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EDITORIAL

Is the World of ART Ready for a *Ménage à Trois*?

Allahbadia Gautam N.

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About the Author

Gautam N. Allahbadia is a MD and is the Editor-in-chief of the Journal of Obstetrics & Gynecology of India as well as the IVF Lite (Journal of Minimal Stimulation IVF). He is the Medical Director of Rotunda—The Center for Human Reproduction, the world-renowned fertility clinic at Bandra, Mumbai, India—as also the New Hope IVF Clinic at Sharjah, UAE. He is a noted world authority on Ultrasound-guided Embryo Transfers and one of the pioneers in Third Party Reproduction in South-East Asia. Dr. Allahbadia was responsible for India's first transethnic surrogate pregnancy involving a Chinese couple's baby delivered by an unrelated Indian surrogate mother. He has over 100 peer-reviewed publications to his credit and is on the Editorial Board of several International journals. Throughout his career, Dr. Allahbadia has been instrumental in developing new fertility-enhancing protocols and propagating the use of Ultrasound in Embryo Transfer procedures. One can read more about his work at www.gautamallahbadia.com.

Introduction

> A *ménage à trois* (French for "household of three") is a domestic arrangement in which three people having romantic and/or sexual relations with each other occupy the same household—*From Wikipedia, the Free Encyclopedia*

Inherited diseases caused by mitochondrial gene (mtDNA) mutations affect at least one in 5000–10,000 children and are associated with severe clinical symptoms [1]. Mitochondrial disease mainly affects children, but is also common in adults due to deteriorating mitochondrial function with age [1]. Novel reproductive techniques designed to replace mutated mtDNA in oocytes or early embryos have been proposed to prevent transmission of disease from parents to their children [2, 3]. The aim is ultimately to prevent children from inheriting genetic diseases caused by mutations in DNA housed by their mitochondria—components of cells, which produce energy. Mitochondrial DNA (mtDNA) mutations are relatively a common cause of progressive disorders that can be severe or even life threatening [4]. There is currently no cure for these disorders; therefore, recent research has been focused on attempting to prevent the transmission of these maternally inherited mutations. These research techniques have been referred to with several terms, including "mitochondria replacement," "mitochondrial manipulation," "oocyte modification," "three-person embryos," "three-parent babies," and "nuclear genome transfer." The "mitochondria replacement" is a new approach that is being researched with the goal of allowing a woman who has mutations in her mtDNA to lessen the risk of passing on inherited mitochondrial disease to her child. The techniques being developed are variations on combining the nuclear DNA from an egg of an affected woman with the mtDNA of an unaffected woman's egg. A resulting child would possess

Allahbadia G. N. (✉), Editor-in-chief
The Center For Human Reproduction, Mumbai, India
e-mail: ivfwaladoc@gmail.com

genes from three adults, and this altered genome would be passed on to succeeding generations.

The techniques being investigated could only be attempted in a minority of the cases of mitochondrial disease. They would not be applicable to mitochondrial disease that is caused by nuclear DNA, which makes up the majority of cases, or would they prevent mitochondrial disease that arises due to spontaneous mutations or deterioration with age [5, 6].

Discussion

Pathological mutations in tRNA genes and tRNA-processing enzymes are numerous, and result in very complicated clinical phenotypes. Mitochondrial tRNA (mt-tRNA) genes are "hotspots" for pathological mutations, and more than 200 mt-tRNA mutations have been linked to various disease states [7]. Often, these mutations prevent tRNA aminoacylation. Mitochondrial tRNA mutations manifest in a wide panoply of diseases related to cellular energetics, including COX deficiency (cytochrome C oxidase), mitochondrial myopathy, myoclonic epilepsy with ragged red fibers (MERRF), and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). Diseases caused by mt-tRNA mutations can also affect very specific tissue types, as in the case of neurosensory nonsyndromic hearing loss and pigmentary retinopathy, diabetes mellitus, and hypertrophic cardiomyopathy. Importantly, mitochondrial heteroplasmy plays a role in disease severity and age of onset as well [7].

This three-person IVF would be used to attempt allowing a small number of women with a rare kind of severe mitochondrial disease to have a healthy and mostly genetically related child. The techniques work by removing the nucleus of an affected woman's extracted egg and putting it into the enucleated egg of another woman, which contains her mitochondria [8]. The child would thus be genetically related to three people, which is why the media often refers to "three-parent babies" or "three-parent in vitro fertilization." The procedure is not currently approved for general use in any country aside from the United Kingdom, which legalized it in February 2015 [9, 10].

There are different techniques to achieve this "mitochondrial replacement."

Pronuclear Transfer (PNT)

Pronuclear transfer begins with an embryo created via IVF, using the intended parents' sperm and eggs [11]. Concurrently, a second embryo is created using a donor egg with healthy mitochondria and the father's sperm. When the embryos are 1 day old, still at the single-cell stage, the pronuclei are removed from the first embryo. The majority of the mother's mutated mitochondria are left behind in the enucleated embryo, which is discarded. Meanwhile, the pronuclei of the second embryo are removed and discarded. The parents' pronuclei are then placed into the second embryo, which has maintained the healthy mitochondria from the donor's egg. This newly constructed embryo can continue to develop and then be transferred into the mother. By optimizing the procedure, Craven et al. found that the average level of carry-over of donor zygote mtDNA after transfer of two pronuclei is less than 2.0 %, with many of the embryos containing no detectable donor mtDNA [11]. They believe that pronuclear transfer between zygotes has the potential to prevent the transmission of mtDNA disease in humans [11].

Maternal Spindle Transfer (MST)

In this technique, nuclear DNA is removed from the intended mother's egg; the rest of the egg is discarded, including the unhealthy mtDNA. The nuclear DNA of an egg from a woman with healthy mitochondria is removed at the same time, leaving her healthy mitochondria in the cytoplasm. The mother's nuclear DNA is placed into the enucleated donor egg, which can then be fertilized with sperm from the father. The resulting newly constructed embryo can then be transferred into the mother. Tachibana et al. [12] investigated the feasibility of mtDNA replacement in human oocytes by spindle transfer (ST; also called spindle-chromosomal complex transfer). Of 106 human oocytes donated for research, 65 were subjected to reciprocal ST and 33 served as controls. Fertilization rate in ST oocytes (73 %) was similar to that of controls (75 %); however, a significant portion of ST zygotes (52 %) showed abnormal fertilization as determined by an irregular number of pronuclei. Among the normally fertilized ST zygotes, blastocyst development (62 %) and embryonic stem-cell isolation (38 %) rates were comparable with controls. All embryonic stem-cell lines derived from ST zygotes had normal euploid karyotypes and contained exclusively donor mtDNA. The mtDNA can be efficiently replaced in human oocytes [12]. Although some ST oocytes displayed abnormal fertilization, remaining embryos were capable of developing to blastocysts and producing embryonic stem cells similar to controls [12].

Nuclear Genome Transfer (NGT)

Nuclear genome transfer is essentially the same as MST. Scientists, working with human eggs, developed a technique that avoided premature oocyte activation and thus

increased success rates [13]. (They did not fertilize the eggs, but did activate them via parthenogenesis.) Paull et al. explored the use of nuclear genome transfer between unfertilized oocytes of two donors to prevent the transmission of mitochondrial mutations. Nuclear genome transfer did not reduce developmental efficiency to the blastocyst stage, and genome's integrity was maintained provided that spontaneous oocyte activation was avoided through the transfer of incompletely assembled spindle—chromosome complexes. Mitochondrial DNA transferred with the nuclear genome was initially detected at levels below 1 %, decreasing in blastocysts and stem-cell lines to undetectable levels, and remained undetectable after passaging for more than 1 year, clonal expansion, differentiation into neurons, cardiomyocytes, or β-cells, and after cellular reprogramming. Stem cells and differentiated cells had mitochondrial respiratory chain enzyme activities and oxygen consumption rates indistinguishable from controls. These results [13] demonstrated the potential of nuclear genome transfer to prevent the transmission of mitochondrial disorders in humans.

Polar Body Genome Transfer (PBT)

Polar body transfer is a new and more experimental technique than the others. It involves transferring polar body genomes instead of the pronucleus or maternal spindle. It is hypothesized that this would reduce carryover of mutant mitochondria since polar bodies contain few mitochondria, but seem to have the same genomic information as the oocyte [14]. Normally, polar bodies cannot survive since they do not have mitochondria, but the theory is that in this case, they would get it from the donor egg [14]. Wang et al. [15] compared the effects of different types of germline genome transfer, including spindle–chromosome transfer, pronuclear transfer, and the first and the second polar body transfers, in mice. Reconstructed embryos support normal fertilization and produce live offspring. Importantly, genetic analysis confirms that the F1 generation from polar body transfers possesses minimal donor mtDNA carryover compared to the F1 generation from other procedures. Moreover, the mtDNA genotype remains stable in F2 progeny after polar body transfer. Their preclinical model demonstrates that polar body transfer has great potential to prevent inherited mtDNA diseases.

Conclusion

Although the donor egg is said to contribute only 0.1 % to the genetic make up of the child, when examining the genetic material of these children, there are still three identifiable genetic parents [16]. This is due to the fact that the donor egg usually comes from a nonmaternal relative. For a child having undergone this procedure to have only two identifiable genetic parents, the donor egg must have come from a maternal relative (this is because mitochondrial DNA mtDNA is inherited maternally; thus maternal relatives will have identical mitochondrial DNA, barring random mutations). Maternal relative egg donation is not commonly used, because if the female egg has a mitochondrial disease, then it is highly likely that the maternal relatives inherited the disease as well.

Three-person IVF would result in inheritable genetic modification. Altering the human germline is considered to be the most objectionable in case of genetic technologies and has constituted a bright line not to be crossed. Many bioethicists, scholars, and advocates from around the world have argued that "mitochondria replacement" does not justify crossing this line [8, 17, 18]. It debates whether the genetic make-up of children born as a result of mitochondrial replacement affect their emotional well-being when they are aware that they are different from other healthy children conceived from two parents [3]. Safety and efficacy of mitochondrial DNA replacement are still unanswered.

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