

Preimplantation Genetic Diagnosis (PGD) **Patient Information**

Preimplantation genetic diagnosis (PGD) is a reproductive technology used with an *in vitro* fertilization (IVF) cycle to increase the potential for a successful pregnancy and delivery. The most common applications for PGD include testing for extra or missing chromosomes (aneuploidy screening), familial structural chromosome rearrangements, and diagnosis of genetic disease in couples of increased risk. PGD also can be used to determine gender for families at risk for an X-linked disease or family balancing.

The PGD Laboratory at Genetics & IVF Institute (GIVF) performed its first clinical cycle in March 1993. Since then, the PGD laboratory staff has performed more than 2000 PGD cycles for hundreds of patients at our clinic in suburban Washington, DC, and more recently for assisted reproductive technology (ART) centers around the US.

USES OF PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

PGD for aneuploidy should be considered for cycles in which women are over 35, men who have a low sperm count, or if testicular sperm is used. Couples who have had several miscarriages, a prior pregnancy with a chromosome abnormality, or have experienced several failed IVF cycles could also benefit from PGD for aneuploidy. When PGD for aneuploidy is used for these couples, each cycle has a better potential outcome, since the miscarriage rate and the risk for a chromosomally abnormal pregnancy is reduced.

PGD for structural chromosome rearrangements is helpful if a member of a couple carries a balanced translocation (exchange of chromosome material between two or more chromosomes), a Robertsonian translocation (the joining of two chromosomes) or other structural chromosome rearrangements. These increase the risk for a pregnancy with an unbalanced chromosome complement, which can cause birth defects, mental retardation, and/or miscarriage. PGD for structural chromosome rearrangements allows couples to decrease these risks and to increase the chance of a healthy ongoing pregnancy.

PGD for gender determination can be used for families with a history of an X-linked genetic disease or for couples who wish to balance the gender of the family.

PGD for single gene genetic defects can be used for inherited disorders such as Huntington disease, spinal muscular atrophy (SMA), cystic fibrosis (CF), and fragile-X syndrome. For couples who are at risk to have children with such diseases, GIVF can provide specialized PGD services. Due to the complexity of genetic testing for these disorders, patients should have a full consultation with a medical geneticist to

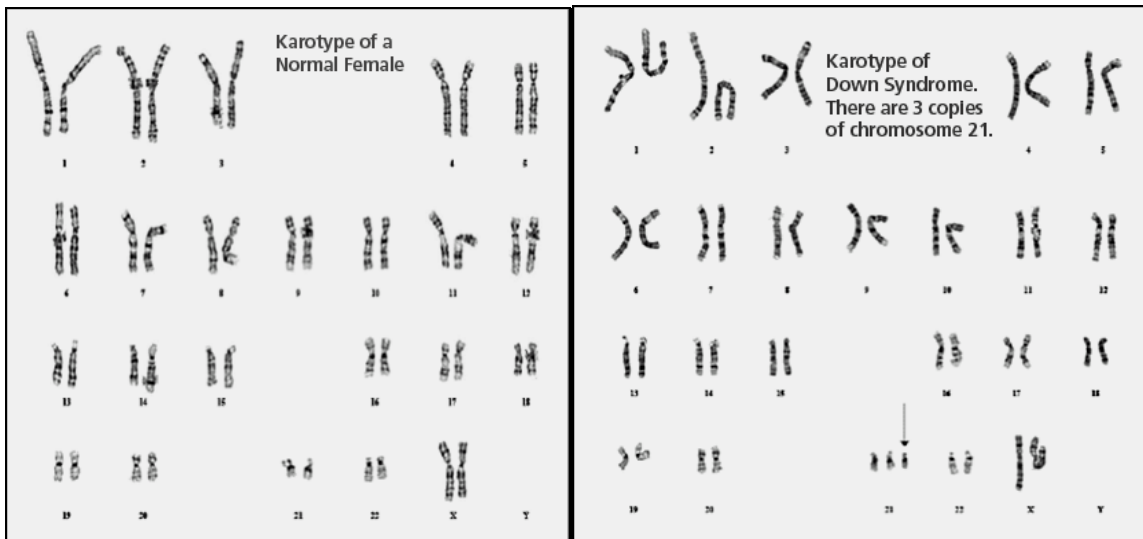
determine if this option would fit their family's needs. Prior to the PGD cycle, the laboratory must be notified in order to develop a patient-specific test for each disorder. In addition to testing for the inherited disorder, aneuploidy screening can also be performed on the same sample.

INTRODUCTION TO CHROMOSOMES

To appreciate how PGD testing can be helpful to couples it is important to understand chromosomes. Chromosomes are the physical structures that contain the DNA and genes necessary for development. Chromosomes are located in the center of the cell, in the area called the nucleus.

A normal human cell should contain exactly 46 chromosomes. There are 23 pairs of chromosomes. The first 22 pairs are identified by number and organized by size. The 23rd pair, the sex chromosomes, determines gender. Females have two of the same sex chromosome, called the X chromosome, while men have two different sex chromosomes, known as the X and Y chromosomes. A normal set of chromosomes is 46,XX for a female and 46,XY for a male.

In a normal conception, both the egg and sperm cells should contribute exactly 23 individual chromosomes, one of each of the 22 numbered pairs and one of the sex chromosome pair. When an egg with 23 chromosomes fuses with a sperm with 23 chromosomes, the correct chromosome number of 46 (23 pairs) is again present, and the fertilized embryo has the best possible chance of developing appropriately.



When an egg or sperm cell misdivides as it is developing, the mature egg or sperm contains more or less than 23 chromosomes. If this egg or sperm is used for fertilization, the resulting embryo will not contain exactly 46 chromosomes (called aneuploidy).

Down syndrome is a common example of aneuploidy. Pregnancies with Down syndrome typically have three copies of chromosome 21 (instead of two, see picture above).

It is known that approximately three out of four (75%) embryos created by IVF will not be capable of producing a live born child. Some embryos will fail to divide, others will not progress, some will fail to implant in the uterus, while others will implant but be unable to carry out early embryonic development. Finally, as in natural pregnancy, approximately 15%-20% of clinically recognized pregnancies will be lost as a miscarriage. While there are many reasons for the failure of an embryo to make a baby, the most common reason is an abnormality of the chromosomes. The most common chromosome abnormalities in miscarriages include: trisomy (3 copies of a chromosome) or monosomy (one copy of a chromosome) for chromosomes 13, 15, 16, 18, 21, or 22; triploidy (3 copies of all the chromosomes); and abnormalities of the sex chromosomes. For many couples, a significant number of the embryos created by IVF will have chromosome abnormalities. The exact percentage of chromosomally abnormal embryos that each couple produces is related to many factors including maternal age, number of unsuccessful IVF cycles, and the quality of sperm used and how it was prepared.

PGD TESTING METHODS

For couples pursuing testing for aneuploidy and structural chromosome rearrangements, a method called chromosomal microarray (CMA) is used. This technology determines the amount of DNA present for each chromosome in a cell or cells from the developing embryo when compared to the DNA from a normal standard. Gains or losses of complete chromosomes or parts of chromosomes are identified. For gender determination, CMA testing or another technique called fluorescence *in situ* hybridization (FISH) can be used. With FISH, fluorescent DNA probes specific for the X and Y chromosomes attach to the sex chromosomes and can be detected using a special microscope.

WHAT HAPPENS DURING AN IVF CYCLE WITH PGD?

Testing can be performed on blastomeres from Day 3 cleavage stage embryos or from trophoctoderm biopsied from Day 5/6 blastocyst embryos. Blastomere testing allows for a fresh embryo transfer while blastocyst testing may require embryo freezing. For blastomere analysis, embryos with normal development on Day 3 will have a procedure called a biopsy where embryos are placed under a powerful microscope and a tiny opening is made in the zona pellucida (a tough outer membrane holding the embryo together). Typically, one cell is removed and the embryo is returned to the incubator. A trophoctoderm biopsy is a technique where by multiple cells are safely taken from the

embryo at a later stage of development. To perform this procedure, a small hole is produced in the zona pellucida on Day 3 and by Day 5/6, as the embryo grows and expands, the trophectoderm begins to protrude through the membrane opening. The trophectoderm represents the outer layer of the embryo which forms the placenta and not the fetus. DNA is prepared from the cell(s) obtained at biopsy for chromosomal microarray or FISH testing. The biopsied cells are frozen and sent by overnight service to GIVF's PGD facility for testing. Results are reported directly to your physician.

On the day of your scheduled embryo transfer, your physician will review the PGD screening information for each embryo and discuss these results with you. This information helps the medical team determine which embryos are most likely to result in a healthy pregnancy. You and your physician can then review your options and decide which embryos should be transferred into the uterus. Your physician can later perform a blood hormone test to confirm whether implantation and pregnancy have occurred.

ACCURACY AND TEST LIMITATIONS

PGD uses the most advanced technology currently available to provide the earliest possible information about the health of an embryo in order to minimize the chances for miscarriage or chromosomally abnormal pregnancies. PGD using CMA technology is a relatively new and technically demanding procedure and thus there is a small chance for inaccuracy.

The first step in CMA testing is the extraction of DNA from a single cell or a small number of cells. About 5% of the time the biopsied cell(s) fails to get into the DNA extraction solution or the DNA is degraded to the point where it cannot be analyzed. In such cases, it is sometimes possible to take another biopsy and get a result. Very infrequently an embryo will have an extra whole set or sets of chromosomes and these may not always be detected by CMA. However, such polyploidy embryos are not compatible with live births. Sometimes there are errors in early division of an embryo creating cells that have different genetic content. This finding is called mosaicism. If an embryo is mosaic and a single cell is tested, that cell may be different from the remaining cells in the embryo. If multiple cells are tested from a mosaic embryo, a very low level of mosaicism may not be detectable by CMA. Errors, therefore, may be due to technical limitations or mosaicism. These could result in a false positive (calling a normal embryo abnormal) or a false negative (calling an abnormal embryo normal). The technical accuracy of CMA, FISH, and single gene disorder testing is about 98%.

There are also some conditions that CMA testing cannot detect. Among these are balanced chromosome rearrangements where there is no loss or gain of DNA, very small deletions or duplications of DNA, or gene mutations for such inherited genetic diseases as cystic fibrosis.

Because the physical structure of the chromosomes is not visualized, PGD is not intended to replace CVS or amniocentesis in an ongoing pregnancy. PGD does, however, lower the chance of an abnormal result on a prenatal test.

CHOOSING EMBRYOS FOR TRANSFER

The combination of normal genetic testing with normal embryo appearance indicates the highest chance of an embryo transfer resulting in a healthy pregnancy. Sometimes embryos that have a normal genetic test will have a physical problem that prevents them from typical development. Alternatively, some embryos that have abnormal genetic tests will appear to be physically normal. Decisions regarding number and selection of embryos to transfer into the uterus are made with the advice of your medical team at your local clinic.

SAFETY

Biopsy: The process of removing a cell from an embryo does not increase the risks for birth defects or mental retardation. Biopsy may slow the development of embryos. Therefore, biopsied embryos may not have progressed as far as unbiopsied embryos. There is a rare chance that biopsy may damage an embryo resulting in the loss of the use of that particular embryo.

Cell removal: Data from many years of PGD in animals and thousands of live births in humans indicate that PGD does **not** lead to an increase in birth defects or chromosomal disorders. Biopsy is done before the embryo's genetic material becomes 'active'. Since it is done so early, each cell inside the embryo is still identical and capable of becoming any part of a baby. Removal of one or two cells of a Day 3 embryo does not appear to affect fetal development.

IMPORTANT POINTS

- PGD reduces the chance for miscarriages and chromosomally abnormal pregnancies.
- PGD for full aneuploidy is available to all couples. However, due to the possibility of test errors it is only medically recommended for couples with increased risks for chromosome abnormalities.
- PGD is most helpful when there are a large number of embryos to test. Many women who desire PGD produce limited number of embryos (four to six). Some couples choose to transfer available embryos without the benefit of PGD.

- It is not possible to correct any chromosome abnormalities detected by PGD.
- The combination of normal genetic testing with normal physical appearance increases the chance of an embryo becoming a healthy pregnancy.
- The use of PGD helps minimize the possibility of higher order multiples since it is no longer necessary to transfer large numbers of embryos to achieve a pregnancy. Embryos with normal PGD results are more likely to be healthy and develop into an ongoing pregnancy.
- Consideration of prenatal diagnosis (chorionic villus sampling or amniocentesis) is still recommended for women 35 and over regardless of PGD results. Women of any age may also wish to consider a newer option called the First Trimester Screen.