

[THE CENTER FOR REPRODUCTION & WOMEN'S HEALTH CARE]

Patient Information

Preimplantation Genetic Diagnosis

Using Embryo Biopsy and Fluorescence in situ Hybridization (FISH), or
Other Procedures Including PCR Single Gene Analysis

This informational pamphlet discusses preimplantation genetic diagnosis (PGD) on your embryo, the purpose of which is to attempt to increase the likelihood of achieving a healthy pregnancy and children following *in vitro* fertilization (IVF). It also addresses other procedures for PGD, such as PCR-Single Cell Gene Analysis, that may be recommended for you. It is important that you read this thoroughly, and have any questions answered by your [The Center for Reproduction & Women's Health Care] physician. In order to undergo preimplantation genetic diagnosis, you will have to weigh the risks, benefits and alternatives discussed, and sign separate consents for (1) In Vitro Fertilization (including stimulation, monitoring and embryo transfer) and (2) Embryo Biopsy and PGD using FISH or (3) Embryo Biopsy and PCR Single Gene Analysis or Other Genetic Tests.

[The Center for Reproduction & Women's Health Care] performs all IVF services and the Embryo Biopsy that is involved in PGD. Genetic testing, however, is performed by independent laboratories for which [The Center for Reproduction & Women's Health Care] is not responsible. FISH is performed by the independent entity RMA-Genetics, LLC. RMA-Genetics, LLC has agreed to use the consent forms given to you by [The Center for Reproduction & Women's Health Care] as evidence of your permission to undergo testing. If you require PCR Single Gene Analysis or Other Genetic Tests performed by another independent laboratory (other than RMA-

PGD involves procedures which allow for the study of specific cells, taken from embryos in the laboratory, as a method of detecting chromosomal abnormalities, prior to the time that such embryos are transferred to the uterus for the possible establishment of a pregnancy. Since both analyses require examination of the embryos in vitro (in a laboratory dish), PGD requires the use of in vitro fertilization (IVF). After genetic analysis, the patient and physician discuss the results and what they may mean for the future development of such embryos to assist the patient in making an informed decision as to whether to undergo embryo transfer, and -- if so -- which embryos to select for use in such transfer.

The purpose of preimplantation genetic diagnosis is to attempt to detect chromosomal abnormalities and potentially avoid the transfer of an embryo which has certain abnormal chromosomes (hopefully preventing a pregnancy with an abnormal fetus, or the possible delivery of a child with genetic defects). Also, by selecting embryos for transfer which, according to the test results, appear to have the best chance for acceptable development, it is possible that the rate of implantation may be increased and the number of miscarriages may be reduced. **Not all genetic defects, however, are ascertainable using PGD, and when undergoing PGD, there is the risk of inaccurate results. PGD offers no guarantee that a diagnosis can be made, that pregnancy will occur, or that a healthy child (free from genetic defects) will result. If you elect to proceed with PGD, you must acknowledge these risks.**

Any understanding of the purposes of PGD or its methods, requires some knowledge of the genetic conditions which form the basis for the need for PGD. *Please note that [The Center for Reproduction & Women's Health Care] strongly recommends that you discuss potential genetic abnormalities with a geneticist and/or with your physician prior to performing the genetic diagnosis on your embryos and again after the diagnosis has been made.*

Information Concerning Genetic Abnormalities that May Affect the Health of a Developing Embryo

During fertilization, the egg provides a set of chromosomes and the sperm provides another set, resulting in two of each chromosome. However, sometimes genetic abnormalities occur in the formation of the sperm or eggs (or both) resulting in embryos with too many or too few chromosomes (**aneuploidy**), or too many or too few parts of specific chromosomes (**translocation or inversion**). For example, the embryo should contain two copies of chromosome 21. When there is an extra copy (three chromosome 21's), the embryo may result in an individual with Down Syndrome (Trisomy 21).

There are essentially three types of genetic conditions which can have significant effects on the development of an embryo: (1) aneuploidy, (2) translocation, or (3) inversion.

Aneuploidy

Aneuploidy is a condition where there are either extra, or too few, chromosomes. When the normal number of chromosomes is present, it is referred to as euploidy. Chromosomes are “numbered” and at least five (5) chromosomes will be tested with PGD, because such chromosomes account for most of the chromosomal abnormalities (aneuploidy) seen in newborns or in the tissue of miscarriages. These abnormalities are also found in up to approximately 50% of human embryos and may contribute to failure of some embryos to implant in the uterus following IVF and embryo transfer. Aneuploidy for these chromosomes can also lead to embryo death, miscarriage, or the live birth of an infant with substantial medical problems. In fact, aneuploidy in a developing embryo may be associated with the following:

1. **Failure to achieve a pregnancy.** Studies have demonstrated that the majority of embryos obtained following IVF that fail to implant in the uterus or miscarriage are **aneuploid** or “**unbalanced.**”
2. **Loss of a pregnancy.** Chromosome abnormalities are the most common reason for spontaneous miscarriages (loss of a pregnancy after it has been established in the uterus).
3. **Offspring born with aneuploidy or an “unbalanced state.”** The medical outcome and the quality of life achieved by children born with these genetic abnormalities will vary depending on which chromosome(s) is affected. *If you have not already done so, you should meet with a medical geneticist and/or a genetic counselor, which will discuss with you the expected outcomes of children born with a genetic abnormality.*

The chance of having a miscarriage or having embryos that fail to develop into a pregnancy increases as the age of the woman increases. There is evidence that the relationship between these two conditions and maternal age may be explained a higher incidence of aneuploidy in the offspring of older women.

While there are many chromosomal aneuploidies, the most common that occur with chromosomes 13, 16, 18, 21, X and Y, and include the following:

- **TRISOMY 21 (one extra chromosome 21, also known as Down’s syndrome)** is the most common chromosome aneuploidy found in live births. Approximately 1 in every 800 babies has it. It is associated with advanced maternal age. Children who have Down syndrome can have varying degrees of developmental delay. Some may be mildly affected, while others may be more severely delayed. Some children with Down syndrome have other associated medical problems, like heart defects or leukemia that may require medical management.

- **TRISOMY 13 (one extra chromosome 13)** In the general population, 1 in every 5,000 babies born has trisomy 13. Trisomy 13 is associated with advanced maternal age. These babies have multiple system abnormalities, including abnormalities of the heart, brain and cleft lip/palate. Babies with trisomy 13 have limited life span. Most of them die within the first few months of life, and those that do survive are severely delayed in growth and development.
- **TRISOMY 18 (one extra chromosome 18)** Approximately 1 in every 3,000 live births results in trisomy 18. It is associated with advanced maternal age. Most babies with trisomy 18 have multiple abnormalities including heart, brain, mental retardation and delayed growth. Most of the babies do not survive more than a few months.
- **XXX (one extra X chromosome resulting in three X chromosomes)** Incidence of XXX is approximately 1 in 1,100. XXX is also associated with advanced maternal age. These are normal appearing, fertile females who may present with learning disabilities.
- **XXY (one extra X chromosome in a male)** This occurs in about 1 in 1,100 births. These males are normal in appearance and are often taller than other male siblings. There are some studies suggesting that these males may have behavioral problems but generally have a normal IQ.
- **XO (missing one X chromosome; also referred to as Turner's syndrome)** This occurs in about 1 in every 5,000 live births. These females have normal IQ, but generally are shorter than expected and may have other physical abnormalities, such as congenital heart defects. Growth can be enhanced with hormone therapy. These women are usually infertile; however, they do have a uterus and can conceive and carry to term with the help of IVF using donor eggs.
- **Monosomes for Chromosomes 13, 15, 16, 17, 18, 21, 22, X and Y (Only one of a particular chromosome is present instead of the typical two chromosomes)** Monosomy 21 (one chromosome 21) is only seen in tissue from miscarriages. The other monosomies have never been seen in miscarriages or

in children. It is therefore, believed that embryos that are missing one of these chromosomes are not able to implant into the uterus.

Translocation or Inversion (“Unbalanced State”)

An “unbalanced state” may result from either translocation or inversion:

a. **Translocation** is the presence of a translocation chromosome (chromosomal rearrangement) that can cause the embryo to have too many copies or parts of one chromosome and too few copies or parts of the other. This results in too many or too few normal genes on a chromosome. This is called an “**unbalanced state**”, meaning that the embryo does not have the proper amount of genetic material. Where one of the patients is known to carry a translocation chromosome involving specific chromosome(s), those chromosomes will be tested utilizing PGD.

An “**unbalanced state**” or **translocation** in an embryo may lead to embryo death, miscarriage, or the live birth of an infant with substantial medical problems. Listed below are very brief descriptions of some of the possible abnormalities that are found when there is the transfer of a balanced or unbalanced translocation or inversion chromosome to one’s offspring.

b. **Inversions** occur when a single chromosome breaks in two places and the material in-between is reconstituted upside down. The presence of this inversion chromosome in eggs or sperm can result in an embryo with too many or too few copies of genes located on particular chromosomes. This can also be considered an “**unbalanced state.**” Where one of the patients is known to carry an inversion of a particular chromosome(s), those chromosomes will be tested utilizing PGD. A pregnancy involving an embryo with too many or too few copies of genes (from this inverted chromosome) may result in the failure of the embryo to grow, miscarriage, or the birth of a severely-affected child with substantial medical problems.

Translocation or Inversion (“Balanced State”)

There may also be “balanced” translocations or inversions, which means that there is a translocation (rearrangement) or inversion (material upside down) but there are the right number of copies of genes. When one partner carries a balanced translocation or inversion chromosome, this intact chromosome can be transferred to their offspring. While the child would be “normal”, that child may transmit this abnormal chromosome to his/her offspring. The FISH technique of analysis (described later) cannot differentiate between the presence of a balanced translocation chromosome and a normal chromosome.

When one partner carries a balanced translocation or inversion chromosome, this chromosome can separate and transfer too few or too many copies of a chromosome to the embryos, which can lead to abnormal genetic conditions. There may be a resultant failure to conceive, miscarriage, fetal death or the birth of a severely-affected child with a genetic disorder.

The Methods For Analyzing Embryos in PGD

PGD, as performed at [The Center for Reproduction & Women’s Health Care] and at the independent laboratory RMA-Genetics, LLC, includes two techniques: **Embryo Biopsy** and **Florescence In Situ Hybridization (FISH)**.

1. Embryo Biopsy- involves the removal of one or two blastomeres (individual cells) from the cleaving (dividing) embryo during the in vitro fertilization process. This is performed by making a small opening in the zona pellucida (the jelly-like membrane surrounding the embryo) followed by the aspiration (suction) of the blastomere from inside the embryo using a very fine needle. The Embryo Biopsy procedure is currently being used by many in vitro fertilization centers worldwide to test for abnormalities in chromosomes or the test for the number of chromosomes. Cells that are biopsied, or removed, are placed on coded and numbered glass slides, and the embryos are returned to the incubator to await the results of the next procedure-Florescence In Situ Hybridization, or FISH.

Embryo Biopsy may result in damage to the embryo, but current data reveals that this damage rate is low. The Embryo Biopsy procedure typically takes some hours to complete; however, the time may vary according to how many of our embryos are being evaluated. The Embryo Biopsy procedure usually begins about three days after oocyte retrieval, but that this time may also vary.

2. FISH- The coded and numbered glass slides are then the subject of FISH, which is done at the independent laboratory of RMA-Genetics, LLC. FISH is a technique used to locate chromosomes in a specific sample of DNA or RNA. The slides are treated with a solution that will mark the chromosomes being tested. Probes, which contain a sequence of chromosomal anomalies, are introduced, and they “attach” to the cells that are being studied, if the same condition is present. Using a special type of microscope (a fluorescent microscope), the number and/or structure of chromosomes can be evaluated to identify abnormalities.

The FISH procedure will take an additional 6 to 12 hours and will also vary depending on the number of slides to be evaluated.

There are risks limitations inherent in the FISH procedure:

1. A limitation of the chromosomes tested: Only a few chromosomes will be tested using FISH-although there are many other chromosomal abnormalities that may occur in developing embryos. RMA-Genetics, LLC will perform FISH and will test as many chromosomes as the technology allows at the time of your test. There may be abnormalities in the chromosomes that are not the subject of testing-which can result in genetic abnormalities in any child ultimately born-which will not be discovered, for the simple reason that the chromosomes involved are not being tested.

2. **Mosaicism**: Some embryos may contain both cells which are genetically normal and, cells which are abnormal. This is called **mosaicism**. Since the Embryo Biopsy results in the removal of only one or two cells, those removed may not reveal an abnormality which exists in the embryo; for this reason, mosaicism can result *in a diagnosis that is incorrect*. This, of course, may result in the transfer of an embryo carrying a chromosome abnormality or the failure to transfer a normal embryo. Based on previous studies, it is estimated that there is about 10% chance that a misdiagnosis may occur due to biopsy of an embryo that contains both genetically normal and abnormal cells.

3. **FISH utilizes equipment** which can malfunction, resulting in an improper analysis.

4. **Contamination of Genetic Material**: FISH is a technique that analyzes a very small amount of genetic material (remember, only one or two cells are removed from the embryo for analysis). As a result, there may be no finding. Also, there is a risk of DNA or RNA contamination-where the DNA or RNA of persons in the laboratory may inadvertently mix with the test specimen. Although all precautions are taken to prevent such contamination-it is a risk of the technique. In such cases, contamination with the DNA of another may lead to a false analysis.

Other Methods of Preimplantation Genetic Diagnosis

The physicians at [The Center for Reproduction & Women's Health Care] may recommend that you have procedures other than FISH. In such cases, the testing for chromosomal defects may be performed at another independent laboratory (other than RMA-Genetics, LLC). In such a case, you will be provided with another consent document from the laboratory performing such testing.

One such test is Polymerase chain reaction (PCR) used to allow analysis of a single cell for a single gene. PCR is a method whereby a single DNA sequence, from a single cell, is artificially amplified (copies are made) through repeated cycles of replication and strand separation. This allows a single DNA sequence to be potentially tested for a gene defect. [PCR-Single Gene Testing]. The risks of such a procedure include all of those listed in connection with embryo biopsy and PGD detailed in this pamphlet.

The Results of the FISH Analysis

The results of the FISH analysis (or other tests) will depend on the nature of the abnormalities that are being tested, and may take several forms. Due to a number of circumstances (including the small amount of genetic material that is involved), no findings may result. Or, the results may reveal either an abnormal, or normal, amount of chromosomes. Our doctors will discuss your options following the results of this chromosome testing. These issues include, but may not be limited to, the following:

- ***What are the consequences of transferring embryo(s) with a chromosome abnormality?*** After considering the effects of the chromosomal abnormality detected, you must consider whether or not to transfer any of the abnormal embryos-or those embryos for which “no finding” results. In the event that none, or too few, of the embryos are normal (or have no findings at all), you might decide to transfer embryos with a chromosome abnormality that may be capable of development and birth, or to transfer embryos for which no findings were made.
- ***What will be done with embryos that are not transferred?*** These may include embryos found to contain abnormal numbers of chromosomes, and those embryos for which no results of chromosome testing could be made. Embryos which, after consultation with your team, you have decided are nonetheless suitable for transfer may be cryopreserved (you must sign a separate cryopreservation consent). With respect to embryos which you have determined to be unsuitable for transfer, you will be asked to chose, on your consent form, to either (1) have them discarded or (2) donate them to [The Center for Reproduction & Women’s Health Care] for research purposes.

When the results of chromosome testing are available, prior to embryo transfer, the results will be discussed and options examined. These options at embryo transfer may include, but are not limited to, the following:

1. If more than two embryos are suitable for transfer, those not transferred following diagnosis can be cryopreserved for transfer in a later cycle.
2. If only one embryo is suitable for transfer, you may choose to transfer only that embryo.
3. If only one embryo appears to have the normal complement of chromosomes tested, you may choose to also transfer an embryo in which no diagnosis could be obtained or one which reveals a genetic abnormality.
4. If no diagnosis can be obtained on any embryos, you may choose to follow the standard IVF/embryo transfer recommendations (for the number of embryos to be transferred).
5. If no diagnosis is obtained from an embryo(s), the embryo could undergo another biopsy and FISH procedure and be cryopreserved for transfer in a later cycle, depending on the results of the chromosome testing.
6. If no embryos are suitable for transfer, future options will be addressed.
7. All embryos unsuitable for transfer may be donated to research or discarded. Discarded embryos or embryos donated for medical research will be evaluated using FISH procedure in a manner similar to that used with the biopsied embryos. The data generated may allow the investigators to improve the process for future couples.

The Risks that Accompany PGD

As with any medical procedure, there are risks and limitations to PGD. These include, but are not limited to, the following:

- Embryos may be damaged during the biopsy procedure resulting in a decrease in their ability to develop. Studies performed show that the chance of this type of damage is low (about 1% of the embryos biopsied are damaged in the procedure). However, it has not been determined if some undetected damage may result in an increased risk of obstetric complications fetal abnormalities, or a reduction of the chance of achieving a pregnancy.
- A diagnosis may not be possible. It is estimated that there is a 10% chance that no diagnosis (“no finding”) will be obtained for any individual embryo from which cells are biopsied.
- The diagnosis that is made on an embryo may be incorrect. Based on previous studies, it is estimated that there is less than a 10% chance that a diagnosis will be incorrect.
- Embryos may be transferred that have abnormalities that were not evaluated. This could include, but is not limited to, aneuploidies on other chromosomes (which were not tested) or defects on chromosomes that cannot be detected with FISH.
- Some embryos may contain some cells that are normal and some that are abnormal (mosaicism). The cells tested, then, may not reveal an abnormality that actually exists in the embryo. This may result in the transfer of a genetically abnormal embryo.
- The number of normal embryos available for transfer may be less than the number of embryos usually transferred in an IVF cycle, thus decreasing the chances of achieving a successful pregnancy.

- As with any technique that utilizes mechanical support systems, equipment failure can occur. Neither the physicians, scientists, employees, staff or consultants of [The Center for Reproduction & Women's Health Care], nor any of the independent testing laboratories (such as RMA-Genetics, LLC), will be responsible for any destruction or damage caused by, or resulting from, any malfunction of equipment, failure of utilities, strike, cessation of services, or other labor disturbance, any war, acts of public enemy, or weather disturbance, any fire, wind, earthquake, flood, or other acts of God.
- With any system that involves handling of microscopic material, human error can occur. It is also possible for a gamete(s), blastomere(s), or embryo(s), to become lost, damaged or contaminated during these procedures.
- The cells and slides may be lost, damaged or compromised during handling, resulting in loss of the ability to provide any diagnosis or the inability to provide a diagnosis in time for a transfer of the fresh embryo; this may require re-biopsy, and cryopreservation of the embryo (for use in a frozen embryo transfer cycle later) while FISH is being performed.
- Due to the small amount of genetic material involved, there could be DNA contamination, leading to a false result.
- Since PGD is a new technology, there may be additional risks to the developing embryo that have not yet been identified.

Benefits

The benefit of PGD is that it might allow the participants to decrease their chances of having embryos with a genetic abnormality, and it may reduce the risk of a multiple pregnancy.

Alternative Treatment

The only known alternative treatment to PGD is no treatment at all- which means to proceed with a pregnancy (through IVF or naturally) without chromosomal analysis (which bears the risk of conceiving a child with a genetic abnormality), to avoid pregnancy, or to use donor sperm and/or donor eggs to minimize the risk of a genetic abnormality.

Confidentiality

Information that is obtained, including answers to questionnaires, history, laboratory data findings, or physical examination will be kept strictly confidential. However, records, just like hospital records, may be subpoenaed by court order or may be inspected by federal authorities. In order to assure that Food and Drug Administration (FDA) regulations are being followed, it may be necessary for a representative(s) of the FDA to review our medical records, or the Society for Assisted Reproductive Technology (SART) in conjunction with the Center for Disease Control (CDC), or consortium collaboration.

Amniocentesis or Chorionic Villus Sampling

Preimplantation genetic analysis is still considered a new diagnostic procedure, and neither [The Center for Reproduction & Women's Health Care] nor any independent testing laboratory (such as RMA-Genetics) can assure you that (1) findings will be made on any of your embryos, or (2) that a correct diagnosis will be made utilizing this technique. [The Center for Reproduction & Women's Health Care] *strongly recommends* that if a pregnancy is achieved, (1) CVS (chorionic villus sampling) during the first trimester, or (2) amniocentesis during the second trimester, be performed to confirm whether the fetus that has developed is free from the genetic or chromosome abnormalities for which the embryo was tested. You will be asked to sign a separate consent form for CVS or amniocentesis, at that time. If a disease causing abnormality is found by CVS or amniocentesis, alternative courses of action, including elective termination of the pregnancy, will be discussed.

If you refuse to undergo CVS or amniocentesis it may deny you important information concerning the health and genetics of the developing embryo, and deny you available medical options. CVS and amniocentesis are necessary to confirm the diagnosis made prior to implanatation.