

PGD (PREIMPLANTATION GENETIC DIAGNOSIS) FACT SHEET

WHAT IS PGD?

Reproductive medicine and genetics are gradually intersecting and merging into a potent mix of technologies. However, the precise role of genetics in assisted reproductive technologies remains somewhat murky.

A PGD treatment cycle requires IVF to form embryos. PGD refers to the technique of evaluating individual cells within an embryo for genetic composition and abnormalities. PGD requires using IVF to produce a growing embryo and then removing 1-2 cells for genetic analysis. The genetic analysis takes 1-2 days, after which we transfer 1-3 of the unaffected or presumed normal embryos into the uterus.

Two different genetics techniques yield the diagnostic information necessary to decide which embryos to transfer.

1. **FISH (Fluorescence In-Situ Hybridization)** refers to the technique of "painting" the whole chromosome with a genetic probe bound to a colored marker. FISH allows us to count the chromosomes based on color staining but it is limited to staining only 8-10 of the 23 chromosomes in the cell.
2. **PCR (polymerase Chain Reaction)** refers to the technique of splitting chromosomes into pieces and then looking for normal and abnormal chromosome fragments. PCR works well with gene disorders but does not help identify the number of chromosomes present.

We offer PGD after diagnostic testing and extensive consultation. We partner with one of the pioneers in PGD technology; Dr. Santiago Munne (Reprogenetics, Inc., www.reprogenetics.com) whose laboratory performs the PGD analysis of the embryos.

WHAT ARE THE INDICATIONS FOR PGD?

GENETIC DISEASE IDENTIFICATION

Some people carry an abnormal gene in their chromosomes but because they only have one copy of the abnormal gene, they do not have the disease (autosomal recessive gene – the most common form). However, if they create a pregnancy with another person who also carries the same abnormal autosomal recessive gene then they have a 25% chance of having an affected child. Other types of genetic disorders occur (autosomal dominant, X-linked recessive, etc.) and a session with a genetic counselor can explain the variations and risks for having an affected child. The genetic basis for some diseases such as cystic fibrosis, spinal muscular atrophy or Huntington's chorea resides in a single gene.

ADVANCED REPRODUCTIVE AGE

As a woman ages, she becomes less fertile and also becomes more likely to miscarry when a pregnancy does occur. The genetic mechanism for most pregnancy losses has been known for decades. **Meiosis** is the process of cell division for reproductive cells (eggs and sperm) in which the "daughter" cells should contain half as many chromosomes as the parent cell. Faulty meiosis or **nondisjunction** occurs more commonly in eggs than sperm and results in gamete cells with the incorrect number of chromosomes. Meiotic nondisjunction increases in frequency as a woman ages such that as many as 60-80% of pregnancies miscarry when a woman reaches her 44th birthday.

PGD, using the FISH technique to count the number of selected chromosomes identifies many chromosomally-abnormal embryos. By selecting the embryos containing the normal number of chromosomes (limited to those chromosomes tested), we can reduce the incidence of miscarriages and the incidence of babies born with abnormal numbers of chromosomes like trisomies (like Down's syndrome) and monosomies (Turner's syndrome). In situations where the woman produces a large number of embryos, we also increase the pregnancy rate from IVF by increasing the probability that the transferred embryos were chromosomally normal.

RECURRENT PREGNANCY LOSS (RPL)

Similar to women of advanced reproductive age, some women repeatedly lose pregnancies because they produce eggs with abnormal numbers of chromosomes. Another group of women and men with RPL carry a genetic abnormality called a translocation. A person with a chromosomal translocation is normal but an examination of their chromosomes reveals that pieces of two different chromosomes switch places. Thus, during meiosis when the chromosomes split in half and one chromatid goes into one "daughter" cell and the other goes into the other "daughter" cell; we end up with a sperm or egg in which the total chromosome complement could be normal, a balanced translocation like the parent, or an unbalanced translocation which will fail as a pregnancy.

PGD FISH assists in identifying the abnormal embryos before we transfer them into the uterus. FISH for the number of chromosomes (again, limited to 8-10 chromosomes) is the techniques used for most RPL cases. FISH probes for the tips of the chromosomes (**telomeres**) are used to identify embryo status for translocation cases.

REPEATED IVF FAILURES

Some patients fail to successfully deliver a baby with even the most intensive and sophisticated treatments. PGD may offer patients with this problem some additional hope. Identification of chromosomally abnormal embryos from the pool of embryos for transfer may improve the probability of a successful IVF treatment cycle. The incidence of aneuploid (abnormal) embryos is as high as 40% and similar to patients with RPL.

FAMILY BALANCING

PGD FISH also allows determination of the sex of the embryo before transfer. We offer a program of sex selection called "Family Balancing" for those families where the number of boys and girls is uneven and the family would like to choose the sex of their next child. We allow patients the option of choosing for the sex that is lower. For example, if a couple has a boy, they may use family balancing to have girl.

Combining PGD with MicroSort, an experimental technique to sort the sperm cells into the X & Y-bearing fractions, increases the number of embryos of the desired sex. MicroSort requires that the couple meet the experimental design requirements (see the MicroSort web site for details).

| Genetic Conditions for Which PGD Procedures Have Been Applied | |
|---|-------------------------------|
| Cystic fibrosis | Epidermolysis bullosa |
| Tay-Sachs disease | Lesch Nyhan syndrome |
| Hemophilia A | Multiple epiphyseal dysplasia |
| Retinitis pigmentosa | Phenylketonuria |
| Sickle cell disease | Thalassemia |
| Gaucher's disease | HLA typing |
| Alport disease | Myotonic dystrophy |
| Alpha-1 antitrypsin | Fanconi anemia |
| Fragile-X | X-linked hydrocephalus |
| Chromosome aneuploidies | X-linked disease by sexing |
| | Translocations |

ETHICAL AND SOCIAL ISSUES

Any technology involving human reproduction touches on very elemental and core ethical issues. For most people, attitudes and feelings toward this technology are anything but ambivalent. Polar extremes are the rule. PGD is no exception. This technology offers couples an opportunity to select embryos that are unaffected by chromosomal abnormalities and those most likely to result in a healthy child. The corollary to this process of selection is that embryos not selected may be discarded.

Ethical objections to genetic selection of embryos also turn on the concern that to open the door to any, selection could lead to inappropriate selection, to gene therapies, or some form of genetic engineering. Even among those favoring these technologies, shared concern exists that strict control should be exerted over a fear that in the future they could be used for questionable or objectionable means. The process of selection of normal embryos draws for many the specter of eugenics and the encouragement of a perfect society. It also draws into the debate the issue of means (avoiding any offspring with genetic defects) justifying an end (manipulation and discarding of embryos).

The discussion intensifies over the issue of social sexing or family balancing (selecting embryos of a desired sex, usually of the opposite sex from an existing child and discarding embryos of the undesired sex). In this setting, genetically normal embryos are discarded for social reasons.

These clinical scenarios touch on extremely sensitive issues for some regarding the sanctity of life and serve as a lightning rod for debate.

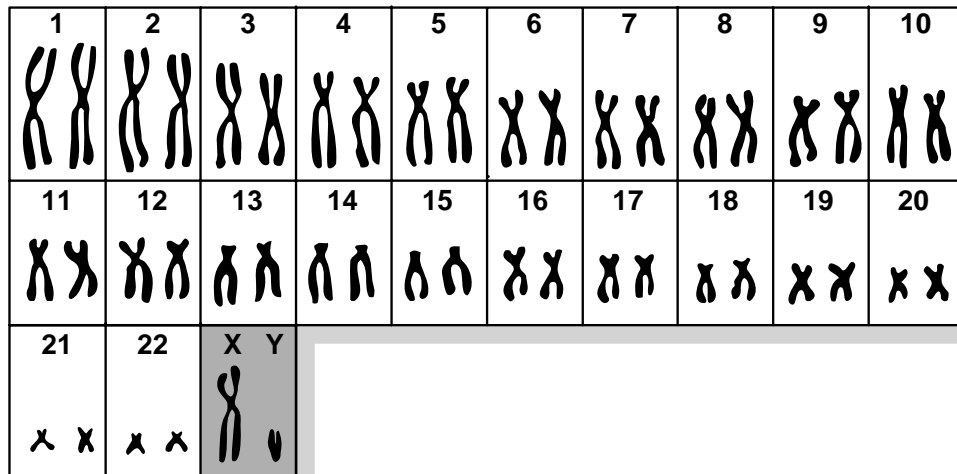
BASIC REPRODUCTIVE GENETICS

These sections return to the basic science underlying the PGD process. Now that you know what the PGD process involves and the indications for PGD, we offer a review the genetics involved for those who would like a deeper understanding.

WHAT ARE CHROMOSOMES?

In order to understand how chromosomes may be abnormal, it is first important to understand the composition and function of chromosomes. The chromosomes are the physical structures that contain the DNA and genes necessary for our development. The 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 chromosomes become visible at a stage of cell division called metaphase. Scientists divide the 23 chromosome pairs into autosomes, which are the same in men and women: 22 pairs identified with numbers (pair #1 being the largest and pair #22 the smallest). The 23rd chromosome pair refers to the sex chromosomes. Women normally have 2 of the same sex chromosomes, called the X chromosome, while men normally have 2 different sex chromosomes, known as the X and Y chromosomes. The shorthand way to refer to a normal set of chromosomes is 46, XX for women and 46, XY for men.



Reproductive cells called gametes (sperms or eggs) form by dividing into two cells through a process called meiosis with the result that each cell contains ½ of the chromosomes. In a normal conception, the egg and sperm cells contribute exactly 23 individual chromosomes each; one of each of the 22 numbered pairs and one from 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.

WHAT ARE CHROMOSOME ABNORMALITIES?

The number of each chromosome refers to its "ploidy" (this number is normally 2, that is to say, we have two of each of the numbered chromosomes as well as two sex-determining chromosomes X and Y.) Normal embryos are euploid (true number); abnormal embryos are called aneuploid. Chromosome screening by PGD is also called aneuploidy screening.

Rather frequently, during meiosis, an egg or sperm cell divides but the mature egg or sperm contains greater or fewer chromosomes than the normal 23. Any embryo resulting from this gamete would contain more or less chromosomes than the normal 46 and it would generally not develop normally with a few exceptions such as Down's syndrome in which an embryo with an extra chromosome 21 sometimes develops into a live baby.

In approximately 70% of spontaneous miscarriages, we find an abnormal number of chromosomes. The most common chromosome abnormalities found in miscarriages include trisomy 16 (3 copies of chromosome number 16); trisomy for chromosomes 22, 21, 15, 18 or 13; triploidy (3 copies of all the chromosomes); and abnormalities of the sex chromosomes.

Another less common chromosome abnormality is called a translocation. A person with a translocation has all the chromosome normal material but pieces between two chromosomes change places on one of the chromatids of each chromosome (each chromosome has two chromatids). The result is that when the sperms or eggs form by meiosis, the cells contain either normal chromosomes, a balanced translocation, or an abnormal chromosome complement because the cell received one normal chromatid and one abnormal chromatid.

While there are many reasons for the failure of an embryo from IVF or natural conception to make a baby, the single most important factor is an abnormality of the chromosomes. Chromosomally abnormal embryos usually fail to implant in the uterus, while others implant but cannot develop normally and the pregnancies miscarry in a few days to a few months. The timing of the miscarriage depends on which or how many chromosomes are abnormal in the embryo.

The percentage of chromosomally abnormal embryos that each couple produces varies depending on their clinical status. Factors such as advancing maternal age (> 35 years), the number of prior miscarriages, the number of failed IVF cycles, and the quality of sperm influence the proportion of embryos that are abnormal.



WHAT ARE SOME PGD LIMITATIONS?

Technical and biological issues reduce the accuracy of the PGD process.

In order to obtain a biopsy result, the cell must contain an intact nucleus which contains the chromosomes. If the cell has no nucleus or if the nucleus ruptures during the biopsy procedure or cell fixation process, that cell cannot be evaluated. The incidence of non-diagnostic cells varies between 5-15% of cells. During normal embryonic cell division, the embryo contains two nuclei. If the cell chosen for biopsy happens to be in the middle of cell division, the result will show four sets of chromosomes and falsely lead to a diagnosis of an abnormal embryo.

Occasionally, two chromosomes might be lying on top of each other, which would underestimate the number of chromosomes and lead to a false interpretation. The corollary situation is when the probe does not bind to a chromosome suggesting a missing chromosome.

Further, some embryos have two separate cell lines called a mosaic. Mosaic embryos may be abnormal but in many cases the abnormal cell line dies off or becomes part of the placenta rather than the baby. Unfortunately, we cannot tell the final outcome. Consequently, a mosaic embryo could be read falsely as normal or abnormal.

In total, we believe that the false normal rate is about 5% and that the false abnormal rate is about the same, 5%