

Mitochondrial DNA disorders: Is there a way to prevent transmission?

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Lay Summary

Mitochondria are powerhouses that convert the food we eat into energy. There are many thousands of mitochondria in the cells of our bodies. Each mitochondrion contains a portion of DNA called genes. If these genes are damaged (mutated) then the patient often develops severe disease leading to disability and death. Mitochondrial genes are inherited only through the mother and mothers often pass the mitochondrial disease on to their children. Unfortunately there is no treatment for mitochondrial disease. Previous studies in mice have indicated that it is possible to prevent the transmission of the mitochondrial disease by moving the pronuclei (which contain all the other genetic information in the cell) from an egg which contains bad mitochondria to another egg, which only contains good mitochondria. These eggs develop normally and the mice generated show no evidence of harmful effects. The purpose of this application is to seek a research licence to conduct preliminary experiments designed to test the feasibility of pronuclear transfer in human eggs. We will also test the feasibility of using a similar approach in immature oocytes.

What are mitochondria?

Mitochondria are small complex structures, which exist in every cell of the body, except red blood cells. Mitochondria produce most of the energy which we all need to grow and live. There are from one to several hundred mitochondria in each cell and each mitochondrion contains complex molecules necessary to carry our energy making reactions [i.e. mammalian mitochondrial DNA encodes the 13 proteins of the oxidative phosphorylation system, which is the system that generates the bulk of the cell's energy (ATP)]. Mitochondria perform many functions necessary for cell metabolism but the energy producing pathways are the most important. These pathways allow us to break down carbohydrate, fat etc and turn it into energy.

What are mitochondrial diseases?

Most of the cell's DNA is contained in the nucleus, but a very small (<1%) is found in the mitochondria. Alterations in the mitochondrial DNA result in a number of relatively rare but very serious diseases. Diseased and healthy mitochondria can exist in the same cell and it is only when the proportion of

diseased mitochondria, compared to healthy mitochondria, reaches a certain amount (>60% of the total mitochondria) that a disease may occur.

Mitochondrial diseases are whole body diseases but the exact features of the disease vary from patient to another. Some patients will have predominately brain or nerve disease. Others will have muscle disease (mitochondrial myopathy), cardiac disease (cardiomyopathies), endocrine, renal or bone marrow disease or a mixture of these or other features.

How are mitochondrial DNA diseases transmitted?

Some mitochondrial diseases are inherited. Mitochondrial DNA is inherited through the maternal side of the family as mitochondria are present in the egg but are not transferred from the male sperm during fertilisation, so the mitochondria of the embryo are derived exclusively from the mother.

What treatments are available?

There are few treatments for mitochondrial diseases and for many patients the disease progresses and can be fatal.

What research has the group in Newcastle been licensed to do?

Researchers at the Newcastle Fertility Centre at LIFE have received a licence to study possible methods of preventing the transmission of mitochondrial DNA disorders.

Studies in mice have indicated that it is possible to prevent the transmission of mitochondrial disease by pronuclear transfer between zygotes. The zygotes (one cell embryos) develop normally *in vitro* and the blastocysts have >20% of donor DNA. In addition, following the transfer of these zygotes to pseudopregnant mice, the offspring were normal and had either no or low levels of donor DNA. The research group in Newcastle believe that these experiments strongly suggest that this approach should be considered in human zygotes. Therefore, they propose to take abnormally fertilised zygotes and transfer the pronuclei between zygotes to determine if they can obtain zygotes with low levels of donor mtDNA. The researchers will also monitor zygote development and determine if the procedure results in either cytogenetic or epigenetic abnormalities.

The overall aim of the research is to develop methods for the prevention of transmission of mitochondrial disease. The project is designed to test the efficacy and safety of human pronuclear transfer. Therefore, the objectives of the project are:

1. To determine whether embryos derived from pronuclear transfer zygotes are capable of development to the blastocyst stage.
2. To determine the extent of mitochondrial DNA carry-over following pronuclear transfer.

3. To evaluate the cytogenetic and gene expression profiles of embryos derived from pronuclear transfer zygotes.

Methodology

Source of pronuclear stage eggs: The researchers will use abnormally fertilised eggs from the IVF/ICSI programme. The Centre's data shows that 9% of all eggs undergo abnormal fertilisation, which is characterised by the presence of either a single pronucleus (monopronuclear) or more than two (usually three) pronuclei. Zygotes with more than two pronuclei are never used for treatment. In exceptional circumstances, monopronuclear eggs will be monitored for the appearance of a second pronucleus. This is only done in cases where no normally fertilised eggs are available for transfer.

Pronuclear removal and transfer: The monopronuclear eggs will be initially used to develop the microsurgical techniques of pronuclear removal and karyoplast fusion. Then tri-pronucleate zygotes from different donors will be paired for reciprocal transfer of two of the three pronuclei. The parental origin of each pronucleus will be determined by its position relative to the polar body. Evidence suggests that the female pronucleus is positioned closer to the polar body. Zygotes will be enucleated using micropipette. The two pronuclei selected for transfer will be fused with the recipient zygote using methods that the Centre has already successfully applied to mouse zygotes.

Assessment of reconstituted zygotes

1. One of the potential problems associated with this technique is that the reconstituted zygotes may contain extra centrosomes. This is especially important in polyspermic zygotes because, in humans, the centrosome is contributed by the sperm. However, removal of cytoplasm surrounding the pronuclei will most likely include the centrosomes, since studies during human fertilisation show that these are closely associated with the pronucleus. Nonetheless, to determine whether a normal bipolar spindle is formed, spindle morphogenesis will be monitored by timelapse DIC microscopy during the first mitotic divisions of the reconstituted zygotes.
2. Reconstituted zygotes will be cultured according to the Centre's standards procedures for human embryos. Embryo development will be monitored and those containing 5-8 cells on day 3 (two days after reconstitution) will be biopsied. Control data for these experiments will be obtained from normally fertilised embryos biopsied under the Centre's R0122 research licence.
3. Biopsied blastomeres will be processed for analysis of mtDNA to estimate the level of carry over from the donor to the recipient zygote. Post-biopsy embryos will be cultured for a further 3-4 days. Development will be monitored daily and arrested embryos (those that fail to divide during a 48h period) will be processed for FISH according to the Centre's established procedures. A combination of probes (13, 18, 21, X and Y) will be used to determine the incidence of aneuploidy. Control data will be

obtained from FISH experiments carried out on normally fertilised embryos under the Centre's R0122 research licence.

4. Embryos that develop to blastocyst stage will be processed either for FISH, using the above probe combination, or for epigenetic studies. Individual blastomeres at this stage will also be isolated for mtDNA analysis to assess variation in the level of heteroplasmy of different cells of the blastocyst. Experiments to detect epigenetic modifications will be done in collaboration with the Babraham Institute, Cambridge. Cytogenetic analysis of embryos from reconstituted zygotes will be compared with normally fertilised embryos from the Centre's R0122 research licence, and epigenetic profiles will be compared with those of normally fertilised embryos studied under the Centre R0145 research licence.

Mitochondrial DNA analysis: For these studies the Centre will determine the mtDNA genotype of both donor and recipient zygotes. Studies have shown that the evolution of human mtDNA is characterised by the emergence of distinct lineages or haplogroups among three major ethnic groups. MtDNA is also highly polymorphic, with two short (300-400bp) hypervariable segments of non-coding control region (known as HVS-1 and HVS-2) containing the highest density of neutral polymorphic variants between individuals. The Centre has extensive experience of haplogroup analysis and of sequencing mitochondrial genomes from blood and solid tissues, and has optimised protocols to for mitochondrial DNA sequencing. To assess the genotype of the two zygotes the Centre will initially perform haplogroup analysis and, if necessary, the researchers will sequence the HVS regions from follicular fluid obtained at the time of the egg collection. Difference detected will then be used to develop either fluorescent primer extension or hot cycle PCR-RFLP assays to analyse heteroplasmy levels in individual cells.