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By Monica Heger

NEW YORK (GenomeWeb) – An ongoing clinical trial of a single-cell sequencing method for preimplantation genetic diagnosis for couples undergoing *in vitro* fertilization has resulted in its first healthy baby, *Clinical Sequencing News* has learned.

Jie Qiao, director of the Medical Center for Human Reproduction at Peking University Third Hospital; Fuchou Tang, an assistant professor at Peking University's Biodynamic Optical Imaging Center; and Sunney Xie at Harvard University are collaborating on the clinical trial.

In the trial, the researchers are testing a single-cell protocol developed in Xie's lab called MALBAC, for multiple annealing and looping-based amplification cycles, on couples undergoing IVF and seeking genetic screening because one of them is a carrier of a known Mendelian disease.

Twenty-four couples are enrolled in the trial, and Qiao told *CSN* that it would last around one year. So far, the team has screened for chromosome abnormalities as well as known single-gene diseases including hereditary multiple osteochondromas, X-linked hypohidrotic ectodermal dysplasia, X-linked severe combined immunodeficiency, hepatolenticular degeneration, and maple syrup urine disease.

In this case, the father suffers from hereditary multiple exostoses, an autosomal dominant disorder characterized by multiple bony spurs or lumps on the bones. The disorder is caused by a frameshift mutation to the EXT2 gene. The father had a 50 percent chance of passing on the disorder.

During the IVF cycle, the researchers collected 18 embryos at blastocyst stage and biopsied a few cells from the embryos at day five and day six.

They first used MALBAC to amplify the genomes of each cell and then used PCR primers to target the known disease causing mutation. Next-gen sequencing was then able to detect both chromosomal aneuploidies and the point mutation simultaneously at approximately 1x coverage.

Xie told *CSN* that being able to detect both chromosomal aneuploidies and known disease-causing mutations simultaneously is important. PCR has previously been used to look for known disease-causing mutations in couples undergoing IVF, in which one partner is a carrier of a genetic disease. However, that method cannot detect chromosomal aneuploidy in the embryos, Xie said, which occurs in about half the embryos from women over 35 and is a frequent cause of IVF failure.

In this case, three embryos were found to be free of both chromosomal aneuploidies and the known mutation, and one was transferred back into the mother. The embryo implanted and grew successfully, and testing of the amniotic fluid cells, and subsequently of the umbilical cord blood genome, revealed that the baby was disease free.

Qiao told *CSN* that the goal is to offer this testing as a service in order to ensure that known genetic disorders are not transferred from a couple to their children and also to screen for chromosomal aneuploidies prior to implantation.

Approximately 13,000 patients go through *in vitro* fertilization at the Peking University Third Hospital's IVF Center, she said.

Prior to 2012, Qiao said the hospital used FISH to detect a "limited number of chromosomal abnormalities," but in the middle of 2012, the center adopted array CGH and SNP array technology to screen for aneuploidies. In the clinical trial, she said that they are comparing the MALBAC method with Sanger sequencing followed by PCR and STR linkage analysis for point mutation of single genes, and with array CGH for aneuploidy detection.

The researchers have also tested the method on polar bodies, which are byproducts of the IVF cycle. The advantage of testing polar bodies is that it is less invasive, but the drawback is that the genome of the fertilized egg cannot be measured directly and must be adduced. Additionally, sequencing polar bodies will not detect any mutations contributed by the father.

According to Xie, the team evaluated polar bodies in the second case and successfully transferred an embryo to the mother, but the baby has not yet been born.

The team previously validated the method on 52 pronucleus cells, 67 polar body 1 cells, and 64 polar body 2 cells from eight healthy volunteers, the results of which they published in *Cell*.

Peking University is not the only institution moving toward sequencing-based PGD. BGI has been testing a single-cell sequencing method for detecting copy number variants in couples undergoing IVF, work that it published in *PLoS One* . According to BGI's Fei Gao, as of October 2013 over 20 babies have been born healthy following pre-IVF single-cell sequencing to screen for chromosomal aneuploidies.

In addition, New Jersey-based Reprogenetics is in the midst of a clinical trial comparing single-cell NGS to standard of care for women undergoing IVF to see whether using NGS to choose chromosomally normal embryos improves pregnancy success rates.

Dagan Wells, a director at the firm and a senior fellow in the Nuffield Department of Obstetrics and Gynecology at the University of Oxford, recently led a study validating the technique on 61 samples and said that Reprogenetics would likely launch a clinical test in the US by the end of the year.

Illumina has also launched its VeriSeq test for preimplantation genetic screening on the MiSeq, which screens embryos for chromosomal aneuploidies.

Meanwhile, the China Food and Drug Administration recently began regulating genetic testing, calling for a halt of tests that don't have the agency's approval, and Qiao said that includes NGS-based preimplantation genetic testing. Currently, however, she said that her team at Peking University is allowed to perform the testing in the context of the clinical trial because it is free to patients and does not require extra biopsy from the same embryo. Upon completion of the trial, however, she said the team would work with China FDA to get the test approved.

Regulations in the US and the EU are less stringent than those in China. Neither the US Food and Drug Administration nor the EU regulates the preconception space.

Monica Heger tracks trends in next-generation sequencing for research and clinical applications for GenomeWeb's *In Sequence* and *Clinical Sequencing News*. E-mail Monica Heger or follow her GenomeWeb Twitter accounts at @InSequence and @ClinSeqNews.

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