Cell Stem Cell
Previews

The Human Egg Is Back

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In a recent issue of *Nature*, Tachibana et al. (2009) conducted chromosomal transfer into recipient primate oocytes to yield live offspring or monkey ESCs with no contribution of donor mitochondria. The success of this technique may increase demand for human oocyte donation for research.

"It will not work." This statement represents the four words that best motivate a scientist. Certainly, it is what I would have said to Mitalipov's team if they had pitched me the idea of transferring the spindle of one egg into the cytosol of another, enucleated egg with the expectation to produce a baby from THAT. Even so, I would have been wrong.

Indeed, in their recent *Nature* article, Tachibana et al. (2009) were able to remove the chromosomes of a primate egg and deliver a replacement set of chromosomes from another donor female, a technique they called spindle transfer (ST). Not only were the authors able to fertilize the ST eggs, they generated blastocysts healthy enough to permit the derivation of embryonic stem cells (ESCs), and when the embryos were transferred into the uterus of a surrogate mother, healthy babies were born. This is not an easy task; there are multiple hurdles that had to be cleared in order to achieve their success.

If we bow to the astronauts that repair the Hubble telescope—and they deserve this respect when they risk their lives by manipulating equipment at a speed of 18,000 miles an hour and with no gravity—we must also bow to the Oregon team that accomplished ST. The authors managed to take a primate egg, probably the most valuable single cell in the body, maintain its viability for hours, subject it to exceptionally precise microsurgery to remove only 11 picolitres of cytosol, introduce the precious cargo from a different oocyte by performing a rendezvous maneuver with the postsurgery egg still in the microscope, fuse them, and subsequently trigger development by injecting a sperm directly into its cytosol. Four healthy monkeys were born after that sequence; what a feat!

What can go wrong during this process? In a nutshell, anything and everything could go wrong. If you are not aware and respectful of the physiology of the egg, the experiment is doomed. For starters, the window of time to remove the chromosomes successfully is small. The egg must maintain high levels of maturation promoting factor (MPF) in order to keep the chromosomes collected together, like a tight bouquet of flowers. If the operation is done prematurely, the chromosomes will not be sufficiently condensed, and too many host mitochondria will also be flushed away. If the operation is done too late, the chromosomes will drift apart as the MPF activity begins to decline, probably compromising the capacity of the sperm to trigger development. Introducing a different set of chromosomes wrapped inside a tiny cell membrane package is not trivial, and delivering them intact inside the enucleated egg is also a challenge. Direct injection carries the risk of losing the genetic material during their aspiration and or ejection from the injection needle. Fusion is the safest option but, if performed with the traditional method of using an electrical pulse, the developmental potential of the egg will be compromised because of premature activation. Instead, the authors relied on one of the oldest "tricks" in the book and used inactivated sendai virus to fuse the two cell membranes. While on paper this progression may seem as simple as a series of well-orchestrated experimental steps, in reality success depends on having top-quality biological material and many, many hours of training at the microscope.

If proven to translate from monkey cells to human oocytes, the proposed use of the technique would be for the treatment—if not eradication—of human mitochondrial diseases in the offspring of an affected biological mother. The scheme necessitates that women are willing to donate their healthy eggs to those that need them, not to mention the experimental progress—and demand for human eggs—that the translation to the clinic will require. Thus, in addition to the technical challenges described above, moving this technique into a routine clinical practice runs into an old and challenging dilemma. Consider the recent historical example: for almost ten years, from 1997 when the cloned sheep Dolly was first announced until the advent of human induced pluripotent stem cells (iPSCs) in 2007 (Wilmut et al., 1997; Takahashi et al., 2007), the field has struggled with the ethical challenge posed by the idea of creating patient-specific ESCs via somatic cell nuclear transfer (SCNT) (Cibelli et al., 2001). Specifically, although SCNT offered hope for those trying to help patients suffering from devastating diseases, the technique raised legitimate worries that the need for human eggs could put the women who supply them at risk, dampening the excitement of having a new tool—albeit a powerful one—to cure patients. Indeed, when Takahashi et al. (2007) and Yu et al. (2007) announced the generation of human iPSCs, a collective sigh of relief was almost audible, as the field embraced an approach that promised to bypass the need for human embryos. Nonetheless, some scientists, and I identify myself among them, believe that human eggs will still be needed for some time to come, in order to help us truly understand their unique properties that permit dedifferentiation and rejuvenation of differentiated cells. Of course, such need will never reach the proportions of what we thought the field faced when SCNT was the only route to generate isogenic ESCs.

Which brings us back to the implications of the new technique described by Tachibana et al. (2009). Will the possibility

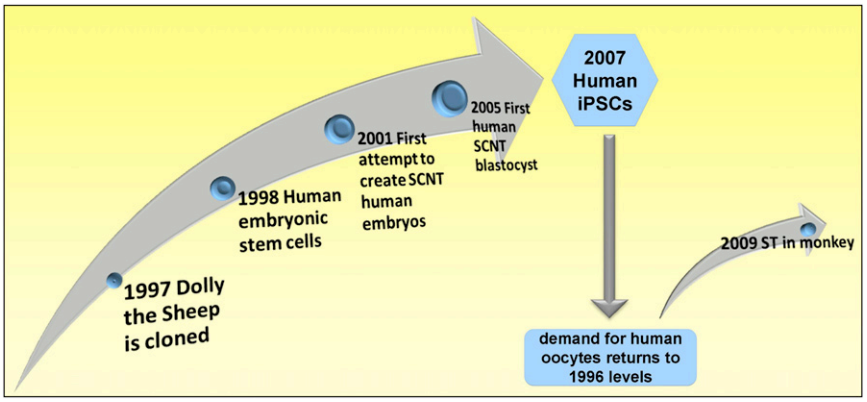

Figure 1. Timeline of Scientific Experiments Fueling the Need for Human Oocytes for Research

of ST reignite the debate over the need for high-quality human eggs? Considering the devastating consequences of mitochondrial diseases and the possibilities that ST can offer to parents in search of a cure, the answer seems self evident. How could there not be a demand to extend their work into a human context? Of course, given that SCNT has yet to be conclusively translated from monkey to human cells, future success of human ST is far from certain. Moreover, the question of how researchers might demonstrate the feasibility and safety of ST with human cells presents significant ethical challenges in and of itself.

Nonetheless, in addition to the promise that ST offers to those suffering from heritable mitochondrial disease, there is at least one other constituency that likely find ST technology a hopeful therapeutic alternative. During 2006, almost 17,000 in vitro fertilization (IVF) cycles were performed with donated eggs for women whose own oocytes were, for some reason, of low quality. Assuming the DNA itself is not the reason for the failures, human ST would likely be the first choice for these patients, if the technology were proven to be effective. Older women could have children carrying their own DNA thanks to the cytosolic contribution of a healthy, young surrogate egg. This procedure could certainly be attempted prior to undertaking IVF with intact donor eggs, resulting in a child related to the donor and not the woman who carried the fetus to term. The long-term safety and efficacy of the ST technique will have to be shown, something that the Oregon team is likely planning to undertake.

Certainly, the debate over the continuing need for donated oocytes that we thought was put to rest in 2007 has just been resurrected (Figure 1). And it caught us almost as unprepared as in 1997, or worse. The legal landscape concerning the compensation of egg donors varies widely, especially across the US, ranging from allowing for payment (New York), to compensation for expenses and lost wages (California), to banning it altogether (Michigan). Perhaps it is time to establish a comprehensive, federal policy that would both protect women and also sponsor the research necessary to test whether a technique such as ST can be used in the clinic.

The repercussions of the ST technique go beyond its practical application in the clinic. For years there has been a "closeted" debate on whether assisted reproductive techniques such as IVF and intracytoplasmic sperm injection (ICSI) could have detrimental health effects on children. The rationale for this concern is based on evidence gathered mostly in laboratory animals, such as the finding that slight changes in the composition of the medium in which mouse embryos are cultured significantly alter its gene expression (Rinaudo and Schultz, 2004). Considering that the first rounds of division in the embryo are driven by molecules already present in the unfertilized egg, its responsibility on the fate of the offspring cannot be underestimated, and yet has been challenging to study. ST can help us answer some of these questions. By exploring the possibility of taking the chromosomes from an egg—deemed of high quality—and transferring it to the cytosol of an egg of low quality (or the opposite), we could not only answer the question of how important is the quality of the egg cytosol on the fate of an embryo, but we could also investigate which critical egg genes predestine the embryo to succeed or fail; all done in primates. But to conduct these important basic science investigations, with the hopes of eventually learning lessons to benefit future reproductive technologies, a source of human oocytes for research will be required.

We bow to the Oregon scientists, while we brace ourselves for the arduous discussions to come as the field works to implement the technique they unveiled. Certainly, the question of what parallel laboratory tests can be undertaken ethically with human eggs and what level of preclinical safety assessment will be sufficient to permit the translation of ST into the clinic remain essential issues to be addressed.

The need for human eggs for research is back. It seems like it never left the stage after all.

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