



# The Genetic Revolution

Christo Zouves, MD

**T**he ability to fertilize egg and sperm outside the human body has changed the face of human reproduction forever. Since the birth of the first IVF baby in 1978, more than 300,000 children have been born through assisted reproduction technology (ART). We can now remove eggs from one female to help another achieve pregnancy; we can remove sperm directly from the testicle, allowing men to father a child who would otherwise not be able to. We can inject a single sperm into an egg to promote fertilization, virtually eliminating severe male factor and most vasectomy reversals. We can transfer the gametes of one couple to a host uterus, allowing implantation and pregnancy to occur.

The technology of manipulating gametes has become more sophisticated, and from injection of a single sperm (ICSI) we have now moved to the molecular level, where we are removing the nucleus and, soon, individual chromosomes and genes.

The newest breakthroughs in assisted reproduction include a renewed focus on the mind/body connection to fertility, advances in genetics including preimplantation genetic diagnosis (PGD), stem cell research, cloning, and gene

therapy, as well as advances in storage, specifically the storage of eggs and ovarian tissue.

## THE GENETIC REVOLUTION

The successful race to map the human genome has spawned faster computers and methods of genetic analysis as well as phenomenal interest in using this new information to better understand,

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prevent and treat disease. Applying this knowledge to the embryo will alter the way IVF is practiced and the future of reproduction and it may well be that one day sex will be for fun only and IVF for reproduction. In addition, as the cost of DNA microchips decreases, we will be able to screen for many genes simultaneously, including those that code

for diseases like cancer, while removing only a single cell from the embryo on day three during the IVF procedure.

## PREIMPLANTATION GENETIC DIAGNOSIS

At the present time, technology like preimplantation genetic diagnosis (PGD) allows testing to be completed in a shorter time and on ever-smaller samples of DNA, allowing for widespread application. Currently, children born in the United States have a 3 to 4 percent chance of having a major birth defect. Some of these abnormalities occur because of a problem with a single gene that is inherited from one or both of the parents, while other abnormalities are related to an abnormal number of chromosomes (aneuploidy). Single gene defects and aneuploidy (age-related chromosomal disorders) can be diagnosed before embryos are transferred to the uterus, and this is PGD.

In addition, an uncommon condition known as translocation, categorized by rearrangements of genetic material between chromosomes, can also be identified through PGD. PGD permits the selection of embryos that are less likely to have chromosomal abnormalities, and

also of embryos that may be free of a known single gene disorder or an unbalanced translocation, thereby increasing the likelihood of a healthy baby and decreasing the chances of having to terminate a pregnancy found to be abnormal through chorionic villus sampling or amniocentesis.

Preimplantation genetic diagnosis is being applied today not only to identify carriers of genetic diseases, but more and more as a valuable means to improve IVF clinical outcomes. PGD represents a new set of tools to help patients and also to widen the potential treatment population to include those carrying genetic diseases even though they may be fertile.

Even in optimal situations, like egg providers under age 30, the percentage of embryos that have normal chromosomes may only be approximately 50 percent. This may explain the frustration that patients and IVF specialists feel when normal-looking embryos are transferred with negative results or recurrent loss, sometimes even after multiple IVF attempts. Chromosomal abnormalities in embryos are therefore responsible for a significant proportion of failed implantations after hormonal, uterine and immunological factors have been excluded.

Couples can benefit from PGD when the woman is 35 or older, by testing for age-related chromosomal disorders, also called aneuploidy, or when there is a single gene defect within a family. Younger women with repeated unexplained miscarriages can also benefit from this test. The purpose is to select and replace only those embryos that appear to be normal so that women may increase the chance of conceiving while reducing the probability of losing the pregnancy or carrying an abnormal baby to term. PGD for aneuploidy can determine the presence or absence of a certain number of chromosomal disorders, but cannot detect

genetic disease or predict congenital malformation.

#### **PGD FOR ANEUPLOIDY**

The majority of PGD procedures are performed for aneuploidy or abnormalities in the number of chromosomes, and this problem increases with increasing maternal age. Studies have also shown that up to 85 percent of aneuploids are caused by the egg, while the sperm may cause the remainder. When PGD is performed for aneuploidy, unfortunately

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we cannot check for single gene defects at the same time unless more than one cell is removed from the embryo. The reason for this is that with single gene analysis, which requires polymerase chain reaction (PCR), the single cell removed needs to be disrupted, whereas for aneuploidy testing, the nucleus is very carefully preserved and fixed on a glass slide so that the individual chromosomes can be analyzed and counted.

Females are born with all the eggs they will have in their lifetime. As a woman advances in age, her eggs are exposed to aging processes that include chromosomal abnormalities. That is why the chance of conceiving a chromosomally abnormal baby increases with age. In complete contrast, sperm in the male are newly made every 65 to 75 days. Chromosomes are stringlike structures found in the center of the cell,

the nucleus. Chromosomes contain genes that are made of DNA, the molecule that contains inherited information. Normal human cells contain 23 pairs of chromosomes, a total of 46. We receive 23 chromosomes from each parent.

If an error occurs leading to the egg or sperm having an extra or missing chromosome, the embryo created by that egg or sperm would have an extra or missing chromosome. This situation is what is called aneuploidy. If the aneuploidy involves chromosomes such as 13, 18, 21, X or Y, the pregnancy may still carry on until birth, even though the fetus has a chromosomal disorder. Trisomy 21 produces the effect called Down's syndrome. The effects of other common aneuploidies include Turner's syndrome and Klinefelter's syndrome. These disorders are nonfatal, in that the pregnancy can be carried to term and result in a live birth, although the baby is abnormal. Overall, the risk of aneuploidy is known to increase with

maternal age, from 1/385 at 30, 1/179 at 35 and 1/63 at 40; at the age of 45, the chance of delivering an affected child is 1/19.

#### **PGD FOR SINGLE GENE DEFECTS**

These defects can be dominant or recessive. Dominant defects are transmitted by one parent alone, with the risk to the affected child being 50 percent (e.g. myotonic dystrophy). Recessive defects occur when both parents have the gene with the risk to the affected child being 25 percent (e.g., cystic fibrosis, sickle cell anemia or Tay-Sachs disease). More than 60 single gene diseases can now be diagnosed with PGD. Most of these genetic syndromes are relatively uncommon.

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three of development; at this stage the embryo usually has six to 10 developing identical cells, each with a full complement of chromosomal material. The embryos remain in culture while the cell is analyzed. PGD is accomplished by making a small opening in the outer shell (zona pellucida) and the blastomere is extracted with a micropipette. Normally, only a single cell is removed from each embryo, as it is expected to be identical to all the other cells, but it may be necessary to remove a second cell depending upon circumstances. In either of the above cases, the analysis of the biopsied cell uses a technique called Fluorescence In-situ hybridization, or FISH, which takes about one day. The cells are fixed to a glass slide and heated and cooled and their DNA is "labeled" with colored fluorescent dyes called probes, one for each chromosome analyzed. At present, the test can check nine chromosomes out of 23. Once the FISH procedure is complete, the geneticist counts the colors using a powerful microscope, thereby distinguishing normal and abnormal cells. This information is then related to the normalcy of the associated embryo being held in culture. After this process the biopsied and analyzed cells are no longer viable in any way, and the slides on which they sit are discarded.

## EMBRYONIC STEM CELLS

Embryonic stem cells or master cells are totipotent, meaning that they have the ability to make any cell or tissue in the body. The ability to produce and harness these cells holds tremendous promise for transplantation and gene therapy and the elimination of many degenerative and debilitating diseases.

The totipotent cells located on the inside of a blastocyst have the ability to develop into a fetus and a baby if transferred into a uterus, or they may be able to develop into any cell in the body if isolated from the blastocyst and cultured in the laboratory. These cells hold the key to preventing or curing many degenerative or genetic diseases, and through the use of these embryonic stem cells, we may one day be able to perform transplantation using only ultrasound

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guidance and a needle to inject the cells into the desired location.

We may no longer require organs from people dying in tragic circumstances, as long as we can unravel the method of directed development in these stem cells to produce specific cell types or organs.

Patients with spinal cord injuries may benefit from direct injection of these cells, which would then facilitate repair of spinal cord tissue. Diabetes affects almost 15 million Americans, and a simple injection of appropriately directed stem cells may be able to cure this devastating condition.

## ETHICAL CONCERNS

Embryonic stem cell research raises ethical questions because of the various methods of obtaining or producing the cells. Given that the cells originate in an embryo, any research involving

destruction of an embryo may be totally unacceptable to certain people who would obviously also be opposed to discarding embryos, abortion or selective reduction.

The logical method of obtaining stem cells would be to use the thousands of embryos now stored by fertility programs throughout the United States. Many of these embryos are destined to be destroyed because their owners have either completed their families or have decided to abandon treatment.

IVF programs should discuss stem cell research with patients as an alternative to destruction when embryos are first stored. These embryos are an extremely valuable resource for preventing and treating disease and they should not be discarded if at all possible.

Another method of obtaining stem cells could be the creation of embryos specifically for the purposes of stem cell production, but this seems unnecessary in light of the large number of embryos that already exist in storage.

A third and more controversial method of creating embryos for stem cell research is cloning or somatic cell nuclear transfer, which would create an identical genetic match for a particular individual requiring stem cells for disease treatment or prevention. This, in my opinion, is the most valid reason to support ongoing cloning research, and it is unfortunate that the controversy surrounding creation of identical individuals impacts cloning research into generation of genetically matched stem cells.

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