Embryo-analysis technique may boost in vitro fertilization success

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(Medical Xpress)—Stanford University School of Medicine researchers have devised a two-part approach to identify developing human embryos most likely to result in successful pregnancies. The technique could transform the lives of infertile couples seeking to use in vitro fertilization, or IVF, to start a family.

The research suggests that fragmentation—a common but not well-understood occurrence in the early stages of human development in which some of the cells in an embryo appear to break down into smaller particles—is often associated with a lethal loss or gain of genetic material in an embryo's cells. Coupling a dynamic analysis of fragmentation with an analysis of the timing of the major steps of embryonic development can significantly increase the chances of selecting an embryo with the correct number of chromosomes, the researchers found.

The findings extend beyond IVF and offer a glimpse into how human reproduction differs from that of many other animals. They also suggest that sperm selection could be much more important than previously believed.

"It is amazing to me that 70 to 80 percent of all human embryos have the wrong number of chromosomes," said Renee Reijo Pera, PhD, professor of obstetrics and gynecology. "But less than 1 percent of all mouse embryos are similarly affected. We're trying to figure out what causes all these abnormalities."

Reijo Pera, who is the director of the Center for Human Embryonic Stem Cell Research and Education at Stanford's Institute for Stem Cell Biology and Regenerative Medicine, is the senior author of the work, published online Dec. 4 in *Nature Communications*. Research associate Shawn Chavez, PhD, is the study's first author.

Regardless of the source of the chromosomal errors, nearly all result in miscarriage. For natural conceptions, this often happens before the woman realizes she is pregnant. Each embryo transfer in IVF, however, is eagerly anticipated and costs thousands of dollars. To improve the odds of a successful pregnancy, clinicians and parents frequently decide to transfer more than one embryo at a time—a decision that has its own risks for mother and any fetuses that may result. For example, instances in which there are multiple fetuses are more likely to result in miscarriages or to threaten the health of the mother.

Recently, Reijo Pera and her colleagues began to investigate ways to better predict embryonic developmental success within one or two days of fertilization. Not only would such an advance decrease the likelihood of miscarriage or the possible need for a selective reduction, it would also reduce the amount of time the embryo would have to be cultured in the laboratory before transfer. (Although it has not been conclusively shown, some researchers are concerned that epigenetic changes may accumulate in a cultured embryo and cause subtle, long-lasting effects in the fetus.)

The study extends previous findings in Reijo Pera's lab indicating that the timing of cell division and other developmental milestones as the embryo progresses from one to four cells can be used to predict with 90 percent accuracy whether the embryo is likely to go on to develop into a 70- to 100-celled embryonic structure called a blastocyst. Achieving blastocyst status, which occurs about five days after fertilization, is a good, but not fail-safe, indication that an embryo might result in a

successful pregnancy. That research was published in *Nature Biotechnology* in October 2010, and is currently the subject of clinical trials in several IVF clinics across California.

In the new study, the researchers decided to look more closely at the chromosomal composition of those four-celled embryos predicted by their previous method to be successful. The 75 human embryos used in the study were originally intended for use in IVF. They were donated for research by infertile couples to the Stanford RENEW Biobank. They are unusual in that they were frozen within hours of fertilization. Clinicians normally monitor the development of fertilized embryos for three to five days in an attempt to identify those that are the best candidates for transfer. Those remaining are then frozen for later use—either in future IVF cycles for that couple, or as research tools to learn more about human development. However, the clinics visited by the couples who donated the embryos for this study supported earlier freezing as a standard practice.

The researchers thawed the embryos and monitored the developmental milestones with time-lapse photography as they progressed over the course of two days to approximately the four-cell stage. They found that only 53 of the original 75 embryos progressed beyond the one-cell stage. They then dissembled the 53 embryos into individual cells, analyzed the chromosomal content of each cell, and compared the findings with each embryo's predicted chance of success. A normal human embryo has 23 pairs of chromosomes; each pair contains one chromosome from each parent.

"We found that, although the parameters we defined earlier do very well in predicting blastocyst success," said Reijo Pera, "about 50 percent of those with normal developmental timing have the wrong number of chromosomes." That is, even though they would likely go on to become blastocysts, they were unlikely to result in healthy pregnancies.

The researchers found that they could increase their chances of picking an embryo with the correct number of chromosomes by combining their previous parameters with an analysis of a perplexing embryonic process called fragmentation, which is thought to possibly represent a breakdown of cellular components within an embryo. (Although embryos exhibiting fragmentation are currently avoided during IVF, some can result in successful pregnancies.) However, there's not been a clear link between fragmentation and chromosomal number until now. There is also some evidence that fragmentation can occur during natural human conceptions, indicating that it is not just associated with IVF.

"We were surprised to find that several embryos had cells that were missing one or more chromosomes with numbers that were not consistent with known types of errors," said first-author Chavez. Added Reijo Pera, "We later realized that some of these chromosomal errors were being generated through the process of fragmentation."

The researchers concluded that, although neither developmental timing nor the presence of fragmentation was a foolproof way to pick a healthy, chromosomally intact embryo, a combination of the two approaches appears much more likely to be successful.

"We found that using the cell-cycle parameters in conjunction with fragmentation dynamics—such as timing, degree and the persistence and resorption of fragments—rather than the incidence of fragmentation itself was most predictive of embryo chromosomal status," said Chavez.

The research also pinpointed a somewhat surprising possible source of at least some lethal errors: the father's contribution. Although the sperm's role is usually written off as a straightforward delivery of presumably unsullied genetic information, Reijo Pera and her colleagues found that it may not be so simple.

"We learned that about 20 percent of human embryos are normal, about 25 percent are carrying errors introduced by the egg, and the remaining 55 percent have errors that could be caused by either the sperm or the egg," said Reijo Pera. "And yet, currently, there is almost no screening process: if a sperm is moving vigorously in a laboratory dish, it's considered to be a suitable candidate for IVF."

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