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Pathway to Parenthood

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PREIMPLANTATION GENETIC DIAGNOSIS AND SCREENING (PGD/PGS)

INTRODUCTION:

The human genome project was a massive collaborative project which successfully provided a detailed map in human DNA. This base of knowledge has provided an important "springboard" from which the wide spread implementation of human-gene therapy for the treatment and prevention of disease, is emerging. Here we will review how it has affected assisted reproductive technology (ART).

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 their 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 and Extended Embryo Culture** i.e. what the embryos look like under the microscope at set intervals e.g. 24 hours, 72 hours and 120 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 pellucida is important. We use group culture of embryos from the time following fertilization by Intracytoplasmic Sperm Injection (ICSI). Four to 6 fertilized eggs are cultured together in a single microdrop of specialized culture medium. This allows the growing embryos to benefit from the secretion of beneficial factors generated by the embryos themselves. As the embryos divide the morphologically normal embryos that have developed to the proper cell stage in a given time frame are regrouped in fresh microdrops of medium. The result is that by the fifth day of culture the best cohort of embryos has been determined. It has also established a rational basis for deciding on the number of embryos/ blastocysts to be transferred in each individual case. However, while this process to identify 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, which 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, the re-introduction of these approaches will be quite likely.

3. **Preimplantation Genetic Diagnosis and Screening (PGD/PGS):** 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 are biopsied during culture in vitro and chromosomal/genetic analysis is carried out on material derived from the embryo(s). PGD is used to diagnose a single gene defect in the embryo, typically for couples that have a single gene mutation and want to avoid having a child that would carry the disease (e.g. Cystic Fibrosis, Thalassemia, Tay Sachs). Embryos shown to be free of the genetic disease under investigation are thereupon selectively transferred to the uterus. PGS refers to screening all of the chromosomes for aneuploidy (an abnormal number of chromosomes). By avoiding the transfer of embryos with chromosomal abnormalities, this decreases the risk of IVF failure, miscarriage or babies born with conditions such as Down's syndrome.

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 disorders. In addition, about 1:50 babies are 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.

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 translucent 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 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.

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 capable, 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.

EMBRYO BIOPSY:

In order for PGD/PGS to be performed, a biopsy of the embryo is performed. This is a microsurgical procedure performed by an expert embryologist. The cells are then placed in special containers and sent

by courier to a Reprogenetics laboratory. The biopsy can be obtained from a polar body, blastomere (taken at the 7-8 cell cleavage stage; day 3) or trophectoderm (taken at the blastocyst stage; day 5 or 6). Trophectoderm biopsy has become the preferred method. This is done by using a laser to make a small hole in the zona pellucida (outer shell) of the embryo and then a fine glass pipette is used to aspirate a few cells. Since the blastocyst has approximately 100 cells, as opposed to 7-8 at day 3, the embryo is less traumatized as compared to a blastomere biopsy on day 3. Also, an advantage is that trophectoderm biopsy involves removing cells from the precursor of the placenta and the fetus is not disturbed. Another benefit is that 3 to 5 cells are removed, rather than just 1, which increases the number of cells for genetic analysis and improves accuracy and reliability. It is important to note, however, that in rare instances the analysis may not be able to be completed for technical reasons, in which case a report will not be available regarding that specific embryo.

ANALYSIS TECHNIQUES USED IN PGD:

Techniques available for use in PGD include fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), single nucleotide polymorphism microarrays (SNP) and array comparative genomic hybridization (aCGH). The preferred method at NYFS is aCGH. This testing allows for detection of all 24-chromosome type aneuploidies, as opposed to other methods which only test a limited number of chromosomes. aCGH screens the entire length of all chromosomes, allowing for the detections of rare chromosomal abnormalities. It does so by comparing the embryo's DNA to a known normal DNA specimen, utilizing thousands of specific genetic markers. The error rate has been estimated to be about 2% (as compared to FISH, which has an error rate of about 5-10%).

EMBRYO TRANSFER:

Results of the PGD/PGS are routinely available 2 to 3 days after biopsy. This means that after biopsy, all embryos are frozen (cryopreserved) using the vitrification technique. This allows the embryos to remain in a safe, stable state until the results are available. The selected embryo(s) would then be transferred later into the uterus during a frozen embryo transfer (FET) cycle. There is an option of rushing the biopsy results to allow for a fresh day 6 transfer. This does involve additional fees. In addition, embryos that do not develop to the blastocyst stage until day 6 would have to be cryopreserved and transferred in a future FET cycle, as delaying a fresh ET until day 7 would not be optimal.

EMBRYO BANKING:

Some patients may decide to do more than 1 IVF cycle prior to transfer to increase the pool of embryos to select from. This process is referred to as "embryo banking." If PGD/PGS is going to be utilized in conjunction with embryo banking, a trophectoderm biopsy will be performed on the blastocysts prior to cryopreservation with each fresh cycle. The biopsy samples will be cryopreserved as well and sent to Reprogenetics. Once the final IVF cycle is completed, all trophectoderm biopsies will be thawed and analyzed, while the embryos remain safe in storage. The report will indicate which embryo(s) are

chromosomally normal, and a decision will be made as to which ones and how many will be transferred in a subsequent FET.

CONCLUSION:

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/PGS in the IVF arena. PGD/PGS 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 risk of miscarriage or need for abortion that may otherwise occur. PGD/PGS is not 100% accurate, and it is important that people understand the limitations of this technology.

In clinical practice, one of the most useful aspects of PGD/PGS, 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 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 rather than later.

The advantages of genetic testing include increased chance of pregnancy, decreased chance of pregnancy loss, decreased chance of multiple pregnancies and avoiding undesired genetic diseases. Sex selection may also be performed with this technology. At NYFS we discuss all of these considerations on an individual basis and make the best plan for each patient.

Rev 1/14

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.