Preimplantation Genetic Testing (PGT)

Patient Information

Preimplantation Genetic Diagnosis (PGT) is a reproductive technology used with an *in vitro* fertilization (IVF) cycle to increase the potential for a successful pregnancy and delivery of a healthy child. The most common applications for PGT include testing embryos for 1) extra or missing chromosomes (aneuploidy screening, PGT-A), 2) familial structural chromosome rearrangements (PGT-SR), and 3) the diagnosis of genetic disease in couples with increased risk of transmitting a genetic disorder (PGT-M). PGT also can be used to determine gender for families at risk for an X-linked disease, family balancing, or the gender of their first child.
The PGT Laboratory at Fairfax Diagnostics performed its first clinical cycle in March 1993. Since then, the PGT laboratory staff has performed more than 5000 PGT 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 TESTING (PGT)

PGT-A 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 for IVF. Couples who have had several miscarriages, a prior pregnancy with a chromosome abnormality, or have experienced several failed IVF cycles could also benefit from PGT-A for aneuploidy. When PGT-A for aneuploidy is used for these couples, each cycle has a better potential outcome, since only chromosomally normal embryo are used greatly reducing the miscarriage rate and the risk for a chromosomally abnormal pregnancy.

PGT-SR for structural chromosome rearrangements is beneficial if a member of a couple carries a balanced translocation (exchange of chromosomal 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. PGT-SR for structural chromosome rearrangements allows couples to decrease these risks and to increase the chance of a healthy ongoing pregnancy.

PGT-M 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, as a few examples. For couples who are at risk to have children with such diseases,

Fairfax Diagnostics Lab can provide specialized PGT-M 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 building needs. Prior to the PGT-M 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 is also performed on each embryo to improve pregnancy outcomes. The goal is to provide embryos for transfer to the prospective mother that are chromosomally normal as well as free of any genetic disorder that could have been inherited from the parents.

INTRODUCTION TO CHROMOSOMES

To appreciate how PGT can be helpful to couples it is important to understand chromosomes. Chromosomes are the physical structures made of DNA that contain the 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 (called autosomes) 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 divides improperly as it is developing, the mature egg or sperm may contain more or less than 23 chromosomes. If this egg or sperm is used for fertilization, the resulting embryo will not contain exactly 46 chromosomes, but more or less than 46, known as 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 chromosome number. 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 chromosomal aneuploidy. 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.

PGT METHODS

For couples pursuing testing for aneuploidy and structural chromosome rearrangements, a method called Next Generation Sequencing (NGS) is used. This technology determines the amount of DNA present for each chromosome in cells removed from the developing embryo. Gains or losses of complete chromosomes or parts of chromosomes are identified by NGS and a dedicated software algorithm. For gender determination, NGS testing can determine the presence the sex chromosomes, XX or XY, for females or males respectively.

WHAT HAPPENS DURING AN IVF CYCLE WITH PGT?

After embryos are created using IVF and grown in the laboratory for five to six days, a biopsy is performed on all appropriate developing embryos at the blastocyst stage. Biopsy involves removing a few cells from the trophectoderm; the outermost layer of cells of the blastocyst that forms the placenta and not the fetus. To perform this procedure, a small hole is made 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 and a few cells can be safely removed. The biopsied cells are frozen and sent by overnight service to Fairfax Diagnostics' PGT laboratory for testing. DNA prepared from these cells is analyzed for chromosomal content (chromosome

number and structure), and if necessary, for genetic disease detection. Results are reported directly to your physician.

On the day of your scheduled embryo transfer, your physician will review the PGT screening information for each embryo and discuss these results with you. This information helps your 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

PGT 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. PGT using NGS technology is a relatively new and technically demanding procedure and thus there is a small chance for inaccuracy.

The first step in NGS testing is the extraction of DNA from a single cell or a small number of cells. About 4% of the time the biopsied cells 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 achieve a result. Very infrequently an embryo will have an extra whole set or sets of chromosomes and these may not always be detected by NGS. 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 compare to other cells in the same embryo. This finding is called mosaicism. If an embryo is mosaic and multiple cells are tested from such an embryo, some normal and some cells containing a gain or loss of a chromosome(s), the NGS technology may show a chromosomal copy number value between 1 and 2 copies, or between 2 and 3 copies, with a value of 2 as normal for autosomes. Results displaying mosaicism are analyzed to determine levels of mosaicism as low, moderate, or high. In our lab, embryos displaying low mosaicism of 25% or less may be considered for transfer. 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 NGS in our lab is approximately 98%.

There are also some conditions that NGS 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 *de novo* (new) gene mutations for inherited genetic diseases that were not previously identified in the parents. Because the physical structure of the chromosomes is not visualized using NGS, PGT is not intended to replace CVS or amniocentesis in an ongoing pregnancy. PGT 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 morphological (physical) 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. Therefore, embryos that have both a normal genetic test result and proper morphology are desirable. Final 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. With the benefit of PGT, couples can consider single embryo transfer with higher confidence of a pregnancy without the consequence of twin or triplets pregnancies.

SAFETY

Biopsy: The process of removing a few cells from a progressing day 5 blastocyst embryo does not increase the risks for birth defects or developmental delay. Removal of a few of the outer trophectoderm cells of the blastocyst does not alter the ability of that embryo to develop into normal pregnancy, since the method avoids disrupting the inner most cells that will form the fetus. There is only a very rare chance that biopsy may damage an embryo resulting in the loss of the use of that particular embryo. Data from many decades of PGT in animals and thousands of live births in humans indicate that PGT does **not** lead to an increase in birth defects, chromosomal disorders, or developmental delay over the general population. Follow-up evaluation of children born does not show any evidence for a detrimental effect of the process on growth or neurological development over the first several years of life. In embryos where chromosomal PGS testing is performed, one can expect fewer pregnancies ending in miscarriages due to chromosomal disorders since most abnormalities are identified prior to embryo transfer.

IMPORTANT POINTS

☐ PGT reduces the chance for miscarriages and chromosomally abnormal pregnancies.

☐ PGT-A 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.

☐ PGT is most helpful when there are a large number of embryos to test. Many women who desire PGT produce limited number of embryos (four to six). Some couples choose to transfer available embryos without the benefit of PGT.

☐ It is not possible to correct any chromosome abnormalities detected by PGT.

☐ The combination of normal genetic testing with normal physical appearance increases the chance of an embryo becoming a healthy pregnancy.

☐ The use of PGT 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 PGT 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 PGT results. Women of any age may also wish to consider a newer option called the First Trimester Screen.