

FULLERTON

Jane Frederick, MD
Daniel Potter, MD
270 Laguna Road, Suite 220
Fullerton, CA 92835
(714) 738-4200
(714) 738-4496 Fax

LAGUNA HILLS

Jane Frederick, MD
Daniel Potter, MD
23961 Calle de la Magdalena
Suite 503
Laguna Hills, CA 92653
(949) 472-9446
(949) 472-3739 Fax

PASADENA

Michele Evans, MD
Bradford Kolb, MD
Jeffrey Nelson, DO
John Wilcox, MD
333 S. Arroyo Parkway, 3rd Floor
Pasadena, CA 91105
(626) 440-9161
(626) 440-0138 Fax

TARZANA

Robert Boostanfar, MD
Michael Feinman, MD
5525 Etiwanda Avenue
Suite 311
Tarzana, CA 91356
(818) 996-2188
(818) 996-2111 Fax

WESTLAKE VILLAGE

Robert Boostanfar, MD
Michael Feinman, MD
1220 La Venta Drive, Suite 103
Westlake Village, CA 91361
(805) 374-1737
(805) 374-1736 Fax

WEST LOS ANGELES

Susan Sarajari, MD, PhD
David Tourgeman, MD
11500 West Olympic Boulevard
Suite 504
West Los Angeles, CA 90064
(310) 481-0881
(310) 481-9017 Fax

CONSULTING SCIENTIFIC and LABORATORY DIRECTOR

Barry Behr, PhD, HCLD

CONTACT US TOLL-FREE:

(866) HRC - 4IVF
(472 - 4483)

VISIT OUR WEBSITE:

www.havingbabies.com

HUNTINGTON

Reproductive Center[®]

MEDICAL GROUP

REPRODUCTIVE ENDOCRINOLOGY & INFERTILITY

PREIMPLANTATION GENETIC DIAGNOSIS (PGD) AND PREIMPLANTATION GENETIC SCREENING (PGS)

by Barry Behr, PhD, HCLD & Victor Ivakhnenko, HCLD

INTRODUCTION

Preimplantation genetic diagnosis (PGD) and screening (PGS) refer to the procedures involved in obtaining genetic makeup of the embryo(s) prior to their transfer into the uterus.

Genetic errors arise from deletions or insertions of genetic material, abnormal numbers of whole chromosomes or genes, and even from misplacement of a single base in the DNA sequence. Genetic abnormalities can range from relatively harmless to severe: from vitamin deficiencies and food allergies to cancer, birth defects and infant mortality. In recent years, significant advances in technology have enabled researchers to trace many disorders and diseases to their roots in the genetic code. Chromosome stretches, or even isolated genes, can now be used as markers to identify individuals at risk for certain illnesses. Additionally, the Human Genome Project, which aims to identify the chromosome location and DNA sequence of every human gene, is providing an ever-expanding catalogue of potential genetic markers. The ability to recognize these genetic warning signs is rapidly becoming most effective tool for prevention, diagnosis and treatment of genetically based disorders.

An estimated 60 percent of all naturally occurring reproductive losses in pregnancies are associated with chromosomal abnormalities in the embryo. A normal embryo has 22 pairs of chromosomes called autosomes and 1 pair of sex chromosomes (XX or XY). Embryos that do not carry the normal pair of each chromosome are called aneuploids. Those that contain three copies of a particular chromosome (Trisomy) are the cause of some genetic disorders such as Down's syndrome (Trisomy 21). Other less common trisomies of chromosomes 13, 16, 18 and 22. Embryos that contain only one copy of a chromosome (Monosomy) are by and large nonviable.

Abnormal aneuploid embryos, either with monosomy (one missing) or trisomy (an extra one), are usually normal in appearance. It is not possible to distinguish these morphologically from other embryos. It is only through genetic analysis that they can be differentiated. Without such an

analysis, many of these embryos are unknowingly transferred to patients.

Depending on the specific abnormality, in IVF pregnancies, research has shown that chromosomal abnormalities such as aneuploidies (extra or missing chromosomes per cell) of the embryo increase either the risk of spontaneous miscarriage, the development of a genetically abnormal child or no pregnancy at all.



Barry Behr, PhD, HCLD

DESCRIPTION OF PREIMPLANTATION GENETIC TESTING

Preimplantation genetic testing is based on the ability of the human eggs and embryos to continue their development into normal pregnancy after microsurgery (embryo or polar body biopsy), since cleavage stage cells of the embryo are pluripotent and removal of one or two cells at this time does not appear to affect further development of the embryo. It involves several obligatory steps: genetic counseling; reproductive counseling and treatment; in Vitro fertilization and genetic laboratory with DNA technologies such as fluorescence In Situ hybridization (FISH) for sex determination and screening for chromosomal abnormalities and polymerase chain reaction (PCR) for single gene diseases.

Preimplantation genetic testing of the embryos entails in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), microsurgical removal of one or two blastomeres at the six- to eight- cell stages after fertilization, molecular (by PCR) in case of single gene diseases or molecular cytogenetic analysis (e.g. fluorescence in situ hybridization FISH) in case of chromosome abnormalities, studies of the biopsied cells, and uterine transfer of unaffected embryos. In cases of X-linked recessive diseases, sexing and selective transfer of female embryos can be performed.

continued on back page

An alternative source of material that has been used, when the disorder tested for is of maternal origin, is the polar body. A polar body is a small section of an egg and contains the complementary set of chromosomes present in the oocyte. Therefore, the genotype of the oocyte can be deduced by examining that in the polar body. The first polar body of an egg has been extruded prior to the egg retrieval and thus before fertilization. This polar body is not necessary for complete embryonic development and is available for analysis. A second polar body is extruded at the time of oocyte fertilization by a sperm. These polar bodies, removed using micromanipulation, can be a valuable source of genetic information. By using fluorescent-tagged genetic probes (Fluorescent in situ hybridization or FISH), we can examine them, thus allowing the chromosomal make-up of the oocyte to be inferred. Studies have shown that the majority of embryo aneuploids (85%) are due to the female oocyte. The remainder is of sperm origin.

FLUORESCENT IN SITU HYBRIDIZATION (FISH) FOR PGS

Large chromosomal abnormalities, such as extra or missing chromosomes (aneuploidies), gender determination and unbalanced chromosomal translocations resulting from a parental balanced translocation can be detected by a laboratory procedure called fluorescent situ hybridization (FISH). For this technique, DNA probes are labeled with colored fluorescent tags that light up so one can see specific chromosomes or genes under a microscope. The reagents are optimized for use with imaging software for probe-signal enumeration. This software allows the simultaneous analysis of up to 12 different target-specific fluorophores in a single cell. However, up until now only 9 chromosomes can be accurately assessed during one analysis using FISH with up to a 10% error rate.

PGD FOR SINGLE GENE DISORDERS

In cases involving more subtle abnormalities, on the scale of single genes or even DNA bases or single gene diseases, highly specialized techniques such as PCR are required. Such methods rely on the fundamental principles of the genetic code, and specifically on the ability to generate a matching, or complementary segment of DNA. Structurally, DNA is composed of two single strands attached to each other to form a double helix. The bases of one strand always bind to the bases (A,T,G & C) of the other in a specific fashion: A pairs with T, and G with C. If one knows the sequence of the bases in one strand, one can deduce the complementary sequence of bases in the other strand.

Based on a known sequence of DNA, a synthetic copy of the matching strand called a DNA probe is created, it will then bind, or hybridize to that specific gene within a chromosome. The mutation in the carrier parent(s) needs to be characterized before PGD is applied.

TIME FRAME AND LIMITATIONS

Both FISH and PCR procedures typically take 24-48 hours to complete. However, since diagnostic tests are performed on a single cell, the possibility of misdiagnosis has to be considered. There are limitations of the test procedures, e.g. allele dropout in PCR, either non-specific or inefficient hybridization in FISH.

NEW DEVELOPMENTS

New techniques like comparative genomic hybridization (CGH) offer the possibility to analyze all 23 pairs of chromosomes simultaneously for aneuploidy, translocations and single gene defects. Unfortunately, this technology is not clinically useful due to the time it takes to generate the results. It currently takes 4-5 days for the results to be obtained using CGH. This requires the biopsied embryos to be cryopreserved after biopsy to allow time for the analysis. There is a single report in the literature that has accomplished this approach successfully. Another technique that is also emerging and that may have application to Preimplantation genetic testing is Gene Chip technology where literally thousands of DNA sequences may be analyzed simultaneously. This technique is a little further off on the horizon.

HISTORICAL DEVELOPMENT

Preimplantation genetic testing was first employed in 1989 with subsequent birth of normal females to couples at risk of various X-linked recessive diseases. The number of genetic diseases potentially diagnosable by PGD is vast. Examples of such disorders that have been reported include: chromosomal translocations, Down syndrome, Turner syndrome, DiGeorge syndrome, alfa-1-antitrypsin deficiency, beta- thalassemia, Charcot-Marie-Tooth disease, cystic fibrosis, Fancony anemia, fragile X syndrome, hemophilia A, Huntington disease, Lesch-Nyhan disease, Marfan syndrome, myotonic dystrophy, sickle cell anemia, and Tay-Sachs disease.

Preimplantation genetic diagnosis (PGD) having been available for over a decade, has clearly become an attractive addition to prenatal genetic diagnosis. Over the past 4 years PGD performed at HRC has resulted in the birth of over 150 healthy children. Presented data illustrates a positive experience with PGD and reflects core indications and success rates for the procedure. However, clinical outcomes could be different within groups of patients depending on multiple clinical variables such as age, mode of treatment etc. For example, clinical pregnancy rates could be as high as 62% in donor/recipient PGD cycles. HRC is in agreement with published data that PGD is a practical procedure with pregnancy and healthy live birth outcome rates equal to those seen with IVF alone.

CONCLUSION

The procedure is considered experimental by the U.S. Food and Drug Administration. However, Huntington Reproductive Center remains committed to keeping pace with the rapid advances in the fields of genetics and human reproduction and making them available to couples as soon as is practically possible. In the past 20 years,

HRC has become one of the largest providers of assisted reproductive treatments in the U.S. Both the physicians and staff of HRC are committed to maintaining the highest standard of care in reproductive medicine in terms of moral and ethical practices.

Significant experience in infertility treatment and embryo culture, highly skilled medical and laboratory personnel make it possible to offer PGD technology to couples at risk of having a genetically abnormal fetus which can help them avoid the birth of an affected child or having to face the painful decision of a pregnancy termination.

