Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases

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The exchange of nuclear genetic material between oocytes and embryos offers a novel reproductive option for the prevention of inherited mitochondrial diseases. Mitochondrial dysfunction has been recognized as a significant cause of a number of serious multi-organ diseases. Tissues with a high metabolic demand, such as brain, heart, muscle, and central nervous system, are often affected. Mitochondrial disease can be due to mutations in mitochondrial DNA or in nuclear genes involved in mitochondrial function. There is no curative treatment for patients with mitochondrial disease. Given the lack of treatments and the limitations of prenatal and preimplantation diagnosis, attention has focused on prevention of transmission of mitochondrial disease through germline gene replacement therapy. Because mitochondrial DNA is strictly maternally inherited, two approaches have been proposed. In the first, the nuclear genome from the pronuclear stage zygote of an affected woman is transferred to an enucleated donor zygote. A second technique involves transfer of the metaphase II spindle from the unfertilized oocyte of an affected woman to an enucleated donor oocyte. Our group recently reported successful spindle transfer between human oocytes, resulting in blastocyst development and embryonic stem cell derivation, with very low levels of heteroplasmy. In this review we summarize these novel assisted reproductive techniques and their use to prevent transmission of mitochondrial disorders. The promises and challenges are discussed, focusing on their potential clinical application. (Fertil Steril® 2014;101:31–5. ©2014 by American Society for Reproductive Medicine.)

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The mitochondria are intracellular organelles that provide an essential supply of cellular energy in the form of adenosine triphosphate generated via oxidative phosphorylation. Mitochondrial disease can be due to mutation in mitochondrial DNA (mtDNA) or mutations in nuclear DNA involved in mitochondrial function. In addition, there is increasing evidence that acquired mtDNA mutations are involved in several chronic age-related diseases, such as diabetes, cardiovascular disease, and Parkinson's disease (for review see reference 1).

The true prevalence of mtDNA disease is unknown. However, it is estimated that approximately 1 in 4,000 children are born in the United States with an inherited with mitochondrial disease (2). Mitochondrial disease often affects high-energy-requiring tissues such as brain, muscle, liver, heart, kidney, and the central nervous system. These diseases are clinically heterogeneous, but symptoms may include deafness, blindness, diabetes, muscle weakness, and heart, kidney, and liver failure. There are a number of well-defined clinical syndromes, but many patients do not fall into easily defined clinical groups.

The mitochondrial genome contains only 37 genes, and mtDNA is maternally inherited. Each cell contains thousands of copies of mtDNA. Normal individuals are homoplasmic; that is, all the mtDNA copies are identical. However, mitochondrial mutations may be either homoplasmic, in which all copies are mutated, or heteroplasmic, whereby the individual contains a mixture of mutated and wild-type DNA. Patients affected by mtDNA disease are usually heteroplasmic. Their tissues and cells have a mixture of wild-type and mutant mtDNA. The clinical phenotype depends on the ratio of mutated to wild-type mtDNA in

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affected cells and tissues. There is a threshold effect; that is, the level of abnormal mtDNA that causes mtDNA disease. This threshold varies by tissue and mutation type but is usually in the range of 60%–90%.

Treatment options are generally limited. Hence, preventive interventions that eliminate the likelihood of transmission of maternally inherited mitochondrial disease to offspring are being actively pursued.

REPRODUCTIVE OPTIONS FOR PREVENTING TRANSMISSION OF MITOCHONDRIAL DISEASE

The transmission of mtDNA is complex and poorly understood. The transmission of heteroplasmic mtDNA is complicated by selective genome replication and *genetic bottleneck*, resulting in marked variation in the levels of mutated mtDNA among the offspring of heteroplasmic mothers (3). A woman with a low level of mtDNA heteroplasmy could transmit much higher levels to her children through the phenomenon known as the *mitochondrial bottleneck*.

Genetic counseling is important to explain the genetic risks involved in spontaneous or assisted reproduction and the limits of prenatal and preimplantation testing. Preimplantation genetic diagnosis (PGD) has limited efficacy for mtDNA disease because of the uncertainty in predicting disease due to heteroplasmy and genetic bottleneck. Preimplantation genetic diagnosis for mtDNA disease has been reported (4–7); however, concerns remain regarding whether mutation loads detected in biopsied blastomeres or trophectoderm accurately represent the entire embryo. There are also uncertainties about correlation between mutation load and disease expression and severity. Another concern is that PGD may only reduce, but not eliminate, the risk of transmitting abnormal mtDNA that may lead to mitochondrial disease in subsequent generations.

Furthermore, PGD is not applicable to patients with homoplasmic mutations or high levels of heteroplasmy. For women with homoplasmic or high levels of heteroplasmic mtDNA mutations, currently the only option to ensure an unaffected child is whole oocyte donation. However, oocyte donation has the limitation of not maintaining the genetic link to the mother.

The limitations of PGD and whole oocyte donation have led to the search for alternative approaches to prevent mitochondrial disease transmission. These approaches involve the exchange of mitochondrial genome between gametes or embryos.

Cytoplasmic transfer was first proposed as a treatment for patients with infertility. Cytoplasmic transfer involves the transfer of a small portion of ooplasm, and hence mtDNA, from one oocyte to another. In 1997 Cohen et al. (8) reported the first cytoplasmic transfer in humans resulting in pregnancies. This approach would likely not prevent the transmission of mitochondrial disease, because it does not remove the mutated mtDNA but rather adds donor mitochondria, creating a heteroplasmic oocyte with both mitochondrial haplotypes. Moreover, the amount of healthy mtDNA that is transferred is relatively small.

Two other promising approaches have emerged more recently. With either method, any resulting child would inherit nuclear genetic material from both parents, whereas the mtDNA would be derived largely or perhaps exclusively from the oocyte provided by the healthy donor. These methods could avoid mitochondrial disease, not just in the resulting child but also in subsequent generations.

One of these approaches, termed *pronuclear transfer*, involves removal of both pronuclei from a zygote containing mtDNA mutations and transfer to the perivitelline space of a donated enucleated zygote. The pronuclei enclosed in a karyoplast are fused with the enucleated zygote by electric pulses or inactivated hemagglutinating virus of Japan. The reconstructed zygote would contain the nuclear DNA material from one zygote, and cytoplasm and mtDNA predominantly from the other (Fig. 1). This has been successfully accomplished in the mouse model, resulting in birth of normal offspring (9).

Craven et al. (10) recently reported pronuclear transfer in human zygotes that were deemed abnormally fertilized. They transferred pronuclei from one zygote into another enucleated zygote. These abnormally fertilized embryos were donated by patients undergoing IVF for fertility treatment. Of the reconstructed embryos, 8.3% developed to the blastocysts stage. Genotype analysis revealed a low mtDNA carryover rate of $<2\%$, but the degree of carryover varied among blastomeres.

An alternative approach, termed *spindle transfer*, uses micromanipulation techniques to transfer the nuclear genetic material (the spindle with maternally derived chromosomes attached) from one unfertilized oocyte to another from which its own nuclear material has been removed (Fig. 2). The reconstituted oocyte is then fertilized to allow embryo development.

Recently Tachibana et al. (11) demonstrated the first successful generation of healthy offspring by spindle transfer in the nonhuman primate model. The spindles were isolated and transferred into enucleated mature oocytes with minimal mtDNA carryover. The reconstructed oocytes were fertilized, and four healthy offspring were born with $<1\%$ carryover mtDNA. Spindle transfer was also successfully carried out after oocyte cryopreservation, resulting in the birth of healthy monkey offspring (12).

Using a similar approach, we also recently reported successful spindle transfer between human MII oocytes, resulting in blastocyst development and embryonic stem cell (ESC) derivation, with very low levels of heteroplasmy (12). Although there was a slightly higher rate of abnormal fertilization, the remaining embryos developed to blastocyst and yielded ESCs similar to control embryos and had normal euploid karyotypes with exclusively donor mtDNA ($<1\%$ mtDNA carryover).

A follow-up study by Paull et al. (13) also demonstrated maternal spindle transfer with human oocytes, although these were parthenogenically activated rather than fertilized. Abnormal oocyte activation was prevented using cooling. The mtDNA carryover rate was $<1\%$. Embryonic stem cells and their differentiated phenotypes showed normal mitochondrial function.

The long-term safety and efficacy of these techniques in humans is unknown, and further clinical research is