Earlier, more accurate prediction of embryo survival enabled by Stanford research

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Two-thirds of all human embryos fail to develop successfully. Now, in a new study, researchers at the Stanford University School of Medicine have shown that they can predict with 93 percent certainty which fertilized eggs will make it to a critical developmental milestone and which will stall and die. The findings are important to the understanding of the fundamentals of human development at the earliest stages, which have largely remained a mystery despite the attention given to human embryonic stem cell research.

Because the parameters measured by the researchers in this study occur before any embryonic genes are expressed, the results indicate that embryos are likely predestined for survival or death before even the first cell division. Assessing these parameters in the clinic could make it easier for in vitro fertilization specialists to select embryos for transfer for a successful pregnancy.

"Until recently, we've had so little knowledge about the basic science of our development," said the study's senior author Renee Reijo Pera, PhD. "In addition to beginning to understand more about our development, we're hopeful that our research will help improve pregnancy rates arising from in vitro fertilization, while also reducing the frequency of miscarriage and the need for the selective reduction of multiple embryos."

Reijo Pera is a professor of obstetrics and gynecology at the medical school and the director of the Center for Human Embryonic Stem Cell

Research and Education at Stanford's Institute for Stem Cell Biology and Regenerative Medicine. The study will be published online Oct. 3 in Nature Biotechnology. Postdoctoral scholar Connie Wong, PhD, and former postdoctoral scholar Kevin Loewke, PhD, are the co-first authors of the research. Loewke is currently the lead engineer at the Menlo Park, Calif., biotechnology company Auxogyn Inc.

The researchers conducted their studies on a unique set of 242 frozen, one-cell human embryos from the Reproductive Medicine Center at the University of Minnesota. The embryos were created at the in vitro fertilization program at Lutheran General Hospital in Illinois over a period of several years prior to 2002, and when the clinic was closed, the patients gave their consent for their embryos to be used in research.

Nowadays it's unusual to freeze embryos so soon after fertilization (about 12 to 18 hours). Instead, clinicians monitor embryonic development for three to five days in an attempt to identify those that are more likely to result in healthy pregnancies after transfer. Despite their best efforts, though, they have only about a 35 percent success rate. As a result, most women elect to transfer two or more embryos to increase the chance of a live birth. However, if multiple embryos implant and develop successfully, a woman and her physician may choose to selectively abort one or more to better the odds for the remaining embryos.

Reijo Pera and her colleagues received a large grant from an anonymous donor 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 be have to be cultured in the laboratory before transfer. (Although it's not been conclusively shown, some researchers are concerned that genetic changes may accumulate in a cultured embryo and cause subtle,

long-lasting effects in the fetus.)

The researchers thawed the embryos, split them into four groups and tracked their development during the first few days using time-lapse video microscopy and computer software specially designed by Loewke, a former Stanford mechanical engineering graduate student, for this study. They followed the cells through the development of a hollow ball called a blastocyst, which typically occurs within five to six days after fertilization. A blastocyst is usually an indication of a healthy embryo.

They found that of the 242 embryos, 100 were able within five or six days to form normal-looking blastocysts — about the same proportion that would be expected to be successful in normal pregnancies. Because they had tracked the embryos' development so closely, they were then able to go back and identify three specific parameters collectively associated with successful blastocyst formation: the duration of first cytokinesis (the last step of a period in the cell cycle called mitosis in which the cell physically divides), the time between first and second mitoses, and the synchronicity of the second and third mitoses. All of these events occur as the embryo progresses from one cell to four cells within the first two days after fertilization.

"It completely surprised me that we could predict embryonic fate so well and so early," said Reijo Pera. If an embryo's values fell within certain windows of time for the three predictive parameters, that embryo was more than 90 percent likely to go on to develop successfully into a blastocyst.

When the researchers looked at the gene expression profiles of individual cells from the embryos, they found that, as had been previously shown, the embryos at first express only genes from the maternally derived egg. By roughly the third day (the eight-cell stage) they begin to express genes specific to embryonic development, and the

relative proportion of embryonic to egg genes increases steadily during the next few cell divisions.

Surprisingly, however, they found that not all cells in an embryo are behaving identically: While some cells may be expressing mostly maternal genes, others in the same embryo are churning out mostly embryonic genes.

Similarly, not all cells in an embryo are dividing in synchrony: The researchers found embryos in which some cells were dividing on schedule while others were seemingly stuck, or paused.

"We've always thought of embryos as living or dying, but in reality we find that each cell in the embryo is making decisions autonomously," said Reijo Pera. "No one has ever looked at this before." She and her colleagues found that embryos in which individual cells varied significantly in their cell-division schedules or gene-expression profiles were less likely to become successful blastocysts.

Together the research indicates that the maternal RNA transcripts — that is, the molecules that carry instructions from the mother's DNA to the embryo's protein-making factories — must be actively degraded in each cell of the embryo, and that this degradation is necessary for the cells to begin to express embryonic genes. Cells that fail to execute some part of this delicate process get out of sync with their neighbors and jeopardize the life of the embryo. The whole endeavor is complicated, and may explain why human embryonic development is so precarious and unique.

The research also highlights the importance of studying human embryos, which currently cannot be supported by federal funds. (Every year since 1996, Congress has approved a provision known as the Dicky-Wicker amendment that prohibits the use of federal funds for research in which

a human embryo is destroyed — even ones that would otherwise be discarded.)

"In mice, about 80 to 90 percent of embryos develop to the blastocyst stage. In humans, it's about 30 percent," said Reijo Pera. "In addition, about one in 100 mouse embryos are chromosomally abnormal, versus about seven out of 10 human embryos. That's why human studies like these are so important. Women, their families and their physicians want to increase the chances of having one healthy baby and avoid high-risk pregnancies, miscarriages or other adverse maternal and fetal outcomes. It's truly a women's health issue that affects the broader family."

The research was funded by an anonymous donor, the March of Dimes and the Stanford Institute for Stem Cell Biology and Regenerative Medicine.

The researchers have developed an automated algorithm for clinical use that could assess these time-lapse microscopy videos and determine with high accuracy which of these very early embryos would be successful by the four-cell stage. That technology has been licensed exclusively to Auxogyn Inc. by Stanford. Reijo Pera and the other coauthors of the manuscript own or have the right to purchase stock in the company.

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