

**Fluorescence In Situ Hybridization Preimplantation Genetic Diagnosis of Embryos
Wake Forest University Health Sciences Clinical Molecular Cytogenetics Laboratory**

Purpose

Human bodies are composed of many cells, and each cell usually contains 46 chromosomes. Chromosomes contain the genetic information (genes) that we inherit from our parents and pass on to our children. Chromosomes are the genetic structures that we can see under the microscope, while genes are too small for us to see. There are two copies of each chromosome in every cell – one inherited from the mother and one inherited from the father. Therefore, the chromosomes can be arranged into 23 pairs. The first twenty-two pairs of chromosomes are the same in males and females and are called “autosomes.” They are numbered from the largest (#1) to the smallest (#22). The twenty-third pair of chromosomes is called the sex chromosomes because it determines whether an individual is male or female. Usually, females have two "X" chromosomes and males have an "X" chromosome and a "Y" chromosome.

When egg cells or sperm cells are formed, the paired chromosomes separate so that each egg cell receives 23 chromosomes and each sperm cell receives 23 chromosomes. Sometimes, for reasons we do not understand completely, a chromosome pair does not separate as we would expect (non-disjunction). When this happens, that egg or sperm cell can have 24 or 22 rather than 23 chromosomes. If this egg cell or sperm cell is used in fertilization, the developing baby will have an extra or missing chromosome in every cell.

Having extra or missing chromosome(s) (aneuploidy) can result in lack of implantation of an embryo, pregnancy loss, and other specific conditions such as infertility or Down syndrome. Patients who are undergoing in vitro fertilization (IVF) and have been determined to have an increased risk of miscarriage, increased risk of having a fetus with a chromosome abnormality, or have had repeated IVF failures have the option of having fluorescence in situ hybridization (FISH) Preimplantation Genetic Diagnosis (PGD). FISH PGD may reduce these risks by assisting the embryologist in selecting embryos more likely to result in a positive pregnancy outcome. Therefore, the purpose of this procedure is to select and transfer into a mother's uterus only the embryos that do not have the recognizable chromosomal aneuploidies for which we are testing.

Genetic Testing and Informed Consent Counseling

FISH PGD aneuploid testing will only provide information concerning aneuploidy of chromosomes 13, 15, 16, 17, 18, 21, 22, X, and Y. If there is a known parental chromosome rearrangement, development of a FISH PGD test can provide information regarding that specific chromosome rearrangement. A copy of all laboratory reports regarding the at-risk parental chromosomal rearrangement is **required** to be sent to the Wake Forest University Health Sciences (WFUHS) Clinical Molecular Cytogenetics Laboratory. A peripheral blood sample from the individual who carries the chromosomal rearrangement is also **required** to confirm the chromosomal rearrangement and to optimize the FISH PGD testing. Any additional genetic alterations associated with a specific disease, but not identified in the patient or her partner, might exist in an embryo and will not be examined. In addition, a letter or clinic note is **required** from a board-certified/eligible genetic counselor or board-certified/eligible clinical geneticist stating the patient has received appropriate counseling regarding: 1) appropriate family history review 2) In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI) 3) embryo biopsy 4) FISH PGD testing 5) prenatal testing. **All required documentation must be sent to the WFUHS Clinical Molecular Cytogenetics Laboratory prior to initiating the IVF procedure.**

Procedures

Patients agreeing to have FISH PGD analysis of their embryos first undergo ovarian stimulation, egg retrieval, and in vitro fertilization. When the embryos are approximately three days old, a single cell is removed (biopsied) from each embryo and prepared for evaluation by the laboratory. The biopsied cells are analyzed using FISH. With this technique, colored probes that are specific for certain chromosomes (13, 15, 16, 17, 18, 21, 22, X, Y or those chromosomes involved in a family specific rearrangement) are used to count the number of

chromosomes present. As mentioned above, each cell should have two copies of each chromosome. If the biopsied embryonic cell is found to have a normal number of chromosomes (within the limits of the nine chromosomes analyzed), then it is likely that the remaining cells of the embryo it was derived from also have a normal number of chromosomes. Embryos found to be normal are transferred to the mother or frozen for possible transfer in the future. Embryos that are found to be abnormal (having missing or extra chromosomes) will not be transferred and will be sent to the WFUHS Clinical Molecular Cytogenetic Laboratory to confirm the previous abnormal results. These embryos will be discarded after confirmational testing.

It is possible that none of the embryos tested will be normal. In such a circumstance, no embryos will be eligible for transfer. It is also possible that FISH PGD testing may fail to produce any results. The patient will then have the choice of whether or not to transfer embryos for which no results were obtained with the understanding that such embryos have the same probability of being normal or abnormal as naturally conceived embryos. It is also possible that a partial result may occur in which one or a few chromosomes of the nine being analyzed may fail. In this case, if the other chromosomes are normal, the embryo can still be considered for replacement pending discussion with your physician. In some cases, an embryo can consist of a mixture of normal and abnormal cells which is called mosaicism. If an embryo is mosaic, FISH PGD testing may give a false negative result (test indicates a normal embryo but it is actually chromosomally abnormal) because the one cell examined had the correct number of chromosomes. The relative risk of having a mosaic embryo is currently unknown.

At present, FISH PGD aneuploid testing for the nine chromosomes being analyzed detects about 85-90% of those embryos with a missing or extra chromosome. In addition, not all of the chromosomes are examined nor is the structure of the chromosomes studied by FISH PGD. Therefore, approximately 10-15% of the embryos analyzed have a chance of being misdiagnosed. As a result, prenatal chromosome analysis through chorionic villus sample (CVS) or amniocentesis is strongly advised to confirm the FISH PGD testing and to identify the number and structure of all the chromosomes. It is recommended that patients who have their FISH PGD testing performed at the WFUHS Clinical Molecular Cytogenetics Laboratory also have any prenatal sample for genetic testing sent to the same laboratory to insure the reliability of the genetic testing. If no prenatal testing is performed to confirm the FISH PGD results, then the risk for misdiagnosis remains at 10-15%.

Risks

Each person in the general population has a 1 in 230 chance for having a balanced chromosome rearrangement. In most cases where a person has a balanced chromosome rearrangement, they do not have any physical, health, or learning differences. However, individuals who have a balanced chromosome rearrangement are at risk for passing on an unbalanced chromosome rearrangement with each pregnancy. Unbalanced chromosome rearrangements can cause pregnancy loss, birth defects, learning problems (including mental retardation), and other potential medical problems. The nine chromosome aneuploid FISH PGD analysis would not typically detect these unbalanced chromosome rearrangements except by chance. Therefore, parents are offered the option of chromosome analysis before they undergo PGD procedures in order to determine if they are at risk. If a balanced rearrangement is identified in a person, then a FISH PGD analysis can be designed to specifically detect the chromosome rearrangement identified in that person.

Some studies have reported that congenital abnormalities, birth defects, genetic abnormalities, mental retardation, and/or other possible differences may occur in children born following in vitro fertilization, cell biopsy, and FISH PGD testing. However, these problems also occur in 3-5% of children resulting from natural fertilization without FISH PGD testing. FISH PGD testing does not detect all chromosome changes. If confirmatory prenatal testing is not performed, it is possible for the fetus to have a chromosome difference.

Benefits

Most chromosomally abnormal embryos either do not implant or spontaneously abort shortly after implantation. Therefore, the risk of a failed IVF cycle increases by not testing for these abnormal embryos prior to transfer. FISH PGD can increase the chance of implantation, increase the likelihood of a successful IVF cycle, and increase the chance of having a live born child without an aneuploid disorder. However, avoidance of genetic disease in any fetus cannot be assured as a result of FISH PGD testing.

Alternatives

Alternatives to having FISH PGD testing performed include continuing with IVF without FISH PGD, using donor gametes for IVF procedures, having only a standard prenatal testing such as CVS or amniocentesis, or not having any genetic testing performed during the pregnancy.