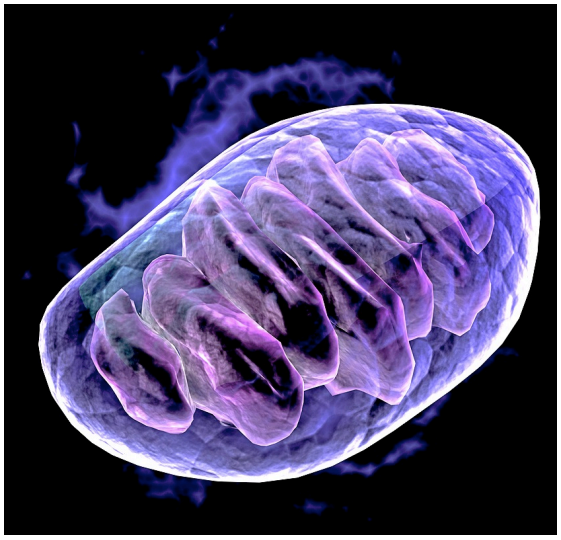
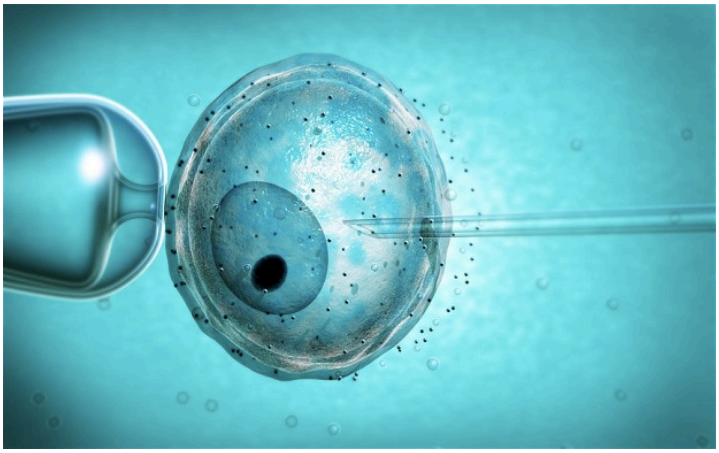
Mitochondrial Donation
or
Three-parent Babies

Biology Paper on an Application of Biotechnology





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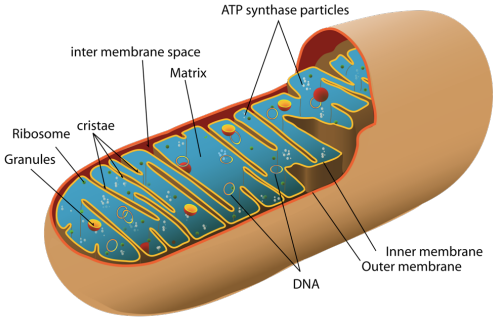
Preface

In this paper we treat the subject of mitochondrial donation. Several different ways of this technique are currently being tested but they all work quite similarly. Simplified, the nuclear DNA from a woman with mitochondrial disease is removed and then put into the enucleated egg or the enucleated embryo of a donor, which contains healthy mitochondria. Thus, the child would be genetically related to three people rather than two. That's why this procedure is often referred to as Three-Parent In Vitro Fertilization by the media. The potential children are nicknamed "three-parent babies".
We chose this topic because it impressively shows that advancements in the field of Biotechnology don't only lead to scientific progress in general but could also greatly affect the development and the way of life of the following human generations and therefore our future on planet earth. We are at a point in history where newly discovered technologies in the domain of genetics slowly allow us to change the natural course of reproduction and affect evolution in a major and irreversible way. These trends go hand in hand with an enormous responsibility and could lead to the dawn of a new era of human life. Therefore it comes with no surprise that these topics are discussed keenly in politics and science.
Although - or perhaps precisely because - the procedure of mitochondrial donation is still in its early stages with a lot of debates and disputes and only a few representative studies, we thought it would be an interesting and relevant approach to the topic of Biotechnology. It turns upside down one of the most fundamental views of family and reproduction that we have – the presence of one father and one mother changes to one father and two mothers.
As stated above, research on mitochondrial donation is far from completed and therefore we chose to focus on two of the most promising methods: Pronuclear Transfer (PNT) and Maternal Spindle Transfer (MST). In addition to the description of the techniques, we also included an interview with an expert as well as a discussion of future research steps and ethical aspects in this paper.

Introduction

Mitochondria are organelles found in the cytoplasm of almost all eukaryotes. They produce ATP and are of special interest in the field of genetics since they possess their own genome (mtDNA), which is independent of the main cellular genome housed in the cell nucleus. In most species, including humans, mtDNA is solely inherited from the mother.



Defects in the system of mitochondria through spontaneous or inherited mutations of mitochondrial DNA or nuclear DNA may damage the heart, brain, liver, kidney, lungs,

skeletal muscles and the endocrine system. Since mitochondria perform different functions in different tissues of the human body there are hundreds of different mitochondrial diseases. The severity of mitochondrial disease varies greatly and can even lead to death in childhood. There is no cure at present and current treatments can only decrease the symptoms but not change the development of the illness. Although mitochondrial disease often affects children, it is also common in adults because mitochondrial function deteriorates with age. Approximately 1 in 5'000 – 10'000 people suffer from a type of mitochondrial disease.

So-called mitochondrial donation or mitochondrial replacement is a new approach that is being researched with the aim of allowing a woman who has mutations in her mtDNA to have healthy and mostly genetically related children. With the help of this procedure a mother could no longer pass on inherited mitochondrial disease to her child. The different methods, which are currently being tested, are variations of combining the nuclear DNA from of an affected woman with the mtDNA of an unaffected woman. A resulting child would therefore possess genes from three 'parents' and this modified genome would be passed on to succeeding generations.

As an alternative treatment, there is usually the possibility of a 'regular' egg donor with a common IVF-procedure or a surrogate mother. However, although the baby would be free from mitochondrial disease, the affected woman would not be genetically related to 'her' child. That would leave mitochondrial donation as the only option in most cases.

Even though these techniques would be the only hope for women with mitochondrial disease to have healthy children who are directly related to them, it's important to keep in mind that they could only be used in a minority of cases. They would not be applicable when mitochondrial disease is caused by nuclear DNA, which makes up the majority of cases, neither would they prevent mitochondrial disease that develops due to deterioration with age or spontaneous mutations. However, they could reduce the cases of inherited mitochondrial disease drastically, which still is a major step forward. In addition, the permission of this method would lay the foundation for further research in the field of prevention of genetically inherited diseases.

Researchers have given notice of some success in animals and in human zygotes, but have not been able to study the development of a three-parent embryo in the human body since clinical trials are not permitted yet. The procedure of mitochondrial donation is currently banned in the United States of America as well as in the United Kingdom because national laws prohibit altering the germ line and therefore legislative changes have to be made before clinical trials may start. Nevertheless, research is expanding in both countries and in the UK legalisation is near.

The *UK House of Commons Science and Technology Committee* held a hearing on Mitochondrial Donation October 22, 2014. As a result, the UK could become the first country where 'three-parent babies' are born since new regulations concerning this topic will be put before parliament in 2015.

Authentication of mitochondrial donation might face a more difficult path in the USA because the *Food and Drug Administration* (FDA) banned a similar technique in 2001. This method, where the egg was injected with sperm from the father as well as cytoplasm from another woman, led to miscarriages and could not be supported by sufficient data to hold up in the FDA hearing at that time.

Nevertheless, in February 2014, a committee of researchers led by Dr Evan Snyder of *Sanford-Burnham Medical Research Institute* (La Jolla, CA) heard evidence from academics on the safety of mitochondrial donation in humans. The hearing was convoked because Dr Shoukhrat Mitalipov, scientist from the *Oregon Health and Science University* in Portland (USA), and Mary Herbert of *Newcastle University* (UK) requested

the FDA's approval to start clinical trials on women with mitochondrial disease. According to Dr Mitalipov and Mary Herbert, their research teams have conducted in vitro and animal research on PNT and MST and were successful with macaque monkeys. However, their report didn't consider several safety aspects. The monkeys were only followed for three years, even though mitochondrial diseases are often shown late in life. Only one generation of monkeys was examined, although these alterations would be passed on to all following generations, and abnormalities were observed in human zygotes, which were non-existent in monkeys. Due to these errors and strong countermovement in the American society, FDA chair Evan Snyder announced that the topic will probably not be addressed again by either the committee or the FDA before more representative animal research has been completed.

Technique

Mitochondria don't only produce ATP and serve as the cells main energy source but they are also responsible for several other cellular activities. They help regulate the self-destruction of cells (apoptosis), produce cholesterol and are responsible for cell growth.
Although most DNA is enclosed in chromosomes within the nucleus, mitochondria also have a small quantity of their own DNA. This genetic material is referred to as mitochondrial DNA or mtDNA, contains approximately 16'000 base pairs and codes for 37 genes, which are all necessary in order for the mitochondria to work properly.
In addition to that, recent research suggests that mitochondria act as much more than just "batteries" and have in fact a certain influence not only on the functions listed above but also on the phenotype of an individual in general. Therefore, it could be possible that mitochondria affect the traits that make us who we are which has to be taken into account when discussing the ethical aspects of mitochondrial donation.

The procedures of Pronuclear Transfer and Maternal Spindle Transfer are both types of In Vitro Fertilizations (IVF). During In Vitro Fertilizations an egg is fertilized by sperm "in vitro" ("in glass") and therefore outside the body. In general, eggs are removed from the woman's ovaries and sperm fertilize them in a fluid medium. Then, the zygote (fertilized egg) or the embryo are implanted in the same or another woman's uterus after they have been cultivated.
Three-Parent In Vitro Fertilization is a specific type of IVF where the baby's mtDNA comes from a third party because the mother's mtDNA is 'faulty'.

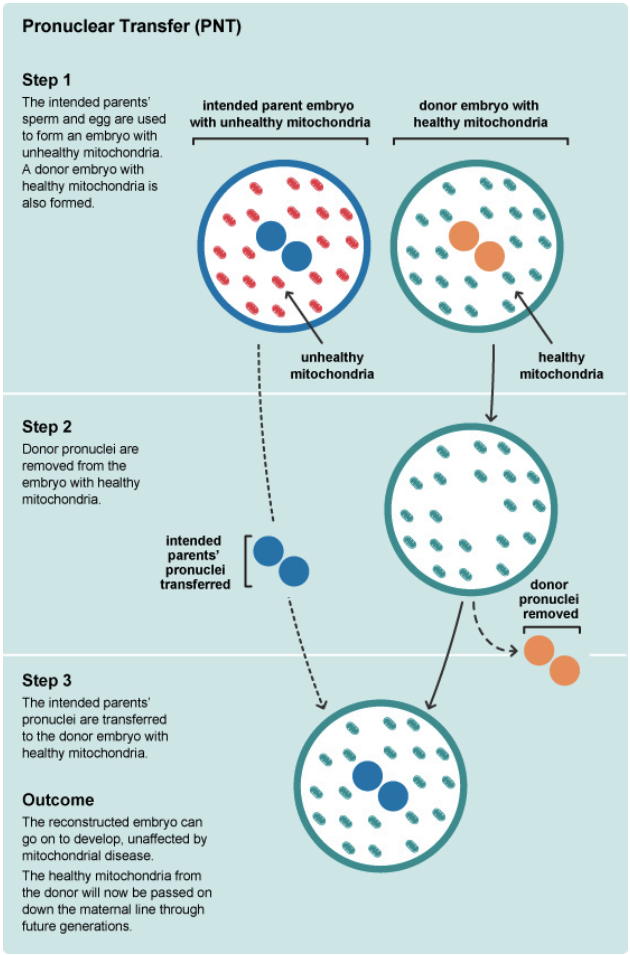
PNT (Pronuclear Transfer)

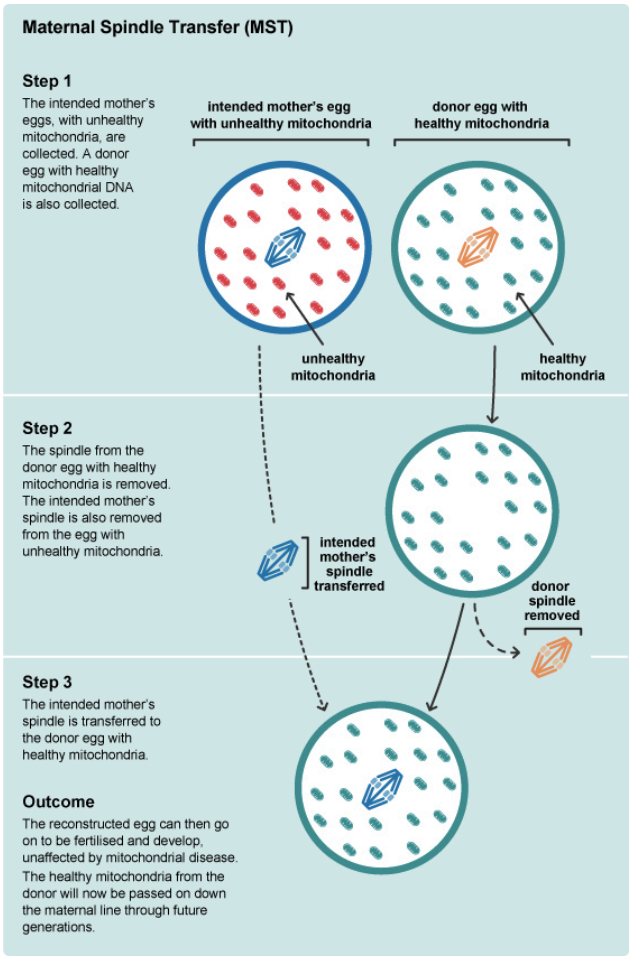
In this procedure, the eggs are removed from the female and then fertilized mechanically by sperm cells in a fluid medium. In the beginning an embryo is created in vitro out of the intended parents' eggs and sperms. Simultaneously, a second embryo is grown with a donor egg and the sperm of the father. After that, the pronucleus of the embryo of the intended parents is taken out and transplanted into the donor embryo with the sane mitochondria. This embryo is then transplanted back into the female body where it develops until birth.

MST (Maternal Spindle Transfer)

In the first phase of the MST technique two eggs are extracted: one from the intended mother and one from a donor. After that, the spindle apparatus of the nuclear DNA of both egg cells is removed. The mother's spindle is maintained and the rest is discarded, whereas the donors spindle is cast-off and the rest is maintained. In the final step the spindle of the mother is transplanted into the sane egg, then fertilized by the father's sperm and finally transplanted back into the mother.

These two graphics show a more detailed version of the two procedures.





Interview on Mitochondrial Donation

Interview Partner:

Dr Lindsay Butterworth, Research Associate
Wellcome Trust Centre for Mitochondrial Research
Institute of Neuroscience
Newcastle University, UK

1. Since when is the Newcastle University involved in research on mitochondrial donation and what technique did the research team focus on exactly?

We obtained a research licence from the Human Fertilisation and Embryology Authority (HFEA) in 2005, which allowed us to perform research into mitochondrial donation using abnormally fertilised human embryos. The technique focussed on was pronuclear transfer.

2. Could you briefly explain how research concerning this topic works in the laboratory? What equipment do you use? What experiments have to be done?

This research is a collaboration between the Wellcome Trust Centre for Mitochondrial Research (WTCMR) and the Newcastle Fertility Centre. All the embryo manipulations are performed in the research laboratory at the fertility centre with equipment used in standard IVF procedures, e.g. an inverted microscope with a micromanipulation stage. Genetic analysis of these embryos is performed within the WTCMR and this is the work I am involved in. These experiments involve looking at the mitochondrial DNA (mtDNA) and I am currently using a technique called pyrosequencing to do this.

3. In your opinion, was it justified that the FDA prohibited the start of clinical tests on women with mitochondrial disease, which was asked permission for by Dr Shoukrat Mitalipov and Mary Herbert in February 2014? Were the accusations reasonable? What was the motivation behind this collaboration between Newcastle University and the Oregon Health and Science University and will it continue nevertheless?

The UK has gone through a very extensive process over many years to get to the point where we may be able to offer mitochondrial donation to women with mtDNA disease. This process has included three independent scientific reviews on the safety of mitochondrial donation, independent ethical reviews and a thorough public consultation. The US has just begun this process by considering the ethical and social implications of mitochondrial donation. I believe this is a very important step before clinical trials can be considered.

4. Legalisation of mitochondrial donation is quite near in the UK. How would this legislative change influence your research?

If mitochondrial donation becomes legal in the UK, the next step will involve regulations being agreed and adopted by the HFEA before any fertility clinic can apply for a treatment licence. Licence applications will need to be submitted and approved; approval will only be granted if the HFEA are satisfied that the science continues to suggest that the methods are safe and efficient. Therefore, my research will continue to focus on the safety of mitochondrial donation.

5. Do you think the procedure of mitochondrial donation is ready to be clinically tested on women with mitochondrial disease and if so, why?

As stated in my answer to question 3, the UK has gone through a very extensive process over many years to get to the point where we may be able to offer mitochondrial donation to women with mtDNA disease. This has included three separate scientific reviews on the safety of mitochondrial donation. These reviews were performed by independent experts in the field who have scrutinised the available evidence and their conclusion was that mitochondrial donation is safe to be used by women with mtDNA disease.

6. Opponents of this technique say that it would lead to the production of 'designer babies' and they also think it's unethical to 'merge' three people into one baby. They believe that we shouldn't 'play God'. How do you react to such accusations and can you refute them?

We are developing mitochondrial donation to give women with mtDNA disease an opportunity to have a child that is genetically related but does not suffer with a severe and sometimes fatal disease. There are other reproductive options that may also be suitable for these women; we believe that with help from specialist doctors, it will always be the choice of the prospective parents as to which option is right for them. These techniques do not alter the nuclear DNA and so will not lead to the production of designer babies.

7. Based on your research, are there any complications that could occur in the field? How high do you think the failure rate of mitochondrial donation will be? What would be reasonable restrictions for the safe application of this technique?

Clinics applying for a licence must comply with the regulations in terms of scientific and clinical practice, including patient support. Long-term follow-up of any children born by this technique will also be important to confirm the safety of mitochondrial donation.

8. Which method is more suitable for practical usage and why: Pronuclear Transfer or Maternal Spindle Transfer?

There are both advantages and disadvantages to Pronuclear Transfer and Maternal Spindle Transfer. Research is ongoing into both techniques. The scientific reviews carried out by independent experts state that there is no evidence to support one technique over the other.

10. In your opinion, is it always wise to prevent transmitted diseases or is it sometimes better not to intervene?

We work closely with patients and families with mtDNA disease and see the pain and suffering they may have to endure on a daily basis. The disease can be severely debilitating and can result in death in early childhood. Mitochondrial donation will prevent transmission of mtDNA disease from mother to child and there are already patients who would like to use this technique if it becomes legal.

11. What is your motivation to invest so much effort into the development of a procedure that could only help in a minority of cases of mitochondrial disease?

As stated in my answer to question 10, we work closely with patients and families with mtDNA disease and see the pain and suffering they may have to endure on a daily basis. The disease can be severely debilitating and can result in death in early childhood. For some women, the current reproductive options available to them will not be suitable and so there is an urgent need to develop other techniques such as mitochondrial donation. I am motivated by the fact that we may be able to help these women have a healthy child.

Discussion

Future Research Steps

As mentioned before, techniques of mitochondrial donation are still actively researched and clinical trials with humans are currently prohibited, even though legalisation is very near in the UK. Therefore, no data on the application of this method can be found at present.
The next step for researchers would be to collect enough representative data concerning the safety of this method on animals in order to convince the parliament and the public that clinical tests on women with mitochondrial disease should be permitted. This goal can only be reached when all important safety aspects are considered during animal testing which means that mistakes, such as the ones that Dr Mitalipov's and Mary Herbert's research team made, have to be prevented. The subjects have to be followed for a sufficient long time and succeeding generations, which are also affected by this technique, must be included in the study.
Once the clinical tests are completed successfully, there are some restrictive guidelines that have to be followed when actual 'three-parent babies' are born in regular hospitals in order to prevent accidents and to study long-term effects. The *UK House of Commons Science and Technology Committee* published some suggestions for the monitoring of future mitochondrial donation procedures, which can be summarized as follows.

1. First of all, they suggest a basic long-term follow-up of the health of the recipient and their progeny.
2. They recommend that there should be a funded, long-term register of any mitochondrial donation procedures performed in the UK. Furthermore, they suggest that researchers should have access to these data.
3. They also think that the origin of mtDNA is medically useful knowledge in relation to the long-term follow-up of those receiving donated mtDNA, and could also be used in future research steps. Therefore, they recommend that a list of donators should be made accessible for researchers as well.
4. Furthermore, they recommend that a cell line is created from the mitochondrial donor at the time of donation. Then, the relationship between the cell nucleus and the donated mitochondria can be studied in the original cellular environment.

Ethical Aspects

It comes with no surprise that the procedure of mitochondrial donation leads to a great ethical discussion since it influences the germ line, may affect all succeeding generations and can lead to a major change concerning reproduction of the human race.

Advantages/Pros

Mitochondrial donation allows women with mitochondrial disease to have sane children. Since mitochondrial disease is incurable, this procedure would give children, which would otherwise probably suffer their whole life, the opportunity to lead a normal and prosperous life.
It can be seen as unethical to force the patient to abandon all hope of having children to whom she is biologically related or to willingly allow children to suffer by prohibiting mitochondrial donation.

Disadvantages/Contras

There are several side effects of mitochondrial donation, which can but don't have to occur, and there are still insecurities about the safety of this procedure because the long-term consequences are not tested yet.
It's still not entirely clear what influence mtDNA has exactly on the development of a child and on the child's identity. It may be seen as unethical to 'merge' three people into one baby. In addition to that, the fact that the child has three parents may lead to an identity crisis as they differ from 'normal' children.
The germ line would be modified during this process and the outcome is not reversible. If there are any genetic changes, they will be inherited to future generations. Opponents of mitochondrial donation suggest that this interference with nature could have unwanted and unpredictable consequences for all succeeding human beings.
Some feel that it's not ethical to 'play God' with the fate of a human, which is the more conservative point-of-view.
Furthermore, the legalisation of mitochondrial donation is seen by most opponents as a 'slippery slope', which may lead directly into a future of eugenics and designer–babies.
Due to limited knowledge about the human genome, experiments concerning this topic in general may be seen as finical.

Evaluation

Even though there are more arguments which speak against mitochondrial donation, the ones for the legalisation of this procedure are much more powerful in our opinion. Most of the contras are only based on personal believes and are not really scientific. A lot of people think that the process of mitochondrial donation is related to cloning or 'designing' babies which is not true. There are no direct changes of nuclear DNA, which result from this method and no explicit genetic modification can be seen. The replaced parts are the unchanged nucleus (spindle apparatus) or the unaltered mitochondria respectively.
However, since the exact influence of mitochondria on the identity and the phenotype in general is not yet entirely clear, it would be careless to legalise mitochondrial donation without any precautions. We think that the ones from the *UK House of Commons Science and Technology Committee* are very well suited to control the responsible handling of this revolutionary technique, which could change the lives of thousands of families for the better. It would be unethical to prevent such a milestone in medical advancement only because conservative views of family dominate in some parts of society. This opportunity should be taken to review concepts of parenthood with a special focus on the number of parents a child might have.

Summary

Mitochondria are organelles, which produce ATP for the cells to use and have their own DNA (mtDNA). In humans, mitochondrial DNA is solely inherited from the mother.
An estimated 1 in 5'000-10'000 people have a gene defect in their mtDNA, which leads to mitochondrial disease. At the moment, there is no cure for the varying symptoms of this illness, which can cause damage to the brain, lungs or liver and may even lead to death in childhood.
Mitochondrial donation is an experimental and not yet legalized method in the field of Biotechnology with the aim to allow women with mitochondrial gene defects to have children who are not affected by the disease but are still biologically related to them. The UK will probably be the first country to legalize this technique - their parliament will discuss the topic in 2015.
We laid our focus on two of the most promising types of mitochondrial donation, which may find application in the next few years: Pronuclear Transfer (PNT) and Maternal Spindle Transfer (MST). Both methods are In Vitro Fertilizations. However, in the PNT method, two embryos are cultivated with the sperm of the father. Then, the pronuclei of both embryos are taken out and the one from the intended mother is placed into the donor embryo with sane mitochondria. In the MST procedure, the spindle apparatus of the nuclear DNA from the intended mother is placed in the donor egg cell, which has its spindle removed and contains healthy mitochondria. Afterwards, the egg is fertilized with the sperm of the father.
This donation of an egg cell with healthy mitochondria makes the donor as well as the intended mother related to the offspring, effectively making it a "three-parent baby".
Since this process affects human reproduction in a major way, it's an important topic for a public ethical debate.
The pro arguments for the legalisation are mainly focussed on the positive impact it would have on those affected by mitochondrial disease. The contras centre on the question whether we should change the genome of human beings as well as the natural constellation of one mother and one father and 'play God'.
However, it's important to realize that, even though the effects of mtDNA on one's identity are not yet entirely clear and there are still no long-term studies, mitochondrial donation is not an actual method of genetic engineering since the genome is not directly manipulated.

Annex

In addition to the references, two questions from the interview, which weren't answered clear enough to use in the paper, are also included.

1. If mitochondrial donation will be used to prevent the inheritance of mitochondrial disease from mother to child, what would be the next step in the fight against inherited diseases? Are there other illnesses which could be prevented through experimental procedures and if so, which ones?

If mitochondrial donation is legalised in the UK, the technique can only be used to prevent the transmission of serious mitochondrial disease (as stated in The Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015).

2. What would be the cost of the procedure for one woman and would it be covered by health insurance?

I have not been involved in these discussions.

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Text

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All links were checked on February 1st for the last time.