Comparative Genomic Hybridization (CGH)

… Preimplantation Genetics of all 23 chromosomes!!

Preimplantation Genetic Screening/Diagnosis (PGS & PGD) consists of the biopsy of a single cell per embryo, followed by its genetic diagnosis through different techniques, and the subsequent replacement to the patient or freezing, of only those embryos classified by genetic diagnosis as normal. Since the early 1990s, the standard preimplantation genetics testing methods, using PGS/PGD techniques, have only allowed for the evaluation of 9 to 12 chromosomes out of a total of 23 chromosome pairs in the human embryo cell.

CGH solves this problem by evaluating all 23 chromosome pairs, allowing completely screened normal embryos to be identified and transferred or frozen. The field of Preimplantation Genetics has been recently revitalized with the appearance of this new CGH technology.

The future of Preimplantation Genetic testing is now available at both our Fertility Centers of Illinois (FCI) Chicago area IVF clinics - FCI Highland Park IVF and FCI River North IVF! Due to our long-standing relationship with ReproGenetics (www.Reprogenetics.com) and our excellent day 5 blastocyst culture methods and embryo freezing (vitrification) techniques, FCI is one of only a handful of IVF programs world-wide able to offer this major breakthrough.

Preimplantation Genetic Diagnosis Background

As women get older, many of their eggs become genetically abnormal, causing infertility but also significantly increasing the risks for miscarriages and genetic birth defects. In vitro Fertilization (IVF) is the best fertility option so egg quality as well as embryo quality can be evaluated visually and then a few of the healthiest appearing embryos can be transferred to the uterus.

Furthermore, assessing some of the chromosomes of each normally developing embryo, for *structural abnormalities (deletions) or abnormal numbers of chromosomes* (aneuploidy), by **Preimplantation Genetic Screening- PGS**, has been available since the early 1990s. PGS can reduce the risk of miscarriage as well as the risk of many genetic birth defects with the subsequent transfer to the patient of those embryos classified by genetic diagnosis as normal. The technique involves the microscopic removal of generally a single cell from a day 3 developing embryo. Most normal embryos on day 3 have 5-8 cells, so removal of one cell *usually* does not disrupt the embryo. Once a single cell (a blastomere) is removed, the cell is fixed on a glass slide for chromosomal analysis… analyzed using a technique called fluorescence in situ hybridization (FISH) Two days later, one or two of the genetically normal embryos are transferred to the uterus on day 5. Several recent studies have confirmed that the *biopsy methods* employed during PGS are critical for providing accurate results without harming the embryos being tested. Day 3 embryo biopsy, combined with PGS, can improve IVF success rates if biopsy methods and PGS analysis are each done by experienced embryologists and geneticists, but may produce negative results if either of these processes is performed in a suboptimal manner by inexperienced hands.

The 9 to12 chromosomes chosen for testing (usually Chromosomes # 13 14 15 16 17 18 21 22 X and Y) account for over 90% of the genetic miscarriages and birth defects. For example, Down's syndrome is caused by an extra # 21 chromosome (Trisomy 21). *Aneuploidy* (any abnormal number of chromosomes….missing or extra) increases dramatically as women age.

The most common situations for recommending PGS include:

- Women age 39 or older (although some women 35-38 ask for the procedure)
- Severe male factor (especially when testicular biopsy is needed to obtain sperm)
- Miscarriages (2 or more genetic or unexplained losses)
- IVF failures (2 or more failures, despite normal appearing quality embryos)

PGS methods utilizing the day 3 biopsy of a single cell (usually representing all cells of the 5-8 cell embryo) are associated with an error rate of 5%, with almost all errors attributable to *mosaicism* (the presence of one or more chromosomally different cell lines within the same embryo). No method based upon screening of a single cell on day 3 can avoid a small error rate due to mosaicism.

In addition, there are numerous genetic diseases secondary to *'single gene disorders'* that result from mutations affecting individual genes on a chromosome. **Preimplantation Genetic Diagnosis, or PGD**, involves assessing a given chromosome for these single gene abnormalities by doing a day 3 embryo biopsy of a single cell. Using polymerase chain reaction (PCR), fluorescent PCR and DNA sequencing, the geneticists in the PGD laboratory can examine each developing embryo to identify the absence or presence of these specific genetic disorders.

The most common indications for recommending PGD include:

- Previous birth of a child with a single gene disorder (examples - Cystic Fibrosis,Tay Sachs, Muscular Dystrophy, Hemophilia, Thalassemia, fragile X or Sickle cell, to name a few)
- Both partners are 'carriers' for a single gene disorder, based on screening tests and therefore at risk for passing on inherited genetic disease to their offspring

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Complete karyotype chromosome analysis (all 23 pairs) of day 5 blastocyst-stage embryos is now clinically available using CGH. This innovative technology uses whole genomic amplification of the DNA from the embryo biopsy, followed by fluorescent green labeling of the sample DNA, then *hybridization* with normal DNA, fluorescently labeled red. Images are collected by specialized software which compares intensities between red and green for each chromosome, generating a molecular karyotype. Thus, CGH can identify an 'imbalance' in chromosomal material and detect all trisomies and monosomies (aneuploidy) and some large structural translocation imbalances.

Advantages of CGH on day 5 blastocyst-stage embryos:

- Multiple cells are analyzed leading to highly accurate results
- Trophectoderm cells (future placenta) are tested…not cells involved in the formation of the fetus. This may reduce any potential damage from the embryo biopsy.
- All 23 chromosome pairs are tested resulting in a complete chromosome analysis.
- Complete elimination of diagnostic errors associated with *mosaicism*. CGH is performed on several cells from day 5 blastocyst embryos compared to one cell biopsy with PGD on day 3 embryos.

After day 5 blastocyst biopsies are performed at FCI, the embryos need to be frozen (vitrified) to allow for the lengthy CGH chromosome analysis at Reprogenetics. Transferring the thawed embryos takes place in a subsequent cycle, at the time of optimal endometrial receptivity. Utilization of this approach requires a significant level of expertise in numerous procedures - day 5 blastocyst cultures, new vitrification (fast freeze) techniques and embryo transfer in a subsequent cycle… and will not be suitable for all IVF centers.

For more information regarding Comparative Genomic Hybridization contact Laurence.Jacobs@integramed.com or go to www.TheInfertilityDoctor.com

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