Ethics of mitochondrial gene replacement: from bench to bedside

The prospect of using mitochondrial gene replacement to conceive children free of mitochondrial disease highlights the need for a sound ethical framework for reproductive genetic technology, say **Annelien Bredenoord** and **Peter Braude**

Medical research in humans is highly regulated and has embedded ethical procedures and standards. However, whereas review and formal oversight have been established for drugs and medical treatments, this is not the case for new reproductive genetic technology. Many questions of safety are wholly or partly resolved by experience from use of the technique—a try it and see approach.[1] Attention has now been drawn to this gap. The European Society of Human Reproduction and Embryology, the US President's Council, and the UK Medical Research Council concluded that professional societies and clinicians should develop a more systematic mechanism for reviewing experimental procedures before they become standard clinical practice.[2-4]

The importance of revisiting research ethics for reproductive genetic technology has been underscored by recent technical successes that may pave the way for the development of mitochondrial gene replacement for carriers of mitochondrial DNA (mtDNA) mutations.[5 6] This novel technique could be a valuable addition to current reproductive strategies but also raises an array of technical and ethical issues,[7] especially in relation to first human application. Here we identify some pressing issues for the purpose of initiating timely debate.

Ethical questions

Moving from animal and other preclinical studies to a first human application is always uncertain and ethically contentious.[8 9] First human use of mitochondrial gene replacement is especially challenging because the technique modifies the germ line and the modification would be transmitted to subsequent generations.[7] How do we evaluate the (intergenerational) risks and benefits? Some of the risks and uncertainties might be clarified and reduced by preclinical research using animals and human embryos. To what extent are we allowed to (or should we) use embryos to determine the safety of mitochondrial gene replacement? A second question is how we should launch the first trial when there are still so many uncertainties regarding mitochondrial activity in the human embryo. We do not know, for example, whether a mixture of mtDNA from two different origins is safe. How much evidence should be required before it is reasonable to consider first human use, and what measures should be taken once the decision has been made to make the jump from bench to bedside? A third question is how to guarantee adequate informed consent given the complexity of technical information, high uncertainty, and competing interests.

Reproductive options for mitochondrial DNA disease

The clinical phenotype of mtDNA diseases is highly variable, affecting patients at any age and in a wide variety of tissues (box 1).[10] There are no treatments to cure these disorders. Therefore, helping carriers of mitochondrial mutations to have healthy children, free of mtDNA disease, has been a focus of attention. Moreover, the special characteristics of mitochondrial genetics make it difficult to estimate the risk of recurrence, including whether, and to what extent, a future child will be clinically affected.

This unpredictability complicates the use of existing reproductive options for carriers of mtDNA mutations. A first option, egg donation followed by in vitro fertilisation, will remove the risk but does not maintain the genetic link with both parents, which is troublesome for many couples. Moreover, recruiting egg donors is difficult, particularly if maternal relatives are at risk of transmitting the same mutation.

A second option is prenatal diagnosis to detect mutation in the fetus, possibly followed by a termination of pregnancy. However, there might not be a clear relation between the severity of the disease and the proportion of defective mitochondria in the cells tested. This makes it difficult to give a definitive diagnosis and set criteria for termination. A third option is preimplantation genetic diagnosis. Here one or two cells are removed from embryos created by in vitro fertilisation, usually at the eight cell stage, and tested for the degree of heteroplasmy before implantation. However, if none of the embryos is mutant free this technique will only reduce rather than eliminate the reproductive risk.[11] Since the relation between proportion of mutations in the embryo and severity of disease in the resulting offspring is not yet understood clearly for all mutations,[12-14] it may be unclear what limits should be set to exclude an embryo from transfer. Furthermore, the technique is not suitable for situations where all of the mtDNA is pathogenic (homoplasmy), with the exception of sex selection when penetrance of the disease is significantly lower in one sex.[15]

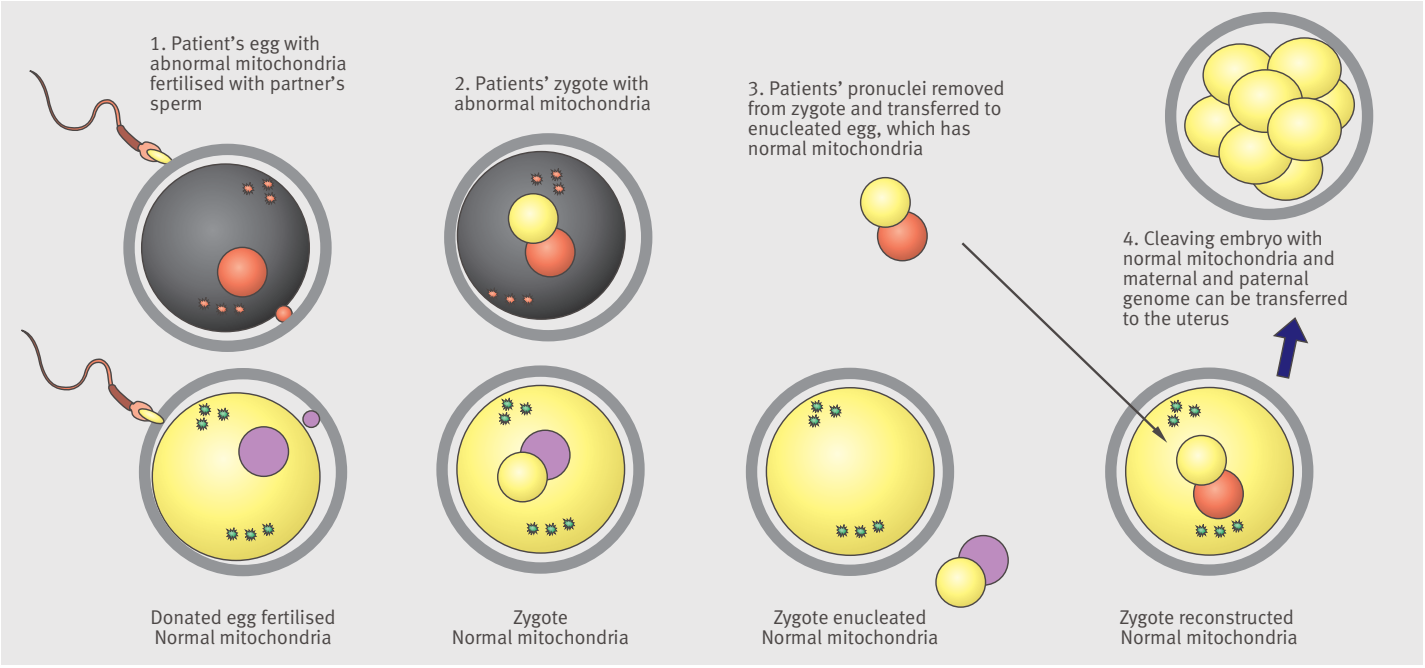
Box 1 | Mitochondrial DNA disorders

All human cells contain two genomes: one in the nucleus and one in the mitochondria. The mitochondrial genome contains only 37 genes, all maternally transmitted. The ratio of genetically aberrant to normal mitochondria transmitted can vary during mitosis and through generations—a situation known as heteroplasmy. People with entirely faulty mitochondria (homoplasmy) or a large proportion of faulty mitochondria may develop a mtDNA disease. These are clinically heterogeneous but usually severe diseases resulting from defects in energy production and include deafness, blindness, diabetes, loss of skills, and heart and liver failure. About 1 in 400 people has a maternally inherited mtDNA mutation.

Although generally morally acceptable, all these reproductive options for carriers of mtDNA mutations have their limitations. Therefore, researchers have sought alternative reproductive options.

DNA replacement technology

Replacement of defective maternal mtDNA by transferring the nuclear genome of the prospective mother to a donated egg should result in healthy offspring carrying the nuclear genome of the prospective parents and the healthy mitochondrial genome from the donor.[7] Researchers in the US have shown the feasibility of nuclear transfer using unfertilised mature oocytes in primates. They transferred the spindle chromosomal complex (containing the nuclear DNA) of a mature oocyte to an enucleated donor oocyte, resulting in three healthy macaque infants.[5] More recently, in a set of proof of principle studies, researchers from Newcastle University used abnormally fertilised human embryos from in vitro fertilisation procedures to investigate the effects of pronuclear transfer on embryo development in vitro. This resulted in human embryos with very low levels of mutant mtDNA, far below the threshold for disease expression.[6]



Mitochondrial gene replacement (pronuclear stage)

In clinical practice, an egg from the prospective mother and a donor would be fertilised using the prospective father's sperm. The two pronuclei in the donor egg would then be removed and replaced with those from the mother's egg, resulting in a zygote with normal mitochondria (figure). The reconstructed zygote would then be allowed to continue developing in vitro before implantation.

Assessing the risks and benefits

To be justifiable, mitochondrial gene replacement should present a favourable balance of risks and benefits in comparison with the available alternatives.[16] The main intended benefit—the birth of a healthy child—may seem clear and worth while. Some might argue, however, that such a radical technology should be used only when a child could not be born using some other means[17]—that is, when a couple would otherwise choose not to reproduce. The availability of reproductive alternatives such as egg donation, prenatal diagnosis, and preimplantation genetic diagnosis, despite their limitations, might argue against the use of mitochondrial gene replacement.

What then about the risks arising from the uncertainty, unpredictability, and irreversibility of germ line modification?[18] How much risk and uncertainty is acceptable in a first human use of mitochondrial gene replacement? We and others have argued that any child born by medically assisted reproduction should have a reasonable chance of an acceptable quality of life. Efforts should be made to reduce the risks as much as reasonably possible.[2 11] Although the results of the US primate studies are reassuring,[5] some effects may not manifest for many years. This provides an argument to delay human application until the primates have reproduced and those offspring been examined. In addition, although primate studies mimic as closely as possible application in humans, clinical trials are essential because animal studies do not predict with sufficient certainty what will happen in humans.[2 19]

In vitro research using human embryos provides another avenue for study, albeit that their use is still contentious in some quarters. The Newcastle researchers used only abnormally fertilised zygotes (those with one or three pronuclei) resulting from in vitro fertilisation for their experiments on pronuclear transfer.[6] However, full evaluation of safety may require the creation of human embryos that carry an mtDNA mutation—for example to examine consistency of mtDNA segregation throughout the cells of the preimplantation embryo or to rehearse and examine the effects of the transfer technique itself. Further information may be gained by examining mitochondrial effects in stem cells grown from such embryos.

The deliberate creation of human embryos for research is much more controversial than using those left over from in vitro fertilisation and legally prohibited in many countries. It is, however, questionable whether there is any fundamental ethical difference between the two. Currently, there is an implied acceptance of creating and discarding surplus or unsuitable embryos as part of trying to establish a successful pregnancy by in vitro fertilisation. In this regard embryos are treated as instrumentally as they are when created for research purposes.[20] In both cases, embryos have equal moral standing, which is likely to be low. This view is also supported by the biology; the early embryo is non-sentient and cannot even be considered a biological individual because twinning can occur until about day 14. The moral value of early embryos in vitro should therefore not outweigh the interests of prospective parents and their future children.[2] As the opportunities for minimising risk (an important international requirement for conducting morally sound medical research in humans) could increase substantially by widening the possibilities for embryo research, the deliberate creation of embryos for research should be allowed under strict conditions.

First human use

Perhaps the most challenging ethical question is when it is justified to make the leap from bench to bedside in the presence of promising preclinical results. When are enough safeguards made to justify introducing mitochondrial gene replacement into the clinic? Although extensive preclinical studies are important preliminary requirements, proof of (long term) safety of mitochondrial gene replacement can in the end be understood only by doing it. While research ethics has made several efforts to better map and clarify the risks and benefits of medical research, less work has been done on how to determine when a favourable risk-benefit balance has been reached. The appraisal of risks and benefits involves much intuition and eventually also depends on a person's position towards risk. In other risky first human applications—such as an oncology trial—it is often the patient who decides to take the risks and participate. A unique complicating factor with reproductive methods is that although the couple uses the new technology, it is the child who will be affected. We could adopt a precautionary approach—"better safe than sorry"—but without some risk, innovations such as in vitro fertilisation and intracytoplasmic sperm injection would never have existed. The difficulty is finding, and defining, an acceptable balance.

Some measures can be taken to enhance a cautious process. For example, the decision to use mitochondrial gene replacement should not be taken by individual researchers but by an expert community after consideration of all the evidence.[21] In addition, the study should be designed so that it will produce useful, scientifically valid results. Instead of the usual linear model in translational science which moves from molecular studies to animal then to humans, commentators have recently favoured a more iterative process that moves back and forth between preclinical and clinical studies.[9] From this perspective, first human use of mitochondrial gene replacement can have added value if it contributes to fundamental knowledge—for example, about mitochondrial genetics—that motivates scientists to perform novel preclinical experiments. However, the primary aim must remain the birth of a healthy baby.

Once the decision has been made to initiate mitochondrial gene replacement, polar body biopsy, preimplantation genetic diagnosis, ultrasonography, and prenatal diagnosis can be used to indicate whether the embryo is developing normally and whether any affected mitochondria have been transferred along with the pronuclei of the recipient woman. Follow-up studies of children conceived by mitochondrial gene replacement will be necessary to determine the long term (intergenerational) safety, although this may raise additional ethical questions such as the acceptability of genetically testing minors.[12 13]

Box 2 | Risks of gene transfer

The only report of germ line modification has been ooplasmic transfer, the injection of donor ooplasm with normal mitochondria into an oocyte purported to contain mutant mtDNA.[22] Developed as a fertility technique for women experiencing repeated embryonic development failure, the first applications resulted in a relatively high number of children with chromosomal abnormalities (2/16 clinical pregnancies), but it was unclear whether this is related to the technique.[1 7 22] Commentators were concerned about mitochondrial heteroplasmy (two out of 15 born children carried mtDNA from donor and recipient) and the possible epigenetic effects of ooplasmic transfer.[1] They criticised its premature introduction, particularly because of the lack of sufficient preclinical research.[1 7 23]

Somatic gene transfer has also proved more difficult than expected. Serious adverse events include the death of an 18 year old participant in a gene therapy trial for an X linked hereditary disorder of the liver in 1999 and the development of leukaemia in four young children after a gene therapy trial for X linked severe combined immunodeficiency in 2003.[24]

Informed consent

Although new genetic technology widens reproductive options for couples, it makes them dependent on experts to make an informed decision, and concepts in mitochondrial inheritance are particularly difficult. Prospective parents should be fully aware that it is an experimental procedure and that neither preclinical data nor monitoring before and during pregnancy can fully predict the effects of the intervention on their child.[16] They should also understand that alternative reproductive options may be available. Our system of review by research ethics committees has been set in place not only to review the scientific robustness and feasibility of a protocol but also to protect patients against overzealous researchers and themselves (desperately wanting a healthy child). Equally, independence of the consent process from those who undertake the research needs to be assured. Although sophisticated oversight might not be in place in all countries that may have the technical expertise to extend the work to the human therapy immediately, rigorous review by a research ethics committee is absolutely warranted.

Due diligence

Earlier adverse experiences with germ line modification and somatic gene transfer serve as a warning for the enormous challenges that may await mitochondrial gene replacement (box 2). Mitochondrial gene replacement involves many controversial issues including creating embryos for research, oocyte donation for genetic therapy and germ line modification,[7] and so the public spotlight will be focused on every step the researchers will take. The 2008 revision of the UK Human Fertilisation and Embryology Act made specific provision for parliament to allow genetic manipulation in embryos for mitochondrial disease. Scientists, regulators, patients, and ethicists should develop, discuss, and refine a framework for a morally sound use of mitochondrial gene replacement. This requires that stakeholders work towards consensus on an acceptable risk-benefit balance. Appropriate preclinical studies and embryo research, a rigorously reviewed protocol, adequate informed consent, long term follow-up, and a transparent public debate are essential.

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