Clinical applications of preimplantation genetic testing

Paul R Brezina,1 2 William H Kutteh1 2

Preimplantation genetic testing is a broad term used to describe the genetic analysis of one or more cells from an oocyte or embryo and the use of the results of the analysis to guide which embryos are transferred to the uterus.1‑3 Currently, preimplantation genetic testing can be performed only within the context of an in vitro fertilization (IVF) cycle.3 Preimplantation genetic testing may be used to evaluate a known genetic disorder present within the parents, a process termed preimplantation genetic diagnosis. Alternatively, it can be used to determine whether aneuploidy exists within an embryo obtained from parents believed to be genetically normal, a process termed preimplantation genetic screening (boxes 1 and 2).2‑4

Continual technological improvements in genetic analysis and assisted reproductive technologies, coupled with an improved understanding of the biologic processes of early human embryology, have resulted in clinical data that suggest a tangible benefit of preimplantation genetic diagnosis and preimplantation genetic screening.2 5‑9 Indeed, studies show that both these techniques are increasingly being used in reproductive medicine worldwide.10 11 This article reviews both aspects of preimplantation genetic testing, defines which patients are appropriate candidates, and summarizes the best technologies available to perform such interventions, with a primary focus on clinical applications in North America. We also discuss some of the limitations of these technologies and list areas of future research.

SOURCES AND SELECTION CRITERIA
We used the terms “preimplantation genetic testing”, “preimplantation genetic diagnosis”, “preimplantation genetic screening”, “PGD”, and “PGS” to search PubMed and Google Scholar from the year 2000 to December of 2014. Bibliographies of articles were also searched for relevant studies. When possible, larger randomized controlled trials were used. However, for some emerging data, only data from meeting abstracts were available. We also looked at abstracts from the recent 69th Annual Meeting of the American Society of Reproductive Medicine (Boston, Massachusetts, October 2013) and the 63nd Annual Meeting of the American Society of Human Genetics (Boston, Massachusetts, October 2013). American meetings were evaluated because this review focuses on current preimplantation genetic testing in North America.

ACRONYMS
CGH: Comparative genomic hybridization
ESHRE: European Society of Human Reproduction and Embryology
FISH: Fluorescence in situ hybridization
IVF: In vitro fertilization
MHC: Major histocompatibility complex
PCR: Polymerase chain reaction
SART: Society for Assisted Reproduction Technology
SNP: Single nucleotide polymorphism

Incidence and prevalence
The true utilization rate of preimplantation genetic testing is difficult to determine internationally. The European Society of Human Reproduction and Embryology Preimplantation Genetic Diagnosis (ESHRE-PGD) Consortium, established in 1997, attempts to track preimplantation genetic testing done on an international scale. According to its most recent report, there were a total of 6160 preimplantation genetic testing cycles from December 2009 to October 2010, of which 3551 (58%) were for screening and 2609 (42%) for diagnosis.14 This is a substantial increase from the previous report of 31 288 total preimplantation genetic testing cycles for the 10 years between January 1997 and December 2007.10
Preimplantation genetic screening: Evaluates whether aneuploidy is present in cell(s) obtained from embryos or polar bodies from parents thought to be genetically normal

Preimplantation genetic diagnosis: Evaluates whether a cell biopsied from a developing embryo contains a genetic abnormality associated with a specific medical disorder known to affect one or both parents

Aneuploidy: Any chromosomal copy number other than diploidy (two chromosomal copies) in all 23 chromosome pairs

Euploidy: Diploidy (two chromosomal copies) in all 23 chromosome pairs

In vitro fertilization: The practice of surgically obtaining eggs, fertilizing those eggs with sperm in a laboratory, then transferring the resulting embryos to a uterus to achieve pregnancy

Uniparental disomy: A condition in which both chromosome pairs in an embryo are identical, thus derived from the same parent

Recurrent pregnancy loss: A medical condition, distinct from infertility, when a woman has two or more clinically recognized pregnancy losses before 20 weeks’ gestation

Comparative genomic hybridization microarray: A test that evaluates the ploidy status of all 23 chromosome pairs by comparing the DNA product of a sample with that of a normal control

Single nucleotide polymorphism microarray: A test that evaluates the ploidy status of all 23 chromosome pairs by comparing the DNA product of a sample with that of a normal reference genome

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis determines whether a polar body obtained from an oocyte or from one or more cells biopsied from a developing embryo contains a genetic abnormality associated with a specific medical disorder known to affect one or both parents. \(^9\) \(^18\) \(^24\) It was first used successfully in 1990 to identify the Y chromosome in the embryos of women with known recessive X chromosome linked diseases through DNA amplification of a Y chromosome specific repeat sequence. \(^5\) Female embryos without Y chromosome material were then transferred. Since then, preimplantation genetic diagnosis has been increasingly used to decrease the chances of propagating known genetic disorders. \(^8\) \(^16\)

In single gene disorders, preimplantation genetic diagnosis is commonly used to detect the specific pathogenic variations within the genetic sequence that are associated with certain phenotypic disease states. \(^9\) \(^17\) \(^23\) Examples include the association of the AF508 mutation and the development of cystic fibrosis. \(^9\) \(^18\) Many such genetic variations have heterogeneous phenotypic presentations in different people owing to variable penetrance and expression. \(^22\) Nonetheless, it is appropriate to offer preimplanta-

the United States, the Society for Assisted Reproduction Technology (SART) reported that 5% of the 165 172 cycles performed in 2012 used preimplantation genetic testing, \(^13\) compared with 4% in 2007. \(^4\) However, ESHRE and SART cannot capture all cycle data and serve primarily as estimates of trends in usage. \(^4\) \(^12\)

Preimplantation genetic screening

Preimplantation genetic screening to all patients is currently considered inappropriate. The value of the universal application of preimplantation genetic screening to all patients is currently unclear. Clinics that currently offer preimplantation genetic screening often recommend that it is used in select patient populations, such as:

- Couples with unexplained recurrent pregnancy loss or those with recurrent aneuploidy in their miscarriages
- Repeated implantation failure during IVF cycles
- Men with severe male factor infertility
- Couples already undergoing preimplantation genetic diagnosis testing
- Couples undergoing IVF who desire single embryo transfer

Box 1 | Use of preimplantation genetic diagnosis

Situations in which preimplantation genetic diagnosis is currently considered appropriate

Autosomal recessive diseases in which both parents are known genetic carriers, such as cystic fibrosis, Tay-Sachs disease, or sickle cell disease

Autosomal dominant diseases, such as Huntington’s disease, in one or both parents

Certain known genetic mutations with important consequences, such as mutation of the BRCA gene

X linked diseases, such as hemophilia

Certain balanced chromosomal translocations or inversions

Situations in which preimplantation genetic diagnosis is not currently considered appropriate

Medical conditions in parent(s) where a definitive genetic cause has not been identified

Evaluation of certain phenotypic traits, such as hair color

Situations in which preimplantation genetic diagnosis is currently considered controversial

Sex selection for the purposes of “family balancing”

HLA matching for the purposes of creating a tissue donor for an existing diseased sibling

Certain cases with severe male factor infertility

Box 2 | Use of preimplantation genetic screening

The value of the universal application of preimplantation genetic screening to all patients undergoing in vitro fertilization (IVF) is currently unclear. Clinics that currently offer preimplantation genetic screening often recommend that it is used in select patient populations, such as:

- Couples with unexplained recurrent pregnancy loss or those with recurrent aneuploidy in their miscarriages
- Repeated implantation failure during IVF cycles
- Men with severe male factor infertility
- Couples already undergoing preimplantation genetic diagnosis testing
- Couples undergoing IVF who desire single embryo transfer
ing by using a modified whole genome amplification protocol. A recent technology, known as karyomapping, which uses single nucleotide polymorphism (SNP) genetic haplotyping, can also be used to diagnose single gene disorders. Because karyomapping uses haplotypes of genotyped alleles, a single gene segregating disorder can be diagnosed without actually knowing the specific gene mutation.

Finally, to confirm that DNA from both the sperm and egg have been properly amplified, ESHRE guidelines recommend using a modified linkage analysis assay that identifies polymorphic markers in both male and female samples of the chromosome where the gene mutation resides. This modified linkage analysis reduces the chances of allele drop-out by confirming polymorphic markers that closely flank the tested gene mutation. Allele drop-out may cause erroneous results owing to the failure of all genetic material to be amplified during PCR and is the leading cause of a single gene misdiagnosis. Some gene expansion disorders, such as fragile X, also use a traditional linkage analysis. This approach incorporates genetic data from multiple generations of the family to identify the mutant X chromosome that contains the pathogenic repeat sequence of interest.

Structural chromosomal aberrations

Preimplantation genetic diagnosis can also be used in parents with known structural chromosomal aberrations. Such aberrations may be present in the form of translocations (either reciprocal or robertsonian) or inversions (mainly pericentric, but to a lesser degree paracentric).

Reciprocal translocation typically involves the breakage and reunion of two different chromosomes with exchange of the acentric terminal segments. Robertsonian translocations involve the fusion of two acrocentric chromosomes and the loss of the short arms of these chromosomes. The short arms of acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22) are thought to contain little genetic information of clinical relevance. Chromosome inversions have two breaks in the same chromosome, either in the same arm (paracentric) or one in each arm (pericentric), with inversion of the segment between the breakpoints.

People with such structural chromosomal aberrations generally have a normal phenotype because all of the necessary genetic coding is present, even though it is not arranged in the standard manner. These aberrations are therefore referred to as “balanced” translocations or inversions. However, the offspring of these people are at higher risk of having “unbalanced” translocations or inversions.

The chances of a child having an unbalanced karyotype depend on the type of parental structural chromosomal aberration and possibly the sex of the parent carrier. Unbalanced translocations in offspring generally result in a failed pregnancy or serious defects after birth.

Structural chromosomal aberrations are present in less than 1% of phenotypically normal adults but are detected in one partner in 2-5% of couples with a history of recurrent pregnancy loss. However, most North American experts and professional societies recommend evaluating parental karyotypes as part of the diagnostic investigation of couples experiencing recurrent pregnancy loss.

Diagnostic platforms

In the setting of recurrent pregnancy loss and an identified structural chromosomal aberration, preimplantation genetic diagnosis for structural chromosomal imbalances is considered appropriate at many reproductive centers and should be discussed with the patient. Fluorescence in situ hybridization (FISH) has traditionally been used in this setting. Most FISH platforms use centromeric and telomeric probes and cannot differentiate between normal chromosomes and balanced rearrangements. FISH does not use DNA amplification so does not introduce errors originating from the amplification process, but it does have serious limitations. These include errors that stem from hybridization, which can lead to the over or under scoring of specific fluorophore signals. Because of the technically demanding nature of the procedure, errors are more likely when the person performing the technique is not skilled in conducting FISH assays.

In addition, FISH generally does not evaluate the ploidy status of chromosomes that are not part of the known structural aberration. In many patients with such aberrations, embryos may be balanced for the chromosomal aberration in question but still harbor aneuploidy on other chromosomes. Data from ESHRE show a disappointing clinical pregnancy rate (<30%) after the use of preimplantation genetic diagnosis, primarily with FISH, for translocations or inversions.

Many genetics laboratories have recently shifted towards the use of microarrays, either SNP microarrays or comparative genomic hybridization (CGH) microarrays, to evaluate the ploidy status of all 23 chromosome pairs when performing preimplantation genetic diagnosis for chromosomal aberrations. Although this approach does not identify balanced chromosomal errors, it does identify unbalanced errors and aneuploidy in all 23 chromosomes pairs. Retrospective and small prospective studies of microarray preimplantation genetic diagnosis for structural chromosomal aberrations have reported encouraging results, with clinical pregnancy rates exceeding 60%.

Other applications

Some other applications remain controversial. Preimplantation genetic diagnosis can be used for HLA typing. The short arm of chromosome 6 holds a cluster of genes that encode the major histocompatibility complex (MHC) and its HLA family of genes. HLA genes encode cell surface proteins that play a crucial role in the immune response. Certain MHC alleles have a greater or lesser frequency in particular haplotypes than would be expected if all MHC alleles were at genetic equilibrium. This phenomenon, known as linkage disequilibrium, may reflect geographic origins and ethnic mating patterns, selection of certain haplotypes over others, the recent origins of some alleles, or the suppression of genetic
a child affected by an illness that would benefit from tissue or organ transplantation can undergo IVF with pre-implantation genetic diagnosis to “find” an HLA matched embryo.2 5 The purpose of this process is for the parents to produce an HLA matched sibling who could serve as a donor for the affected child. Although this practice is relatively uncommon, it is beset with considerable ethical, moral, and legal questions. Currently, there is no consensus among experts or professional societies that preimplantation genetic diagnosis for HLA typing is appropriate in most instances.2 5 46 47

recombination operating in some haplotypes. Because of the close proximity of these MHC genes, HLA patterns are often inherited “en block” from parent to child. In most cases, two siblings with the same parents have a 25% chance of having the same HLA genes.46 47

HLA encoded proteins dictate much about how the immune system responds to a given cell surface. Consequently, people who receive transplanted human tissues or organs from HLA matched donors have a significantly better clinical course than those who receive non-HLA matched tissues or organs.1 3 46 47 Currently, parents with a child affected by an illness that would benefit from tissue or organ transplantation can undergo IVF with pre-implantation genetic diagnosis to “find” an HLA matched embryo.2 5 The purpose of this process is for the parents to produce an HLA matched sibling who could serve as a donor for the affected child. Although this practice is relatively uncommon, it is beset with considerable ethical, moral, and legal questions. Currently, there is no consensus among experts or professional societies that preimplantation genetic diagnosis for HLA typing is appropriate in most instances.2 5 46 47

Fig 1 | The same chromosomally normal (diploid) embryonic DNA evaluated by comparative genomic hybridization (CGH) microarray and next generation sequencing. The CGH microarray image shows a diploid genome with a relatively equal ratio of green and red fluorescence in all 23 pairs of chromosomes. The next generation sequencing image also shows no deviation upwards or downwards in chromosomes 1-22. It shows actual copy number and is consistent with a euploid karyotype in chromosomes 1-22. For the sex chromosomes, one copy of X and one copy of Y is seen, represented by a deviation downward for these chromosomes.
Sex selection is another controversial application. Also known as “family balancing,” this practice involves couples, many of whom are not infertile, undergoing an IVF cycle followed by preimplantation genetic diagnosis for selection of a particular sex. The embryos of the desired sex are then preferentially transferred into the uterus. This practice is currently legal in many countries, such as the US, but is illegal in others, such as China. The fate of the embryos that are not selected remains an ethical and moral dilemma; many couples resolve this predicament by anonymously donating the embryos to an infertile couple. There is no consensus among experts or professional societies that preimplantation genetic diagnosis for family balancing is appropriate in most instances.

The propensity to develop medical disorders can increasingly be linked to certain genetic patterns. Similarly, phenotypic traits, such as hair color, are associated with certain identifiable sequences. As genetic diagnostic capabilities continue to improve and our understanding of how genetics affects phenotype and the development of disease, it is possible that preimplantation genetic diagnosis could be used in these situations. The ethical, moral, and legal implications of such testing are not well defined.

Clinical guidelines
Although carriers of a recessive condition and those with a balanced chromosome rearrangement do not have a genetic disease, their offspring may have an increased risk of being affected. Preimplantation genetic diagnosis can help these people obtain the same chance of having a healthy child as the general population. The technique is one of several reproductive options available, including gamete donation and adoption.

The use of preimplantation genetic diagnosis to avoid the propagation of an identified parental disorder of genetic origin is recognized by international professional societies as an appropriate medical procedure. It is likely to become more common in the future, particularly in nations with considerable healthcare resources. However, in many countries, such as the US, financial resources to pay for such testing may not be available to all patients. Regardless of the patient’s perceived ability to pay for the procedure, preimplantation genetic diagnosis should be explained to eligible patients by an appropriate healthcare professional. Box 1 outlines the appropriate, inappropriate, and controversial uses of preimplantation genetic diagnosis.

Future directions
The number of patients eligible for preimplantation genetic diagnosis will probably increase in the coming decades as the number of diseases with an identifiable genetic cause continues to rise. Currently, many of the mutations that are evaluated by preimplantation genetic diagnosis lead to specific syndromes, such as cystic fibrosis. However, many common conditions, such as breast cancer, hypertension, and diabetes, are now known to be associated with certain genetic sequences or mutations. In the future, preimplantation genetic diagnosis might be used to detect genetic sequences or mutations that predispose towards certain diseases.

Preimplantation carrier screening
Preconception carrier screening for genetic disorders (including many uncommon autosomal recessive disorders) is also becoming more common. Over the past decade many professional societies have recommended that such testing is offered to all patients regardless of ethnicity. This universal screening will result in the identification of more patients with genetic abnormalities that would be suitable for preimplantation genetic diagnosis. Originally, guidelines recommended screening certain ethnic groups for specific genetic disorders on the basis of the frequency of the carrier state and the severity of the disorder; however, such ethnic guidelines are being challenged by professional societies, including the American College of Obstetrics and Gynecology.

Preimplantation genetic screening
Preimplantation genetic screening evaluates whether aneuploidy is present in cell(s) obtained from embryos or polar bodies from parents thought to be genetically normal. Unlike preimplantation genetic diagnosis, this practice is controversial. Aneuploidy is defined as any chromosomal copy number other than diploidy in all 23 chromosome pairs. Aneuploidy is common in developing human embryos, and examples include trisomy (an extra chromosome copy) and monosomy (a missing copy). Current data suggest that aneuploidy occurs in many, perhaps most, embryos. Aneuploidy is the leading single cause of early pregnancy loss and it often leads to developmental arrest of the embryo before uterine implantation even occurs.

Preimplantation genetic screening attempts to identify embryos with euploid (diploid) karyotypes, which are selected for uterine transfer, thereby increasing the efficiency of IVF per embryo transfer. Because the procedure is a diagnostic intervention, it does not necessarily improve the pregnancy and miscarriage rates per IVF retrieval, including cumulative transfer of all embryos through multiple frozen embryo transfers. However, proponents of this procedure maintain that it may increase pregnancy rates per embryo transfer performed, resulting in a shorter time to conception, a decreased chance of miscarriage per pregnancy obtained, and in some cases a financial benefit through increased efficiency. Opponents of the procedure maintain that insufficient evidence exists to justify the broad application of this technique.

Available tests
FISH
FISH was the first genetic test used in preimplantation genetic screening. FISH has several advantages, including a rapid turnaround time for evaluating samples (4–10 h). In addition, FISH does not require DNA amplification, so errors introduced by amplification, such as allele drop-out, are not present. FISH results can be erroneous, however, because of hybridization errors or errors in the subjective interpretation of fluorescent signals.
Another serious limitation of FISH is its inability to evaluate the ploidy status of all 23 chromosome pairs.\textsuperscript{5,42,77}

Microarrays

CGH and SNP microarrays are increasingly used for preimplantation genetic screening. Microarray platforms have the advantage of simultaneously evaluating the ploidy status of all 23 chromosome pairs. However, CGH and SNP microarrays are distinct technologies and each has its own advantages and disadvantages.

CGH microarrays compare the DNA product of a sample with that of a normal control. CGH on metaphase chromosomes was an early application of this technology.\textsuperscript{5,40,79-81} DNA from the biopsied embryonic cells and a normal DNA sample are amplified and the resulting DNA products hybridized with a series of site specific fluorophores on a microarray chip.\textsuperscript{5,83-85} A computer then compares the intensity of each fluorophore in the sample versus the control to determine the ploidy status of the sample.\textsuperscript{5,85}

CGH microarrays are relatively quick to perform, with results usually available 12 hours after receipt of the cells by the genetics laboratory.\textsuperscript{5,40} Disadvantages include possible errors introduced during amplification,\textsuperscript{5,31,83} and the inability of CGH to detect triploidy—that is, the presence of trisomy in all chromosomes. In addition, CGH cannot detect uniparental disomy, a condition in which both chromosome pairs are identical, because CGH microarrays use ratio labeling without obtaining the genotype.\textsuperscript{5}

SNP microarrays also amplify embryonic DNA obtained from embryo biopsies before analysis.\textsuperscript{5,17} This DNA product is then hybridized on to a microarray chip at certain SNP specific sites. The hybridized sites have activated fluorochromes that give out light signals read by a computer scanner. The intensity of these light signals allows genotypic determinations for each given SNP, which are then graphically displayed as a histogram.\textsuperscript{5,59} Unlike CGH microarrays, SNP microarrays do not use a known normal DNA sample but compare results with a normal reference genome (human hapmap).

An advantage of SNP microarrays for preimplantation genetic screening is their ability to detect relatively small deletions and duplications while simultaneously evaluating aneuploidy in all 23 chromosome pairs.\textsuperscript{5,59,86,87} In addition, because SNP microarrays obtain a specific allelic genotype, comparison with the parental DNA can identify whether the DNA comes from the mother or father.\textsuperscript{88,89} SNP microarrays can also detect uniparental disomy.\textsuperscript{88,89} However, SNP microarrays may require several days to generate a result. Despite the theoretical advantages of SNP over CGH, clinical pregnancy data from retrospective and prospective trials suggest that the two tests are comparable when used in preimplantation genetic screening.\textsuperscript{8,45,74,78-80,90-92}

Real time PCR

Real time PCR detects copy number variations along a given chromosome and compares this result with a known normal control.\textsuperscript{5,78} This technology can rapidly evaluate 23 chromosome pair aneuploidy (4-6 hours).\textsuperscript{78} However, real time PCR tests a relatively small number of loci along each chromosome and is laborious, making it difficult to evaluate multiple samples simultaneously.\textsuperscript{5} This technique cannot detect structural chromosomal aberrations or uniparental disomy,\textsuperscript{5} although it can identify triploidy.\textsuperscript{5} Encouraging clinical pregnancy rates have been seen with the use of real time PCR in preimplantation genetic screening.\textsuperscript{8,45,74,78-80,90-92}

Comparisons between CGH, SNP, and real time PCR analysis have shown that each technique provides accurate preimplantation genetic screening results.\textsuperscript{5,45,74,78-80,90-92}
**STATE OF THE ART REVIEW**

**EMBRYOGENESIS**

<table>
<thead>
<tr>
<th>Days after fertilization</th>
<th>Embryo stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2 cells</td>
</tr>
<tr>
<td>1</td>
<td>Cleavage stage</td>
</tr>
<tr>
<td>2</td>
<td>Compacting morula stage</td>
</tr>
<tr>
<td>3</td>
<td>Expanded blastocyst stage</td>
</tr>
<tr>
<td>4</td>
<td>Blastocyst stage</td>
</tr>
</tbody>
</table>

**Fig 3** The course of early embryogenesis from a two cell embryo the day after fertilization, to a cleavage stage embryo three days after fertilization, a compacting morula stage embryo four days after fertilization, and finally an expanded blastocyst stage embryo five to six days after fertilization with a clear distinction between the trophectoderm and inner cell mass populations.

**Fig 4** (A) Cleavage stage embryo and (B) blastocyst stage embryo.

**Next generation sequencing**

Next generation sequencing can also be used for 23 chromosome pair preimplantation genetic screening (figs 1 and 2). This technique amplifies embryonic DNA and compares millions of fragmented DNA sequences with a reference genome (human hapmap hg19). This technique can evaluate specific DNA sequences along each chromosome and also determine single (or multiple) gene mutations. Next generation sequencing can therefore be used concurrently for preimplantation genetic screening and preimplantation genetic diagnosis when parental genetic mutations are present.

An increasing number of genomic variations have been associated with certain disease states. These may or may not be pathogenic, and further research is needed to determine their clinical significance. The proper use of preimplantation genetic screening with concurrent determination of these other sequences is currently unclear. The broad diagnostic applications of next generation sequencing make the increasing use of this technology likely in the future.

**Clinical efficacy**

**Questionable efficacy of preimplantation genetic screening with FISH**

A prospective randomized trial published in 2007 cast doubt on the ability of preimplantation genetic screening to improve clinical pregnancy rates. Preimplantation genetic screening using FISH analysis of eight chromosomes was performed on cleavage stage embryos from women aged 35-41 undergoing IVF. The study found that screening had a deleterious effect on the ongoing pregnancy rate, which was 25% in women undergoing preimplantation genetic screening and 37% in women not undergoing the procedure (rate ratio 0.69, 0.51 to 0.93). The live birth rate was also lower in the preimplantation genetic screening group (24% v 35%; 0.68, 0.50 to 0.92) compared with controls. Similar results have been reported in several subsequent clinical trials that evaluated preimplantation genetic screening using FISH on cleavage stage embryos. Furthermore, some have questioned whether FISH can consistently identify sporadic chromosomal aneuploidy from a single cell.

These trials led to recommendations by professional societies internationally to discourage the use of such screening.

**Evaluation of all blastocyst stage biopsies**

Since the 2007 trial, the testing platforms and methods of embryo biopsy used by many clinics to perform preimplantation genetic screening have been improved. For example, embryo biopsy is increasingly being performed at the blastocyst (figs 3 and 4) stage of development rather than the cleavage stage. Clinical data show that biopsy at the cleavage stage confers a substantial insult to developing embryos, resulting in slower development and a higher chance of embryo death.

Data also suggest that the level of mosaicism within embryos may be higher at the cleavage stage than the blastocyst stage of development. Such mosaicism increases the embryonic misdiagnosis rate even when cellular diagnosis is correct. In addition, some experts believe that the removal of a euploid cell from a mosaic cleavage stage embryo may result in a higher aneuploid cellular load, which could have further deleterious effects. Clinical trials suggest that pregnancy rates are higher when trophectoderm biopsy for preimplantation genetic screening occurs at the blastocyst stage rather than the cleavage stage. Previous data that showed no benefit with preimplantation genetic screening using biopsy at the cleavage stage rather than the blastocyst stage.

**Evaluation of all 23 chromosome pairs**

Aneuploidy occurs on all 23 chromosome pairs in early human embryos. The presence of a single error generally results in failure to produce a viable pregnancy. Therefore, technologies that determine the ploidy status of a limited number of chromosomes, such as FISH, are inferior to those that simultaneously evaluate all 23 chromosome pairs. Because FISH typically evaluates fewer than half of all 23 chromosome pairs, it could easily miss an aneuploidy error on untested chromosomes.
Data have shown no benefit of screening with FISH diagnostic platforms that do not evaluate the ploidy status of all 23 chromosome pairs.\(^{97,99}\)

Multiple preimplantation genetic screening testing platforms, including SNP and CGH microarrays and next generation sequencing, are currently used to detect aneuploidy in all 23 chromosome pairs. Retrospective and prospective clinical data on preimplantation genetic screening show that pregnancy rates are consistently higher for genetic analysis platforms that evaluate all 23 chromosome pairs compared with FISH technology.\(^{2,5,44,75,78,81,91,110,111}\)

**Current clinical data**

As mentioned previously, preimplantation genetic screening on a cleavage stage cell embryo by FISH is deleterious.\(^{77,99}\) In the post-FISH era, however, retrospective and prospective trials have reported improved clinical outcomes, both in terms of increased pregnancy rates and decreased miscarriage rates, using screening with trophectoderm biopsy and evaluation of 23 chromosome pair aneuploidy.\(^{8,74,78,80,93,110,112,113}\) Despite this, some experts maintain that there is still not enough evidence to support the broad application of preimplantation genetic screening.\(^{96}\)

Some experts point out that many of these trials evaluated an idealized patient population with a relatively high egg yield at the time of oocyte retrieval. This could result in a blunting of the true benefit to a broader population because it would mitigate the impact of false positive (normal embryos that were diagnosed as abnormal) results.

Retrospective and prospective data discourage the use of screening with FISH.\(^{76,97,99,117}\) Although each technique used to evaluate 23 chromosome pair aneuploidy has its advantages and disadvantages, currently there is no clinical evidence of the superiority of one method in terms of increased pregnancy rates.\(^{8,80}\) Trophectoderm biopsy of blastocyst embryos is preferred over cleavage stage or polar body biopsy.\(^{7}\) Therefore, if a clinical decision is made to proceed with preimplantation genetic screening, trophectoderm biopsy and a genetic platform that can detect aneuploidy in all 23 chromosome pairs should be used.

Data suggest that IVF pregnancy rates are higher in transfer cycles with frozen embryos rather than fresh embryos, especially in the context of aggressive controlled ovarian hyperstimulation.\(^{115-118}\) A concern among some experts is that part of the success seen with trials of preimplantation genetic screening may be an artifact of frozen embryo transfer being commonly used after blastocyst biopsy.\(^{76}\)

However, a recent prospective randomized controlled trial compared clinical pregnancy rates using preimplantation genetic screening with 23 chromosome pair evaluation with fresh transfer to a control group and found significantly higher implantation rates (79.8% vs 63.2%; relative risk 1.26, 1.04 to 1.39) and delivery rates per treatment cycle (66.4% vs 47.9%; 1.39, 1.07 to 1.60).\(^{78}\)

Healthcare costs are consistently lower for single embryo transfer than for multiple embryo transfer owing to reduced obstetrical and neonatal costs.\(^{119,120}\) Recent prospective trials have shown a benefit for preimplantation genetic screening when single embryo transfer is used in patients with a good prognosis.\(^{74,78,79,80}\) In addition, data suggest a cost benefit for preimplantation genetic screening in women with a history of recurrent pregnancy loss when compared with the use of IVF alone.\(^{81}\)

**Clinical guidelines**

Preimplantation genetic screening is commonly used in reproductive medicine. According to ESHRE, of 27 630 IVF cycles that used preimplantation genetic testing over a 10 year period, 16 806 (61%) were performed for screening. Nonetheless, because of the past shortcomings with cleavage stage biopsy and FISH, many experts and professional societies are unwilling to endorse the use of preimplantation genetic screening. Currently, the official recommendations from many professional societies discourage its use.\(^{61,62,66}\) However, as data supporting the use of preimplantation genetic screening accumulate, reevaluation of the technology on a large and international scale seems likely.\(^{6}\)

It is still unclear which clinical populations might benefit from preimplantation genetic screening. Because no published guidelines recommend screening, there are no accepted guidelines that define the appropriate patient population.\(^{7}\) The ESHRE preimplantation genetic diagnosis consortium, which reviewed 10 years of data, reported that the most common indications cited by physicians for performing preimplantation genetic screening for aneuploidy were advanced maternal age, repeated implantation failure in IVF cycles, recurrent pregnancy loss, and severe male factor infertility.\(^{10}\)

The 2007 study evaluated women aged 35-42 years, not women with recurrent pregnancy loss.\(^{26}\) A recent prospective randomized controlled trial compared clinical pregnancy rates in infertile women aged 21-42 years using preimplantation genetic screening with 23 chromosome evaluation versus fresh transfer to a control group and found significantly higher implantation rates and pregnancy rates in the screening group.\(^{78}\)
In the past, preimplantation genetic screening was principally offered to certain patient subsets including those affected by advanced maternal age, repeated implantation failure in IVF cycles, unexplained recurrent pregnancy loss, recurrent pregnancy loss secondary to parental chromosomal aberrations, recurrent fetal aneuploidy, or severe male factor infertility. However, clear evidence for limiting preimplantation genetic screening to these groups is lacking. Recent data previously discussed seem to show a broad benefit for infertile couples in general. More data and time are probably needed before experts reach a consensus regarding the application of preimplantation genetic screening. Box 2 outlines the appropriate uses of preimplantation genetic diagnosis.

**Preimplantation genetic testing: risk of misdiagnosis**

As with all clinical diagnostic tests a chief concern is misdiagnosis. All causes of misdiagnosis may lead to false positive and false negative results. A false positive result has a deleterious effect on achieving pregnancy because a healthy embryo is not transferred. In the case of a false negative result, an abnormal embryo is selected for uterine transfer, possibly resulting in a child with a genetic defect. There are two categories of misdiagnosis within preimplantation genetic testing—cell misdiagnosis and embryo misdiagnosis in the setting of correct cell diagnosis. These misdiagnoses occur as the result of biological, technological, and methodological factors (box 3).

**Cell misdiagnosis**

Cell misdiagnosis refers to a genetic result (diagnosis) that is different from the true genetic code of the analyzed sample. Similar to many other types of genetic diagnostic testing, this type of error occurs for several reasons. Causes range from human error, such as mislabeling or contamination, to errors introduced through the diagnostic methods and techniques. All highly complex laboratories have multiple safeguards and protocols to minimize these errors, so the rate of cellular misdiagnosis is thought to be relatively low in most leading laboratories.

Many types of preimplantation genetic testing require the amplification of extremely small quantities of DNA from either one or a few embryonic cells. Consequently, if this process is not successful a quantifiable result is not obtainable. This phenomenon, known as failed amplification, occurs in less than 5% of samples. Nonetheless, genetics laboratories are constantly improving DNA amplification techniques to reduce the number of samples with failed diagnosis readings.

**Embryo misdiagnosis in the setting of correct cell diagnosis**

As already mentioned, embryonic mosaicism is thought to be common in early human embryos. Although the rate of mosaicism is minimized by the use of trophectoderm biopsy, the rate of karyotypic discordance between the trophectoderm and inner cell mass is 2-4%. Therefore, even with the best technology and optimal timing of preimplantation genetic biopsy, the biology of early human embryogenesis can result in discordance between the fetal karyotype and the analyzed cells obtained at biopsy. In these cases, the cell analyzed is correctly diagnosed. However, the result is not clinically accurate. Similarly, embryos with high levels of genetic mosaicism, such as those with Turner’s syndrome, could be misdiagnosed in the setting of correct cell diagnosis.

Importantly, trophectodermal cells are trophoblastic in origin and preimplantation genetic testing of these cells is analogous to chorionic villus sampling, which is accepted as a standard of care procedure. Hence, preimplantation genetic testing (with testing on chorionic villus cells) will always have a level of clinical misdiagnosis, even in the presence of accurate cellular genetic analysis. Those delivering medical care, including physicians, geneticists, and counselors, must advise patients of these inherent risks to preimplantation genetic testing.

Throughout this process it is strongly recommended that a healthcare professional with expert knowledge about preimplantation genetic testing and genetics counsels prospective patients about these risks before proceeding with preimplantation genetic testing.

**Looking ahead**

Several well designed prospective trials have shown that preimplantation genetic screening is useful when used to evaluate ploidy status in all 23 chromosome pairs in trophectodermal cells. However, more data are needed...
to define the extent of this benefit and determine which patient populations would benefit most. Many reproductive medicine centers, including ours, are currently conducting in-house prospective trials to answer these questions. However, we are unaware of any large, multicenter, and well funded studies looking at these questions. Such studies will probably be needed before any consensus is reached on how to use preimplantation genetic screening responsibly.

Several relatively non-invasive technologies have also been introduced that attempt to identify optimal embryos for uterine transfer, thus increasing the efficiency of IVF. Metabolomics is the practice of analyzing the biochemistry of fluid surrounding a developing embryo. Certain chemical patterns of embryonic metabolites have been hypothesized to be associated with healthy embryos. Although the results of research on metabolomics in the context of IVF are promising, clear data consistently showing predictive efficacy in a prospective manner are lacking. Similarly, some centers have evaluated whether certain cellular division patterns on time lapse videography may help identify which embryos should be used for uterine transfer. Emerging data suggest that some patterns may confer a selection benefit when specific criteria are used.

Preimplantation genetic screening, metabolomics, and time lapse videography all attempt to increase the efficiency of IVF through selecting the best embryos for uterine transfer. The process of early embryogenesis is dynamic. Many embryos that are generated during IVF are not compatible with life as a result of aneuploidy or other serious developmental defects. Science is now capable of detecting some of these problems. Preimplantation genetic screening directly identifies aneuploidy through genetic analysis, while metabolomics and time lapse videography attempt to evaluate indirect evidence of embryo development and health. As the science surrounding these technologies continues to advance, in the future, not one, but a combination of these approaches will probably be used to optimize the efficiency of IVF.

Conclusion
Preimplantation genetic testing has given the field of reproductive medicine a dynamic tool. As science advances and the correlation between genetic code and disease states will probably be used to optimize the efficiency of IVF.

The authors would like to acknowledge William G Keams, Amelila Bailey, Raymond W Ke, Jianchi Ding, James Klosky, and Jennifer Brezina for their help in reviewing this manuscript.

Contributors: PRB and WHK primarily searched the literature. PRB wrote the first draft of the manuscript; WHK advised on the content of the manuscript regarding generalist recurrent pregnancy loss, helped in the literature search, and contributed to the writing of the manuscript. PRB oversaw all genetic aspects of this review and contributed to the writing and editing of this manuscript. PRB is guarantor.

Competing interests: We have read and understood BMJ policy on declaration of interests and have none to declare.

Provenance and peer review: Commissioned; externally peer reviewed.

For personal use only
STATE OF THE ART REVIEW

81. Resekova N, Tobler K, Kearns WG, Werner EF. In vitro fertilization (IVF) with a 3-chromosome pair preimplantation genetic screening (PGS) is cost-effective to achieve a live birth compared to IVF alone for recurrent pregnancy loss (RPL). Fertil Steril 2011;96:536-40.


86. Christiansen MS, Brenna PR, Berner AT, Du L, Siegel A, Kearns WG. Chromosomal duplications (≤200 KILOBASES (KB)) are more common than deletions ≥200 KB in developing human embryos as identified by 23 chromosome single nucleotide polymorphism (SNP) microarray. Fertil Steril 2011;96:3 (suppl) S21-2.


89. Tobler KJ, Brenna PR, Berner AT, Du L, Boyd B, Kearns WG. Two different microarray technologies for preimplantation genetic diagnosis (PGD) and screening (PGS), due to reciprocal translocation imbalances, demonstrate equivalent clinical pregnancy rates. Fertil Steril 2013;100:suppl 5, S36.


