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FERTILITY CENTER
Pathway to Parenthood

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PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

INTRODUCTION

The human genome project was a massive collaborative project which successfully provided a detailed map in human DNA. This base of knowledge will provide an important "springboard" from which the wide spread implementation of human-gene therapy for the treatment and prevention of disease, will inevitably emerge. We are on the verge of nothing less than a biomedical revolution, the likes of which has not been encountered before. Information obtained from the human genome project will lead to profound changes in how medicine is practiced. It will change the way we are born, how we exist, how we die, and how we view ourselves in relation to our destiny. Before us lies a difficult transition. Most Americans have begun to wonder what the cost will be and while many are profoundly divided and even ambivalent regarding genetic research and its applications, most feel strongly that the introduction of human genetic engineering for the purpose of curing disease, is well justified. So too, the potential to eradicate certain lethal diseases through pre-implantation diagnosis of an embryo prior to its transfer back into the body, is important.

Compared with other mammalian species, humans have a low natural fecundity (conception rate). For example in cows, the probability of any single fertilized egg establishing a viable conception is 65-70%, while in healthy, young, fertile human females it is only 15-20% per month. Central to the comparatively poor rate of human fertility, is intrinsic abnormalities within the oocyte (egg) and/or embryo. This likely accounts for more than 60% of failed human conceptions. The combined use of fertility drugs with In Vitro Fertilization and Embryo Transfer (IVF/ET), by providing access to multiple oocytes, increases the availability of "potentially viable" embryos for transfer to the uterus, thereby significantly improving fecundity. Unfortunately, standard microscopic techniques for predicting which embryo(s) are most likely to implant, are far from optimal. Such limitations, coupled with pressure to maximize the chance of pregnancy, have typically resulted in a tendency to transfer too many embryos at a time. While such practice, has led to an improvement of U.S., IVF birth rates which currently range from 20%-55% (depending upon medical expertise and patient demographics), the transfer of multiple embryos at one time has resulted in an unacceptably high rate of high-order multiple births (triplets or greater). This in turn has resulted in an alarming escalation in the incidence of prematurity-related neonatal complications that are all too often both life threatening and life enduring.

In a concerted effort to reduce the incidence of high-order multiple births (HOM) (triplets or greater), reproductive specialists have attempted to reduce the number of embryos transferred back into the patients. In order to achieve this objective (i.e. maximizing pregnancy rates while minimizing HOM pregnancy rates), accurate laboratory methods must be incorporated into practice in order to restrict the number of embryos to transfer. Therefore, strategies that would improve the accuracy for selecting

healthy "competent" embryos for transfer are used. Presently 3 main methods are used to achieve this goal. These are:

1) **Morphologic Grading of the Embryos** i.e. what the embryos look like under the microscope at set intervals e.g 24 hours, 48 hours and 72 hours. Here we are looking for the size and shape of the cells, alignment of cells, evidence of fragmentation etc. Different labs have different grading systems .e.g A.B.C. or 1.2.3. Also, the thickness of the zona pellucid is important. We use a *"Graduated Embryo Scoring (GES)"* system in which each individual fertilized egg, after fertilization by Intracytoplasmic Sperm Injection (ICSI), is placed in a separate well containing a specialized culture medium. This allows for serial microscopic observations relating to the development of each individual egg/embryo over a period of 3 days. Using weighted scoring criteria such as pronuclear disposition, nucleolar organization, cellular fragmentation, distinctiveness of the mononucleate blastomeres and the rate of cell (i.e;blastomere) cleavage (i.e.; division) etc. (out of maximum of 100 points), we are able to predict the competency of each embryo, much more accurately. This has enabled us to limit the number of embryos transferred to those 2 or 3 in the overwhelming majority of cases where one or more embryos has a high GES scores (i.e. >70/100), on day 3. The remaining embryos are cultured in a sequential medium for an additional 2-3 days, in the hope that some will develop into healthy looking expanded blastocysts and thus qualify for being transferred to the uterus or for cryopreservation (freeze-storage), for future dispensation at the sole discretion of the patient involved. The GES system has made it possible to establish a relatively sound basis for advising patients with regard to having their embryos transferred early (on the 3rd day following fertilization) or waiting 2-3 days longer to have blastocysts transferred. It has also established a rational basis for deciding on the number of embryos/ blastocysts to be transferred in each individual case. However, while GES identification of the best embryos probably avoids the inadvertent transfer of many embryos with chromosomal/genetic abnormalities, it comes nowhere near eliminating this problem.

2) **Use of biologic markers:** Embryos are very active organisms and secrete a variety of proteins into the surrounding fluid. These can be measured. An example of this is s-HLAG. Unfortunately, the promise these markers showed in some early studies, has failed to withstand the test of time or be validated by many labs. Accordingly, these tests are used infrequently or have been abandoned. As the science improves, to evaluate these proteins better, the re-introduction of these approaches will be quite likely.

3) **Preimplantation Genetic Diagnosis (PGD)**

PGD is a technique used for the early diagnosis of chromosomal/genetic disorders, prior to the onset of pregnancy. It incorporates the latest techniques in assisted reproduction and molecular genetics. Embryos or oocytes are biopsied during culture in vitro and chromosomal/genetic analysis is carried out on material derived from polar body(ies) and/or blastomere(s). Embryos shown to be free of the genetic disease under investigation are thereupon selectively transferred to the uterus. Staining by ,multicolour fluorescence in situ hybridisation (FISH) is used to diagnose numerical and certain structural abnormalities of the chromosomes .

Approximately 3.5 million babies are born annually in the United States. About 1 in 500 of these is afflicted by a sex-linked disorder (when a genetically-defective Y (male) or X(female) chromosome is transmitted to offspring). Another 1 in 300 newborns has an autosomal genetic disorder ,an abnormality of 1 or more genes involving the 44 remaining autosomes (non-sex chromosomes). This means that approximately 1 in 20,000 babies born annually in the U.S. will have one or other genetic or chromosomal disorder. In addition, about 1: 50 babies is born with an identifiable major genetic abnormality. In other words, more than 70,000 babies are afflicted by severe genetic disease, annually

in this country. Many couples who parent a child with a severe birth defect will subsequently elect not to have another child or to adopt. These facts and figures offer a glimpse at the magnitude of the challenge confronting the medical profession, government, and society in general

The advent of IVF/ET technology which involves stimulation with fertility drugs, followed by harvesting eggs from the woman's ovaries, their fertilization in a Petri dish and incubator and the subsequent transfer of one or more of the resulting embryos to the uterus a few days later - - provides a unique opportunity to diagnose and/or exclude genetic and chromosomal disorders that have the potential to impact adversely on pregnancy outcome and the very quality of life after birth. The procedure is referred to as pre-implantation genetic diagnosis (PGD) which involves extracting 2 polar bodies soon after fertilization or embryonic cells (blastomeres) 3days after fertilization. The biopsied material is then subjected to genetic testing using FISH. Presently, technology exists to evaluate 12 of the possible 23 chromosomes. Undoubtedly, additional chromosomes will be added and within the next few years, it is likely that more chromosomes will be added. (in fact, as you read this, the number 12 may already be outdated). If found to be normal, 1 or more chromosomally/genetically healthy embryos are transferred to the uterus. As such, the emergence of in vitro fertilization, along with access to PGD, has provided a unique ability to prevent many diagnosable chromosome/genetic disorders prior to the initiation of pregnancy and thereby, provide many desperate couples who might transmit a potential genetic catastrophe to their offspring, with real hope. Further, the results obtained using PGD have given us deeper insights into the frequency of chromosome abnormalities and to our surprise, they are more common that we originally expected, prior to the introduction of this technology.

Additional Information on the development of the embryo in vitro:

The human oocyte (egg), is similar in structure to a chicken egg……only of course, it is much smaller (about the size of a grain of sand). It has a "shell" ,the zona pellucida, a thin white membrane on the outside, the viteline membrane which is separated from the zona pellucida by a potential space, the periviteline space. Inside the viteline membrane is the "white"of the egg,.. the ooplasm that contains many microorganelles (e.g.; the mitochondria, endoplasmic reticulum, microsomes etc). Then there is the "yolk" or nucleus, in the center of the egg" which in the human contains (among other structures), 46 chromosomes (i.e.; 1 pair of (XX) sex chromosomes plus 22 pairs of non-sex chromosomes or autosomes). Following the onset of the spontaneous luteinizing hormone (LH) preovulatory surge or following the administration of LH or hCG to induce ovulation each chromosome pair divides into two identical halves, through a process known as meiosis. One half (comprising 23 chromosomes) is extruded and pushed out to the periphery of the oocyte, into the periviteline space. This is the first "polar body". Immediately following fertilization of the egg by a sperm, the 2nd polar body forms. Both polar bodies are located in the perivteline space where they are readily accessible to direct biopsy..

The mature egg usually begins to divide within 24-48 hours of fertilization occurring. Its rate of cleavage(division) is believed to be indicative of its "competency" to produce a viable embryo and its potential to implant into the uterine lining. The most competent embryos (the ones that are apable , upon fertilization of producing a viable pregnancy) are believed to be those that progress to 8-9 cells (blastomeres) within 72 hours of fertilization. Embryos that have divided more rapidly are probably less competent and those that comprise less than 7 cells (blastomeres) within 72 hours are believed to be relatively "incompetent, having little potential to produce a viable pregnancy. The chromosomal make-up of the blastomeres reflects the added contribution of the sperm chromatin (DNA).

Between 72 and 96 hours following fertilization, the blastomeres of the embryo begin to divide so rapidly that they "compact" . At this stage the embryo is referred to as a "morula" (mulberry). In the

ensuing 24-48 hours the cells begin to differentiate into a blastocyst which has an inner fluid cavity, an outer layer known as the trophectoderm that subsequently develops into the root system (placenta) and membranes surrounding the baby and an inner collection of cells known as the "inner cell mass" which develops into the fetus itself.

Since the immature egg comprises two x chromosomes (XX) it follows that after normal meiosis (reduction division), there will be only one X chromosome in the nucleus and one in the first polar body. In contrast, blastomeres comprise the chromosomes of both the egg and the sperm. Since the sex chromosome make-up of the immature sperm is X+Y, it follows that during sperm-meiosis, the mature sperm will contain either an X or a Y chromosome. When a mature Y-carrying sperm fertilizes an egg, the resulting embryo will be male and if it contributes an X chromosome, the embryo will be female… Thus it is the sperm rather than the egg that determines the sex of the offspring.

BLASTOMERE BIOPSY:

Blastomere biopsy involves the removal of one or more blastomeres at the 7-8 cell stage of cleavage (day 3 post-fertilization). Its performance is indicated in the following circumstances:

1. To diagnose single or multiple gene disorders (see PCR below)
2. To diagnose certain sex-linked genetic disorders
3. To diagnose embryo aneuploidies involving the sex-chromosomes
4. To confirm the diagnosis of aneuploidy in cases where a polar biopsy produces a questionable result

Blastomere biopsy has two distinct advantages:

- It allows investigative access to the Genes that located on the chromosomes. By using a technique known as Polymerase Chain Reaction (PCR) and applying certain gene-specific markers, it is becoming possible to identify an ever increasing number of genetic disorders such as Tay Sachs disease, cystic fibrosis, familial breast cancer, multiple polyposis that leads to colon cancer, early onset Alzheimer's etc) in the embryo and thus by avoiding the transfer of such affected embryos, preventing the disease from occurring in the offspring.

- Application of a chromosome staining technique known as fluorescence in situ hybridization (FISH) it allows for the diagnosis of both egg and sperm-induced embryo aneuploidies (see below).

Embryo aneuploidy is largely determined by the egg , not by the sperm. In fact more than 90% of human embryo aneuploidies can be traced back exclusively to an egg aneuploidy which is potentially diagnosable through polar body biopsy. But there are a few circumstances where sperm chromosome defects can produce aneuploidy in the embryo. Therefore, in the following situations it might be advisable to test the sperm by performing a Sperm DNA Integrity Assay (SDIA) to try and detect an inherent sperm DNA defect:

a. repeated unexplained poor embryo quality in a young woman,
b. unexplained recurrent IVF failure in spite of having transferred embryos derived from eggs that had a normal chromosomal make-up (euploidy) by polar body or blastomere biopsy,
c. cases of repeated failed IVF failure following successful fertilization by intracytoplasmic sperm injection (ICSI) done for male infertility.

d. Recurrent pregnancy loss due to previously diagnosed embryo aneuploidy

The prediction of genetic disorders using blastomere biopsy requires the prior anticipation of an increased risk of the disorder occurring in the offspring, is very limited by the lack availability of genetic markers for all but a handful of genetic diseases and has little potential for widespread application or benefit.

In contrast, egg/embryo aneuploidy is responsible for a significant percentage of infertility, IVF failure and miscarriages, these factors combined with the advantage of selectively transferring fewer "normal" embryos so as to reduce the risk of high order multiple pregnancies, supports an increased role for PGD with FISH in the IVF arena.

STAINING TECHNIQUES USED IN PGD

The following represents available staining techniques: that are used in conjunction with PGD:

- **Fluorescence in situ hybridization (FISH);** which evaluates the structural integrity of chromosomes. This technique when applied to polar body and /or blastomere (day 3) biopsied material, allows for recognition of structural chromosomal defects of aneuploidies involving 5-6 chromosomes (#s 13, 15 ,16, 18, 21, 22 +). Down's syndrome (trisomy 21) which involves an extra chromosome 21 (trisomy-21) is one of the most commonly encountered aneuploidies in the new born. Certain structural aneuploidies such as occurs with selective translocations can also be diagnosed in this way. The detection of sex-chromosome aneuploidies requires blastomere biopsy since the contribution made by sperm- X or Y chromosome can not be evaluated from examination of the polar body(ies). This applies equally to, abnormalities such as those involving the Y (male) chromosome. These are the so-called "sex-linked disorders" (e.g.; Hemophilia, Duchene Muscular Dystrophy etc) that only affect male offspring. Thus the selective transfer of X-X (female) embryos to the uterus, would prevent the occurrence of these disorders.

- **Polymerase Chain Reaction. (PCR)** technology involves identification and amplification of one or more gene loci on the chromosome. PCR provides the capability of PGD of a growing list of many single and multiple gene disorders, including conditions such as Tay-Sachs disease and cystic fibrosis, Early Alzheimer's disease, familial breast cancer, multiple polyposis coli, etc.

In the future, additional methods will become available for use in the lab to help determine which embryos are most likely to implant. This will therefore allow the replacement of fewer embryos, as mentioned previously.

CONCLUSION.

While progress in the development and application of PGD in clinical practice has been limited, the technology provides the possibility for thousands of couples at risk of producing offspring with genetic disorders to deliver children free of severe chromosomal/genetic disease, while reducing the need for abortion that most likely would otherwise be performed.

PGD is not 100% accurate and it is important that people understand the limitations of this technology. There is a concept known as Mosaicism, which implies that not all cells in an early embryo are uniform and have the same chromosomal make-up. Therefore, PGD may yield a result of a normal embryo that is in fact abnormal and vice versa. This is the reason why either CVS or amniocentesis is needed for patients who have PGD. In addition, as mentioned above, not all 23 chromosomes can be evaluated.

In clinical practice, one of the most useful aspects of PGD, relates to the evidence it provides couples who are trying to decide whether to do another cycle with own egg treatment versus moving to donor egg treatment. If PGD demonstrates 100% abnormalities in the eggs or embryos, even though one cannot guarantee there is not a normal egg(s) lurking in the ovaries (recall a woman is born with all the eggs she will ever have), this does provide rather compelling evidence that the couple should move on to egg donation sooner than later. PGD gives tangible verifiable evidence that is most useful when counseling patients.

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This handout is intended as an aid to provide patients with general information. As science is rapidly evolving, some new information may not be presented here. It is not intended to replace or define evaluation and treatment by a physician.