

Preimplantation Genetic Diagnosis: Technology and Clinical Applications

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ABSTRACT

Preimplantation genetic diagnosis (PGD) is a method by which embryos formed through in vitro fertilization (IVF) can be tested for single-gene disorders or chromosome abnormalities prior to embryo transfer. This enables couples to significantly improve their chances of having a healthy child. PGD is an important addition to conventional prenatal diagnosis for genetic disorders. PGD is a complex combination of various technologies that requires close collaboration of a team of specialists for optimal patient care. This review article will cover patient management, assisted reproductive technologies including IVF and PGD as well as indications for PGD. Clinical vignettes from The Froedtert Hospital and Medical College of Wisconsin Reproductive Medicine Clinic PGD Program will be presented, including the first single-gene disorder PGD performed in Wisconsin. These vignettes highlight the importance of a detailed family history, use of PGD for cases of recurrent miscarriage, and the use of PGD for spinal muscular atrophy.

INTRODUCTION

Preimplantation genetic diagnosis (PGD) is a technique allowing for analysis of the genetic make-up of embryos formed through in vitro fertilization (IVF). Results of this analysis lead to selection of embryos for uterine transfer to establish a pregnancy. Although the first successful PGD by Alan Handyside in 1990 was for embryo gender determination in families known to be at risk for an X-linked disease, there is now a growing list of indications for PGD.¹ Current indications for PGD include a variety of chromosomal disorders,

single gene disorders, and Human Leukocyte Antigen (HLA)-matching of embryos.²⁻⁴

Prior to the advent of PGD, couples at risk for pregnancies with genetic disorders were offered prenatal testing through amniocentesis or chorionic villus sampling. While these methods do allow for diagnosis of genetic abnormalities during pregnancy, couples are faced with difficult decisions following an abnormal result. PGD enables couples to avoid issues including pregnancy termination and giving birth to an affected child by initiating pregnancies with unaffected embryos.

It is currently estimated that over 7000 PGD cycles have been carried out worldwide.⁵ Of the 101 assisted reproductive medicine clinics in the United States with 200 or more stimulation cycles per year, 65 report offering PGD. Following embryo biopsy, 30 of these 65 centers perform analysis in their own laboratories while the remaining send the cells to another laboratory for analysis [Strawn, Bick, Milwaukee, Wis, unpublished data, November 2005]. The Froedtert Hospital and Medical College of Wisconsin Reproductive Medicine Clinic has the only PGD laboratory in Wisconsin.

PATIENT MANAGEMENT

Due to the complex nature of PGD, a multidisciplinary team approach is critical to the success of this undertaking and should include reproductive endocrinologists, medical geneticists, nurses, genetic counselors, embryologists, cytogeneticists, and molecular biologists.

Counseling by a reproductive endocrinologist is an important first step for any patient considering PGD. This consultation provides patients with information about IVF, including the risks and benefits of this procedure.^{6,7} At this time, a detailed medical history is obtained and laboratory tests are performed to help predict the likelihood of a successful IVF cycle.

Genetic counseling is another key component for patients contemplating PGD. During the counseling session, a genetic risk assessment is performed based on the patient's history, her partner's history, and their

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family histories. Based on this assessment, there is a discussion of the disorder(s) in the family, the severity of such condition(s) and the chance for having affected children. All of this information is useful in determining whether PGD would be a reasonable option for a patient's particular situation.

Clinical Vignette

A genetic counselor met with a patient and her husband in our clinic to discuss PGD. At an earlier clinic visit, the patient was asked about her family medical history and she stated there were no concerns. A 3-generation family medical history obtained by the genetic counselor revealed that the patient's mother was diagnosed with "cystic kidneys" in her late 30s, and required a kidney transplant at age 75. The patient reported that she had a renal ultrasound that also revealed cystic kidneys. The counselor then explained that these findings are suggestive of adult-onset polycystic kidney disease, discussed the natural history and autosomal dominant inheritance of this genetic condition and recommended further evaluation. This case emphasizes the importance of obtaining a detailed family history when couples are considering their reproductive options.

If PGD is possible, the patient is provided with information detailing the testing that can be used on single embryonic cells to diagnose or rule out a particular condition in that embryo. At this time, limitations of such testing are discussed, which include resulting an abnormal embryo as normal or a normal embryo as abnormal. Furthermore, patients are reminded of alternative reproductive options including use of donor gametes, prenatal diagnosis, accepting genetic risk without further testing, adoption, and remaining childless.

It should be noted that the central principle guiding these consultations is respect for patient autonomy.⁸ With the information provided during the reproductive and genetic consultations, patients are able to decide whether IVF-PGD is an option they wish to pursue. Presently, patients are able to make these reproductive and genetic decisions despite increasing government regulations of assisted reproductive technologies.^{9,10}

IN VITRO FERTILIZATION AND INTRACYTOPLASMIC SPERM INJECTION

For those patients who decide to proceed with PGD, medications are given to promote oocyte development and to prepare the endometrium for embryo transfer into the uterine cavity.¹¹ Conventional IVF is performed

by combining an egg with several thousand motile sperm on the day of egg retrieval in hopes that fertilization occurs.¹² Numerous sperm are present around the fertilized egg following conventional IVF. This is not an ideal situation for embryo biopsy as contamination from excess sperm can lead to incorrect PGD results. To eliminate this risk, a procedure known as intracytoplasmic sperm injection (ICSI) is used, which entails injecting a single sperm into an egg to fertilize it.¹³

EMBRYO BIOPSY

The most commonly used method for performing PGD involves testing individual cells (blastomeres) obtained on the third day after in-vitro fertilization at approximately the 8-cell stage. This is performed by first creating a hole in the zona pellucida and then removing 1 or 2 blastomeres for analysis (Figure 1).¹⁴ Transfer of selected embryos (based on PGD results) into the uterus takes place on day 4 or 5 post-fertilization.

Two alternative methods are currently used to carry out PGD in some centers. One of these methods involves examining the genetic material of the first and second polar bodies. Polar bodies contain excess genetic material that is excluded from the female pronucleus. Thus, by establishing whether there is an abnormal gene or chromosome arrangement in the polar bodies, it is possible to infer the maternal genetic contribution to the embryo. This technique is limited in the information it can provide, as it does not test for paternal contribution to the embryo.¹⁵ Furthermore, whereas blastomere biopsy data can be replicated by studying 2 cells, polar body biopsy data cannot be replicated unless it is followed by blastomere biopsy.

The other method is blastocyst-stage biopsy.¹⁶ This procedure is performed approximately 5 days after insemination. At this stage of embryo development, differentiation of the trophoctoderm (which gives rise to the placenta) and the inner cell mass (which gives rise to the fetus) has occurred. Laser assisted biopsy of the human blastocyst allows for several cells from the trophoctoderm layer to be removed thereby improving accuracy of PGD results.¹⁷ These results are available in time for a day 6 transfer.

ANALYSIS OF GENETIC MATERIAL FROM BIOPSY

Different testing methods can be used for PGD depending on the indication for PGD. The 2 most commonly used techniques are fluorescence-in-situ-hybridization (FISH) and polymerase chain reaction (PCR). FISH is used for PGD when testing for aneuploidy, chromo-

some rearrangements, and gender determination in the setting of X-linked disease. This particular method involves fixing the biopsied cells to slides and hybridizing with fluorescently labeled, chromosome specific DNA probes. Individual cells can be probed with a mixture of probes, stripped, and probed with a second mixture as needed to diagnose abnormal cells.¹⁸

PCR is used for PGD when testing for single-gene disorders and HLA-typing. PCR is a method of DNA amplification. It can be performed on individual cells derived from the embryo biopsy to create millions of copies of a few, carefully chosen short DNA sequences within the genome. To increase the signal from the first round of PCR, the initial PCR product can be used in a second round of PCR. When 2 rounds of PCR are used, this is referred to as nested or hemi-nested PCR.¹⁸

ACCURACY OF PGD

Although PGD methods are constantly being improved, there still remain several pitfalls that can result in misdiagnosis of an embryo. Currently, FISH testing is limited by the number of probes that can be applied to a single biopsied cell. This limitation is due to the risk of hybridization failure and FISH artifacts that can result when multiple probes are applied to a cell.¹⁹ Thus, FISH cannot test for aneuploidy of all chromosomes at this time. Presently, most PGD programs test for numerical abnormalities involving chromosomes 13, 18, 21, X and Y as these can result in an affected live-born child. Most centers also test for chromosomes 15, 16 and 22 as these are commonly found in spontaneous abortions. Some programs are testing for additional chromosomes involved in miscarriage and/or IVF failure.^{20,21} As aneuploidy FISH testing is limited by the number of probes used, it is often referred to as aneuploidy screening or preimplantation genetic screening (PGS).

Mosaicism is another concern for PGD results. Mosaicism refers to a situation in which there are 2 cell lines present with different genotypes or karyotypes. The rate of mosaicism is approximately 33% in early-cleavage-stage embryos.²²

PCR for single-gene disorders can also be problematic because of allele drop-out (ADO) and preferential amplification (PA). ADO occurs when 1 allele fails to be amplified. PA occurs when 1 allele is poorly amplified. Both of these situations can lead to inaccurate results if a mutant allele is not detected.^{23,24}

In view of the aforementioned issues, confirmation of PGD results by amniocentesis or chorionic villus sampling is recommended.

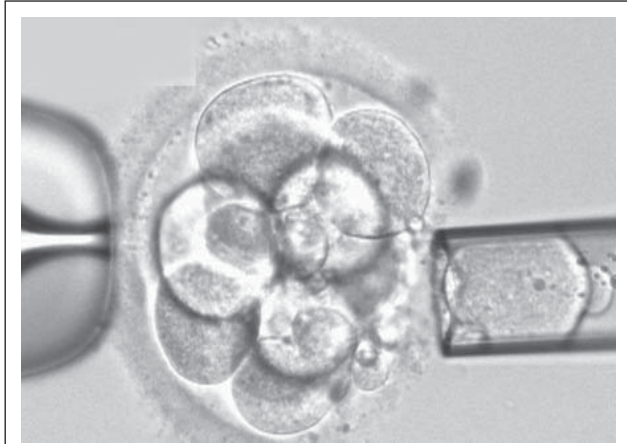


Figure 1. Biopsy of a human embryo. The holding pipette on the left abuts the zona pellucida of the embryo. The biopsy pipette on the right is inside the opening in the zona pellucida. One cell is inside the pipette.

INDICATIONS FOR PGD

It is known that the rate of aneuploidy rises with maternal age.²⁵⁻²⁷ Aneuploidy screening in IVF patients with advanced maternal age (over age 35 at delivery) is the most frequent indication for PGD using FISH.²⁸ This type of PGD can also be offered to couples who have had a previous child and/or pregnancy with aneuploidy.²⁹

PGD aneuploidy screening can be useful in cases of recurrent miscarriage. Recurrent miscarriage (RM) is typically defined as 3 or more consecutive miscarriages prior to 20 weeks gestation. There are several known causes of RM including uterine anomalies, maternal conditions such as diabetes, acquired and/or inherited thrombophilia, and parental chromosomal translocations.³⁰ However, for patients who do not have any of the aforementioned risk factors, de novo chromosome abnormalities may be the underlying etiology of their recurrent miscarriage.³¹ PGD can be used in this setting to increase the chance of transferring chromosomally normal embryos. In 2005, Munne et al showed that for couples with RM, using PGD to select embryos for transfer significantly decreased the miscarriage rate from 37% to 16.7%.²⁰

In some couples with recurrent miscarriage, PGD to test for evidence of recurrent aneuploidy can be used as a diagnostic test. A study by Rubio et al found that 26.9% of couples with recurrent miscarriage had chromosomal aberrations in all embryos in a given cycle and percentages were similar in subsequent cycles.³² Thus, patients with high rates of aneuploidy diagnosed by PGD-FISH may want to consider an egg donor for future IVF cycles rather than undergoing additional IVF cycles with their own eggs, as aneuploidy is typically maternal in origin.

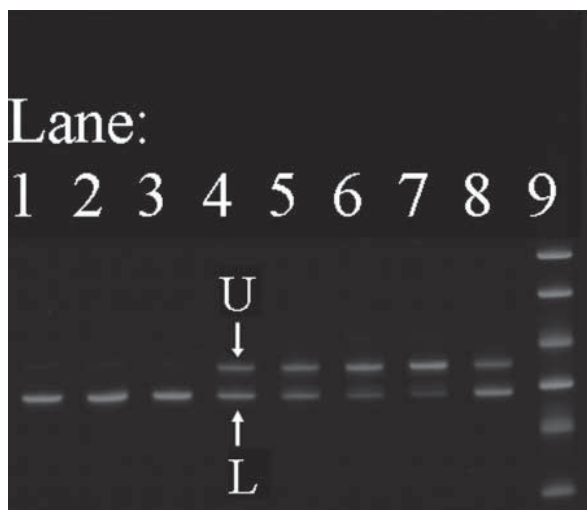


Figure 2. Spinal muscular atrophy (SMA) preimplantation genetic diagnosis. Approximately 94%-95% of SMA cases are due to a homozygous deletion of the entire SMN1 (SMN-T) gene. This gel shows the analysis of Exon 7 of the SMN-T and SMN-C genes. Presence of the upper band (U) indicates that the SMN-T gene is present. Presence of the lower band on the gel (L) indicates that the SMN-C gene is present. Each lane represents the analysis of 1 cell of an embryo (a blastomere). When possible, 2 cells are studied from each embryo. Lanes 2 and 3, lanes 4 and 5, and lanes 6 and 7 are from the same embryo. Only a single cell could be studied in 2 embryos (lane 1 and lane 8). Lane 9 is the size marker ladder. Lanes 1, 2, and 3 show that these 2 embryos are affected with SMA. Lanes 4 through 8 show that these 3 embryos do not have SMA.

PGD aneuploidy screening is currently offered to couples experiencing recurrent implantation failure (RIF). RIF is typically defined as 3 or more consecutive IVF cycles without a clinical pregnancy.³¹ It has been suggested that these couples may be predisposed to creating aneuploid embryos. Data published in 2005 by Tarranissi et al indicated that PGD aneuploidy screening for RIF is associated with improved outcome in younger women, but is not associated with improved outcome in women >40 years of age.³³ Individuals identified as having high rates of aneuploid embryos may want to consider using eggs from an egg donor. This may not only save them time but may also reduce the number of IVF cycles necessary to achieve a live birth.

Clinical Vignette

A 33-year-old white female patient in our clinic had a history of 3 failed intrauterine insemination (IUI) cycles and 3 failed IVF cycles. The patient did have 1 pregnancy following an IVF cycle, which resulted in miscarriage, and karyotyping of the products of conception revealed trisomy 16. She was subsequently offered PGD aneuploidy screening for recurrent implantation failure and history of

a trisomy 16 conception. Seven mature eggs were retrieved and 6 fertilized following ICSI. One of 6 embryos arrested prior to biopsy. Five embryos were biopsied and PGD results were abnormal for all 5 embryos. Donor egg was recommended and the patient delivered healthy twins using eggs from a donor. This highlights the value of PGD aneuploidy screening as a diagnostic tool.

Some men with severe male factor infertility and a normal karyotype can have chromosomally abnormal sperm.³⁴ For example, in 1 study, 27 men with oligoasthenoteratospermia (abnormalities in sperm numbers, motility, and morphology) and 11 men with non-obstructive azoospermia (absence of sperm in the ejaculate) underwent testicular sperm extraction. Aneuploidy analysis of sperm from these men found that 79% had a significantly increased rate of chromosomal abnormal sperm.³⁵ PGD aneuploidy screening of embryos created from men with these sperm abnormalities could be considered. For example, a PGD aneuploidy screening study in 2006 analyzing 94 embryos involving 52 men with teratozoospermia (morphologically abnormal sperm) found that 47 of 94 embryos (50%) were chromosomally abnormal.³⁶ This finding suggests a role for PGD in couples with severe male factor infertility.

Constitutional chromosome abnormalities can be found in approximately 5% of infertile males, most notably translocations and Klinefelter syndrome (47, XXY).³⁷ Studies have also shown that 6% of women with recurrent miscarriage and 1.25% of women with secondary infertility have a chromosome abnormality.^{38,39} PGD-FISH for aneuploidy screening can be applied to cases of Klinefelter syndrome. However, translocations and other chromosomal rearrangements often require FISH probes other than those in the typical aneuploidy screen. PGD has been shown to be highly successful in these situations, despite the technical challenges of customizing FISH probes to each patient situation. A recent study of 45 balanced translocation carriers that underwent PGD found a reduction in spontaneous abortions from 87.8% to 17.8%, and improvement in live-birth rate from 11.5% to 81.4% in this cohort.⁴⁰

PGD is currently available for over 100 different single-gene disorders using PCR.¹⁵ Some disorders such as spinal muscular atrophy are the result of a recurrent mutation.⁴¹ As a result, a single assay can be used in most affected families (Figure 2). However, for most genetic disorders, families harbor unique mutations. In these cases, a customized assay must be developed to identify the family-specific mutation(s).

Clinical Vignette

A patient seen in our clinic had a history of a child who was diagnosed with spinal muscular atrophy type I (SMA type I) during infancy. SMA type I is an autosomal recessive disorder involving degeneration of anterior horn cells in the spinal cord and brain stem nuclei. Such degeneration leads to progressive muscle weakness, lack of motor development and poor muscle tone. Death typically occurs by age 2 due to respiratory distress. The patient underwent an IVF cycle in our clinic for the purpose of PGD for SMA. A total of 9 eggs were retrieved and 8 fertilized. Embryo biopsy was performed on day 3 and PGD for SMA identified 2 affected embryos. Two unaffected blastocysts were transferred on day 5. A twin pregnancy resulted and chorionic villus sampling confirmed that both fetuses were unaffected with SMA. This highlights the dramatic risk reduction afforded by PGD to couples at high risk for having a child with a genetic disorder.

PGD has been developed for a variety of X-linked disorders such as Duchenne/Becker muscular dystrophy and hemophilias A and B.⁴² When a specific mutation has not been identified or when an assay is not available, PGD to identify female embryos for transfer can be used to eliminate the risk of an affected male pregnancy. This type of testing cannot distinguish between carrier and non-carrier female embryos. This is acceptable to most couples because female carriers of X-linked disease are generally asymptomatic. However, PGD for gender selection requires half of the embryos to be discarded simply because they are male. The number of embryos discarded based on gender can be greatly reduced by altering the ratio of X-bearing to Y-bearing sperm in the fertilizing sperm population. This is possible using a technique called MicroSort®. MicroSort® is flow cytometric sperm sorting based on the detection of differential fluorescence emitted by fluorescently labeled X and Y chromosome-bearing sperm. Currently in clinical trial, this method averages approximately 90% X-bearing sperm or 75% Y-bearing sperm depending on the sort parameters. Thus, using sperm sorted for the X-chromosome in IVF-ICSI, the majority of embryos biopsied and tested for gender are female.⁴³

PGD can also be used to identify embryos that are an HLA-match to a sibling in need of an HLA-matched hematopoietic progenitor cell transplant (HPCT) when no match is available (Figure 3).⁴ At birth, umbilical cord blood from the matched infant can be used for transplan-



Figure 3. Preimplantation genetic diagnosis for HLA matching is accomplished by linkage analysis utilizing short tandem repeats (STRs) within the HLA locus. STRs are short sequences of DNA, normally of length 2-5 base pairs, that are repeated numerous times. The number of repeats can vary in a population of individuals. This gel shows STR analysis of single human embryonic cells (blastomeres) at the HLA locus D6S2819. Each lane is an analysis of a single cell. Lanes 2 and 3, lanes 4 and 5, and lanes 6 and 7 are biopsied cells from 3 different embryos. Lane 1 is the size marker ladder. Each of the 3 embryos show 2 different sized STR alleles. By matching the STR allele sizes of the embryos with the child in need of an HLA matched donor, it is possible to select an embryo that is an HLA match.

tation. The first successful PGD for HLA-matching for HPCT involved treatment of a child with Fanconi anemia in the United States.⁴⁴ PGD for HLA-matching has since been carried out by a number of international groups. PGD for HLA-matching has been performed for families with children affected by sporadic diseases such as aplastic anemia and leukemia. It has also been performed for inherited disorders such as Fanconi anemia and Wiscott-Aldrich syndrome.⁴⁵ Fanconi anemia is a group of disorders characterized by progressive bone marrow failure, an increased risk of malignancy, and various physical abnormalities. Most types of Fanconi anemia are autosomal recessive. Wiscott-Aldrich syndrome is an X-linked recessive disorder of the hematopoietic cells that leads to an increased risk of lymphoma. In the United States, PGD for HLA-matching is available in a limited number of centers including the Reproductive Genetics Institute (Chicago, Ill), Genesis Genetics Institute (Detroit, Mich), Children's Hospital & Research Center (Oakland, Calif), and The Froedtert Hospital and Medical College of Wisconsin Reproductive Medicine Clinic (Milwaukee, Wis).

Increasingly, physicians and patients are working to avoid multiple births following IVF by limiting the number of embryos transferred to a single embryo.⁴⁶ PGD for aneuploidy screening has a role in this effort. Use of aneuploidy screening can significantly improve the

chance of identifying euploid embryos, thereby increasing the chance that a single embryo transfer will result in a chromosomally normal live-born. A retrospective analysis of IVF cycles involving men with obstructive azoospermia by Donoso et al, confirms the value of PGD for aneuploidy screening in single embryo transfer cycles.⁴⁷ The authors show that the choice of the embryo would have been altered by PGD-aneuploidy screening in 36.6% of single embryo transfer cycles.

USAGE AND OUTCOME OF PGD

Due to the invasive nature and the cost of assisted reproductive technologies, it is unlikely that IVF-PGD will replace standard methods of prenatal diagnosis. While standard prenatal diagnosis is covered by insurance companies, assisted reproductive technologies typically are not covered benefits in Wisconsin. Increasingly though, insurance companies are beginning to recognize the importance of assisted reproductive technologies including PGD. As such, many insurance companies are providing partial or full coverage.

The European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium was established in 1997 to collect data of PGD outcomes. They systematically assess reasons for referral, PGD cycles performed, resultant pregnancies, and outcome of babies born. The most recent data set published in 2006 analyzes cycles in 2003 with the associated births in 2004. The published data consist of 2909 PGD cycles, 550 pregnancies and 411 babies.⁴⁸ Overall, the data show that pregnancy courses and outcomes following PGD are comparable to pregnancies following IVF with ICSI but without PGD. Embryo biopsy does not appear to affect the course of the pregnancy, newborn characteristics (birth weight, length, and gestational age at delivery), nor the rate of congenital malformations.

SUMMARY AND FUTURE DIRECTIONS FOR PGD

Since its advent in 1990, PGD has become a well-accepted method to test embryos formed through IVF in couples at risk for genetic disease. This allows couples to start a pregnancy with confidence that they have dramatically improved their chance of having a healthy child. While issues of cost and time commitment to the PGD process limit its general use presently, new technologies such as whole genome amplification and laser-assisted blastocyst biopsy promise to simplify the technique, improve the accuracy, and reduce the cost of PGD.^{17,49}

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