MEDICINE

Mitochondrial Replacement, Evolution, and the Clinic

Mitochondrial replacement therapy might bear health risks, especially for males.

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Mitochondrial diseases [often caused by mutations in mitochondrial DNA (mtDNA)] can manifest in a range of severe symptoms, for which there are currently no cures (*1*). The diseases are passed from mothers to offspring. Intense research efforts have recently focused on a germline therapeutic strategy to prevent the inheritance of disease-causing mitochondria. However, although there has been increased government interest, especially in the United Kingdom, for using this approach to treat patients, there are reasons to believe that it is premature to move this technology into the clinic at this stage.

Early experiments in mice produced disease-free oocytes by means of mitochondrial replacement (MR): the fertilized nucleus from an oocyte laden with mitochondria carrying mtDNA mutations was injected into the cytoplasm of an enucleated donor oocyte that carried mutation-free mitochondria (*2*). A breakthrough in primates came when four macaque babies were born after MR-assisted in vitro fertilization (IVF) (*3*) and when human embryos survived intact after MR to the blastocyst stage (*4*). Consequently, the British government commissioned the Human Fertilisation and Embryology Authority (HFEA) to collect evidence as to the suitability of MR as a therapeutic approach. The HFEA urged further experiments before clinical use of MR (*5*). The U.K. Nuffield Council on Bioethics initiated an ethical review of MR and concluded it was ethically acceptable (*6*). This view was supported by the Medical Research Council and Wellcome Trust of the United Kingdom, who suggested that MR will not affect characteristics normally associated with individual identity (*6*). MR was compared with replacing the batteries of a camera (*6*), an analogy picked up by the popular press.

Subsequently, the four macaques born after MR were shown to be healthy at 3 years of age (*7*), and technical difficulties have been reduced in human blastocysts (*7*, *8*). These results were used to urge the U.S. Food and Drug Administration to review its funding policy on gene therapy (*7*).

The HFEA then was asked to initiate a public consultation into the question of whether MR-assisted IVF "should be made available to couples at risk of having an affected child" (*9*). The results, published in March 2013, note general support within the United Kingdom for permitting MR; if applied under strict regulation, such as case-by-case approval of licensed hospitals, ethical concerns about the implementation of MR were considered to be outweighed by arguments in favor of MR (*10*). In June 2013, the British government announced they will produce draft regulations on therapeutic MR for further public consultation, to be debated in parliament in 2014 (*11*).

Much of the scientific debate has focused on genetic factors that are well known to affect mitochondrial disease, which typically develops only if the relative amount of mutated, relative to healthy, mtDNA in any given tissue surpasses a critical threshold (*1*, *10*). These frequencies can shift rapidly at embryogenesis. If any mutant mtDNA remains in an enucleated oocyte after MR, the children might be at risk of developing the disease or passing the pathogenic mutation on to their offspring. As a result, the HFEA considered whether only male embryos should be allowed to develop post-MR, to ensure that no mutations are inadvertently transmitted after the treatment. This body of research was carefully considered by the HFEA, and we do not review it here (*10*).

Rather, we draw attention to theory and experimental findings that appear to have been overlooked in the scientific and public forums of this debate. Studies on model organisms, ranging from mice to fruit flies, indicate that MR can profoundly change the expression profiles of nuclear genes and affect a range of important traits such as individual development, cognitive behavior, and key health parameters. These studies also suggest that males of reproductive age are particularly sensitive to MR-induced effects.

Natural genetic differences in the mtDNA sequence exist from one individual to another,

Health outcomes of intraspecies experimental MR in animals

Studies using invasive techniques

Human (*Homo sapiens*)
Development to blastocyst stage
Normal gene expression and metabolic profile in cell lines

Macaque (*Macaca mulatta*)
Normal embryo development and metabolic profile at juvenile age

Mouse (*Mus musculus*)
Altered respiration and growth in mouse hybrid cell line

Studies using genetic crossing techniques

Mouse (*Mus musculus*)
Normal survival to adulthood
Reduced growth, exercise ability, learning in adult ♂

Fruit fly (*Drosophila melanogaster*)
Altered juvenile viability
Altered nuclear gene expression in adult ♂
Altered, aging mostly in adult ♂
Altered, often reduced, fertility in adult ♂

Seed beetle (*Callosobruchus maculatus*)
Altered development time and metabolic rate
Altered fertility in adult ♂
Altered survival in adult ♀

Copepod (*Tigriopus californicus*)
Reduced juvenile viability
Reduced mitochondrial function and ATP production in adults

Health outcomes of intraspecies experimental MR in animals. Studies either invasively removed the nucleus by placing it into the enucleated donor oocyte (blue shading) or replaced mitochondria by repeatedly crossing foreign mitochondrial genotypes into nuclear backgrounds (green shading) (see table S1 for details). All species share the same 37 mitochondrial encoded genes. Studies of health effects of MR on vertebrates that have reached reproductive ages are lacking.

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broadly denoted as mtDNA haplotypes. Putatively healthy mitochondrial haplotypes differ in their effect on the expression of key health and performance parameters. In particular, energy production critically hinges on extensive cross-talk between genes dispersed across the nucleus and the mitochondria (*12*). Because phenotypes with less-than-ideal cross-talk are disfavored by natural selection, coordinated mitochondrial-nuclear interactions become highly specific over evolutionary time. If MR disrupts such specific, highly coordinated mito-nuclear allelic interactions, adverse health outcomes might occur.

This prediction is supported by experimental studies reporting the expression of disease phenotypes when genetic backcrossing was used to replace mitochondria and to create intergenomic mismatches in mice. Altered respiratory metabolism and reduced performance, learning, and exploratory capacity in males were reported when mitochondrial-nuclear genomic interactions were experimentally mismatched (see the table and table S1). Females were not tested in those studies. The health effects were observed after mismatches between mitochondrial and nuclear genomes in mice of different inbred strains of the same species. Furthermore, experimental mismatches of putatively healthy mitochondrial and nuclear genomes from different populations within the same species in invertebrate models, suggest that the reduced performance reported in mice extends to a range of organisms [see the table and supplementary materials (SM) for additional information].

MR-assisted IVF immediately places the offspring's mtDNA (acquired from a donor) alongside a novel set of maternal and paternal nuclear alleles—a different situation from the case of sexual reproduction, in which the offspring's mitochondrial DNA is invariably inherited together with and exposed to a haploid maternal genome (see SM). Maternal inheritance of mitochondria means natural selection can only shape mitochondrial gene evolution directly through females. This facilitates the accumulation of mtDNA mutations that are harmful to males, if these mutations have favorable, benign, or only slightly deleterious, effects on females (*13*–*15*). Male-limited mitochondrial diseases such as Leber's disease, or forms of impaired fertility, might be clinical manifestations of this evolutionary hypothesis. This specific prediction has experimental backing: MR in fruit flies had little effect on nuclear gene expression in females but changed the expression of roughly 10% of genes in adult males (*14*). The mitochondrial haplotypes responsible for these male-specific effects were naturally occurring, putatively healthy variants. Hundreds of mitochondrial-sensitive nuclear genes identified in that study had a core role in male fertility. For example, one of the five combinations in which mitochondrial-nucleus interactions were disrupted by mismatching was completely male-sterile but female-fertile (*14*). In other fly studies, MR resulted in male-biased modifications to components of aging and affected the outcomes of in vivo male fertility (see the table). Together, these results suggest that core components of male health depend on fine-tuned coordination between mitochondrial and nuclear gene complexes, and thus the HFEA conclusion that "there is no evidence for any mismatch between the nucleus and any mtDNA haplogroup, at least within a species" [(*10*), 6.17 on p. 17] is incomplete and unsubstantiated.

Two points may deserve careful consideration prior to any change in legislation. First, studies in humans have only tracked health through to the blastocyst stage and in macaques to 3 years of age (see the table). The results from mice and invertebrates suggest that many deleterious effects of MR would not be revealed until adulthood. Without preclinical trials that take a longer-term approach, the current suggestion that families allow "very long term follow-up of their children and families in order to acquire further knowledge about the outcomes of these techniques" [(*6*), p. xvi] would place an experimental risk on families. It would seem fruitful to monitor fertility and health outcomes through to sexual maturity among the macaques born after MR-assisted IVF. Awaiting the macaques' health outcome upon sexual maturity in ~2 years seems a relatively low-cost endeavor. Equivalent studies assessing health post-MR in the mouse model [also recommended by (*10*)] would help to further quantify the potential costs and benefits of MR therapy.

Second, the possibility that MR outcomes may be improved by matching mtDNA haplotypes of donor and recipient (*10*) warrants experimental attention. The proof-of-principle MR study on macaques was based on oocytes of donors and recipients from the same troop (*3*, *7*). The resulting high genetic relatedness between donor and recipient at both the mtDNA and the nuclear level predicts low levels of mitochondrial-nuclear mismatch, probably to a degree that makes it unrepresentative for prospective donors and recipients from the targeted general human population. Comparing the genetic relatedness between recipients and donors for both successful and failed MR outcomes in the studies of macaques (*3*, *7*) should be relatively simple.

In conclusion, recent technical advances suggest that MR-assisted gene therapy could soon be available to help female sufferers of mitochondrial diseases have healthy children, and this is clearly an exciting prospect. Changing legislation that would facilitate this technique will require a debate over the extent to which the results and principles outlined here are ethically and clinically relevant to MR in humans. MtDNA diseases vary from mild symptoms and learning disabilities to severe disabilities and premature death. Assessing the costs and benefits of MR treatment requires that prospective patients are as fully informed as possible. The difference across patients in the severity of expected offspring symptoms in the event that MR treatment is not taken will shape the decision of choosing the treatment versus waiting for the outcomes of further research. Some families who are predicted to be, or who have previously conceived offspring that were, severely afflicted by mtDNA diseases are more likely to be prepared to take the risk. Others whose children are expected to suffer less detrimental symptoms, cognition problems or infertility, may wish to wait for further empirical clarification of the risks involved.

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Supplementary Materials

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