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Preimplantation Genetic Testing

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

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Disclosure statements have been received from all members of the committee.

Abstract

Objective: To review the techniques and indications of preimplantation genetic testing, including preimplantation genetic diagnosis and screening.

Options: Limited to an introductory discussion about the genetic aspects of preimplantation reproductive techniques.

Outcomes: This update does not discuss in detail the adverse outcomes that have been recorded in association with assisted reproductive technologies.

Evidence: The Cochrane Library and Medline were searched for articles relating to preimplantation testing that were published from 1990 to February 2008, using the following terms: preimplantation genetic diagnosis, preimplantation genetic screening, and in vitro fertilization. Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies. Additional publications were identified from the bibliographies of these articles. Randomized controlled trials were considered evidence of the highest quality, followed by cohort studies.

Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections, clinical trial registries, and from national and international medical specialty societies.

Values: This update is a consensus of the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada. The recommendations were made according to guidelines developed by the Canadian Task Force on Preventive Health Care.

Benefits, Harms, and Costs: This update educates readers about new genetic concepts, directions, and technology. The major harms and costs identified are those of assisted reproductive technologies.

Sponsor: The Society of Obstetricians and Gynaecologists of Canada.

Conclusions: 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 has been proposed to improve the effectiveness of in vitro fertilization in women of advanced maternal age or in couples with recurrent miscarriage or implantation failure, but the benefits of this approach are debated.

Recommendations

The recommendations were made according to guidelines developed by the Canadian Task Force on Preventive Health Care.

1. Before preimplantation genetic diagnosis is performed, genetic counselling must be provided 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. (II-2B)
3. Invasive prenatal 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 negative result. (II-2B)
4. Before preimplantation genetic screening is performed, thorough education and counselling must be provided to ensure that patients fully understand the limitations of the technique, the risk of error, and the lack of evidence that preimplantation genetic screening improves live-birth rates. (III-A)
5. Available evidence does not support the use of preimplantation genetic screening as currently performed to improve live-birth rates in patients with advanced maternal age, recurrent implantation failure, or recurrent pregnancy loss. (I-D)

Key Words: Preimplantation genetic diagnosis, preimplantation genetic screening, genetic counselling, gene defects

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INTRODUCTION

Amniocentesis and chorionic villus sampling are currently the mainstays of traditional prenatal genetic testing, and they allow the detection of a wide range of genetic abnormalities throughout pregnancy. Both procedures involve the sampling of fetal cells: amniocytes from the amniotic fluid in the case of amniocentesis, and trophoblast cells from chorionic villi for CVS. DNA can be extracted from the cells and tested for mutations in specific genes, or the chromosomes can be analyzed through karyotyping.[1]

An alternative to CVS and amniocentesis, which can be offered to couples known to be at high risk of transmitting a genetic disorder, is preimplantation genetic diagnosis. This involves the diagnosis of a genetic disease before a pregnancy has been established. Patients requesting PGD undergo IVF treatment with multiple embryos generated and tested prior to implantation, giving an increased probability that a disease free embryo will be identified. In most cases, the embryos are cultured for three days, by which time they are usually composed of 6 to 10 cells. Either one or two cells (blastomeres) are then removed from each embryo and subjected to genetic analysis. While the cells are tested, the embryos remain in culture. If the biopsied blastomere is shown to be unaffected for the genetic disorder, it can be inferred that the embryo is also free of the disease. Only unaffected embryos are transferred to the mother's uterus, and so any pregnancy resulting from the IVF procedure should be unaffected for the tested disease. The main limitation of PGD is the low efficiency of IVF. Only 20% to 30% of couples achieve a pregnancy per IVF cycle, and the success rate for couples undergoing PGD is similar.[2]

The first clinical use of PGD was in the early 1990s, when it was used to determine fetal sex for couples who were at risk of transmitting an X-linked recessive disorder.[3] Since that time, the number of diseases that can be diagnosed using this technology has increased dramatically, as have the patient groups who use PGD to attempt a healthy pregnancy.

At present, PGD is considered as an alternative to prenatal diagnosis,[4] while the related methods known as preimplantation genetic screening are employed to increase success rates of ART.

Preimplantation genetic diagnosis is currently proposed for

1. Carriers of single gene disorders, dominant or recessive, autosomal, or X-linked.
2. Carriers of structural chromosome abnormalities, including reciprocal or Robertsonian translocations, inversions, and others.

Preimplantation genetic screening is currently proposed for

1. Couples with repeated implantation failure following assisted reproduction treatments to enhance pregnancy success with transfer of normal karyotypic embryos.
2. Couples with repeated unexplained miscarriages.
3. Women of advanced maternal age, in order to avoid chromosomally abnormal offspring and to improve the success of in vitro fertilization procedures.

Diagnosis of Single Gene Defects

The first methods to be applied to the PGD of X-linked disorders were PCR-based and involved the amplification of a repeat sequence on the long arm of the Y chromosome. This allowed the determination of embryo sex, and the transfer of females.[3] Soon after these early PGD cases, PCR-based protocols were developed for inherited diseases such as cystic fibrosis and α-1-antitrypsin deficiency. These tests involved the amplification of the DNA fragment that contained the causative mutation and its detection, using mutation analysis techniques.[5,6]

As time has progressed, PCR strategies have become more sophisticated, leading both to an increase in the number of disorders for which PGD could be employed and to improved accuracy rates. Currently, approximately 200 diseases can be diagnosed via PGD-PCR, including numerous pediatric conditions and some forms of inherited cancers, such as retinoblastoma and the breast cancer susceptibility gene (BRCA2).[7] Additionally, PGD has been applied to new indications that have not traditionally been the subject of prenatal testing, such as HLA-antigen matching.[8,9] Table 1 shows the different diseases for which PGD was carried out between 1998 and 2004, according to the ESHRE data.[10] Although the ESHRE data present only a partial record of the PGD cases conducted worldwide, they are indicative of general trends in the field of PGD.

ABBREVIATIONS

ADO	allele dropout
ART	assisted reproductive technology
CVS	chorionic villus sampling
ESHRE	European Society for Human Reproduction and Embryology
FISH	fluorescent in situ hybridization
IVF	in vitro fertilization
PCR	polymerase chain reaction
PGD	preimplantation genetic diagnosis
PGS	preimplantation genetic screening

The development of PCR protocols for PGD can be technically very demanding, as the DNA content of single blastomeres is small (5–10 pg). A large number of amplification cycles are therefore needed for the mutation to be visualized. The large number of PCR cycles leads to a risk of contamination, by either extraneous or parental DNA. As all PCR-based PGD strategies analyze minute amounts of genetic material, any contaminating DNA will lead to an increased risk of misdiagnosis. A way around this setback is the amplification of additional hypervariable DNA fragments along with the alleles used for the diagnosis. This approach is effectively similar to DNA fingerprinting, and enables the detection of contamination by an external DNA source by identifying alleles that are non-embryonic in origin. If two alleles from the same parent are present, this indicates either that the contaminating DNA is of parental origin[11] 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 intracytoplasmic sperm injection instead of IVF alone 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.

An additional problem, common to all single-cell based PCR tests, is a phenomenon known as allele dropout, which can be defined as amplification failure affecting only one of the parental alleles present in the single cell.[12] The incidence of ADO varies, but in extreme cases it has affected 20% of amplifications, and in the past has led to several misdiagnoses.[13]

The simultaneous amplification of one or more polymorphic markers, located on the same chromosome and near the disease-causing gene, is a way to ensure that a PCR-based PGD approach will be error-free, as far as ADO is concerned. This strategy, termed multiplex PCR, effectively enables diagnosis by scoring the mutation itself, or the polymorphic allele(s) that are inherited with it, as it is very unlikely that ADO will affect both amplified fragments in the same reaction.[14]

It is very likely that as molecular genetics and associated technologies advance, PGD-PCR strategies will become simpler and more accurate. This will lead to a significant increase in the number of disorders diagnosed. Effectively, PGD will find more widespread use, benefiting many more couples who are at risk of transmitting an inherited disease to their children.

Table 1. Most common clinical applications of PGD for single gene disorders in the ESHRE consortium 1998–2004 (data collection I-VII)

Disease	Number of cycles
Cystic fibrosis	403
β-thalassaemia	220
Spinal muscular atrophy	147
Sickle-cell anaemia	50
Huntington disease	261
Myotonic dystrophy type 1	317
Duchenne or Becker muscular dystrophy	79
Hemophilia	38
Fragile-X syndrome	108
Others	406
Total	2029

Cycles performed for calendar years 1998 to 2004, adapted from Harper et al.[10] 2008 (current data reported by ESHRE).

Diagnosis of Structural Chromosome Abnormalities

Although the use of PGD for the diagnosis of monogenic disorders is growing rapidly, the most common indication for preimplantation embryo testing remains the risk of chromosomal imbalance (aneuploidy). Unlike PCR-PGD where embryonic blastomeres are placed in microcentrifuge tubes, PGD for chromosome abnormalities involves as an initial step the spreading and fixation of a single cell with its subsequent cytogenetic analysis. Classical cytogenetic techniques (e.g., G-banding) are not applicable at the single cell level, as they require chromosomes at the metaphase stage of the cell cycle. Embryonic blastomeres, however, are mainly in interphase. To overcome this problem, PGD protocols commonly employ a molecular cytogenetic method, fluorescent in situ hybridization. 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.[15]

Among groups seeking PGD for chromosomal analysis in order to achieve a healthy pregnancy are carriers of a structural chromosome abnormality. Patients who are balanced carriers of such abnormalities have a dramatically elevated risk of producing gametes with an incorrect number of chromosomes. They therefore frequently have complex reproductive histories, involving subfertility or complete infertility, multiple spontaneous miscarriages, or the birth of children with congenital abnormalities.

PGD is most frequently employed for two types of structural chromosome anomalies: reciprocal and Robertsonian translocations. Two different FISH strategies have been used for blastomere analysis during the PGD of these two types of structural anomalies. The first approach uses

Table 2. 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
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	E. There is good evidence to recommend against the clinical preventive action
	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 these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.[37]

†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the The Canadian Task Force on Preventive Health Care.[37]

probes that span the breakpoints of a translocation,[16] and the second uses probes that flank the breakpoints.[17] In both schemes, a distinct combination of signals is seen in interphase nuclei, each corresponding to one of the four chromosomes involved in the rearrangement (two normal and two derivatives). Chromosome imbalance due to segregation of translocated chromosomes is detected by both schemes. However, the relative simplicity of the "flanking probe" approach, along with the commercial availability of sub-telomeric probes specific for each chromosome arm, has made it the more popular strategy for PGD of reciprocal translocations.

A similar FISH strategy was devised for the PGD of Robertsonian translocations. This involves the application of two probes, each one hybridizing to one of the two chromosomes that form this rearrangement. In cases where one of the chromosomes participating in this type of translocation could result in a viable trisomic pregnancy, such as chromosome 21, it is advisable to use two probes for this chromosome to ensure its visualization during diagnosis.[18]

FISH strategies have been developed for other less common chromosome abnormalities, such as pericentric inversions[19] and deletion of long arm of chromosome 22 leading to Di George syndrome,[20] and for patients with gonadal mosaicism for a trisomic cell line.[17] The wider availability of commercial probes has increased the number of FISH PGD cases that are feasible, and hence the number of patients that could be treated.

A limitation associated with the PGD of chromosome abnormalities is the level of mosaicism, defined as the presence of two or more cytogenetically distinct cell lines within the resulting embryos.[21] Reports suggest that for some translocations, as many as 70% to 100% of embryos may be abnormal, which will greatly reduce the chance of a pregnancy.[22] However, as couples in this situation may be unable to establish or maintain a pregnancy naturally, PGD is still the most promising option.

Preimplantation Genetic Screening

In recent years, PGD technology has been increasingly used to screen the embryos of infertile patients undergoing IVF treatment for chromosomal abnormalities. Aneuploidy is extremely common in human embryos and leads to developmental arrest, implantation failure, and spontaneous abortion. The inadvertent transfer of chromosomally abnormal embryos is believed to explain a significant proportion of failed IVF cycles. By screening for aneuploidy and ensuring the transfer of chromosomally normal embryos, it has been suggested that a variety of IVF outcomes (including implantation and pregnancy rates) can be improved. This approach is known as preimplantation genetic screening.[23]

Many PGD centres offer PGS to couples with one or more of the following indications: advanced maternal age (cut-off varies between 35 and 40 years of age, depending on the centre), three or more previous unsuccessful embryo transfers with regular IVF procedures (repeated implantation failure), or repeated spontaneous loss of pregnancies with

normal parental karyotypes (recurrent miscarriage). As described previously, PGD tests for the diagnosis of single gene disorders or structural chromosome anomalies are generally patient specific. However, in the case of PGS, an identical probe combination and protocol is employed for all patients. PGD laboratories that offer this service examine 6 to 15 chromosomes per embryo.[24,25] This restricted number of chromosomes is attributed to a technical limitation of FISH: there are only five spectrally distinct fluorochromes available for probe labelling; therefore only five chromosomes can be simultaneously assessed.

Early PGS protocols examined chromosomes 13, 18, 21, X, and Y, (which are sometimes compatible with viable pregnancies), thus screening for aneuploid syndromes (i.e., trisomy 13, 18, 21, Turner [45,X], and Klinefelter [47,XXY]). This probe combination led to a reduction in the incidence of aneuploid syndromes but did not result in any statistically significant improvement of implantation rates.[26] Current protocols involve the combination of up to five probes in a single experiment and also investigate up to 15 chromosomes in two sequential FISH rounds.[24] Preliminary data obtained with such protocols have demonstrated a doubling in implantation rates and a significant increase in pregnancies per retrieval for women of advanced maternal age and/or couples who have had recurrent miscarriage.[25,27,28] To date, however, no notable benefits have been seen for younger women and couples with repeated implantation failure, and it remains controversial whether one or two blastomeres should be biopsied from individual embryos and examined via PGS.[29,30] Moreover it has been shown that aneuploidy could affect any chromosome during preimplantation development, and this, along with embryo mosaicism, could reduce the efficiency of current PGS strategies.

While it is generally accepted that PGS succeeds in reducing miscarriage rates and the incidence of aneuploid syndromes, there are conflicting data concerning the efficacy of PGS in raising implantation and birth rates. Recently, Mastenbroek et al. reported the results of a large, multicentre, randomized, double-blind trial demonstrating that a planned one-cell biopsy with FISH for nine chromosomes is not an effective means of improving pregnancy outcomes for women 35 to 41 years of age.[31] Given these findings, PGD for aneuploidy screening should not be performed solely because of advanced maternal age. Adequately powered randomized trials are needed to assess whether the same is true when this procedure is used for recurrent unexplained miscarriage and recurrent implantation failure; its use for these conditions should be restricted to research studies pending evidence of effectiveness.[32]

Limitations, Counselling, and Risks Associated With ART

In the report of the PGD consortium of ESHRE, a total of 18 misdiagnoses have been reported from 1998 to 2004, 9 after PGD using PCR and 9 after PGD or PGS using FISH.[10] In all cases of misdiagnosis, unprotected sex during the PGD cycle could be responsible, since any embryos generated in vivo would not be tested. Patients should be advised to avoid unprotected intercourse during PGD/PGS cycles.[33] The problems that lead to PCR misdiagnoses are well known: contamination or ADO. Furthermore, embryo mosaicism and, notably, the presence of haploid cells could cause misdiagnoses.[10] In a recent study, embryos that would have been discarded in patients undergoing PGD for chromosome abnormalities were fixed, and FISH reanalysis was performed.[34] The positive and negative predictive values were only 83% and 81%, respectively. FISH errors and mosaicism are primarily responsible for the errors associated with FISH analysis.

Couples considering PGD must receive extensive and comprehensive counselling that includes information relating to the following[35]

1. The role of PGD as an alternative to prenatal diagnosis that can in some cases avert the conception of an embryo affected by an inheritable disease.
2. The risks associated with assisted reproductive technologies.[36]
3. The option of choosing not to proceed with IVF and PGD.
4. The risks associated with embryo biopsy and extended culture.
5. 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.
6. 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.
7. The technical limitations and pitfalls of PGD, including the risk for misdiagnosis and the need for subsequent prenatal diagnostic testing via chorionic villus sampling or amniocentesis to confirm the results obtained with PGD.
8. Options relating to prenatal diagnostic testing (chorionic villus sampling, amniocentesis, ultrasonography with or without additional blood tests, no prenatal testing), and their associated risks.

9. The possibility that no embryos may be transferred if all are affected and the possibility that unaffected embryos that carry the recessive or X-linked disorder may be transferred.
10. The disposition of embryos (e.g., discard, cryopreserve, use in research, or donate) not transferred or for which testing yields no conclusive result.
11. Alternative methods for avoiding risk of disease (e.g., use of donor gametes, adoption).
12. The availability of PGD in Canada: couples should be aware that preimplantation genetic screening is available through certain fertility clinics across Canada, but thus far (unlike in several European countries), this service is not covered by the government, and significant costs are associated with the procedure.

CONCLUSIONS

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 screening has been proposed to improve the effectiveness of in vitro fertilization in women of advanced maternal age or with recurrent miscarriage or implantation failure, but this approach has led to conflicting results.

Recommendations

The recommendations were made according to guidelines developed by the Canadian Task Force on Preventive Health Care (Table 2).

1. Before preimplantation genetic diagnosis is performed, genetic counselling must be provided 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. (II-2B)
3. Invasive prenatal 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 negative result. (II-2B)
4. Before preimplantation genetic screening is performed, thorough education and counselling must be provided to ensure that patients fully understand the limitations of the technique, the risk of error, and the lack of evidence that preimplantation genetic screening improves live-birth rates. (III-A)
5. Available evidence does not support the use of preimplantation genetic screening as currently performed to improve live-birth rates in patients with advanced maternal age, recurrent implantation failure, or recurrent pregnancy loss. (I-D)

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