Preimplantation Genetic Testing (PGT)

What is PGT?

Preimplantation genetic testing (*PGT*) is a technique that allows embryos to be genetically tested prior to being transferred into a woman's uterus for the purpose of establishing a healthy pregnancy. PGT allows embryos at risk for chromosomal or genetic conditions to be identified as healthy before they are selected for embryo transfer. In other words, the embryos that are known to be unaffected by the condition being tested can be selected for transfer, giving the best chance for a healthy pregnancy to be achieved.

PGT can only be performed in conjunction with in vitro fertilization (IVF). Since IVF involves fertilization of the eggs in a laboratory, the resulting embryos can be tested before being placed into the uterus. There are several types of PGT, which are described below:

Preimplantation Genetic Testing-Mutation (PGT-M) can be used to diagnose a wide variety of detrimental genetic conditions (e.g. Tay Sachs, cystic fibrosis, thalassemia etc.) in an embryo, facilitating transfer only of an embryo that is not affected by that genetic condition. Most often, the persons who need to use PGT-M are those who are coupled with another carrier of that identical mutation. This is because the vast majority of genetic mutations are recessive, meaning that they only manifest as disease if a child is born carrying both mutations (one from each parent). Almost all couples seeking care with a reproductive specialist will have had screening for *recessive mutations*, which is called *carrier screening.* Couples who find themselves in a situation where each partner carries the same recessive gene have a *25% risk of bearing a child with that genetic disease*. Recessive genetic diseases are serious; they can be devastating to the family as well as to the affected child. PGT-M provides such couples a way of essentially eliminating the chance of a child being born with a recessive genetic disease. For such couples, embryos will be tested for the mutant gene and those affected with the disease will not be transferred to the uterus. If an embryo is not affected with the disease, but carries one copy of the recessive disorder ("carrier embryos") it, too, may be transferred, most often without consequence. (It should be mentioned that some at-risk couples find out during pregnancy about a mutation in the fetus and have to face the awful decision to go through with or terminate their pregnancy. PGT is a proven way to prevent such decisions for needing to be made.)

Some couples understand the need for PGT-M before they begin any attempt to conceive, when preconception screening has identified them both as co-carriers. (Some couples know they are carriers because they already have had a sick child. With carrier screening readily available, there is no good reason why couples should ever have to be in this situation. Optimally, all couples are tested before they begin

trying to conceive.) Some persons carry a *dominant mutation* (e.g. Huntington's disease or neurofibromatosis). They are, by definition, affected by the genetic mutation and they, too, will need PGT-M if they wish avoid the *50% risk of transmitting the disease to their child*. This is true regardless of whether or not the partner carries the same mutation. The same is true for some genetic mutations that affect the X-chromosome. Women who carry mutations in their X chromosome, or those who have been found to have a *Fragile-X* chromosome, often require PGT-M to screen out the *Fragile X Syndrome,* a genetic disorder that is the most common transmissible cause of mental retardation.

Some couples are at risk for having offspring with genetic disease even though they are not carriers of damaged DNA. These include situations where one parent is carrying an addition or deletion of chromosomal material. There are 23 pairs, or a total of 46 chromosomes in every cell of the human body. As home to all our DNA, the chromosomes provide the essential instruction manual (*genetic code*) that allows us to develop and to be born. Nature does not tolerate any deviations from this number, although occasionally those born with a small chromosomal *deletion* or *addition* do reach adulthood and wish to have children. In such situations, PGT-M or its subtype, PGT-SR, which detects structural rearrangements, is indicated.

One common example is when one partner carries a *balanced chromosomal translocation*, which is a known cause of *recurrent pregnancy loss*. The purpose of PGT-SR in such cases is to evaluate embryos for the chromosomes involved in the translocation. Embryos containing unbalanced chromosomal rearrangements will not be transferred as they could lead to miscarriage or other abnormalities. Not all PGT-SR differentiates normal embryos from those with a balanced translocation but both can equally lead to a normal pregnancy and a healthy child.

Preimplantation Genetic Testing-Aneuploidy (PGT-A) does not detect any specific genetic condition but, more simply, screens for the correct number of chromosomes in the embryo, which is called *euploidy*. An abnormal number of chromosomes, which is called *aneuploidy*, can lead to failed implantation and miscarriage or, less commonly, developmental and cognitive impairment, as seen in children with Down Syndrome.

Situations that are amenable to PGT

PGT-M may benefit couples who are known carriers of chromosomal or genetic conditions, since they are high risk of having embryos affected by these conditions.

PGT-A is offered to couples who have experienced multiple pregnancy losses or multiple IVF failures. PGT-A may also benefit women who are over 35 years of age. Finally, PGT-A may be offered to men with severe *oligospermia* (poor sperm counts) *non-obstructive azoospermia* (absence of sperm not caused by any blockages) or other forms of severe male infertility. This is not a comprehensive list of indications for PGT-A. Should your physician deem that your overall fertility situation would be improved with PGT-A, the rationale, or *indication for PGT*, will be discussed with you.

What are chromosomes?

Our bodies are made up of billions of cells, and within each of our cells our genetic material is packaged into chromosomes. Chromosomes are made of DNA and contain the information that allows our bodies to develop and function normally. The nucleus of each cell contains 23 pairs of chromosomes, or 46 in total. We inherit

these chromosomes from our parents; optimally, during a reproductive encounter the egg and sperm will each contain 23 chromosomes so that, when they fuse to form an early embryo, it then contains a total of 46 chromosomes. All healthy humans begin life with as one cell containing 46 chromosomes; together, they contain approximately 3 billion molecules called, arranged as *pairs of nucleotides*, that make up the DNA strand that will control our physical features such as height, eye color, skin tone, etc. As development occurs, the chromosomes and their DNA content replicate continuously and virtually without error. The sequence of those *base pairs* is what scientists refer to as our *genome.*

What is a chromosomal abnormality?

Chromosomal abnormalities are either extra or missing genetic material or a rearrangement in chromosome structure. Most chromosomal abnormalities are severe and result in miscarriage or failure to conceive. If a baby with a chromosome problem is born, he or she could have developmental problems and/or mental retardation. However, some chromosome abnormalities are very mild and may cause no health problems at all.

What is aneuploidy?

As already noted, physically normal individuals have 46 chromosomes in each of their cells. A *chromosomal aneuploidy* arises when the individual develops from an embryo that had greater or fewer than 46 chromosomes, i.e. when one or more of the chromosomes is missing or present in an extra copy. For example, individuals with Down Syndrome all have one extra chromosome in each of their cells, or 47 chromosomes in total. Chromosomes are arranged in numbered pairs for the purpose of identification, based mainly on their size. The pairs are numbered 1-22; the last pair is the sex chromosomes, X and Y. The extra chromosome that causes the physical and developmental features of Down syndrome is always chromosome 21, and hence it is technically referred to as *Trisomy 21*. Other aneuploid conditions that are rarely diagnosed in newborns are Trisomy 13 and Trisomy 18. Sometimes aneuploidy involves the sex chromosomes – girls may be born with one instead of two X chromosomes (*Turner Syndrome*, 45X) and some boys are born with an additional X chromosome (*Klinefelter Syndrome*, 47XXY). All of these are very rare conditions and they are not inherited from parents – they occur as genetic accidents during the formation of eggs or sperm in the parents, who are perfectly healthy. These phenomena cannot be predicted except through PGT-A. These children will experience severe reproductive disorders as adults. The features of each condition depend upon which chromosome is extra or missing. It should be noted that aneuploidy may affect any chromosome(s) in the embryo. The vast majority of these are not compatible with human development and therefore embryos with these aneuploidies will either not implant or will result in a miscarriage.

Does the risk of aneuploidy increase with maternal age?

As a woman's age increases, the chance of aneuploidy in her pregnancies also increases. Because women are born with all the eggs they will have in their lifetime, a woman's eggs are as old as she is. It is thought that older eggs are more likely to contain "mistakes" in their chromosome number, i.e. extra or missing chromosomes. These extra or missing chromosomes cause aneuploidy in the embryo. A 30 year old woman has a 1/385 chance of giving birth to a child with an aneuploidy, such as Down syndrome. This chance increases to 1/204 at age 35, 1/64 at age 40 and 1/19 at the age of 45.

It is important to note that these statistics refer to children who have been born. The frequency of aneuploidy and other abnormalities in embryos is actually much higher. The above statistics do not include all abnormal embryos, because these embryos are less likely to implant in the uterus or go to term. Most result in miscarriage. For women between the ages of 35 and 39 years old, 30% or more of embryos can be aneuploidy. As maternal ages rises above 40 years old, this number can rise to 75% or more. As already noted, the vast majority of aneuploid embryos will either not implant or, if they do implant, will result in miscarriage. This completely explains the association of advanced reproductive age with impaired fertility and increased risk of miscarriage.

During a regular IVF cycle, it is impossible for the embryologist to determine whether a given embryo is aneuploid without testing the embryo via PGT-A. Aneuploid embryos and chromosomally normal embryos are indistinguishable from one another visually. It is possible for aneuploid embryo visually to look very healthy.

Does the risk of aneuploidy increase with paternal age?

Paternal age is not associated with an increased risk for aneuploidy. In the average man, sperm is made every 65-75 days. Since sperm is constantly being regenerated unlike a woman's eggs, we don't find higher rates of aneuploidy with increasing paternal age. (Some health issues have been described in children who were fathered at an *advanced paternal age*, but these are not usually chromosomal in origin.)

What procedures are involved in PGT?

The purpose of preimplantation genetic testing is to allow selection and transfer of only chromosomally normal, disease-free embryos during an IVF cycle. The goal of PGT is to achieve more pregnancies, reduce the number of pregnancy losses, and reduce the transmission of chromosomal disorders to offspring. The procedures involved in PGT are described below:

1. IVF: The first step involves the same procedures as a regular IVF cycle. The ovaries are stimulated to produce multiple eggs. When the eggs are mature, they are retrieved. These eggs are then fertilized in the laboratory and the resulting embryos are monitored as they develop. Only embryos that develop to the *blastocyst stage*, which generally occurs by the 5^{th} or 6^{th} day after egg retrieval, can be biopsied. *Not all embryos will reach this stage and become suitable for biopsy.* This is important because, in the case that no embryos reach the blastocyst stage, the cycle will be deemed to have failed even with no embryo transfer taking place. Embryos that do not develop to blastocysts are, in any event, most likely to be chromosomally abnormal.

2. Embryo biopsy: To analyze an embryo, it must be biopsied. An opening is first made in the covering of the blastocyst, called the *zona pellucida*. A small cluster of cells (3-5) is taken from a developing blastocyst; this is called a *trophectoderm biopsy*. The trophectoderm is the precursor of the cells that become the placenta, and generally contains the same chromosomal content as the *inner cell mass*, which is the precursor of the fetus. By removing only trophectoderm cells, harm to the future fetus is avoided. Because analyzing the contents of the biopsied cells takes time, the blastocyst must be frozen after it is biopsied. This is so that, when it is eventually transferred to the patient, the blastocyst will be synchronized properly to her endometrial (uterine) lining. The

freezing process, called *vitrification*, generally will not affect the implantation potential of the embryo after it is thawed. Not all blastocysts meet the criteria for biopsy or subsequent freezing only those that meet the laboratory criteria are biopsied. Genetic analysis is performed by external clinical genetics laboratories that work in conjunction with the GENESIS embryology lab.

3. Genetic analysis: The biopsied cells are analyzed using genetic techniques that detect gene mutations, chromosomal rearrangements or aneuploidy. In order to screen for gene mutations (PGT-M), a specific testing strategy must first be developed for each case by the testing lab. This process, termed "*probe development*," must be completed prior to the onset of the IVF cycle. For chromosomal issues, including rearrangements and aneuploidy, the biopsied cell's DNA is analyzed using gene sequencing. Various sequencing platforms currently in use permit the simultaneous testing of all chromosomes, allowing chromosomally normal (*euploid*) cells to be distinguished from cells with extra or missing genetic material (*aneuploid*). The analysis normally takes 7-10 days to complete. For couples undergoing testing for PGT-M, the analysis can take 2-3 weeks, depending on the complexity of the mutation.

4. Embryo transfer: In general, we recommend that the embryo transfer occur with a subsequent menstrual cycle following your fresh IVF cycle. In other words, after the embryos are biopsied, they are frozen while we await the results. Once the results have returned and we have confirmed that there is at least one normal embryo to transfer, we then schedule a frozen embryo transfer to occur with a future menstrual cycle. Because of the high efficiency of vitrification, embryos are expected to have the same implantation potential whether the transfer occurs a month after retrieval or many months or, even years, later.

Who does GENESIS collaborate with to perform PGT?

If you choose to undergo PGT, your IVF cycle and the embryo biopsy will be performed at GENESIS. The biopsied cell(s) will be sent to an external lab for analysis. GENESIS collaborates with several providers, six of which are listed below. Your clinical situation and your insurance will determine which lab is used.

1. Igenomix, Jersey City, NY
   www.igenomix.com
2. Cooper Genomics, Livingston, New Jersey, NY
   www.coopergenomics.com
3. NateraGenetics, Cambridge, MA
   https://www.natera.com
4. Reproductive Genetics Institute, Chicago, IL
   www.reproductivegenetics.com
5. Invitae, San Francisco, CA
   www.invitae.com
6. Foundation for Embryo Competence, Basking Ridge, NJ
   www.feclabs.org

How are the embryos genetically tested?

Before testing can be done on the cells biopsied from an embryo, the genetic material (DNA) of the embryonic cells is amplified using a technique called *polymerase chain reaction* (PCR). This amplification produces enough DNA for the testing to be performed. The amplified genetic material is then generally tested for

aneuploidy using next generation sequencing (NGS) microarray. Both of these testing methods determine the amount of DNA derived from each chromosome, revealing whether or not the correct number of chromosomes is present.

NGS, the latest technological breakthrough, detects substantially more markers on each chromosome, making it more reliable than with older techniques.

What are the advantages of PGT?

PGT-A and PGT-SR will reduce the chance of having a child with aneuploidy. PGT-M will prevent the transmission of genetic disease from at-risk couples to their child. However, no test is 100% accurate and PGT-based testing is not as robust as standard testing strategies performed during pregnancy. Because of this limitation, **we recommend that all resulting pregnancies are tested via CVS (chorionic villous sampling) or amniocentesis** in order to confirm the PGT diagnosis.

PGT-A may increase implantation rates. In general, pregnancy rates after IVF decrease dramatically with maternal age. Particularly for women over 35, pregnancy rates after IVF are low. The decrease in pregnancy rates with maternal age is partly caused by a corresponding increase in aneuploid embryos. Aneuploid embryos are known to have significantly lower implantation rates than normal embryos. By performing PGT-A for aneuploidy and transferring only chromosomally normal embryos, we are able to increase both implantation rates and live birth rates in this demographic category.

PGT for aneuploidy can also reduce pregnancy losses/miscarriages. In women over 35, approximately 35% of pregnancies will miscarry, and this percentage rises with increasing age. Aneuploidy is the cause in more than 50% of these losses. Studies have shown that PGT-A reduces the miscarriage rate by at least half. Many studies that have examined the use of PGT-A to selectively transfer chromosomally normal embryos in women over 35 have shown an increased likelihood of pregnancies going to term.

What are the risks of the embryo biopsy?

Blastocyst biopsy techniques require removing a few cells from the developing embryo for analysis and are associated with very low risks. The risk of accidental damage to the embryo due to the biopsy is around 1%. No part of the future fetus will be lacking as a result of the biopsy because the biopsied area of the embryo is not actually from the part of the embryo that will eventually grow into the fetus, but rather from the part that grows into the placenta. Data from many studies indicates that embryo biopsy does not lower implantation rates, and as already noted the selection of euploid embryos by this technique will increase implantation rates, especially in women older than 35 years old. The balance between potential biopsy damage and beneficial effects of PGT seems to be positive.

Is there a risk of misdiagnosis?

Unfortunately, with any testing procedure there is always a risk of misdiagnosis. The accuracy of Day 5 biopsy for PGT-A is greater than 95%. The error rate of NGS testing for aneuploidy is thought to be approximately 2%, although other forms of errors could occur during the overall process. There are several types of misdiagnoses, including a false negative result or a false positive result. Not uncommonly, the diagnostic procedures can fail, leading to a “no result.” On occasion, biopsied cells that show no result might call for re-biopsy of the embryo.

Accuracy of the diagnosis is also somewhat confounded by a condition known as *mosaicism* that can occur in a small fraction of embryos. Typically, all of the cells of a single embryo have the same chromosomal make-up. A mosaic embryo is one with cells that have different chromosome complements. In other words, some of the embryo's cells may be chromosomally normal and some may be aneuploid, or different aneuploidies can occur in cells of the same embryo. The embryo's cells are described as "mosaic" because they can be thought to resemble a mixture of different mosaic tiles.

It is thought that mosaicism results in a difference between the biopsied cell and the remainder of the embryo in perhaps 2-5% of tested embryos. A mosaic embryo, with a mixture of normal and aneuploid cells, could be misdiagnosed as being normal if the cell chosen for biopsy and analysis happens to be chromosomally normal. Due to the chance of misdiagnosis, **we recommend that all resulting pregnancies are tested via CVS (chorionic villous sampling) or amniocentesis in order to confirm the PGT diagnosis.**

Mosaicism is a natural phenomenon present is 30% of embryos and independent of age. A few studies on mosaic embryos show that mosaic embryos, mostly those with a low percentage of aneuploid cells, have the potential to be used successfully. If the pregnancy does not miscarry, testing later in the pregnancy will often reveal a fetus with completely normal chromosomes, and perfectly healthy children have been born from such mosaicism. However, this is also an increased risk of failed implantation and, even if implantation does occur, of miscarriage. The reasons for this, clearly, are related to the abnormal chromosomes. However, there is also a chance that the fetus may be carrying the mosaicism, and in this case there is a significant chance that the child would be affected with a variety of physical and cognitive disabilities. Again, this is the reason that any transfer of a mosaic embryo that results in an ongoing pregnancy must be followed by prenatal genetic assessment of the fetus **via CVS (chorionic villous sampling) or amniocentesis in order to confirm the PGT diagnosis**

What can go wrong?

a. The embryo can get damaged during the biopsy procedure.
b. DNA in the cells may fail to amplify using PCR. If this process fails, it is not possible to obtain an answer. This is a rare event.
c. All embryos are found to be abnormal or of the unwanted genetic type and no embryo transfer can take place.
d. Incorrect embryo diagnosis due to mosaicism: We estimate, on the basis of our experience and worldwide figures, that the risk of an incorrect diagnosis will be around 5%. For this reason, patients becoming pregnant from the transfer of embryos diagnosed by this procedure are still encouraged to have pre-natal diagnosis of the fetus at approximately 12-14 weeks of pregnancy.
e. No embryos grow to the blastocyst stage, in which case PGT cannot be performed, the embryos are discarded and the cycle therefore fails.

In summary, it is possible that all embryos tested may be abnormal, leaving no normal embryos available for transfer. It is also likely that, after transfer, there may be no normal embryos remaining for cryopreservation. The genetic diagnostic procedures can fail completely or partially, resulting in embryos at the time of transfer that do not have results available. Conversely, PGT can provide important information regarding embryos that have been biopsied and cryopreserved. Embryos that are found by the PGT analysis to be aneuploid or carriers of genetic disease can

be ignored/discarded, while those that are found to be euploid and genetically healthy can confidently be kept in storage for future use.

What is the cost of PGT?

Please discuss the current fees for PGT with our financial counselors. The PGT fees are in addition to the cost of IVF and embryo transfer. The overall fees will include the cost of the biopsy procedure performed at GENESIS as well as the genetic testing fees from the laboratory performing those procedures. Insurance companies typically do not cover the cost of PGT-A. It is usually an out of pocket expense. Many companies will cover PGT-M and PGT-SR given the obvious medical necessity that prompts the IVF treatment.

One final note

If you are planning an IVF cycle with PGT-M, you must have had a consultation with the genetic counselor at Genesis. She will act as a liaison between you and the genetics laboratory that will perform the testing. As stated above, probe development for the mutation in question can take four to eight weeks or more. Under no circumstances will the start of your IVF treatment begin prior completion of probe development. This is in order to ensure that your IVF and PGT treatments will proceed as smoothly – and successfully – as possible.

If you are planning to have PGT-A done as part of your IVF cycle, you must have had a consultation with either the genetic counselor or, more commonly, the Director of Laboratories at Genesis, *before the start of your IVF treatment*. All genetic labs require that you be fully informed of the benefits and risks of PGT, so this separate consenting process is required. The laboratory will expect that a document testifying to your informed consent is on hand prior to running the PGT analysis. You must also keep in mind that, even when PGT has been planned, on occasion the PGT will not be performed. Successful outcome of PGT is heavily dependent embryo development in the laboratory. Embryos that do not develop to the blastocyst stage cannot be biopsied.