanced maternal age, repeated implantation failure and repeated miscarriage with normal karyotypes in

the parents. The technique was called a number of things, including PGD for aneuploidy screening (PGD-AS). Unfortunately, the unhelpful term of PGS has stuck.

PGS is very different from PGD (Table 1) and it is more controversial. The patients are infertile or subfertile and the main aim is to aid current selection methods used in IVF. In this situation, PGS has to be shown to improve delivery rates compared to other IVF selection methods. The only way this can reliably be achieved is through randomised controlled trials (RCT) (Harper et al. 2010b; Harper et al., unpublished).

The early studies reporting on were non-controlled, non-randomised studies and they usually reported pregnancy rates, rather than delivery rates, as the final outcome. In 2004, the first RCT on PGS was reported (Staessen et al. 2004) and was followed by a study by Mastenbroek et al. (2007). Both studies were heavily criticised but there are now ten RCTs at the cleavage stage (Staessen et al. 2008; Meyer et al. 2009); Mersereau et al. 2008; Blockeel et al. 2008; Staessen et al. 2004; Stevens et al. 2004; Debrock et al. 2010; Hardarson et al. 2008; Mastenbroek et al. 2007; Schoolcraft et al. 2009) and one at the blastocyst stage (Jansen et al. 2008) all using FISH to examine a varying number of chromosomes, but none have shown an increase in delivery rates (some show a significant decrease in delivery rates). This work has been discussed extensively and the position statement from the ESHRE PGD Consortium stated that 'until results of RCTs using a different biopsy stage and arrays can demonstrate a significant increase in delivery rates, there is no evidence that routine PGS is beneficial for patients with advanced maternal age' (Harper et al. 2010b).

Embryo biopsy

For two decades, the prevalent method of embryo biopsy has been cleavage stage biopsy where usually one, but sometimes two, blastomeres have been biopsied from the cleavage stage embryo on day 3 of development (Harper et al. 2010a; Harton et al. 2010a). The use of this technique was based on a study by Hardy et al. (1990). The initial method used acid Tyrodes to drill a hole in the zona pellucida and aspiration to remove the blastomeres. There has been much debate on whether one blastomere or two should be biopsied for PGD and PGS. Two blastomeres will allow a more accurate diagnosis but taking two cells is more invasive to the embryo and may affect implantation. Since PGS is trying to improve implantation, usually only one blastomere is biopsied. There is only one study comparing one and two blastomeres biopsy and the data is not significant (Goossens et al. 2008).

For many years, this method remained unchanged. 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 other change was the introduction of Ca^{2+} Mg^{2+} free biopsy media that reduced the junctions between blastomeres and made the biopsy easier (Dumoulin et al. 1998).

Polar body biopsy was first reported by Verlinsky and colleagues where they originally biopsied only the first polar body (preconception diagnosis) (Verlinsky et al. 1992). It was soon realised that both the first polar body and the second polar body were required for an accurate diagnosis (Verlinsky et al. 1998). The main limitation of polar body biopsy is that it only allows identification of the maternal genes and chromosomes. Therefore, it cannot be applied if the male is carrying a chromosome abnormality or dominant single gene disorder. Polar body biopsy has been used in countries whose laws forbid 'embryo' biopsy, such as Germany and more recently, Italy. The polar bodies can be removed simultaneously (Montag et al. 2009) or sequentially (Strom et al. 1998), both procedures having advantages and disadvantages. Simultaneous removal calls for fewer manipulations as both polar bodies are removed at the same time, but it may be difficult to distinguish between the first and second polar body (which might be necessary for some diagnoses) and the first polar body may degenerate. Sequential biopsy requires two biopsy stages but has the advantage of knowing which polar body is removed.

Blastocyst biopsy is growing in popularity since more centres successfully culture embryos to the blastocyst stage. Following pioneering work from Muggleton Harris and others (Monk et al. 1988), it was applied clinically several years ago (Kokkali et al. 2005; McArthur et al. 2005). Blastocyst biopsy can be performed in two ways. A hole can be drilled on day 3 and the embryos left in culture so that some of the trophectoderm cells herniate, which can be biopsied on day 5. The problem with this method is that inner cell mass cells may herniate instead of trophectoderm. In the second method, the hole is drilled on the morning of day 5, away from the inner cell mass to ensure that only trophectoderm cells herniate. The blastocyst can be returned to culture for a few hours to allow herniation and if required, trophectoderm can be gently aspirated. The cells are cut from the embryo using a laser. The blastocyst will collapse but usually rapidly reforms, sealing the hole where the cells have been removed (Kokkali et al. 2005; McArthur et al. 2005).

Recent advancements in IVF have enabled blastocyst biopsy to become a highly viable stage to biopsy for PGD. Blastocyst culture and transfer are now routine in many IVF units. This has resulted in a higher yield of blastocysts and higher quality. One of the previous problems with

blastocyst biopsy was that it gave a relatively short time for the diagnosis as transfer had to occur by day 6, giving just 24 h for the diagnosis compared to 48–60 h after cleavage biopsy. The introduction of vitrification as a method to cryopreserve blastocysts (Youssry et al. 2008), and the reported high survival rates even after blastocyst biopsy (Kokkali et al. 2005; McArthur et al. 2005), has resulted in a shift to blastocyst biopsy and vitrification, allowing an unlimited amount of time for the diagnosis. Vitrification is an ultra rapid cooling and warming method that prevents ice crystal formation. During a normal IVF cycle, there is a juggle between obtaining a good number of oocytes and the preparation of the endometrium for implantation. With the high survival rate after vitrification, there is a growing amount of data showing that transfer of vitrified embryos to the uterus during a 'non-stimulated' cycle, when the thickness of the endometrium can be optimised, results in a high pregnancy rate (Zhu et al. 2011; Chang et al. 2011).

Blastocyst biopsy usually gives between 5–10 cells and therefore more DNA than cleavage stage biopsy (1–2 blastomeres). This makes diagnosis easier and reduces the misdiagnosis rate. Some of the techniques currently being introduced, such as micro-arrays, utilise very expensive technology. Since only approximately 50–60% of embryos reach the blastocyst stage, biopsy at this time results in less embryos to process which is more time and cost effective. This is especially relevant in comparison to polar body biopsy where at least 2–4× the number of samples will have to be analysed. In an attempt to reduce the prevalence of multiple pregnancies, single embryo transfer (SET) is becoming commonplace in IVF (Cutting et al. 2008). Blastocyst transfer aids this process.

It is well reported that the cleavage stage embryo shows high levels of chromosomal mosaicism (Harper et al. 1995; Munné et al. 1995b) and recent data indicates a high levels of mosaicism also in blastocysts (Fragouli et al. 2011). Chromosomal mosaicism within the preimplantation embryo makes PGD highly problematic, as the cells removed may not be representative of the rest of the embryo. This biological phenomenon has to be taken into account, especially in diagnosis examining chromosomes as it could lead to false positive and negative results.

Whether there are two polar bodies, 1–2 blastomeres or 5–10 trophectoderm cells, the major advances in PGD over recent years have been the methods of diagnosis.

Diagnosis

Examining chromosomes

Fluorescent in situ hybridisation (FISH) uses fluorescently tagged DNA probes that bind to their complementary sequence and can be visualised under a fluorescent microscope. The first chromosome analysis for PGD used FISH to identify the X and Y chromosomes (Griffin et al. 1994; Munné et al. 2004). This method was rapidly applied to detect inherited chromosome abnormalities, such as translocations (Fridstrom et al. 2001; Mackie Ogilvie and Scriven 2002) and non-inherited abnormalities for PGS (Munné et al. 1995a, b; Verlinsky et al. 1995).

The biopsied cells are spread, usually using HCl/Tween (Coonen et al. 1994; Harper et al. 1994) or methanol:acetic acid (Munne et al. 1993) and FISH performed using appropriate probes. Three colour FISH for interphase nuclei has been shown to be relatively efficient, but as more probes are added, the efficiency decreases (Ruangvutilert et al. 2000). For translocations and other inherited chromosome abnormalities, protocols usually use three probe FISH with probes for the chromosomes involved in the translocation (Mackie-Ogilvie and Scriven 2002). For aneuploidy screening, it is important to examine as many chromosomes as possible and up to 15 probes have been used (Baart et al. 2007).

Since FISH is limited by the number of chromosomes that can be examined, many groups are replacing FISH with array-comparative genomic hybridisation (see below).

Molecular diagnosis

The PCR is designed to enrich a DNA sample for one specific fragment, amplifying it to a level at which it can be visualised and subjected to further genetic analysis. It has become one of the most important methods in genetic testing and refinement of the PCR protocol for single cell analysis has proven highly successful. The sensitivity of PCR-based protocols has been increased 1,000-fold compared to conventional non-radioactive methods by the use of fluorescent primers and detection of the resultant PCR products on fluorescent DNA sequencing apparatus.

A growing number of genes have been analysed by PCR–PGD and a variety of mutation detection strategies employed. Minisequencing (Fiorentino et al. 2006) and real-time PCR assay with fluorescence resonance energy transfer (FRET) hybridization probes followed by melting curve analysis (Vrettou et al. 2004) are the most common methods used in recent protocols. The best protocols take into consideration the principal problems; amplification efficiency, contamination and allele dropout. It is now understood that an absence of amplification (PCR failure) should never be taken as an indication that an embryo is free of mutation; amplification is unsuccessful in approximately 10% of isolated blastomeres regardless of their genotype.

Some protocols for the analysis of triplet repeat mutations rely upon large expansions being refractory to PCR

amplification. Diagnosis of normal embryos is based upon the PCR amplification of two alleles with repeat sizes in normal range. Affected embryos only have one allele that amplifies by PCR (Kakourou et al. 2008). If the couple are not informative for the triplet repeat (have the same number of triplet repeats for their normal alleles) and are also not informative for linked markers, then triple-primed PCR can be used to amplify the expanded repeat to distinguish embryos with an expansion from those that are transferable (Kakourou et al. 2010). Overall, the use of fluorescence has reduced the problems of contamination and time taken for the genetic analysis to be performed. Allele dropout, the phenomenon where only one of the two alleles present in a cell is amplified to a detectable level, generally affects 5–20% of single cell amplifications (although in some instances the frequency is higher) and is a problem that is yet to be fully understood (Ray and Handyside 1996; Wells and Sherlock 1998). This is an important consideration in the diagnosis of dominant disorders or recessive diseases where only one mutation can be detected. ADO may appear to have occurred in cells that are monosomic for chromosome being tested by PCR.

In a multiplex PCR reaction, several loci can be investigated at once if the primers are labelled with different fluorescent tags and by designing primers to give PCR products of different size ranges. In all cases of molecular diagnosis, multiplex PCR of the mutation locus together with flanking linked polymorphic markers provides an additional means of determining the genotype of the embryo hence reducing the risk of misdiagnosis due to ADO (Abou-Sleiman et al. 2002; Harton et al. 2010b).

Protocols based solely on the analysis of multiple microsatellite markers linked to and flanking the mutation site provide an indirect method for diagnosis by the identification of alleles in phase with the mutation (Dhanjal et al. 2007). These protocols require the availability of DNA from at least two individuals in the family with the mutation so that the phase alleles can be identified by linkage analysis. However, in the case of de novo mutations phase alleles can be identified using a combination of mutation detection and haplotyping of linked STR markers by single sperm or polar body multiplex PCR (Rechitsky et al. 2011).

Linkage-based protocols can be applied to more than one family with different germline mutations in the same gene. Multiplex linkage analysis forms the basis of HLA typing of embryos (Fiorentino et al. 2004). Inclusion of informative STR markers located on several chromosomes into the multiplex protocol allows the identification common aneuploidies. The selection of euploid embryos may improve the overall pregnancy of PGD for single gene disorders (Rechitsky et al. 2006).

All protocols that involve direct multiplex PCR of the single template of genomic DNA in a single cell requires extensive optimization and testing to ensure the efficiency and accuracy of amplification of each locus tested. This is time consuming and labour-intensive. The availability of different polymerases and multiplex PCR kits has reduced the time taken to optimise protocols for use in single cells.

PGD for mutations in the mitochondrial genome is complex due random genetic drift during oogenesis resulting in heteroplasmy where primary oocytes have a mixture of mitochondria with and without the inherited mutation. A bottleneck that operates at oogenesis determines the mutational load in primary oocytes. The size of the bottleneck appears to vary for different mitochondrial mutations and also between individuals. Determining a suitable threshold value for mutational load in embryos at PGD is difficult as the mutational load is also subject to random genetic drift in somatic tissue during development, which will affect embryo survival and overall phenotype. PGD for mitochondrial mutations can be considered to be risk reducing rather than complete removal of the mutation and couples need to be carefully counselled about the limitations of PGD for these disorders. PCR with restriction enzyme analysis has been used for the detection of specific mitochondrial mutations showing skewed meiotic segregation and the selection of embryos with a low mutational load (Steffann et al. 2006; Monnot et al. 2011). PGD for mutations in the mitochondrial genome requires extensive workup to allow semiquantitative assessment of mutational load in single cells.

Whole genome amplification (WGA)

There is a variety of methods aimed at non-specific amplification of the entire genome (WGA) (reviewed by Hughes et al. 2005). Using these techniques a single genome can be amplified numerous times, thus providing sufficient DNA templates for many independent PCR amplifications including mutation detection and polymorphic markers, which could test for ADO and contamination or be used in protocols based only on linkage analysis. WGA removes the need to optimise multiplex PCR reactions to a single cell level so the diagnostic procedure would be easier and could be applied to any patient as informative markers could easily be selected. WGA also provides a supply of sample DNA that can be reassessed, allowing confirmation of diagnosis or the analysis of other genes. WGA can be PCR-based or non-PCR-based. Complete coverage of the genome is rarely achieved by any WGA method; therefore, PGD protocols using WGA DNA should be based on linkage analysis of many STR markers. This enables diagnosis to be made even if some loci fail to amplify or if ADO occurs during WGA. The use of WGA is not widely used as it adds another step to

the PGD protocol and limits diagnosis to linkage based strategies.

Primer extension preamplification (PEP) utilises 15 base oligonucleotide primers of random sequence to initiate DNA synthesis by PCR throughout the genome. At least 70% of the genome is amplified more than 30 times (Zhang et al. 1992; Arneson et al. 2008). A second form of PCR-based WGA is degenerate oligonucleotide primed PCR (DOP-PCR) which uses primers with partially degenerate sequence so that the primers can initially anneal throughout the genome and priming in later cycles can be directed towards the fixed sequence in the primer (Telenius et al. 1992; Wells and Sherlock 1998). This method amplifies a similar proportion of the genome to PEP, but to a much more significant level.

Currently, the most common PCR-based method used is GenomePlex, which combines PEP and DOP. It uses degenerate oligonucleotide primers coupled with universal adaptors for a linker adapter PCR of fragmented template. This method results in a million-fold amplification of DNA from a single cell giving approximately 5 μg of DNA.

Multiple displacement amplification (MDA) is a non-PCR isothermal, strand-displacing amplification method (Dean et al. 2002). It yields about 1–2 μg DNA from as little as 1–10 copies. It achieves this by using exonuclease-resistant primers and Ø29 DNA polymerase. MDA generates DNA products, which are about 10 kb in length and the amplification is reported to be uniform (Burtt 2011); however, artefacts may be introduced into the WGA
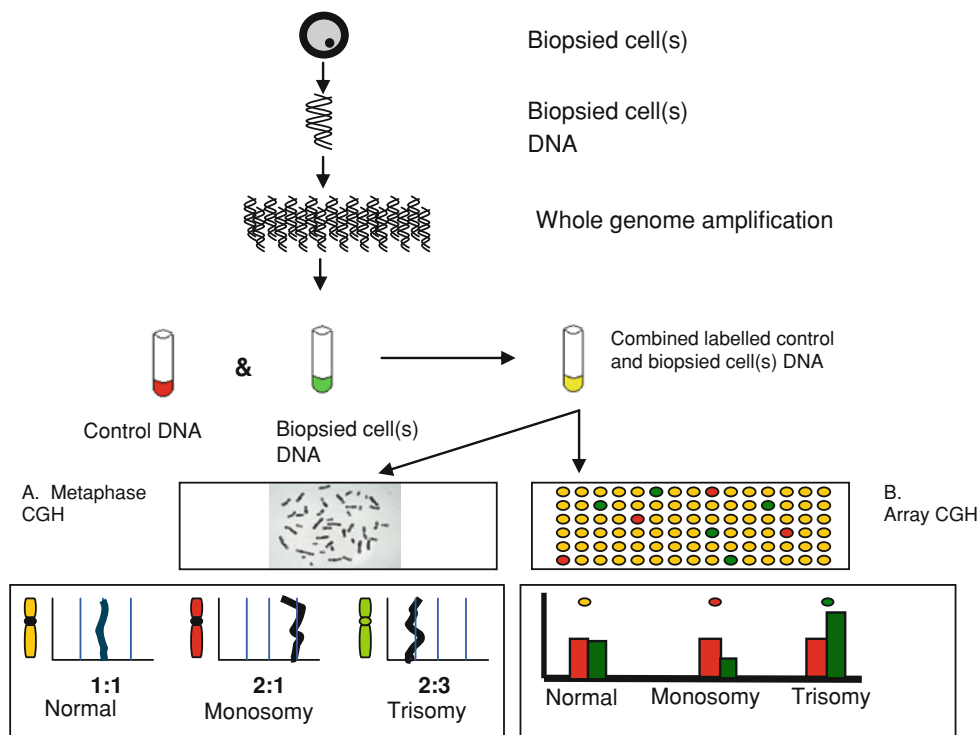
product (Glentis et al. 2009). MDA followed by haplotype analysis for linkage-based diagnosis has been applied clinically and is known as preimplantation genetic haplotyping (Hellani et al. 2005 and Renwick et al. 2010).

Comparative genomic hybridisation: metaphase and arrays for testing chromosomes

Comparative genomic hybridisation is a technique that bridges the gap between molecular genetics and cytogenetics (Kallioniemi et al. 1992). In CGH, DNA from the test sample and DNA from a normal control DNA are amplified separately using a WGA approach (Fig. 1). The amplified DNA is differentially labelled with one of two fluorochromes, for example red for the test DNA and green for the control DNA. Following labelling, both DNAs are mixed together in equal proportions and are allowed to compete to hybridise to either metaphase spreads from a normal male control cell line (m-CGH) or onto an array platform containing small pieces of chromosome (a-CGH) (Fig. 1).

In m-CGH, specialised computer software analyses the ratio of red-to-green fluorescence along the length of each metaphase chromosome in about six different metaphase sets and plots the ratios on an ideogram. Gains in red indicate that the test sample is deficient in that region or chromosome and gains in green indicate that the test has extra copies of that region or chromosome. The process is time-consuming and technically challenging, taking up to

Fig. 1 Comparative genomic hybridisation *A.* metaphase CGH and *B* array CGH. First, the biopsied material undergoes whole genome amplification and the embryonic DNA is labelled in green fluorescence. A control sample is labelled in red fluorescence. The samples are then co-hybridised onto either *A* a metaphase spread or *B* an array platform. In both cases, a computer analyses the ratio of *red*-to-*green* fluorescence. Courtesy of Thalia Mamas and Leoni Xanthopoulou, UCL Centre for PG&D, UCL, Adapted from Harper and Harton 2010

72 h to perform the laboratory bench work with additional time required for the detailed analysis of the data.

Applying this technique to single cells has taken many years of research. Initial studies used a variety of single cells (Wells 1999; Voullaire et al. 1999), and the technique was then successfully applied to blastomeres (Voullaire et al. 2000; Wells and Delhanty 2000) and the blastocyst stage (Schoolcraft et al. 2009). M-CGH takes at least 72 h to perform and so two different approaches have been applied clinically for aneuploidy screening for IVF patients. Wilton et al. (2001) and Schoolcraft et al. (2010) have overcome this problem by freezing the biopsied embryos. Wells et al. (2002) have used a rapid m-CGH method on polar bodies.

Array-CGH uses the same principal but instead of metaphase spreads, an array platform is used. It has been possible to apply a-CGH for PGD on polar bodies (Gera-edts et al. 2010), cleavage stage embryos (Hellani et al. 2008) and trophectoderm cells (reviewed in Harper and Harton 2010).

Instead of using metaphase chromosomes, well-defined genomic clones, such as BAC, PAC or YACs are arrayed onto a slide (Fiegler et al. 2007; Vanneste et al. 2009; Le-Caignec et al. 2006). Each slide may consist of 100 to 1,000s of clones covering the entire genome that increases the resolution to 100–200 kb (Pinkel et al. 1998; Pollack et al. 1999; Heiskanen et al. 2000; Bruder et al. 2001; Hodgson et al. 2001; Wessendorf et al. 2002). The analysis is performed using laser scanning and advanced software with results presented as separate logarithmic signal ratio values for each clone. In PGD or PGS, CGH array platforms that are commonly used are of low resolution to avoid the problems of copy number variation of unknown relevance and target diagnosis to large chromosomal aneuploidies.

Since the analysis is fully automated, the whole procedure can be performed within 24 h. For day 3 biopsy, it is possible to perform the embryo transfer on day 5 of embryo development in a fresh cycle (Hellani et al. 2008). However, for some groups, especially those performing blastocyst biopsy and/or transport PGD (where the diagnosis is done in a specialist PGD centre at a different location to the IVF unit), it may be preferable to freeze the biopsied embryos whilst the diagnosis and analysis is performed.

When arrays are applied to translocations, it is important that the smallest region of imbalance that may arise in embryos can be detected on the array. Therefore, the number of clones present on the array in the region that is telomeric to each breakpoint should be checked to ensure that the region is sufficiently represented on the array to detect an imbalance (Fiorentino et al. 2011, Alfarawati et al. 2011). Couples need to be counselled so that they are aware that chromosomal aneuplodies other than those involved in the translocation will be detected on the array.

This will be an ongoing issue for PGD as new technology detects more than just the abnormality being diagnosed.

There are some shortfalls of using CGH-based technology. CGH cannot detect polyploidies, such as triploidies, as there is no imbalance in the total DNA content. In addition, CGH tests cannot detect balanced translocations or inversions as the total amount of DNA in the sample is the same as in the control sample. These systems cannot detect changes in DNA sequences (point mutations, intragenic insertions or deletions, triplet repeat expansion, etc.) or gains or losses in regions of the genome not covered by the array.

If arrays are going to be applied clinically in PGD or PGS cycles, the WGA and array platform must first has to be validated. This can be achieved by individually testing single cells isolated from euploid control samples and samples with known aneuploidies to ensure the consistent results on the array for each single cell analysed. (Harton et al. 2010b; Mamas et al., submitted). Single cells are easily obtained and can include buccal cells, lymphocytes and fibroblasts but the DNA quality varies depending on the cell type (Glentis et al. 2009).

Single nucleotide polymorphism arrays

SNP arrays make use of the 10 million SNPs across genome but a limitation has been the discovery of over 1,000 copy number variants (CNVs) with unknown clinical significance. SNP arrays are being developed for PGD. They can be used for PGD of single gene disorders by linkage analysis as well as for aneuploidy (Treff et al. 2010; Handyside et al. 2010; Brezina et al. 2011) but the couple and sometimes their parents, have to be tested to obtain their haplotype. The amount of information obtained is immense, and potentially it can also detect predispositions to common diseases, physical characteristics and late onset disorders. There are three problems when using SNP arrays; we do not know the relevance of some CNVs, parental DNA needs to be tested for linkage analysis, and information may be obtained regarding predispositions in parents and their embryos (which might not be requested).

SNP arrays are based mainly in molecular genetics and rely on a WGA step to amplify the single cell or small number of cells removed from a developing embryo (Treff et al. 2007; Vanneste et al. 2009; Kearns et al. 2008). Newer methods of performing WGA like MDA and GenomePlex are used for most applications of SNP arrays. These WGA methods tend to allow for better overall coverage of the genome and are less inclined to preferentially amplify some parts of the genome while leaving others unamplified or under-amplified.

SNP-based arrays offer other options for testing that are not available on CGH-based systems. SNP-based arrays,

due to the data that is collected and the way it is analysed, allow for simultaneous testing of specific genetic diseases and aneuploidy in each embryo (Handyside et al. 2010. This will allow for the selective transfer of genetically and chromosomally normal embryos for patients undergoing IVF with PGD for monogenic diseases. Simple haplotyping of SNPs surrounding and embedded in disease-causing genes allows for selection of embryos that have not inherited the affected chromosome. In addition, simple haplotyping of various markers around the genome allow for analysis of live-born children to determine which embryo or embryos implanted in any given ART cycle transfer. This last 'test' may help IVF centres develop technology to assess an embryo's implantation potential that can be added on to tests for genetic and chromosomal normalcy allowing for increased implantation and delivery rates in IVF centres around the world. With most countries moving toward SET, a set of tests that pinpoints the one 'best' embryo for transfer would be a welcome addition.

A number of groups around the world are currently validating SNP arrays and analysis software for clinical use in PGD. SNP arrays are just beginning to be utilised clinically in PGD (Brezina et al. 2011).

Conclusion

The ESHRE PGD Consortium has reported on 10 years of data collection (Harper et al. 2010a). Blastocyst biopsy is increasing in popularity and since vitrification shows excellent survival rates (Vajta and Kuwayama 2006) it is now possible to freeze all the embryos post biopsy giving more time to perform the diagnosis (Harton et al. 2010a). For the diagnosis, FISH is rapidly being replaced by array-CGH, whole genome amplification is becoming routine and SNP arrays are entering the PGD arena. These improvements have resulted in an increase in the number of diseases that can be diagnosed by PGD but also more controversial uses of PGD. PGS cycles have increased over the years and account for more cycles than all other indications added together. The number of PGD cycles for monogenic disorders have been slowly increasing in number and it is predicted that this increase will continue (Harper et al. 2010a).

Table 2 summarises the methods that can be used for PGD for single gene defects and chromosome detection. SNP arrays can be performed for each indication, but are currently time consuming, expensive to run and often difficult to analyse. If aneuploidy and single gene defects need to be diagnosed, it may be cheaper and more reliable to perform WGA, array-CGH and a specific PCR for the condition carried by the couple. It is also possible that new technology will supersede arrays. The use of array-CGH

Table 2 Methods of diagnosis

Single gene defects	Chromosomes
Multiplex PCR—Mutation detection e.g. minisequencing and linkage analysis of STR markers	Interphase FISH
Multiplex PCR—linkage analysis of STR markers	Multiplex PCR
WGA and multiplex PCR—linkage analysis of STR markers	Array-CGH
SNP arrays—linkage analysis	SNP arrays

and SNP arrays will increase over the next few years (Harper and Harton 2010) but new technology is continuously being developed.

The evolution of genetic testing in the wider field, as well as PGD, has seen the merging of the disciplines of cytogenetics and molecular genetics. It is key that diagnostic labs have appropriate training programmes for PGD clinical scientists and that PGD laboratories are accredited to ISO 15189 or equivalent (Harper et al. 2010c). Embryologists need to perform the embryology and clinical scientists need to perform the genetic analysis. PGD centres should participate in external quality assessment schemes to ensure that they meet international standards (Harper et al. 2010c).

Whole genome scanning technology will bring challenges and opportunities, including ethical implications. Array CGH sexes embryos which brings into discussion the issues surrounding social sex selection which is illegal in the EU (Pennings and de Wert 2003; Sharp et al. 2010). SNP arrays will identify unexpected predispositions that may require appropriate counselling. PGD for HLA matching and late onset disorders has resulted in much ethical debate (Pennings and de Wert 2003; de Wert et al. 2007). The discussions will be ongoing as it is possible to diagnose non-disease-causing traits. However, current methods of PGD still require patients to go through expensive and time-consuming IVF procedures, with a relatively low success rate. For the majority of patients, this technique will only be used for serious conditions. For PGS, data from the RCT using array-CGH will be welcome to determine if this procedure has any benefit for infertile patients.

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