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Technical Update: Preimplantation Genetic Diagnosis and Screening

This technical update has been prepared by the Genetics Committee and approved by the Executive and Board of the Society of Obstetricians and Gynaecologists of Canada.

PRINCIPAL AUTHORS

Elias M. Dahdouh, MD, Montreal QC

Jacques Balayla, MD, Montreal QC

François Audibert, MD, Montreal QC

GENETICS COMMITTEE

R. Douglas Wilson, MD (Chair), Calgary AB

François Audibert, MD, Montreal QC

Jo-Ann Brock, MD, Halifax NS

Carla Campagnolo, MSc, London ON

June Carroll, MD, Toronto ON

Karen Chong, MD, Toronto ON

Alain Gagnon, MD, Vancouver BC

Jo-Ann Johnson, MD, Calgary AB

William MacDonald, MD, Squamish BC

Nanette Okun, MD, Toronto ON

Melanie Pastuck, RN, Cochrane AB

Karine Vallée-Pouliot, RM, Montreal QC

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Abstract

Objective: To update and review the techniques and indications of preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS).

Options: Discussion about the genetic and technical aspects of preimplantation reproductive techniques, particularly those using new cytogenetic technologies and embryo-stage biopsy.

Outcomes: Clinical outcomes of reproductive techniques following the use of PGD and PGS are included. This update does not discuss in detail the adverse outcomes that have been recorded in association with assisted reproductive technologies.

Evidence: Published literature was retrieved through searches of The Cochrane Library and Medline in April 2014 using appropriate controlled vocabulary (aneuploidy, blastocyst/physiology, genetic diseases, preimplantation diagnosis/methods, fertilization in vitro) and key words (e.g., preimplantation genetic diagnosis, preimplantation genetic screening, comprehensive chromosome screening, aCGH, SNP microarray, qPCR, and embryo selection). Results were restricted to systematic reviews, randomized controlled trials/controlled clinical trials, and observational studies published from 1990 to April 2014. There were no language restrictions. Searches were updated on a regular basis and incorporated in the update to January 2015. Additional publications were identified from the bibliographies of retrieved articles. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies.

Values: The quality of evidence in this document was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care. (Table 1)

Benefits, harms, and costs: This update will educate readers about new preimplantation genetic concepts, directions, and technologies. The major harms and costs identified are those of assisted reproductive technologies.

Summary: Preimplantation genetic diagnosis is an alternative to prenatal diagnosis for the detection of genetic disorders in couples at risk of transmitting a genetic condition to their offspring. Preimplantation genetic screening is being proposed to improve the effectiveness of in vitro fertilization by screening for embryonic aneuploidy. Though FISH-based PGS showed adverse effects on IVF success, emerging evidence from new studies using

Table 1. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

Quality of evidence assessment*	Classification of recommendations†
I: Evidence obtained from at least one properly randomized controlled trial	A. There is good evidence to recommend the clinical preventive action
II-1: Evidence from well-designed controlled trials without randomization	B. There is fair evidence to recommend the clinical preventive action
II-2: Evidence from well-designed cohort (prospective or retrospective) or case–control studies, preferably from more than one centre or research group	C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making
II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category	D. There is fair evidence to recommend against the clinical preventive action
	E. There is good evidence to recommend against the clinical preventive action
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	L. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making

*The quality of evidence reported in here has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.[89]

†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the Canadian Task Force on Preventive Health Care.[89]

comprehensive chromosome screening technology appears promising.

Recommendations

1. Before preimplantation genetic diagnosis is performed, genetic counselling must be provided by a certified genetic counsellor to ensure that patients fully understand the risk of having an affected child, the impact of the disease on an affected child, and the benefits and limitations of all available options for preimplantation and prenatal diagnosis. (III-A)
2. Couples should be informed that preimplantation genetic diagnosis can reduce the risk of conceiving a child with a genetic abnormality carried by one or both parents if that abnormality can be identified with tests performed on a single cell or on multiple trophectoderm cells. (II-2B)
3. Invasive prenatal or postnatal testing to confirm the results of preimplantation genetic diagnosis is encouraged because the methods used for preimplantation genetic diagnosis have technical limitations that include the possibility of a false result. (II-2B)
4. Trophectoderm biopsy has no measurable impact on embryo development, as opposed to blastomere biopsy. Therefore, whenever possible, trophectoderm biopsy should be the method of choice in embryo biopsy and should be performed by experienced hands. (I-B)
5. Preimplantation genetic diagnosis of single-gene disorders should ideally be performed with multiplex polymerase chain reaction coupled with trophectoderm biopsy whenever available. (II-2B)
6. The use of comprehensive chromosome screening technology coupled with trophectoderm biopsy in preimplantation genetic diagnosis in couples carrying chromosomal translocations is recommended because it is associated with favourable clinical outcomes. (II-2B)
7. Before preimplantation genetic screening is performed, thorough education and counselling must be provided by a certified genetic counsellor to ensure that patients fully understand the limitations of the technique, the risk of error, and the ongoing debate on whether preimplantation genetic screening is necessary to improve live birth rates with in vitro fertilization. (III-A)
8. Preimplantation genetic screening using fluorescence in situ hybridization technology on day-3 embryo biopsy is associated with decreased live birth rates and therefore should not be performed with in vitro fertilization. (I-E)
9. Preimplantation genetic screening using comprehensive chromosome screening technology on blastocyst biopsy, increases implantation rates and improves embryo selection in IVF cycles in patients with a good prognosis. (I-B)

INTRODUCTION

Given the advent of new cytogenetic techniques, the practice of prenatal diagnosis has seen major advances in both obstetrical and reproductive sciences over the last decade.[1] Though amniocentesis and CVS have been the mainstays of traditional prenatal testing, improvements in preimplantation genetic diagnosis have revolutionized the world of genetic diagnosis, particularly among patients carrying single-gene disorders or chromosomal translocations.[2,3] Preimplantation genetic testing is the use of reproductive technologies for the genetic analysis of embryos prior to transfer and implantation.[2] This technology was first developed in the late 1980s, when PCR was used to determine the sex of embryos from patients carrying X-linked disorders.[3,4] Such practice allowed for the transfer of only select, unaffected embryos, and thus avoided elective pregnancy termination following conventional prenatal testing.[2,3]

In patients with an hereditary genetic disorder such as a known heritable genetic mutation (single-gene disorder) or when a chromosomal abnormality is carried by either biological parent, the genetic profiling of oocytes and embryos prior to implantation using molecular biology or

cytogenetic techniques is known as *preimplantation genetic diagnosis*.[1] Though controversial, PGD has also been used for gender selection, compatible HLA typing (aiming for a "saviour sibling"), and identifying hereditary cancers with variable penetrance (e.g., BRCA 1, 2 status) and late-onset genetic diseases (e.g., Huntington's Disease), may therefore play an important role in certain clinical and social scenarios.[5–7]

Another application of preimplantation genetic testing exists in the treatment of infertility.[8] Indeed, modern demographics and delayed childbearing have led to the increased use of IVF as a method of conception. Despite numerous advances, live birth rates following eSET IVF cycles range from 27% to 35%, depending on the age group and methodology used.

Factors such as embryo status (chromosomal complement), endometrial receptivity, and transfer efficiency must be considered as potential etiologic causes of this low implantation rate.[9–11] The most likely cause of the low pregnancy rate observed in women undergoing IVF, especially in those of advanced maternal age and recurrent pregnancy loss, is the increased incidence of numerical chromosomal abnormalities (aneuploidy) despite normal embryonic microscopic morphology.[11,12] Consequently, transfer of euploid embryos has been proposed as a way to increase implantation and live birth rates and to decrease early pregnancy loss.[13] The process of embryo genetic testing using cytogenetic techniques for the purposes of de novo aneuploidy screening is known as *preimplantation genetic screening*.[13]

Both PGD and PGS require IVF with or without ICSI, embryo biopsy for DNA sampling, genetic testing, and selected embryo transfer. DNA can be extracted from the oocytes (polar bodies) or from embryonic cells as one blastomere from a cleavage-stage embryo or 5 to 10 trophectoderm cells from a blastocyst-stage embryo.[12,14] The genetic material is then tested for either single-gene mutations, using molecular biology techniques (PCR, PCR-multiplex),[15] or for chromosomal translocation and de novo aneuploidy, using cytogenetic techniques such as FISH or CCS.[13,16,17] The latter is the emerging new cytogenetic technique that consists of identifying the whole chromosomal complement (24 chromosomes).[18,19] CCS can be accomplished through microarray technology such as aCGH and SNP or through qPCR.[17–21] As the cells are being tested, the embryos remain in IVF media culture. If the biopsied cell or cells are shown to be unaffected for the genetic disorder in PGD or to carry a euploid embryo in PGS, then that particular embryo is considered an apt candidate for transfer into the uterus.[15,17–21] The main limitations of preimplantation genetic testing are its low efficacy in achieving implantation and low live birth rates. This might be explained by technical difficulties encountered during IVF procedures, embryo biopsy techniques, embryo culture, and genetic diagnosis.[22] Such low pregnancy rates may be further explained by the transference of a chromosomally abnormal embryo (aneuploid) despite its having tested as free of the genetic disorder in question, such as a single-gene mutation or chromosomal translocation. Testing for a specific gene mutation can be currently be performed in combination with 24-chromosome aneuploidy screening and this is the ideal way to increase pregnancy outcomes in PGD.[23]

The practices of PGD and PGS are complex and invasive, and they might be associated with false-positive or false-negative results. Therefore, a multidisciplinary approach, including the input and coordination of professionals from ART, genetics, high-risk pregnancy, ethics, and psychology must be applied.[24,25]

PGD is currently used to decrease the transmission of genetic disorders to the offspring, and is therefore proposed for carriers of single-gene disorders (dominant and recessive, autosomal or X-linked) and carriers of structural chromosome abnormalities, including but not limited to reciprocal and Robertsonian translocations, inversions, deletions, and insertions.

ABBREVIATIONS

aCGH	array comparative genomic hybridization
ADO	allele dropout
ART	assisted reproductive technology
CCS	comprehensive chromosome screening
CGH	comparative genomic hybridization
CVS	chorionic villus sampling
eSET	elective single embryo transfer
ESHRE	European Society for Human Reproduction and Embryology
FISH	fluorescence in situ hybridization
HLA	human leukocyte antigen
ICSI	intra-cytoplasmic sperm injection
IVF	in vitro fertilization
Mbp	megabase pairs
NGS	next-generation sequencing
PB	polar body
PCR	polymerase chain reaction
PGD	preimplantation genetic diagnosis
PGS	preimplantation genetic screening
PGS-SS	preimplantation genetic screening for sexual selection
qPCR	quantitative real-time PCR
SNP	single nucleotide polymorphism
WGA	whole genome amplification

PGS is currently used in assisted reproduction treatments to enhance pregnancy success with the transfer of euploid embryos, and is therefore proposed for women of advanced maternal age, couples with repeated implantation failure, couples with repeated unexplained miscarriages, and couples with severe male factor infertility.

Recommendations

1. Before preimplantation genetic diagnosis is performed, genetic counselling must be provided by a certified genetic counsellor to ensure that patients fully understand the risk of having an affected child, the impact of the disease on an affected child, and the benefits and limitations of all available options for preimplantation and prenatal diagnosis. (III-A)
2. Couples should be informed that preimplantation genetic diagnosis can reduce the risk of conceiving a child with a genetic abnormality carried by one or both parents if that abnormality can be identified with tests performed on a single cell or on multiple trophectoderm cells. (II-2B)
3. Invasive prenatal or postnatal testing to confirm the results of preimplantation genetic diagnosis is encouraged because the methods used for preimplantation genetic diagnosis have technical limitations that include the possibility of a false result. (II-2B)

DEVELOPMENT OF CYTOGENETIC TECHNIQUES

The earliest trials of PGD involved the use of karyotyping and PCR for the sexing of preimplanted human embryos and the analysis of PBs for Mendelian disease.[4] By the mid-1990s, the use of cytogenetic techniques such as FISH allowed for the preimplantation diagnosis of certain aneuploidies and chromosomal translocations, a process then greatly aided by the sequencing of the human genome. The FISH technique was later shown to impose important technical limitations: only a select number of chromosomes was apt for analysis (maximum of 12 probes); interpretation was often cumbersome because hybridization failure, signal overlap, and splitting affect the accuracy of the output; and more importantly, numerous studies showed no difference in clinical outcomes for this method.[13,26,27] Given these drawbacks, other new cytogenetic techniques were developed, such as aCGH, SNP microarray, and qPCR, which allow for CCS, the screening of all chromosomal material.[18,19,28,29]

Array CGH

The principal aCGH requires labelled DNA from both test and control samples; the labelled DNA is then hybridized to a DNA microarray. The analysis is performed by scanning and imaging the array, then measuring the intensity of both hybridization signals relative to each probe. Finally, a computer program analyzes the data and generates a plot.[30] Originally, the analysis was performed with a microscope using metaphase CGH.[31,32] For accuracy and practical reasons, metaphase CGH was quickly replaced by aCGH. The evaluation by aCGH determines whether any quantitative deviations (extra or missing DNA sequences) exist in the DNA of the test case. Therefore it can detect chromosomal copy number (e.g., trisomies or monosomy) and unbalanced chromosome translocations.[10,33] Balanced chromosome rearrangements such as translocations or inversions (in which genetic material is only rearranged, not lost or gained) cannot be identified by aCGH.

SNP Microarray

An SNP is a DNA sequence variant in which, at a particular position or locus, one of two or more nucleotides may be present on different chromosomes within a population. To date, almost 40 million SNPs have been validated across the genome—mostly in non-coding regions. Most SNP arrays detect 660 000 to 2 million SNPs across the length of all chromosomes. For molecular cytogenetics, analysis of the ratio of the intensity of both alleles at heterozygous loci allows high resolution detection of duplications in, and deletions from, whole chromosomes in small regions. In deletions, loss of heterozygosity is detected by the absence of the heterozygous band.[34,35] SNP arrays also have the advantage that the parental origin of any abnormalities can be investigated by genotyping the parents, allowing the detection of uniparental disomy among others. Because SNP-based approaches provide extra theoretical resolution and parent-of-origin information, they may be particularly suited to certain applications such as PGD of single-gene defects or translocation chromosome imbalance combined with comprehensive detection of aneuploidy. In addition, SNP microarray can distinguish between balanced and normal chromosomes in embryos from a translocation carrier.[34–36]

qPCR

An alternative method for 24-chromosome copy number analysis that uses real-time qPCR was developed and extensively validated.[37] In this method, a preamplification step, followed by a high-order multiplex PCR reaction in a 384-multiwell plate format, is used to amplify at least two sequences on each arm of each chromosome. Real-time qPCR is then used for the rapid quantification of each product, allowing comparison across the genome. The multiplex PCR is performed on the sample directly to avoid amplification bias from whole-genome amplification and ensure accurate copy number analysis;

therefore it is applicable to multiple-cell trophectoderm samples only.[35]

Both aCGH and SNP microarray require WGA prior to application. The qPCR technology has recently been investigated in PGS and has shown improvement in implantation and live birth rates when used in IVF cycles.[17]

The different cytogenetic techniques used in PGD and PGS are outlined in Table 2.

STAGE OF EMBRYO BIOPSY

Embryo biopsy for either PGD or PGS purposes can be done at different embryo developmental stages during IVF procedures. The technique can be accomplished through biopsy of the oocyte (one or two polar bodies), cleavage stage embryo (one blastomere cell), or blastocyst stage embryo (5 to 10 trophectoderm cells).[38]

Polar Body Biopsy

This biopsy technique is usually performed in countries where embryo biopsy is considered illegal (e.g., Italy, Germany, Austria).[39] PB removal requires access to the perivitelline space of the oocyte by creating an opening of the zona pellucida,[39] which can be accomplished by mechanical or laser dissection.[40] This procedure can be done sequentially by removing the first and second PBs at separate times, or ideally, by a simultaneous approach in which both PBs are removed concurrently (8 to 14 hours after ICSI). Although PB analysis provides important prognostic information for couples about the origin of aneuploidies, there is still ongoing debate on the need to perform this type of biopsy. A recent study showed that both first and second PB are prone to meiotic errors.[41] Unfortunately, this technique carries drawbacks when used during PGD, especially its limitation to diagnosis of genetic or chromosomal abnormalities carried by maternal DNA alone. In PGS, PB biopsy is still a matter for debate because of questions pertaining to its cost-effectiveness (the high number of oocytes needed to be tested), the high incidence of post-meiotic chromosome abnormalities that cannot be detected by PB biopsy approach, and the questionable diagnostic accuracy of PB biopsy given the possible self-correction of meiotic aneuploidy.[41]

Cleavage-Stage Biopsy

Opening of the zona pellucida can be accomplished by acidic tyrode solution, by mechanical dissection, or by laser dissection. Cleavage-stage biopsy is typically performed on day 3 of in vitro development by extracting one blastomere.[20,38] Extracting two blastomeres has been previously shown to have detrimental effects on embryo development and thus should be avoided.[42] The major drawback of blastomere biopsy is the risk of mosaicism, which might be responsible for the false-positive or false-negative results encountered with preimplantation genetic techniques.[43,44] However, this technique is compatible with fresh embryo transfer on day 5 to day 6 of embryo development, given that genetic results will usually be available 1 to 2 days after blastomere biopsy.[43]

Blastocyst-Stage Biopsy

The blastocyst-stage biopsy technique consists in removing 5 to 10 trophectoderm cells on day 5 or day 6 of embryo development.[45] Opening of the zona pellucida is accomplished on day 3 of embryo development by mechanical or laser penetration. These embryos are then put in an extended IVF culture for blastocyst stage, and blastocyst biopsy is performed by extracting herniated trophectoderm cells. Retrieval of 5 to 10 trophectoderm cells from a 100- or 150-cell blastocyst corresponds with a lower proportion of cell loss (3.3% to 10%) than the removal of one or two blastomeres from a 6- to 8-cell embryo, which reduces the cell content by 12.5% to 33%.[46] Blastocyst biopsy also provides more starting DNA templates than day-3 biopsy, which would theoretically lead to improved sensitivity and specificity of PGD and is associated with lower rates of mosaicism. This technique is cost-effective, because fewer embryos are tested, and it has been associated with increased chance of live birth in the last decade.[45] However, embryologists working in PGD-PGS units should be experienced with blastocyst embryo culture and vitrification if frozen embryo transfer is to be performed. Recently, trophectoderm biopsy has been shown to have no impact on blastocyst reproductive potential when compared with cleavage-stage biopsy, in which 39% reduction in implantation rate was reported.[47] Although the live birth rate per transfer may increase with this technique, it should be kept in mind that with extended embryo culture, a higher rate of patients will not reach embryo transfer; therefore couples should be carefully counselled about these technical limitations and the procedure's higher cost.[48] See Table 3 for a comparison of the advantages and drawbacks of different embryo-stage biopsies.

Recommendation

4. Trophectoderm biopsy has no measurable impact on embryo development, as opposed to blastomere biopsy. Therefore, whenever possible, trophectoderm biopsy should be the method of choice in embryo biopsy and should be performed by experienced hands. (I-B)

Table 2. Description of cytogenetic technologies used in PGD and PGS

Acronym	FISH	aCGH	SNP microarray	qPCR
Name	*Fluorescence in situ hybridization*	*Array comparative genomic hybridization*	*Single nucleotide polymorphism arrays*	*Real-time quantitative polymerase chain reaction*
Technique	• Cells are arrested in mitosis and then fixed on a glass microscope slide using acetic acid and methanol. • Fluorescent DNA probes of a few hundred kbp in length are used that match regions of the chromosomes containing the DNA sequence in question. • Detection is immediate via fluorescence microscopy.	• Patient and control DNA are labelled with fluorescent dyes and applied to the microarray • Both DNAs compete to attach to or hybridize on the microarray • The microarray scanner measures the fluorescent signals. • Software analyzes the data and generates a plot.	• After amplification, the DNA is labelled with red and green fluorescent molecules, with one version of the SNP in red and the other version of the SNP in green. • This DNA is then assessed for intensity of signal and number of SNP calls. • It is then compared with a control population by powerful computer software to allow for diagnosis of inheritance.	• A preamplification step, followed by a high-order multiplex PCR reaction in a 384-multiwell plate format, is used to amplify ≥2 sequences on each arm of each chromosome.
Indication	Aneuploidy screening and chromosomal translocation	Aneuploidy screening and chromosomal translocation	Aneuploidy screening and chromosomal translocation	Aneuploidy screening
Advantages	• applicable to metaphase and interphase cells • can identify a range of structural abnormalities including deletions, duplications, aneuploidy, and the presence of derivative chromosomes • enables detection of translocation products • better resolution than traditional chromosome banding	• complete chromosome analysis • detects copy number changes at a level of 5–10 kbp of DNA sequences • detects structural variations at resolution of 200 base pairs • identifies microdeletions and duplications • 24-hour process, rapid turnover	• simultaneous testing of specific genetic diseases and aneuploidy • possible to analyze hundreds of thousands of loci across the genome using a single array, with an average spacing as close as 5 kbp, enabling high-resolution analysis • the genotype information allows the parental origin of any abnormalities to be identified • may detect balanced chromosomal translocations, inversions, and whole genome changes	• biopsy and analysis can be completed in only 4 hours, facilitating the fresh transfer of single euploid blastocysts in the same cycle • less DNA material required relative to regular PCR
Limitations	• small mutations, chromosomal inversions, and uniparental disomies cannot be detected • probes not available for all chromosomes • technical problems with interpretation (overlapping)	• balanced reciprocal translocations, inversions, Robertsonian translocations, reciprocal insertions, and triploidy will not be detected • levels of mosaicism of 20% or less will not be detected • relatively expensive	• SNP array analysis of DNA extracted from a cell population cannot indicate the heterogeneity within the sample • may not be compatible with a fresh embryo transfer (72-hour process) • relatively expensive	• amplification may fail and ADO may take place • limited number of samples, currently 2 on each plate, that can be run on the available equipment
Use in preimplantation genetic testing	PGS, PGD	PGS, PGD	PGS, PGD	PGS

kbp: kilo base pair

Table 3. Advantages and drawbacks of different embryo-stage biopsies

Biopsy stage	Polar body (oocyte)	Day 3 blastomere	Day 5–6 trophectoderm
Advantages	• no effect on development • ample time for genetic testing • excellent for maternal origin • avoids legal and ethical concerns	• low number of cells required • all indications • time for genetic test	• low number to test • more cells available • all indications • less mosaicism
Drawbacks	• high number tested • sequential biopsy • no information on mutations of paternal origin	• mosaicism • ADO • possible lower implantation rates	• blastocyst culture • needs vitrification • expertise required

PGD OF SINGLE-GENE DEFECTS

The first PGD performed used PCR-based methods for an X-linked disorder. This allowed the determination of embryo sex and the transfer of unaffected females.[49] Soon after these early PGD cases, PCR-based protocols were developed for inherited diseases such as cystic fibrosis and α-1-antitrypsin deficiency. These were based on the amplification of the DNA fragment that contained the causative mutation and its detection.[50,51]

PCR strategies have become more complex, leading both to an increase in the number of disorders for which PGD could be employed and to increased accuracy rates. The number of diseases currently diagnosed via PGD-PCR is approximately 200 and includes some forms of inherited cancers such as retinoblastoma and the breast cancer susceptibility gene (BRCA2).[52] PGD has also been used in new applications such as HLA matching.[53,54] The ESHRE PGD consortium data analysis of the past 10 years' experience demonstrated a clinical pregnancy rate of 22% per oocyte retrieval and 29% per embryo transfer.[55] Table 4 shows a sample of the different monogenetic diseases for which PGD was carried out between January and December 2009, according to the ESHRE data.[22] In these reports a total of 6160 cycles of IVF cycles with PGD or PGS, including PGS-SS, are presented. Of these, 2580 (41.8%) were carried out for PGD purposes, in which 1597 cycles were performed for single-gene disorders, including HLA typing. An additional 3551 (57.6%) cycles were carried out for PGS purposes and 29 (0.5%) for PGS-SS.[22] Although the ESHRE data represent only a partial record of the PGD cases conducted worldwide, it is indicative of general trends in the field of PGD.

Development of PGD-PCR protocols can be technically challenging because the DNA content is small (5 to 10 pg/mL). This requires a large number of amplification cycles for the mutation to be visualized, which can lead to a high risk of contamination, either by extraneous or parental DNA. A way around this setback is the amplification of additional hypervariable DNA fragments along with the alleles used for the diagnosis. This approach is in effective similar to DNA fingerprinting, and it enables the detection of contamination by an external DNA source through identifying alleles that are non-embryonic in origin. The presence of two alleles from the same parent indicates either that the contaminating DNA is of parental origin[56] or that the specific embryo is trisomic, carrying two copies of one of the parental chromosomes. In both cases such embryos are eliminated from transfer. Additionally, the use of ICSI instead of IVF eliminates the risk of sperm or cumulus cell contamination and is routinely used for all PGD-PCR cases. Denuding the oocyte of cumulus cells is also standard practice for PCR-based PGD.[57]

Another problem common to all single-cell based PCR tests is a phenomenon known as allele dropout. ADO can be defined as amplification failure affecting only one of the parental alleles present in the single cell.[58] ADO's incidence varies, but in extreme cases has affected 20% of amplifications[59] and in the past has led to several misdiagnoses.

The simultaneous amplification of one or more polymorphic markers, located on the same chromosome and near the disease-causing gene can ensure that a PCR-based PGD approach will be free of ADO-related error. This strategy (multiplex PCR) effectively enables diagnosis through scoring either the mutation itself or the polymorphic allele(s) inherited with it, because it is very unlikely that ADO will affect both amplified fragments in the same reaction.[60]

Generally, the most reliable PCR-PGD protocols employ multiplex PCR. In addition to amplification of a DNA fragment encompassing the mutation site, extra fragments containing linked polymorphisms are amplified to avoid misdiagnosis due to ADO, and at least one highly polymorphic marker is amplified to detect possible contamination.[61] Another strategy used to decrease ADO is blastocyst biopsy, with frozen embryo transfer for PGD of monogenic diseases. It has been associated with higher

Table 4. Sample indications for PGD in single-gene disorders (ESHRE Consortium)[22]

Monogenic disorder	Mode of transmission	Cases, n*
B-thalassemia	AR	153
Cystic fibrosis	AR	149
Huntington's disease	AD	136
Fragile-X	X-linked	124
Myotonic dystrophy	AD	124
Spinal muscular atrophy	AR	58
Neurofibromatosis type I	AD	45
Duchenne's muscular dystrophy	X-linked	42
Marfan syndrome	AD	27
Hemophilia A	X-linked	17
Tuberous sclerosis	AD	15

AR: autosomal recessive; AD: autosomal dominant

*Total cases = 2580 cycles to OR

genotyping and implantation rates and lower amplification failure and ADO than traditional blastomere biopsy.[62,63]

Recommendation

5. Preimplantation genetic diagnosis of single-gene disorders should ideally be performed with multiplex polymerase chain reaction coupled with trophectoderm biopsy whenever available. (II-2B)

See Table 4 for an outline of indications for PGD in single-gene disorders

PGD OF CHROMOSOMAL TRANSLOCATIONS

The two common types of chromosomal translocations, Robertsonian and reciprocal, usually result in normal phenotypes when balanced. However, they still carry associated reproductive risks, such as infertility, spontaneous abortion, and the delivery of babies with mental retardation or developmental delay.[64] The transfer of chromosomally normal and/or balanced embryos following PGD is reported to significantly reduce the risks of affected pregnancy and miscarriage. For decades, this was performed by FISH or PCR-based PGD methods. Unlike PCR-PGD, in which embryonic cells are placed in microcentrifuge tubes, PGD for chromosome abnormalities involves as an initial step the spreading and fixation of a single cell and its subsequent cytogenetic analysis.[57] Classical cytogenetic techniques (e.g. G-banding) are not applicable at the single cell level because they require chromosomes at the metaphase stage of the cell cycle. The majority of embryonic blastomeres, however, are found to be in interphase. To overcome this problem, PGD protocols commonly employ the molecular cytogenetic method of FISH. This technique involves the hybridization of chromosome-specific DNA probes, labelled with different colours, to nuclei or chromosomes spread on microscope slides. The method is rapid and performs equally well whether applied to metaphase or to interphase nuclei.[65]

However, the FISH technique requires preclinical validation before each IVF cycle and is limited to a certain number of chromosomes. Several drawbacks may be encountered including hybridization failure, signal overlap, and splitting that can affect the accuracy of the interpretation.[66] PCR-based protocols could offer improvements in terms of test performance, automation, turnaround time, sensitivity, and reliability.[67] Both methods may allow identification of aneuploidies simultaneously, but only for a limited number of chromosomes.[68] This may lead to the transfer of aneuploid embryos and might explain the relatively low clinical results of early PGD in some couples.[68] The FISH technique can be used only at the single-cell level and is therefore incompatible with PGD in blastocyst-stage biopsy.

aCGH and SNP microarray with trophectoderm biopsy are now used worldwide for PGD in couples carrying balanced reciprocal or Robertsonian translocations. They do not require preclinical validation before each IVF cycle and allow simultaneous screening for unbalanced translocation derivatives and aneuploidy of all 24 chromosomes.[69,70]

Fiorentino et al. first reported 28 cycles of PGD using aCGH at the cleavage embryo stage for chromosomal translocations.[70] A high percentage of embryos (93%) were successfully diagnosed. Embryos suitable for transfer were in 60% of started cycles. A 70% pregnancy rate and a 64% implantation rate per transfer cycle were achieved.

Colls et al. recently validated aCGH for translocations by reanalyzing all diagnosed embryos with FISH-PGD. The smallest detectable fragments we re ~6 Mbp for blastomeres and ~5 Mbp for trophectoderm. The error rate for array CGH was 1.9%. Retrospective analysis of their 926 FISH-PGD cycles for translocations showed that all the translocated fragments were < 6 Mbp, and thus could be properly diagnosed by aCGH.[71]

Treff et al. reported successful application of SNP array for PGD to distinguish between normal and balanced chromosomes in embryos from translocation carriers. 67% (12/18) of started cycles had suitable embryos for transfer.[72] The clinical pregnancy rate per transfer was 75% and a high 45% implantation rate was obtained. Recent results from a retrospective study comparing SNP-PGD

(169 couples) and FISH-PGD (406 couples) showed that the procedure using the SNP array combined with trophectoderm biopsy and subsequent frozen embryo transfer significantly improves the ongoing pregnancy rate for translocation carriers (69% versus 38%) and slightly decreases the miscarriage rate.[69]

Overall, it is evident from the aforementioned studies, that aCGH and SNP microarray used for chromosomal translocations are theoretically better approaches than FISH because they allow simultaneous screening for translocations and aneuploidy in all chromosomes. In addition, they have been associated with favourable clinical outcomes and should soon become the standard of care in PGD for couples carrying chromosomal rearrangements.

Recommendation

6. The use of comprehensive chromosome screening technology coupled with trophectoderm biopsy in preimplantation genetic diagnosis in couples carrying chromosomal translocations is recommended because it is associated with favourable clinical outcomes. (II-2B)

PREIMPLANTATION GENETIC SCREENING

This technique has recently been used to improve clinical outcomes in IVF cycles by screening embryos for chromosomal aneuploidies. At least 40% to 60% of human embryos are abnormal, and that number increases to 80% in women 40 years or older. These abnormalities result in low implantation rates in embryos transferred during IVF procedures, from 30% in women < 35 years to 6% in women ≥ 40 years.[33] In a recent retrospective review of trophectoderm biopsies, aneuploidy risk was evident with increasing female age. A slightly increased prevalence was noted at younger ages, with > 40% aneuploidy in women ≤ 23 years. The risk of having no chromosomally normal blastocyst for transfer (the no-euploid embryo rate) was lowest (2–6%) in women aged 26 to 37, then rose to 33% at age 42 and reached 53% at age 44.[11] IVF efficiency depends on two factors: embryonic chromosomal status and endometrial receptivity. Endometrial receptivity might be improved with reduced ovarian stimulation, responsible for the adverse high estrogenic effects observed during fresh embryo transfer, or by transferring embryos in a frozen cycle.[73,74] On the other hand, an embryo's potential depends mainly on its chromosomal status. Ideally, the highest implantation rate and potential could be anticipated with the transfer of one single euploid embryo into the uterus. Several techniques have been developed to assess embryonic chromosomal status through comprehensive chromosomal screening. Since a large randomized trial[13] and a recent meta-analysis[75] demonstrated that FISH-based PGS yields worse outcomes than no PGS, many centres and recommendations have discouraged its practice.

The use of CCS (assessing all 24 chromosomes) with concurrent trophectoderm biopsy and subsequent fresh or frozen embryo transfer appears thus far to provide favourable clinical outcomes in PGS practice when applied to patients with a good prognosis. aCGH and SNP microarray have recently been validated for PGS and applied to biopsied blastomeres and trophectoderm analysis.[30,76]

Early reports from Voullaire et al. and recent data showed that implantation and pregnancy rates using metaphase CGH seemed improved over FISH in PGS analyzing blastomeres.[77] Clinical application of this new technology used at the blastocyst stage in the study by Schoolcraft et al. has yielded improved clinical outcomes, as implantation rates were higher (72.2%) than in embryo transfer cycles using blastocyst morphologic criteria alone for embryo selection (46.5%).[45] New preliminary IVF outcome data from the same group was recently published for normally responding patients following single embryo transfer. It showed clearly that implantation rates were significantly higher in the group using frozen single blastocyst transfer following CCS (65.1%) than with either frozen single blastocyst transfer (52.6%) or day-5 fresh single embryo transfer (49.2%) based on morphology alone.[78] The improvement in IVF success for patients who underwent single blastocyst transfer following CCS was independent of maternal age. Therefore chromosomal aneuploidy screening represents a promising way to reach the full objective of routine eSET practice.

Implantation rates in all three available randomized trials were higher when CCS was combined with trophectoderm biopsy than when traditional IVF care was given.[17,79,80] In the two studies in which the same number of embryos were transferred in the CCS-PGS and control groups, both ongoing pregnancy rates beyond 20 weeks[79] and delivery rates[17] were improved. In one randomized study from Forman et al., when a single euploid blastocyst was transferred (following CCS-PGS), a dramatic decrease in multiple pregnancy rates was observed, while the pregnancy rate remained equivalent that with use of 2 untested blastocysts.[18]

In addition, a recent systematic review of randomized controlled trials by Dahdouh et al. showed that the application of CCS coupled with trophectoderm biopsy in PGS is associated with improvement in IVF success rates

(increased ongoing pregnancy rate beyond 20 weeks) and enhanced embryo selection.[81] However, these results were derived from patients with a good ovarian reserve who had blastocysts available to biopsy, and therefore the success rates of PGS using this technology may be overestimated and are not generalizable to other patient populations.[81]

Recommendations

7. Before preimplantation genetic screening is performed, thorough education and counselling must be provided by a certified genetic counsellor to ensure that patients fully understand the limitations of the technique, the risk of error, and the ongoing debate on whether preimplantation genetic screening is necessary to improve live birth rates with in vitro fertilization. (III-A).
8. Preimplantation genetic screening using fluorescence in situ hybridization technology on day-3 embryo biopsy is associated with decreased live birth rates and therefore should not be performed with in vitro fertilization. (I-E).
9. Preimplantation genetic screening using comprehensive chromosome screening technology on blastocyst biopsy increases implantation rates and improves embryo selection in IVF cycles in patients with a good prognosis. (I-B).

WHAT NEXT FOR PGD/PGS?

A new genetic technique, known as karyomapping, has been developed and provides a promising tool in PGD for single-gene disorders without requiring any prior patient or disease specific test development. The technique, a universal method for genome-wide analysis of genetic disease based on mapping crossovers between parental haplotypes, consists of a comprehensive approach for the simultaneous detection of monogenic and chromosomal disorders.[82,83]

Given that the sequencing of the entire genome has been developed, NGS is now being applied in PGS and in PGD for single-gene mutations and chromosomal translocations.[84] Treff et al. used a targeted NGS strategy and a multiplex PCR reaction that included both the mutation site and the chromosome-specific target sequences required for qPCR.[85] This strategy reduced the read depth necessary for accurate sequencing of the mutation site, which reduces both the time required and the cost. In parallel, qPCR of the multiplex PCR products provided rapid analysis of the chromosome copy number. Using this approach with trophectoderm samples from a series of blastocysts, both whole-chromosome aneuploidy and translocation chromosome imbalance have been also tested.[86] Before the introduction of NGS into routine clinical practice, this promising new technology should be extensively evaluated in the PGD/PGS setting.

COUNSELLING, LIMITATIONS, AND RISKS ASSOCIATED WITH ART

In 2007, the American Society of Reproductive Medicine published a committee opinion on counselling for couples undergoing PGD.[87] Counselling for couples considering PGD is required and should include information relating to the following key points:

- the risks associated with assisted reproductive technologies;[88]
- the option of choosing not to proceed with IVF and PGD;
- the risks associated with embryo biopsy and extended culture;
- for carriers of autosomal and X-linked disorders, the relevant patterns of inheritance and the impact of the disorder on the quality of life for an affected child;
- for carriers of balanced chromosomal translocations or other structural chromosomal abnormalities, a review of the possible patterns of segregation during meiosis and the increased risk for conceiving offspring having an unbalanced chromosomal composition;
- the technical limitations and pitfalls of PGD, including the risk for misdiagnosis and the need for subsequent prenatal diagnostic testing via CVS or amniocentesis to confirm the results obtained with PGD;
- options relating to prenatal diagnostic testing (chorionic villus sampling, amniocentesis, ultrasonography with or without additional blood tests, no prenatal testing) and their associated risks.
- the possibilities that no embryos may be transferred if all are affected and that unaffected embryos that carry the recessive or X-linked disorder may be transferred;
- the disposition of embryos for which testing yields no conclusive result;
- the disposition of embryos not transferred (e.g., discarding, cryopreservation, research, or donation) as and when appropriate; and
- alternative methods for avoiding risk of disease (e.g., use of donor gametes).

Availability in Canada

Finally, couples should be aware that although PGS is available through certain infertility clinics across Canada, unlike in several European countries, government medical

plans do not cover this service, which is associated with significant costs. Some provincial health authorities, such as that in Quebec, have begun to cover the cost of PGD cycles for couples with known genetic anomalies such as single-gene disorders and chromosomal translocations in certain university-based hospitals; however, HLA typing and other controversial indications for PGD are currently excluded from coverage for this service.

SUMMARY

PGD for single-gene disorders and chromosomal translocations is an alternative to prenatal diagnosis for the detection of genetic disorders in couples at risk of transmitting a genetic condition to their offspring. Ideally, detection should be performed by multiplex PCR genetic analysis on trophectoderm cells. The introduction of new CCS technologies (aCGH, SNP microarrays, and qPCR) holds great promise for cytogenetic techniques to achieve the expected clinical benefit failed to be demonstrated by FISH-based methods. These methodologies allow simultaneous identification and disregarding of embryos with a specific chromosomal anomaly (unbalanced translocations) and aneuploidy in PGD and selection of the most competent (euploid) embryo for transfer in PGS. More robust evidence is still needed from ongoing RCTs before its clinical use on a routine basis is applied in the PGD-PGS setting.

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