Medical News & Perspectives

Sanctioned UK Trial of Mitochondrial Transfer Nears

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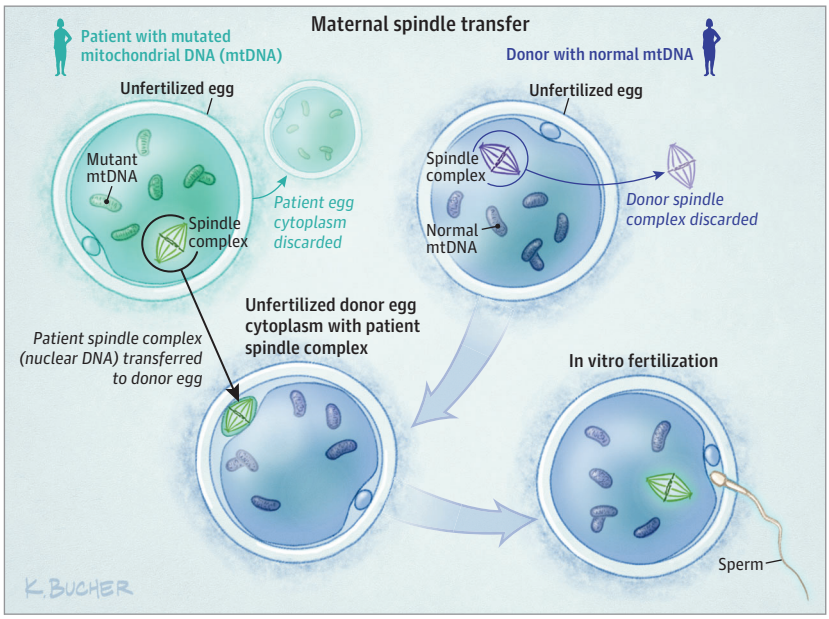
Several years ago, a ray of light appeared in pediatric care when researchers devised a way to reset a child's mitochondrial destiny.

Mitochondria—the tiny organelles floating by the hundreds in cellular cytoplasm that children inherit exclusively from their mothers—figure in a large number of burdensome, often fatal disorders, whose full extent medical science has only recently recognized. Mitochondria have their own DNA (mtDNA), separate from that contained in cell nuclei (nDNA), and their 37 genes are susceptible to heritable mutations that can interfere with the mitochondria's essential job of converting food and oxygen into energy.

However, by using in vitro fertilization technology to replace, at the embryo stage, the mitochondria of a mother whose mtDNA is known to be compromised with healthy mitochondria from a female donor, researchers found they might short-circuit the inheritance pattern and possibly give many children a life free of mitochondrial disease.

Even though the United Mitochondrial Disease Foundation says the procedure, called mitochondrial replacement therapy (MRT), may benefit many of the 1000 to 4000 US neonates born each year with a mitochondria-related vulnerability to life-altering disease—strokes, seizures, blindness, deafness, diabetes, gastrointestinal problems, inability to walk or talk, and failure to thrive—it nevertheless has generated ethical and political controversy in the United States. In keeping with its long-standing opposition to human embryo research, Congress has barred the US Food and Drug Administration (FDA) from even accepting applications to try MRT despite an FDA-commissioned Institute of Medicine (IOM) (now the Health and Medicine Division of the National Academies of Sciences, Engineering, and Medicine) report that last February endorsed the procedure as "ethically permissible" with certain qualifications—particularly that it only be tried in male embryos to avoid affecting the germline.



Meanwhile, the United Kingdom has green-lighted MRT. Both houses of Parliament and the Human Fertilisation and Embryology Authority (HFEA)—the agency that oversees work involving human embryos in the United Kingdom—gave their provisional blessing in 2015 to an official trial of the procedure. But the agency placed final approval on hold last summer following research articles that threw a wrench into the mix. After due consideration, a HFEA expert panel recommended going forward in late November, and the Authority subsequently gave its approval.

Significantly, the United Kingdom's endorsement sanctions alteration of not only male but also female embryos in what would become the world's first known germline gene therapy. Any changes introduced into a female embryo's mtDNA will be passed on and likely enter the human gene pool.

Given that many bioethicists have expressed deep misgivings about such germline tampering, Sir Douglass Turnbull, MBBS, PhD, whose research group at Newcastle University is likely to go forward with the UK's first MRT procedure, doesn't share their sentiments. "I look after more patients with mitochondrial disease than anyone around and my big-

gest concern is safety," he said. "But my patients are handing down mutated mitochondria. My view is [trying to correct] that is ethical."

Technical Stumbling Blocks

The HFEA hesitated last summer when major players in the drive to bring MRT to fruition published work that included some qualms. The first article last June in *Cell Stem Cell* described an in vitro experiment in which Dieter Egli, PhD, and his colleagues at the New York Stem Cell Foundation sought to determine what happens to the minute amount of maternal mtDNA that inevitably hitches a ride when researchers create an embryo with the mother's nDNA and the donor's healthy mtDNA. Such a mix of 2 people's mtDNA genomes, or haplotypes, which does not occur in normal reproduction and has unknown consequences, is a form of what is called heteroplasmy. The team created 8 mitochondrial replacement stem cell lines and then tracked the fate of the carryover mtDNA that accompanied the mother's nDNA. Among the different cell lines, up to 2.2% of total mtDNA was carryover, but the average rate was 0.2%. In most cases, the mother's mtDNA disappeared as the stem cells divided. But in a few instances of what is called genetic "drift," the maternal mtDNA reconstituted itself and began taking over the cells. A few cell colonies wound up containing only carryover DNA that accompanied the mother's nDNA.

The message: If mutant maternal mtDNA slips through during the transfer of nDNA into an embryo, it will coexist with the donor's healthy mtDNA. Eventually the mother's damaged mtDNA may outcompete the donor mtDNA and perhaps cause the very pathology the procedure was designed to eliminate.

Days after the Egli group's article appeared, Turnbull's group published a letter in *Nature* that, while admitting that mtDNA carryover could be a problem, took a more positive tone. The group described what amounted to the first preclinical trial of a long-studied technique called pronuclear transfer (PNT). The technique uses the father's sperm to fertilize 2 oocytes, one from the mother and another from a donor. The nucleus from the donor zygote then is removed and replaced with the nucleus from the parental one. The result was a zygote with the donor's mitochondria and the parents' nDNA. The team found that it could produce higher-quality blastocysts for implantation by performing the nuclear transfer immediately after fertilization rather than waiting until the sperm and egg have actually fused, as they had been doing with disappointing results. This success contributed to their optimism. Importantly, they also reported that mtDNA carryover had been reduced from 4% to less than 2% and concluded that "PNT has the potential to reduce the risk of mtDNA disease, but it may not guarantee prevention."

In November, a third report was published in *Nature* by a group headed by Shoukhrat Mitalipov, PhD, who directs the Center for Embryonic Cell and Gene Therapy at the Oregon Health & Science University in Portland. Mitalipov, who applied to the FDA to perform MRT in the United States before the ban went into effect, has developed an alternative technique called maternal spindle transfer (MST), in which the nucleus of the mother's oocyte is transferred to an enucleated donor oocyte *before* fertilization with the father's sperm. The technique has been vetted over a number of years in rhesus macaque monkeys.

According to its report, the Mitalipov team used spindle transfer to perform a dry run of MRT, using oocytes from 11 healthy donors and 5 mothers with known pathogenic mtDNA mutations. The result was "efficient" in vitro replacement of the mutated mtDNA and production of embryos that contained less than 1% of carryover maternal mtDNA. But the team's success was marred by the same phenomenon that troubled Egli: Some of the resulting embryonic stem cell lines gradually lost donor mtDNA and reverted to the mutated maternal form. The problem, the team noted, seemed to arise from the difference in mitochondrial haplotypes.

"We observed substantial differences in cell proliferation and growth rates among different clones," the team wrote. "Those with higher maternal mtDNA levels exhibited significantly faster growth rates." This imbalance, they speculated, was due to certain genetic polymorphisms that gave a selective advantage to the maternal mtDNA. To address the problem, the team suggested leveling the playing field by "selecting compatible donors harbouring [polymorphisms] similar to the maternal mtDNA." This would presumably offset the maternal mtDNA's upper hand.

In any case, Mitalipov said in an interview that he did not consider genetic drift to be sufficient reason to abandon MRT. "It does happen with cell lines," he said. "I don't know about in children. But there is no treatment that works 100% of the time. Cancer comes back in some cases, but we don't ban the whole treatment as a result."

Turnbull, who was knighted last summer for his 30 years of work in mitochondrial disease, also questioned whether in vitro results would apply to the procedure in vivo. "We too found in our paper that [the heteroplasmy in] 1 stem cell line went up," he said during an interview. "The question is the relevance of what happens in an embryonic stem cell to what happens in actual embryo development."

Egli said his article was not intended to halt MRT approval. "It was meant as a cautionary note," he said. "This technique leads to a mixture of mitochondria from 2 people. We don't know what the consequences are. They could be anywhere from none to what animal work has suggested, which is neurological problems, learning difficulties." To minimize the problem, Egli agreed with Mitalipov that researchers should make sure the mtDNA from mother and donor are "closely related" in any future clinical trial.

"This is a good technique that will most likely work and the baby will be healthy if the carryover is low," Egli said. But he said the kinks should be worked out before approval. A slipshod trial with a bad result " would be a big setback for this field," he noted.

Despite the carryover problem, the HFEA's expert panel ended months of deliberation in late November by recommending that MRT with either the pronuclear or spindle transfer technique be approved for "cautious" use in "specific circumstances." The HFEA itself then approved the recommendation in mid-December and opened the door to applications, with a clinical trial of MRT likely to take place in 2017. Turnbull said his team was ready to apply for a license to try it.

But while it may be the first governmentally sanctioned trial, it would not be the first known use in humans. Someone has beaten everyone to the punch.

"There are no rules"

In late September, *New Scientist* magazine revealed that John Zhang, MD, a New York City fertility specialist, traveled to relatively

unregulated Mexico to perform the technique. He did so on behalf of a Jordanian couple who lost their first 2 children to Leigh syndrome, a progressive neurological condition often linked with faulty mitochondria. In breaking the news of the outcome—an apparently healthy boy born last April—the magazine quoted Zhang as saying he chose Mexico because "there are no rules." His scientific colleagues reacted with surprise and criticism.

"It's unfortunate this was done in such a closed way," said pediatrician Marni Falk, MD, director of the Mitochondrial-Genetic Disease Clinic at the Children's Hospital of Philadelphia and a member of the IOM committee that last February gave conditional ethical approval for a US human trial. Noting that mitochondrial disease often is not apparent at birth but presents later in life, she questioned whether a long-term follow-up plan exists for the child and if the family had given proper informed consent. Zhang reported in an abstract published in *Fertility and Sterility* and presented in October to the American Society for Reproductive Medicine that his procedure was performed "under IRB-approved protocol with proper consent." He did not identify the institutional review board (IRB) that approved the protocol, however. Nor did he specify any follow-up plan.

"Without oversight and the ability to follow this child, it's bad medicine and bad science," added Lainie Ross, MD, PhD, associate director of the MacLean Center for Clinical Ethics at the University of Chicago.

Zhang, who did not respond to *JAMA*'s interview requests, wrote in his abstract that he used the MST technique because PNT involves the destruction of a fertilized embryo and the Jordanian couple, both Muslims, rejected it on religious grounds. According to the abstract, the average mtDNA carryover rate in the implanted blastocyst was 5.10% (SD, 1.11%), although the average level of carryover mtDNA in the full-term baby's hair, foreskin, urine and other neonatal tissues was less than 1.60% (SD, 0.92%).

Falk noted, however, that these tissues do not tell the full story because mitochondrial flaws affect the organs with the greatest energy needs. "It's easy to test hair and urine, but hard to test the heteroplasmy level in the heart or brain," she said. "It can be quite different in different tissues because of the random way mitochondrial DNA replicates itself. If you don't follow the child over time, you can have severe problems later with the heart and other organs."

Egli said the Zhang team's carryover of maternal mtDNA was "rather high" in any case, contending that the optimum would be less than "0.5%," which he called "feasible." He noted that the problem may have been genetic incompatibility between mother and donor. "There you had a Middle Eastern couple, whereas the egg donor was probably a woman from Mexico. If so, you had the coinheritance of 2 very different genotypes. There is no precedent for that. We don't know what happens."

It's anyone's guess when, if ever, Congress will remove the rider it attached to the 2016 omnibus spending bill that banned MRT in the United States.

Mitalipov, who would like to be the first US investigator to perform the procedure if the ban were lifted, said he has lobbied hard to remove the prohibition. "I'm not a politician," he added. "We tried to make a strong case, but we didn't have enough muscle."

The problem is not scientific, he said. "The procedure is ready to go. It may not be ready to put it into widespread clinical application, but it is ready for trial with a limited number of patients."

But Falk said she is dubious that the ban will be lifted any time soon. "I feel like nothing is moving forward," she said.

Writing in a Viewpoint published in *JAMA* last July, I. Glenn Cohen, JD, of Harvard Law School's Petrie-Flom Center for Health Law Policy, and Eli Y. Adashi, MD, MS, of the Warren Alpert Medical School at Brown University, said Congressional opposition to MRT runs "counter to the moral doctrine of beneficence," but, like Falk, were dubious about the procedure's prospects in the United States. They said that the history of such legislative riders does not bode well.

"For instance, the Dickey-Wicker Amendment (which prohibits public funding of human embryo research) has just marked its 20th anniversary, and the Hyde Amendment (which prohibits public funding of abortions) is rapidly approaching 40 years as a policy rider."

Their hope, they wrote, is that the birth of disease-free children in the United Kingdom will change congressional hearts and minds. "Failing such, progress in the prevention of mitochondrial DNA diseases will remain the domain of a biomedical enterprise an ocean away." ■

Note: The print version excludes source references. Please go online to jama.com.