

Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception

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Executive summary

Mitochondria are small structures present in human cells that produce a cell's energy. They contain a small amount of DNA that is inherited exclusively from the mother through the mitochondria present in her eggs. Mutations in this mitochondrial DNA can cause a range of rare but serious diseases, which can be fatal. However, there are several novel methods with the potential to reduce the transmission of abnormal mitochondrial DNA from a mother to her child, and thus avoid mitochondrial disease in the child and subsequent generations.

The Human Fertilisation and Embryology (HFE) Act 1990 (as amended) only permits eggs and embryos that have not had their nuclear or mitochondrial DNA altered to be used for treatment. However, the Act allows for regulations to be passed by Parliament that will allow techniques that alter the DNA of an egg or embryo to be used in assisted conception, to prevent the transmission of serious mitochondrial disease.

The Secretary of State for Health asked the Human Fertilisation and Embryology Authority (HFEA), in February 2011, to carry out a scientific review to scope "expert views on the effectiveness and safety of mitochondrial transfer". In order to carry out this task, the HFEA established a small panel, with broad-ranging scientific and clinical expertise, to collate and summarise the current state of expert understanding on the safety and efficacy of methods to avoid mitochondrial disease through assisted conception.

This report presents the panel's assessment of the safety and efficacy of the three techniques that have the potential to avoid mitochondrial disease. Preimplantation genetic diagnosis (PGD) involves removing and examining one or more cells from an early embryo to identify those that are unlikely to lead to a mitochondrial disorder in the resulting child. PGD for mitochondrial diseases is licensed in the UK. Maternal spindle transfer and pronuclear transfer are two techniques, currently at the research stage, that involve transferring the mother's, or both parent's, genetic material into an unfertilised donor egg or a fertilised donor embryo that contains healthy mitochondria. Neither maternal spindle transfer nor pronuclear transfer is permitted for treatment under the HFE Act 1990 (as amended) because each alters the mitochondrial DNA of the egg or embryo.

The panel concludes that PGD can only reduce, not eliminate, the risk of transmitting abnormal mitochondrial DNA that may lead to a mitochondrial disease. PGD is suitable for some, but not all, patients who suffer from mutations in their mitochondrial DNA. The panel makes recommendations for centres carrying out PGD for mitochondrial disease to reduce the level of uncertainty around the diagnosis.

The panel concludes that the techniques of maternal spindle transfer and pronuclear transfer are potentially useful for a specific and defined group of patients whose offspring may have severe or lethal genetic disease, and who have no other option of having their own genetic child. As in every area of medicine, moving from research into clinical practice always involves a degree of uncertainty. The evidence currently available does not suggest that the techniques are unsafe. Nevertheless, these techniques are relatively novel, especially applied to human embryos, and with relatively few data to provide robust evidence on safety. The panel therefore urges that additional research be undertaken to provide further safety information and knowledge about the biology of human mitochondria. The panel proposes a (minimum) set of experiments that it feels is critical. Although optimistic about the potential of these techniques, the panel recommends a cautious approach and advises that this research is carried out, and the results taken into account, before the techniques can be considered safe and effective for clinical use.

1. Introduction, scope and objectives

1.1 Introduction

- 1.1.1** Mitochondrial malfunction has been recognised as the significant cause of a number of serious multi-organ diseases. The underlying defects can be due to mutations in nuclear DNA affecting gene products required within mitochondria, or to mutations in DNA carried within the mitochondria themselves. Table 1 at Annex A lists clinical disorders that are associated with mutations in mitochondrial DNA (mtDNA). Although relatively rare, the seriousness of these diseases and the unusual inheritance pattern of mtDNA mutations have made them a focus for research into preimplantation methods to reduce or avoid a disease in offspring.
- 1.1.2** Preimplantation genetic diagnosis (PGD) can be used to test embryos that might be carrying mutations in their nuclear DNA so that only unaffected embryos are selected for implantation. Unlike most nuclear DNA mutations, mtDNA is solely maternally inherited, and any mutations it acquires are therefore likely to be passed on to all offspring. This presents particular challenges when it comes to avoiding transmission of disease to subsequent generations. For some women who are known to carry mtDNA mutations, current methods of diagnosis including PGD, can reduce, but not eliminate, the risk of a child being born with mitochondrial disease. However, these methods are not applicable to all cases. Moreover, even if unaffected themselves, girls born after the use of this procedure may themselves still be at risk of having affected children, as some abnormal mitochondria may be present in their oocytes (eggs). It is against this backdrop that recent research has explored alternative methods of maternal spindle transfer (MST) and pronuclear transfer (PNT) that have the possibility of eliminating, and not just reducing, the risk of disease due to mtDNA mutations.

1.2 Scope and objectives of this review

- 1.2.1** The terms of reference for the panel are “to collate and summarise the current state of expert understanding on the safety and efficacy of methods to avoid mitochondrial disease through assisted conception”. Accordingly, this review focuses exclusively on

the safety and effectiveness of these techniques, and does not consider the ethical and legal issues that are raised by such techniques.

1.2.2 The methodology of the review is set out at annex B and the evidence reviewed is listed at annex C. The panel looked in particular at the current use of PGD and the recent advances in MST and PNT research that may be applied to diseases caused by mtDNA mutations. The review focuses on mtDNA mutations and only peripherally on mitochondrial disease due to mutations in nuclear DNA, for which MST and PNT techniques are not relevant. However, the panel recognises that these are also serious diseases that deserve attention. The panel also recognises that oocyte donation can be used to avoid mtDNA associated mitochondrial disease and to eliminate it from descendants. However, this option is not one favoured by patients who desire to have their own genetic children and is beyond the scope of this scientific review.

1.2.3 This report is structured as follows: section 2 provides an overview of mitochondrial biology and disease; section 3 considers and makes recommendations on the use of PGD to avoid mitochondrial disease; sections 4 and 5 consider the effectiveness and safety of MST and PNT, and suggest further research. The review makes a number of conclusions and recommendations throughout, which have been highlighted in bold text. These are summarised in section 6 of the report. In addressing its terms of reference, the panel has tried to set out the issues in as clear manner as possible. However, as the biology of mitochondria is complex, the language used is necessarily technical in parts. A glossary is supplied at annex D.

2 Background information on mitochondrial biology and disease

2.1 Mitochondria are the energy-producing organelles in the cytoplasm of every mammalian cell. Mitochondria produce much of the energy in a cell, in the form of adenosine triphosphate (ATP) via oxidative phosphorylation reactions involving the electron transfer chain (ETC). Energy is required for cells to synthesise proteins and other molecules, to

move and to proliferate. Mitochondrial function and replication involves both DNA contained within the mitochondria (mtDNA) and within the nucleus. The small mtDNA genome encodes 13 genes essential for the ETC, the remaining components being encoded by about 67 genes residing on chromosomes in the nucleus. The mtDNA also carries transfer RNA (tRNA) genes required for mitochondrial protein synthesis.

Mitochondria have other important roles in cellular physiology, notably in programmed cell death (apoptosis) and steroid synthesis, although these depend on genes encoded entirely within the nucleus.

2.2 Mutations in any of the 1500 nuclear genes required for mitochondrial function can lead to severe disease. These mutations are inherited in the same way as any other nuclear genetic traits. About 1 in 5000 births, and a likely higher proportion of fetuses, has a mtDNA mutation. The inheritance and regulation of mtDNA, however, is different from genetic information that is located on chromosomes within the nucleus of the cell. There are several compounding reasons for this:

2.2.1 Maternal inheritance: mtDNA is transmitted solely from the mother via the cytoplasm of the oocyte¹. In contrast, nuclear genes, apart from genes specific to the X and Y chromosomes, are inherited both maternally and paternally (one copy from the mother and one from the father).

2.2.2 mtDNA copy number: The number of mitochondria varies dramatically between cell types during different stages of development. Adult (somatic) cells typically contain from a few hundred to several thousand mitochondria and this generally reflects demand for ATP in these cells. Tissues, such as muscle and brain, therefore have high numbers of mitochondria, and it is these tissues that tend to show the strongest phenotypes when mitochondria are defective. The numbers of mitochondria in the germ line varies according to the stage of development; thus oocytes have more than 100,000, each cell of a blastocyst has about 1000, and there may be as few as 10 per primordial germ cell

¹ Sperm contain an active mitochondrion, which is essential for their motility, but this is programmed for destruction immediately after fertilisation. There have been very rare cases noted where paternal mitochondria have persisted naturally, or after certain somatic cell nuclear transfer experiments, but these are sufficiently exceptional that the panel does not consider this to be a significant issue.

during very early development of the embryo post implantation. Only after this do mtDNA copy numbers increase, in a cell type specific manner.

2.2.3 mtDNA replication: mtDNA replication and transcription depend on regulatory proteins encoded by nuclear genes. It is not clear how mtDNA copy number is regulated, and whether this is strictly dependent on demand, as abnormal mtDNA that does not function properly can be replicated to the same degree as normal mtDNA. It is also apparent that mtDNA replication does not occur in the early preimplantation embryo, with some evidence in the mouse suggesting that it begins at the blastocyst stage. The high numbers of mitochondria in oocytes are almost certainly essential to enable embryonic cells to inherit sufficient numbers of mtDNA copies that will be needed at later stages of development.

2.2.4 Heteroplasmy: Where abnormal mtDNA coexists in a cell with normal mtDNA, the cell is called heteroplasmic². Where all copies of mtDNA in a cell are either all abnormal or all normal, the cell is called homoplasmic. The severity of most mitochondrial genetic diseases is dictated by the proportion of abnormal to normal mitochondria within the cells. The proportion may also change over time. Thus many of these diseases may manifest late or increase in severity with age. There may also be variations in heteroplasmy between tissues, perhaps resulting in specific manifestations of the disease according to the most affected organ or system. Homoplasmy for abnormal mtDNA is generally associated with more severe disease.

2.2.5 Mitochondrial bottleneck: Where an early embryo is heteroplasmic, a mitochondrial 'bottleneck' arises because of a combination of low mtDNA copy number per cell and small founding cell populations for each tissue type during early embryogenesis. This means that, simply by chance, it is likely that different cell types will have different proportions of abnormal and normal mtDNA. It is possible that replication of mtDNA can further skew the proportions in subsequent development, especially if one form has a

² Because of errors in replication, variant mtDNA sequences are common in all cells. Therefore heteroplasmy is the norm. However, most of these variants or polymorphisms will be benign. For this reason, the term heteroplasmy generally refers to situations where both disease-causing and normal mtDNA are present.

replicative advantage³. A heteroplasmic mother can produce oocytes (and children) that have dramatically different proportions, from 0-100%, of normal and abnormal mtDNA, compared to each other and to her. This germline mosaicism occurs because the germline is thought to arise from very few cells at a time when mtDNA copy number is especially low. Some women carriers can have low or even undetectable heteroplasmy within their adult somatic tissues but very high abnormal loads or even homoplasmy in all their oocytes⁴. It is also possible that de novo mutations in mtDNA may occur in the germline at any stage.

2.2.6 Nuclear-mitochondrial interactions: Mitochondrial dynamics and function depend on nuclear-encoded genes. Therefore defects in the interaction between nucleus and mitochondria can lead to defects in replication, transcription or activity of mitochondria, or perhaps to the triggering of apoptosis (programmed cell death) or autophagy (where the cell begins to digest part of itself). Any of these processes could also in theory contribute to altered segregation of abnormal and normal mitochondria. This incompatibility can explain why certain cytoplasmic hybrids, where the nuclear and mtDNA genomes within a cell are from different species, fail to thrive.

2.2.7 Differing phenotypes: Specific disease-causing mtDNA mutations vary significantly in the degree of mitochondrial dysfunction, and therefore the severity of the phenotype they produce. This variation is further compounded by the degree of heteroplasmy and the possible differing levels of heteroplasmy in different tissues. There have been some reports that certain mtDNA mutations lead to preferential replication of abnormal mtDNA ie, there is selection for abnormal mtDNA even though it might be expected that they should be selected against. These instances are unusual and have only been noted for a few rare types of mtDNA mutation.

³ For example, Certain D-loop mutations are likely to compromise replication efficiency.

⁴ Similar bottlenecks can also explain why genotyping some tissues, notably blood, can be unreliable. At any one moment, the majority of blood cells may be derived from very few, or perhaps even a single haematopoietic (blood) stem cell.

2.3 Much of the information on mtDNA copy number, replication and the bottleneck has been obtained from studying mouse and other animal embryos. Further research on mitochondrial biology in early human embryos, and in the germline in particular, is encouraged and should be supported by research funding agencies.

3 Review of preimplantation genetic diagnosis to avoid mitochondrial disease

3.1 Background

3.1.1 Preimplantation genetic diagnosis (PGD) involves the removal of one or more cells from an early embryo as a biopsy for genetic diagnosis to identify those embryos that are suitable for transferring to the uterus in a treatment cycle. PGD is currently carried out to test for both nuclear DNA mutations and mtDNA mutations. However, nuclear mitochondrial disease and mtDNA disease have different modes of inheritance and therefore the benefits of PGD differ.

3.2 Effectiveness and safety of PGD for nuclear mitochondrial disease

3.2.1 PGD for nuclear genetic diseases examines well-understood patterns of chromosomal inheritance. The presence or absence of a nuclear mutation will help to determine disease susceptibility. PGD is possible for mitochondrial diseases resulting from mutations in nuclear DNA, providing that the affected gene is known. Such nuclear mutations are responsible for about 80% of cases of mitochondrial diseases in childhood, but as they often lead to early death, their prevalence is only about 50% in adult cases. However, given the number of nuclear genes involved in mitochondrial function, accurate diagnosis of the nuclear mutation(s) causing the disease is still difficult and PGD is only possible if the causative mutations are known. Without knowing the nuclear gene, the possibility remains that the disease is due to a mutation in mtDNA; this may be the wrong diagnosis. Sequencing the whole mtDNA genome from the affected child or the mother could in principle exclude mtDNA mutations, though

heteroplasmy may make the results unclear. In addition, finding that the mtDNA is normal will not help identify the affected nuclear gene.

3.2.2 Further work is required to develop reliable assays for mitochondrial disease caused by mutations in nuclear DNA, especially as PGD may be the preferred alternative for those who wish to prevent transmission of such diseases without having to consider prenatal diagnosis and possible termination of an affected pregnancy.

3.3 Effectiveness and safety of PGD for mtDNA disease

3.3.1 PGD can be used to select embryos with levels of abnormal mtDNA that are low enough to be unlikely to manifest a disease in the resulting child or adult. Again, the exact mutation will have had to be previously identified. However, even with this knowledge, PGD is not appropriate for all patients with abnormal mtDNA. It cannot be used where there is a high heteroplasmic or homoplasmic level of abnormal mtDNA, estimated to be present in about 20% of patients at risk. Where a mother is heteroplasmic, PGD cannot eliminate all abnormal mtDNA being passed on to the resulting child. It can only ever reduce the risk of the child having a mtDNA disorder by transferring embryos with as low a level of abnormal mtDNA as possible. Therefore the accuracy of the embryo biopsy and the level of abnormal mtDNA per embryo are important when assessing the effectiveness and safety of PGD for mtDNA disorders.

3.3.2 Embryo biopsy: The available evidence suggests that mtDNA is not replicating during early division of cells in an embryo. Mitochondria are therefore likely to be partitioned between these early cells at random and, because there are many per cell at this stage, theory would suggest that one blastomere should be representative of all others. However concern about non-uniformity (mosaicism) found between cells tested for nuclear chromosome abnormalities in embryos⁵ led to two cells being removed in initial clinical PGD procedures for mtDNA diseases in order to avoid misdiagnosis. So far,

⁵ L. Wilton, A. Thornhill, et al. (2009). "The causes of misdiagnosis and adverse outcomes in PGD." Hum. Reprod. 24(5): 1221-1228

data from these two cell biopsies has been reassuring⁶, as has analysis of each cell within non-replaced affected embryos, which suggests that mitochondrial mosaicism is unusual. However routine removal of two cells has been shown to compromise the chances of pregnancy following PGD⁷. An alternative, that has more cells available for testing, is to wait until the blastocyst stage (day 5 or 6 of embryo development) where a group of cells from the outer trophectoderm layer can be removed with higher chances of pregnancy. This method is not thought to compromise embryo viability, but any delay in genotyping may require the embryo to be frozen and then thawed before transferring at another time. Although the available evidence suggests that the proportion of abnormal mtDNA in trophectoderm cells is equivalent to that in the inner cell mass, the data are based on relatively few embryos.

3.3.3 Threshold level of abnormal mtDNA: PGD for mtDNA disorders does not result in embryos free from abnormal mtDNA; it identifies embryos that have a level of heteroplasmy that is unlikely to result in mitochondrial disease, which in some instances may be as high as 40%. A critical issue is the level of abnormal mtDNA, assessed at biopsy, which should exclude an embryo from being suitable for transfer. This needs to be set based on the best estimate of mitochondrial proportions associated with health rather than disease. This may differ for different mutations and different clinical presentations. It may not always be possible to set a precise proportion of heteroplasmy that will guarantee the offspring to be disease-free into adult life, whilst at the same time finding sufficient embryos that fulfill the criteria for safe replacement. The only embryos available may exceed the proportion previously agreed for safe transfer. In addition, any female born after this procedure, even without apparent mitochondrial disease, will be at risk of having affected children. This has led some to propose the idea of combining the PGD test for abnormal mtDNA with sex selection, so that only sons are born.

⁶ Professor Hubert Smeets, Maastricht UMC, oral presentation to panel

⁷ A. De Vos, C. Staessen, et al. (2009). "Impact of cleavage-stage embryo biopsy in view of PGD on human blastocyst implantation: a prospective cohort of single embryo transfers." *Hum. Reprod.* 24(12): 2988-2996

3.3.4 Rather than aiming specifically at avoiding transmission of the mutation and thus disease prevention, PGD for mitochondrial heteroplasmy can only be aimed at risk reduction.

3.3.5 Centres that carry out PGD for mtDNA disorders should:

- offer prenatal diagnosis or PGD as the first option for patients who have an identified mutation and a low to medium level of heteroplasmy
- have appropriate experience and expertise in the diagnosis and management of mtDNA disorders, knowledge of the different mutations and the proportion of heteroplasmy likely to lead adult disease
- provide appropriate experienced genetic counselling, in order to decide upon and document the threshold level of abnormal mtDNA measured at PGD above which embryos would not be replaced in order to minimise the risk of the child being affected with the disorder
- be encouraged to consider single embryo transfer to enable follow up of the fate of the embryo transferred⁸
- encourage patients to consider prenatal testing following PGD, to assess the consistency in levels of heteroplasmy
- record the levels of abnormal mtDNA in the woman, the embryo (trophectoderm or cleavage stage biopsy) and in the subsequent child to assess the degree of correlation

4 Review of maternal spindle transfer and pronuclear transfer to avoid mitochondrial disease

4.1 Background

4.1.1 In cases where PGD is not appropriate, such as cases where the woman has high levels of heteroplasmy or is homoplasmic, transmission of mtDNA disease can be

⁸ Using single embryo transfer will require centres to have an effective cryopreservation programme in order to freeze the remaining suitable embryos that were not transferred.

avoided by using healthy donated oocytes. However, whilst this guarantees the disease is not transmitted, it also means that any resultant child will not be genetically related to the mother. The panel reviewed two other methods that allow the transmission of both parent's nuclear DNA but involve replacing abnormal mitochondria with normal mitochondria: maternal spindle transfer (MST) and pronuclear transfer (PNT). A third technique, cytoplasmic transfer, where some ooplasm containing mitochondria from a donor oocyte is injected into an oocyte with compromised mitochondria, was attempted clinically more than a decade ago with the intention of improving development of embryos from poor quality oocytes, but not specifically to overcome mitochondrial disease. The technique has been discontinued⁹. In mitochondrial disease, it is unlikely to allow sufficient substitution of abnormal with normal mitochondria and the panel did not consider this possibility further as a method to avoid mtDNA disease.

4.1.2 MST uses micromanipulation techniques to transfer the woman's nuclear genetic material (the spindle with chromosomes attached) from her oocyte into an enucleated oocyte donated by a woman with normal mtDNA (Figure 1). The reconstituted oocyte would then need to be fertilised with sperm from the patient's partner. PNT uses similar micromanipulation techniques to transfer the couple's nuclear genetic material (the maternal and paternal derived pronuclei) from a fertilised oocyte (zygote) into an enucleated donor zygote with normal mtDNA (Figure 2). MST takes place between mature metaphase II oocytes. PNT takes place between fertilised oocytes, after the stage where the egg has been penetrated by sperm but prior to the first embryonic division. Both techniques are therefore carried out prior to the formation of an embryo with the maternal and paternal chromosomes together within the same nucleus¹⁰. With either method, any resulting child would inherit nuclear genetic material from both parents, while the mitochondria would be derived from the egg donor. If efficient, so that

⁹ There was concern about a US clinical trial on cytoplasmic transfer, which was halted as it seemed to be associated with a high frequency of chromosome abnormalities in resulting embryos. However, this could have been due to the age of the mothers, and to poor egg quality, etc, rather than the method itself. Several children were born, but it has not been possible to obtain information about their health. (From written evidence submitted to panel by Dr Jacques Cohen)

¹⁰ MST occurs pre-fertilisation and PNT occurs after syngamy but prior to the breakdown of the pronuclear membranes

there is little or no transfer of abnormal mtDNA, this method could avoid mitochondrial disease not just in the resulting child, but also in subsequent generations.

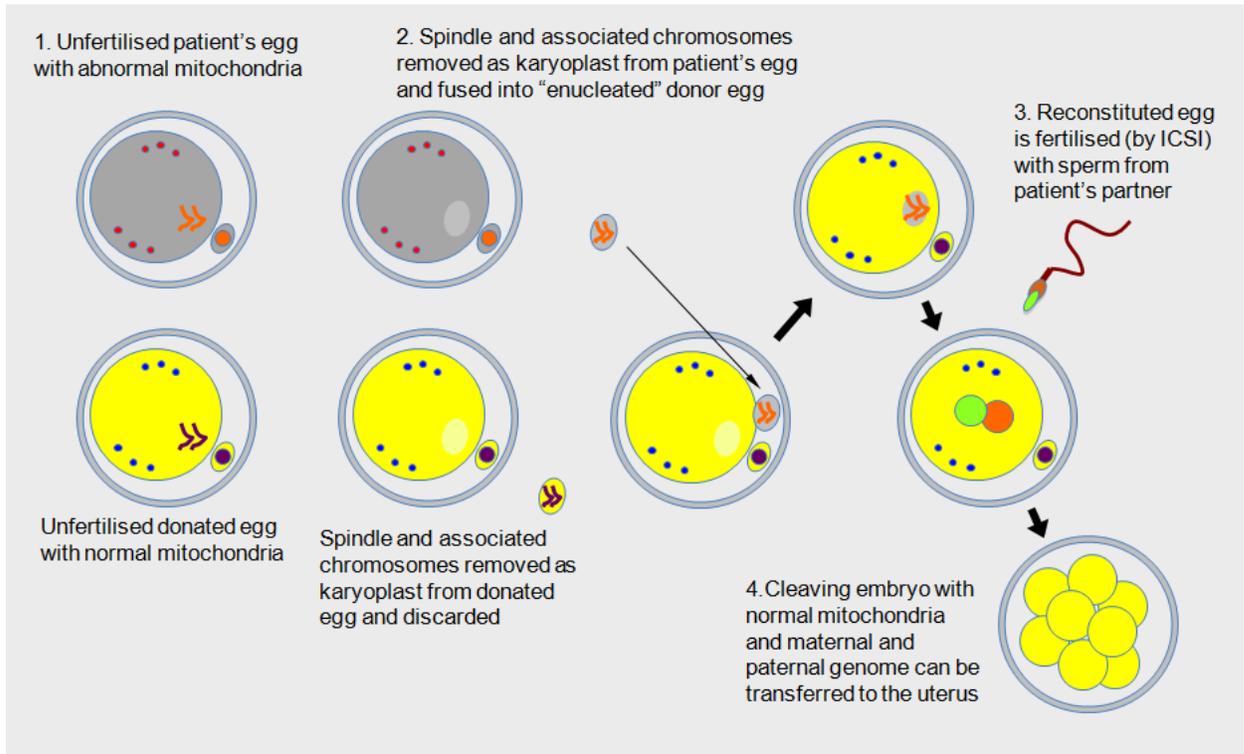


Figure 1. Maternal spindle transfer technique

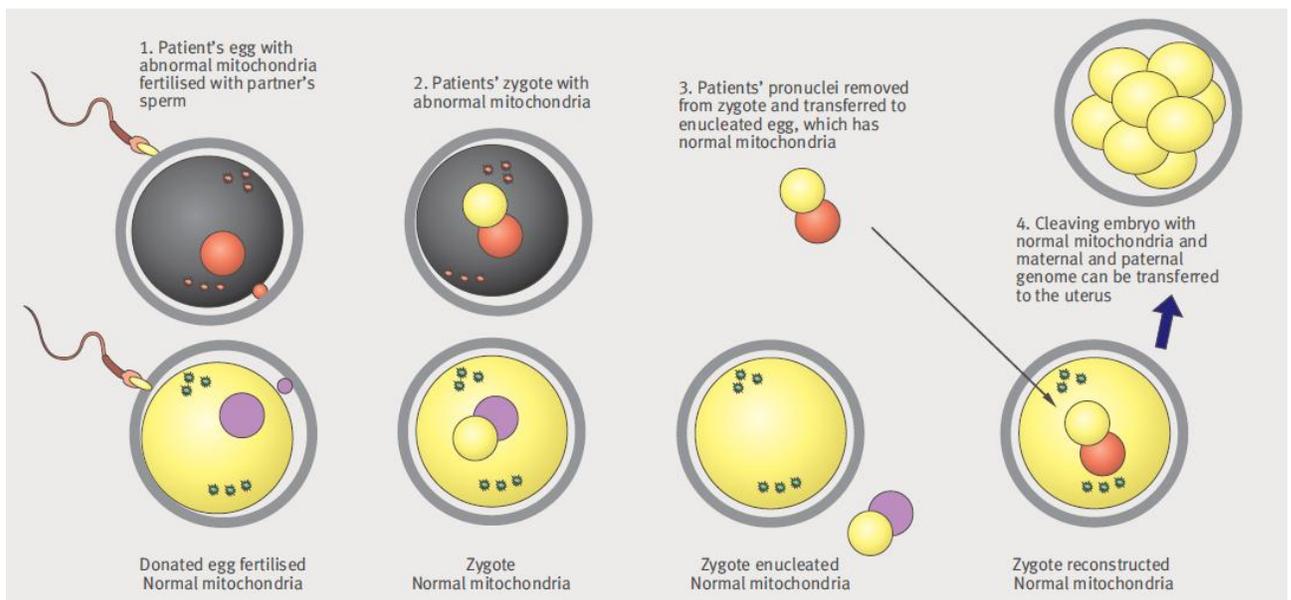


Figure 2. Pronuclear transfer technique¹¹

¹¹ Bredenoord, A and P. Braude (2010) "Ethics of mitochondrial gene replacement: from bench to bedside" BMJ 341. Image reproduced with permission of Author

4.1.3 Although similar methodology is employed, it is important to stress that neither MST nor PNT is equivalent to reproductive cloning (somatic cell nuclear transfer, or SCNT). Any children resulting from MST or PNT would have arisen from fertilisation and be genetically unique. They would be the genetic child of the woman receiving treatment and her partner. MST and PNT do not involve reprogramming cells or nuclei as SCNT does, which is a relatively inefficient process and associated with significant risks of abnormal development¹².

4.2 Effectiveness of MST and PNT

4.2.1 There have been many experiments conducted using MST and particularly PNT in animals. PNT has been carried out since the mid 1980s in mice. All the available evidence suggests that it is very efficient and reproducible when conducted with normally-fertilised zygotes, giving rates of normal development and offspring similar to those obtained with unmanipulated zygotes. MST has also been conducted as a control in some SCNT experiments in a wide range of animals, and again the evidence suggests that it is reasonably efficient.

4.2.2 Several proof of principle studies with respect to the possible use of MST and PNT methods for treating mitochondrial disease have been carried out using animal models, including mice and rhesus macaque monkeys, and with human oocytes and abnormal human zygotes. With the exception of one mouse study¹³ these have not involved abnormal mitochondria; instead, researchers have used substrains or subspecies, or relied on the presence of different mtDNA haplogroups, so that sequence differences in

¹² The panel examined substantial evidence about SCNT, including studies on heteroplasmy where mitochondria in the somatic cell persisted, at sometimes high levels, in the cloned embryo and offspring. This was usually associated with fusion of the somatic cell with an enucleated oocyte. This can introduce significant numbers of mitochondria that are in an active and replicating state, together with associated mitochondrial replication factors made by the somatic cell nucleus. In contrast, these factors are probably absent in mitochondria in mature oocytes or zygotes, as these mitochondria do not replicate until later. MST and PNT do not involve somatic cells.

¹³ Sato A., T. Kono, et al. (2005). "Gene therapy for progeny for mito-mice carrying pathogenic mtDNA by nuclear transplantation." PNAS 102: 16765-16770. The study made use of a strain of mouse carrying mitochondria with a mtDNA deletion. They used PNT and showed that they could transfer PN from affected zygotes with minimal transfer of abnormal mtDNA and rescue the resulting offspring from defects in OxPhos.

mtDNA can be used to look at carryover and persistence of mtDNA with the spindle or pronucleus.

- 4.2.3** Studies in rhesus macaque monkeys¹⁴ suggest that MST is efficient, allowing replacement of almost all mitochondria and the birth of four healthy offspring that show no abnormalities after two years¹⁵. Rates of development to blastocyst stages and pregnancy rates were essentially the same as for control unmanipulated embryos.
- 4.2.4** Studies using PNT with human zygotes are more difficult to gauge. The published study to date¹⁶ used only abnormally-fertilised human oocytes, for example those containing three pronuclei. Embryos were reconstituted so that they had a normal complement of one maternal and one paternal pronucleus. The efficiency of blastocyst formation was about half that of control blastocysts. However, as the control blastocysts were not always good quality embryos and the PNT embryos were from abnormally fertilised oocytes, it is difficult to assess the efficiency of blastocyst formation. Nevertheless, all evidence presented to the panel (both published and unpublished) suggested that the blastocysts obtained after PNT and MST were as normal as controls, including cell numbers in the trophoblast and inner cell mass, and markers of cell type and stage.
- 4.2.5** Overall MST appears less efficient than PNT, which may reflect problems in visualising the spindle and perhaps in transferring all the chromosomes. In mature metaphase II oocytes, the chromosomes are lined up attached to the spindle in preparation for the second meiotic division, rather than being enclosed in a pronuclear membrane, as is the case for PNT. Chromosomes may, therefore, be left behind, although this can be checked using fluorescent dyes that label DNA or chromatin. However, more recent studies have made use of new polarised light microscopy techniques, and these seem

¹⁴ Tachibana M., M. Sparman, et al. (2009) "Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature* 461, 367-372; Tachibana et al. (2010) "Chromosome transfer in mature oocytes." *Nature Protocols*, 5(6):1138-1147. Dr Mitalipov, Oregon University, oral presentation to panel.

¹⁵ Although the experimental numbers are small, it would be both costly and ethically unacceptable to extend these studies to many macaques.

¹⁶ Craven L., H. A. Tuppen, et al. (2010) "Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease." *Nature* 465(7294):82-5

to permit far more reliable visualisation and removal of the spindle, with all its associated chromosomes.

4.3 Safety of MST and PNT

4.3.1 The panel considered the following safety issues of the MST and PNT techniques: the carryover of mtDNA from the affected oocyte or zygote; the reagents used in the techniques; and the nuclear-mitochondrial interactions involved.

4.3.2 mtDNA carryover: Carryover of mtDNA from the affected oocyte or zygote might be expected with both techniques as the spindle or pronuclei are enclosed in a 'karyoplast' during the manipulation technique, that contains nuclear DNA, cell membrane and a small amount of surrounding cytoplasm. In theory, carryover of abnormal mtDNA may be an issue if abnormal mtDNA is preferentially replicated and if there is a marked difference in segregation across tissues. However evidence presented to the panel was reassuring¹⁷. Carryover was usually considerably less than 2% of the total mtDNA in the reconstituted embryos from both MST and PNT, with MST appearing to have a lower carryover than PNT. In several cases the level was undetectable (although the sensitivity of the methods used would not have detected very low amounts). The evidence suggests that the likelihood of preferential replication of abnormal mtDNA and marked differences in segregation across tissues is remote. The maximum levels of carryover of mtDNA in MST and PNT is markedly less than the level of abnormal mtDNA in an embryo that may be accepted for transfer following PGD. Nevertheless, without evidence from the use of MST or PNT in humans, the possibility of carryover of even a small percentage of abnormal mtDNA, means that any girls born from these techniques should be considered to have a (theoretically) low risk of transmitting the disease to their offspring.

4.3.3 Reagents: To carry out the micromanipulation techniques in MST and PNT, it is necessary to use a number of reagents not used in conventional assisted conception or

¹⁷ Professor Doug Turnbull, Newcastle University, oral presentation to panel; Dr Mitalipov, Oregon University, oral presentation to panel.

PGD. Notably, these included nocadazole and cytochalasin B, which depolymerise microtubules and microfilaments, making the cytoplasm and cell membranes less rigid and therefore less prone to damage when removing karyoplasts. Both drugs can disrupt chromosome segregation and cell division. However the panel was reassured that there is substantial evidence to show that these agents wash out easily and, as long as that is done, they should not persist to stages that might be affected. To reintroduce the isolated karyoplasts into the enucleated recipient oocyte or zygote, it is necessary to use cell fusion. Inactivated Sendai virus, which is both gentler and more efficient than other methods¹⁸, was found to be necessary in both the rhesus macaque monkey and human studies. It is essential that the Sendai virus is properly inactivated and that this procedure is performed in a separate facility. As long as care is taken, the use of this fusogen should be entirely safe. Prior to MST or PNT methods being used clinically, the safety of all the procedures will need to be subject to proper safety and risk assessment, as with any similar procedure.

4.3.4 Nuclear-mitochondrial interactions: A concern has been raised that there might be a failure of correct nuclear-mitochondrial interaction following MST or PNT because the donor mtDNA may be of a haplogroup different from that with which the maternal nuclear genome has been functioning. Mitochondria from separate human lineages can be classified according to similarities or differences in their DNA sequence into many different haplogroups. The more evolutionary distant the separation of two maternal lineages, the greater the differences between mitochondrial haplogroups. This is as typified by comparisons between European and African mtDNA. However, there is no evidence for any mismatch between the nucleus and any mtDNA haplogroup, at least within a species. Fifty per cent of nuclear genes are paternally inherited and are consequently alien to the mtDNA; backcrossing can replace the nuclear DNA entirely in a few generations. Furthermore, mitochondrial disease has not been noted to be more frequent amongst mixed-race children. However, if further concerns were raised, it would be possible to match mtDNA haplogroups from the egg donor and the mother.

¹⁸ A variety of electrical and chemical methods (electrofusion or polyethylene glycol) are typically employed in similar experiments involving rodents and domestic animals. However, the original method as developed in mice by McGrath and Solter made use of inactivated Sendai virus.

- 4.3.5 MST and PNT have the potential to be used for all patients with mtDNA disorders, which may make them preferential to PGD in the future. In patients with homoplasmy or high levels of heteroplasmy, these are the only techniques that would make it possible for them to have a genetically related unaffected child.**
- 4.3.6 There is currently insufficient evidence to recommend one transfer technique over the other.**
- 4.3.7 Although potentially useful clinical techniques, further safety experiments need to be done before introducing them into clinical practice.**
- 4.3.8 Once assessed as safe to use in clinical practice, the panel strongly recommends that permission is sought from the parents of the children born from MST and PNT to be followed up for an extensive period.**

5 Further research

- 5.1** From the evidence received, the panel has not identified anything that indicates that the MST and PNT methods are unsafe. Nevertheless, these techniques are relatively novel, especially as applied to human embryos, and with relatively few data. The panel therefore recommends that additional studies be undertaken both on basic research to improve the knowledge about the biology of human mitochondria and on research aimed specifically at providing further safety information on MST and PNT.
- 5.2** Basic research is needed into how the mitochondrial bottleneck functions and the critical parameters involved in the segregation of normal and any specific abnormal mitochondria amongst cell types in humans, as this is generally not well understood. For example, in the long term it may eventually be possible to influence or control replication of abnormal mtDNA in the early embryo to affect its segregation or inheritance in

subsequent development. This research may aid decisions about threshold levels when carrying out PGD, although it may be less relevant when considering the use of PNT or MST.

5.3 The panel categorised experiments that it feels are critical to assessing the effectiveness and safety of MST and PNT techniques (paragraph 5.4) and experiments that will provide useful information on MST and PNT or mitochondria and disease (paragraph 5.5). The former research will also inform which of the two techniques is likely to be the most appropriate for clinical use. Many of the latter experiments, whilst of potential importance for basic research and for possibly exploring alternative methods whereby abnormal mtDNA can be selected against, will not necessarily inform the decision as to whether it is safe to proceed to clinical application of MST and PNT methods.

5.4 The following (minimum) set of experiments is critical. These experiments should be undertaken and the results taken into account before MST and PNT techniques can be assessed as safe to use clinically:

- **MST using human oocytes that are then fertilised (not activated)**
- **PNT using normally-fertilised human oocytes and development compared to normal ICSI-fertilised human oocytes**
- **PNT in a non-human primate model, with the demonstration that the offspring derived are normal**

5.5 The following additional research is recommended to provide useful information on mitochondrial disease and the MST and PNT techniques:

- Removing the spindle or pronuclei and replacing them back into the same oocyte/zygote to better identify the impact of the manipulation technique
- Karyotype analysis and comparative genomic hybridisation/copy number variation arrays of embryos derived from MST or PNT
- Detailed analysis of epigenetic modifications and gene expression, with a range of markers for blastocyst cell types or embryos derived from MST or PNT

- MST on unfertilised human oocytes that have abnormal mtDNA
- PNT on fertilised oocytes that have abnormal mtDNA
- Studies on human embryonic stem (ES) cells derived from blastocysts that are heteroplasmic for abnormal mtDNA and blastocysts created through MST and PNT, where the oocytes had abnormal mtDNA¹⁹
- Similar experiments using induced pluripotent stem (iPS) cells derived from patients carrying different mtDNA mutations²⁰
- Further studies on the mtDNA carryover in a non-human primate model into the possible heteroplasmy of tissues in the fetus. The possibility of carryover of even a small percentage of abnormal mtDNA, means that any girls born from MST or PNT should be considered at risk of transmitting the disease to their offspring
- Further studies on vitrifying zygotes created through PNT

6 Conclusions

6.1 This section summarises the conclusions made in the report.

6.2 Much of the information on mtDNA copy number, replication and the bottleneck has been obtained from studying mouse and other animal embryos. Further research on mitochondrial biology in early human embryos, and in the germline in particular, is encouraged and should be supported by research funding agencies.

6.3 Further work is required to develop reliable assays for mitochondrial disease caused by mutations in nuclear DNA, especially as PGD may be the preferred alternative for those who wish to prevent transmission of such diseases without having to consider prenatal diagnosis and possible termination of an affected pregnancy.

¹⁹ ES cells have a low number of mitochondria that do not need to function. Differentiated cells derived from the ES cells, such as muscle, can have high numbers of mitochondria. These can be put in conditions requiring oxidative phosphorylation. It is also possible to derive primordial germ cells *in vitro* to explore aspects of the mitochondrial bottleneck and whether certain abnormal mtDNA have a replication advantage.

²⁰ It is not possible for iPS cells to provide information on mitochondrial behaviour in the early embryo.

6.4 Rather than aiming specifically at avoiding transmission of the mutation and thus disease prevention, PGD for mitochondrial heteroplasmy can only be aimed at risk reduction.

6.5 Centres that carry out PGD for mtDNA disorders should:

- offer prenatal diagnosis or PGD as the first option for patients who have an identified mutation and a low to medium level of heteroplasmy
- have appropriate experience and expertise in the diagnosis and management of mtDNA disorders, knowledge of the different mutations and the proportion of heteroplasmy likely to lead adult disease
- provide appropriate experienced genetic counselling, in order to decide upon and document the threshold level of abnormal mtDNA measured at PGD above which embryos would not be replaced in order to minimise the risk of the child being affected with the disorder
- be encouraged to consider single embryo transfer to enable follow up of the fate of the embryo transferred²¹
- encourage patients to consider prenatal testing following PGD, to assess the consistency in levels of heteroplasmy
- record the levels of abnormal mtDNA in the woman, the embryo (trophectoderm or cleavage stage biopsy) and in the subsequent child to assess the degree of correlation

6.6 MST and PNT have the potential to be used for all patients with mtDNA disorders, which may make them preferential to PGD in the future. In patients with homoplasmy or high levels of heteroplasmy, these are the only techniques that would make it possible for them to have a genetically related unaffected child.

6.7 There is currently insufficient evidence to recommend one transfer technique over the other.

²¹ Using single embryo transfer will require centres to have an effective cryopreservation programme in order to freeze the remaining suitable embryos that were not transferred.

- 6.8** Although potentially useful clinical techniques, further safety experiments need to be done before introducing them into clinical practice.
- 6.9** Once assessed as safe to use in clinical practice, the panel strongly recommends that permission is sought from the parents of the children born from MST and PNT to be followed-up for an extensive period.
- 6.10** The following (minimum) set of experiments is critical. These experiments should be undertaken and the results taken into account before MST and PNT techniques can be assessed as safe to use clinically:
- MST using human oocytes (eggs) that are then fertilised (not activated)
 - PNT using normally-fertilised human oocytes and development compared to normal ICSI-fertilised human oocytes
 - PNT in a non-human primate model, with the demonstration that the offspring derived are normal

Annex A: Clinical disorders due to mutations in mtDNA

TABLE 1. Clinical disorders due to mutations in mtDNA

Mitochondrial DNA disorder	Clinical phenotype	mtDNA mutation	Affected gene	Status	Inheritance
Kearns-Sayre syndrome	Progressive myopathy, cardiomyopathy, ophthalmoplegia	A single large deletion	Several	Heteroplasmic	Usually sporadic
CPEO	Ophthalmoplegia	A single large deletion	Several	Heteroplasmic	Usually sporadic
Pearson syndrome	Pancytopenia, lactic acidosis	A single large deletion	Several	Heteroplasmic	Usually sporadic
MELAS	Myopathy, encephalopathy, lactic acidosis, stroke-like episodes	3243A>G 3271T>C Individual mutations	<i>TRNL1</i> <i>ND1</i> , <i>ND5</i>	Heteroplasmic	Maternal
MERFF	Myoclonic epilepsy, myopathy	8344A>G 8356T>C	<i>TRNK</i>	Heteroplasmic	Maternal
NARP	Neuropathy, ataxia, retinitis pigmentosa	8993T>G	<i>ATP6</i>	Heteroplasmic	Maternal
MILS	Progressive brain-stem disorder	8993T>C	<i>ATP6</i>	Heteroplasmic	Maternal
MIDD	Diabetes,	3243A>G	<i>TRNL1</i>	Heteroplasmic	Maternal

	deafness				
LHON	Optic neuropathy	3460G>A 11778G>A 14484T>C	<i>ND1</i> <i>ND4</i> <i>ND6</i>	Heteroplasmic or homoplasmic	Maternal
Myopathy and diabetes	Myopathy, weakness, diabetes	14709T>C	<i>TRNE</i>	Hetero- or homoplasmic	Maternal
Sensorineural hearing loss	Deafness	1555A>G Individual mutations	<i>RNR1</i> <i>TRNS1</i>	Homoplasmic Hetero- or homoplasmic	Maternal
Exercise intolerance	Fatigue, muscle weakness	Individual mutations	<i>CYB</i>	Heteroplasmic	Sporadic
Fatal, infantile encephalopathy; Leigh/Leigh-like syndrome	Encephalopathy, lactic acidosis	10158T>C; 10191T>C	<i>ND3</i>	Heteroplasmic	Sporadic

CPEO, chronic progressive external ophthalmoplegia; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes; MERFF, myoclonic epilepsy and ragged-red fibres; NARP, neurogenic weakness, ataxia and retinitis pigmentosa; MILS, maternally inherited Leigh syndrome; MIDD maternally-inherited diabetes and deafness; LHON, Leber hereditary optic neuropathy. TRN genes encode tRNAs; RNR1 encodes 12S ribosomal RNA, all other genes encode proteins required for OxPhos.

Adapted from Taylor and Turnbull (2005)²²

²² R. Taylor and D. Turnbull (2005) "Mitochondrial DNA mutations in human disease." *Nat Rev Genet.* 6(5): 389–402 Author manuscript; available in PMC 2007 January 4.6(5):389-402.

Annex B: Methodology of review

1. The Human Fertilisation and Embryology Authority (HFEA) agreed on 16 February 2011 to a request from the Secretary of State for Health to carry out a scientific review to scope “expert views on the effectiveness and safety of mitochondrial transfer”.
2. In order to carry out this review, the HFEA established a small panel to collate and summarise the current state of expert understanding on the safety and efficacy of methods to avoid mitochondrial disease through assisted conception. Panel members were selected for their broad-ranging scientific and clinical expertise, and for having no direct interests in the outcome of the review.
3. Membership of the panel:
 - Professor Neva Haites (co-chair), University of Aberdeen
 - Dr Robin Lovell-Badge (co-chair), Medical Research Council National Institute for Medical Research
 - Professor Peter Braude, Kings College London
 - Professor Keith Campbell, University of Nottingham
 - Professor Sir Richard Gardner
 - Professor Anneke Lucassen, Human Genetics Commission
4. The panel put out a call for evidence on 28 February 2011. It asked for scientific evidence on the safety and effectiveness of methods to avoid mitochondria disease through assisted conception from experts in any relevant field by 15 March 2011. The call for evidence was sent directly to more than 30 experts in the field and to 25 professional bodies. Recipients were invited to circulate the call to colleagues. The call for evidence was also published on the HFEA website²³.
5. When submitting evidence, individuals were asked to indicate whether they were interested in discussing their evidence with the panel. Any researcher who expressed interest was

²³ <http://www.hfea.gov.uk/6372.html>

invited to present their evidence. Places were limited to a maximum of two people per submission.

6. The following people presented evidence to the panel at a workshop on 25 March 2011:
 - Dr Mary Herbert and Professor Douglas Turnbull, Newcastle University
 - Dr Rhiannon Lloyd, Institute of Zoology, Zoological Society of London
 - Dr Shoukhrat Mitalipov, Oregon Health and Science University
 - Professor Joanna Poulton, Nuffield Department of Obstetrics & Gynaecology, John Radcliffe Hospital, Oxford
 - Dr Shamima Rahman, National Commissioning Group for Rare Mitochondrial Disease of Adults and Children, National Hospital for Neurology, University College London Hospital
 - Professor Hubert Smeets, Department of Clinical Genetics, Maastricht UMC

7. The presentations were followed by an open discussion with all the participants. The panel reviewed the submissions of written evidence and the evidence presented at the workshop. The panel requested additional information and clarifications from researchers as necessary.

8. Annex C lists the written evidence reviewed by the panel.

Annex C: Evidence reviewed

1. Statements/letters

- A statement (with reference to literature) from Dr Rhiannon Lloyd, Leverhulme Early Career Research Fellow, Zoological Society of London
- A letter from Dr David King, Director of Human Genetics Alert
- A letter from David Thorburn, Head of Mitochondrial Research Group, Murdoch Children's Research Institute, Victoria, Australia
- A statement from Dr Shamima Rahman & Professor Michael Hanna (National Commissioning Group funded service for Diagnosis and Management of Rare Mitochondrial Diseases in Adults and Children, Centre for Neuromuscular Diseases, National Hospital for Neurology, UCLH) and Professor Peter Clayton & Professor Francesco Muntoni (Great Ormond Street Hospital and the UCL Institute of Child Health, London)
- A statement from Dr Marita Pohlschmidt, Research Director, Muscular Dystrophy Campaign
- A statement from Dr Robert McFarland and Professor Robert Taylor, NCG Rare Mitochondrial Diseases Service, Newcastle University
- Summary of evidence (including slides presented to the HFEA Scientific and Clinical Advances Advisory Committee in May 2010) from Lyndsey Craven, Laura Irving, Alison Murdoch, Mary Herbert, Doug Turnbull, Newcastle University
- A statement from Nathan Treff with reference to four published papers

- A confidential statement (with reference to literature) from Professor Joanna Poulton and Dr Dagan Wells, Nuffield Department Obstetrics & Gynaecology John Radcliffe Hospital, Oxford
- A statement about the use of cytochalasin and nocodazole from Professor Martin Johnson, Professor of Reproductive Sciences, University of Cambridge
- A statement from the Medicine and Healthcare Products Regulatory Agency (MHRA) on cytochalasin B
- A statement from researchers at Newcastle University providing further information about the agents and techniques used in their research
- A statement on cytoplasmic transfer from Dr Marcos Chiaratti, Universidade de São Paulo, Brazil
- A statement from Dr Jacques Cohen about cytoplasmic transfer
- Information from the Clinical Molecular Genetics Society on the number of prenatal tests carried out for mtDNA disease

2. Unpublished abstracts and manuscripts

- Confidential manuscript on the feasibility of PGD for mtDNA disorders - Professor Hubert Smeets, Professor in Clinical Genomics, Department of Clinical Genetics, Maastricht UMC
- Confidential manuscript (with supplementary tables and figures) - Transfer of the meiotic metaphase II spindle between human oocytes shows potential to prevent transmission of mitochondrial DNA disease – Newcastle University and collaborators

- Abstract – Mitochondrial DNA replacement in a primate model – Dr Shoukhrat Mitalipov, Division of Reproductive Sciences, Oregon National Primate Research Center, Oregon Stem Cell Center and Departments of Obstetrics & Gynecology and Molecular & Medical Genetics, Oregon Health & Science University
- Abstract - Pre-implantation Genetic Diagnosis for the mitochondrial DNA m.8993T>G mutation yields a healthy child and evidence of selection for germ cells with high abnormal loads – Professor David Thorburn, Head of Mitochondrial Research Group, Murdoch Childrens Research Institute, Victoria, Australia
- Abstract - Successful application of preimplantation genetic diagnosis to reduce the transgenerational risk of transmitting a mitochondrial DNA disorder – Dr Nathan Treff, Director of Molecular Biology Research, Reproductive Medicine Associates of New Jersey

3. Published articles and reports

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Annex D: Glossary

Activate	To artificially trigger the processes that occur when an egg is fertilised
Adenosine triphosphate (ATP)	Molecule which transports energy in cells. It is involved in reactions of the electron transfer chain (ETC)
Apoptosis	Programmed cell death
Autophagy	Where a cell begins to digest part of itself
Backcross	Formally, breeding a hybrid individual with one of its parents. In this context, where a hybrid or mixed race individual breeds with one of its parental types, such as race or strain
Biopsy	Procedure of obtaining cells eg, from an embryo, in order to test them
Blastocyst	A 5-6 day old embryo, with both an outer trophoctoderm cell layer and an inner cell mass
Blastomere	A cell of an embryo prior to the blastocyst stage
Chromatin	The combination of DNA and other proteins in the nucleus, which packages the chromosomes
Chromosome	Structure in a nucleus that carries DNA

Chromosomal inheritance	The process for passing on the DNA in the nucleus to subsequent generations
Comparative genomic hybridisation array	Screening method to detect abnormal chromosomes
Cytoplasm	The jelly-like substance that fills a cell and contains the structures within it, including mitochondria
Cytoplasmic hybrids	An adult cell or nucleus that has been fused to a cell with its nucleus removed. The cells can be from different individuals or species
Cytoplasmic transfer	The technique of injecting cytoplasm from a donor egg into a recipient egg, for example one that has compromised mitochondria
De novo mutation	A spontaneous new mutation (as opposed to one that is inherited)
Depolymerise	To break a polymer, composed of many similar molecules (whether chemicals or proteins), into its constituent parts
Electron transfer chain (ETC)	The chain of chemical reactions that produce energy in a mitochondrion
Embryogenesis	The process by which an embryo forms and develops
Embryonic stem (ES) cells	Cells, derived from an embryo and cultured in a laboratory, that have the potential to form all different cell/tissue types

in an animal or human and can replicate themselves indefinitely

Epigenetic

Changes in phenotype that can be inherited, but are not caused by the underlying DNA

Founding cells

The small number of cells that give rise to a larger number of cells/tissues later in development

Gene expression

The process by which a gene results in a phenotype

Genetic counselling

The process that helps individuals, couples, or families to understand genetic information and issues that may have an impact on them

Germ cells

Any cell that will give rise to sperm or egg cells

Germline

The sequence of cells that give rise to sperm or egg cells that will pass genetic information on to a child

Germline mosaicism

A difference in the mutations within germ cells, compared to the individuals adult cells. This may lead to a disease in offspring where the mother is not affected

Heteroplasmy

Where two or more different mtDNA types coexist in a single cell, commonly used (as in this report) where one type is abnormal, and the other normal

Homoplasmy

Where all the mitochondria in a cell contain the same mtDNA, which can either be all abnormal or all normal

Induce pluripotent stem (iPS) cells	Adult cells that have been reprogrammed to act like embryonic stem cells
Inner cell mass	The mass of cells contained within a blastocyst stage mammalian embryo that will give rise to all cell and tissues types of the fetus
Karyoplast	A structure made up of the nuclear genetic material, surrounded by a small amount of cytoplasm, enclosed in a cell membrane, used to transfer a spindle or pronuclei during MST or PNT experiments
Karyotype	The number and appearance of chromosomes in a cell
Lineage	Term used to describe cells with a common ancestor
Maternal spindle transfer (MST)	Technique to transfer the nuclear genetic material from a woman's egg into a donated egg with its genetic material removed
Meiotic division	Type of cell division necessary for sexual reproduction
Metaphase II oocytes	Mature oocytes or unfertilised eggs (that have undergone the first meiotic cell division)
Microfilaments	Thin protein structures that contribute to the 'skeleton' and to the dynamics (movement) of the cytoplasm
Micromanipulation	Use of physical techniques to add or remove cellular components

Microtubules	Structural components of the cytoplasm, contributing to its 'skeleton' and involved in many cellular processes including cell division
Mitochondria	Small structures present in cells that produce the cell's energy (see Introduction)
Mitochondrial bottleneck	The term describing how, during the development of the embryo, the low number of mitochondria per cell and the small number of cells that go on to form each tissue type means that by chance different cell types will have different proportions of mitochondrial DNA of different types
Mitochondria carryover	The proportion of mitochondria that is carried over from the original egg or zygote during MST or PNT
Mitochondrial DNA (mtDNA)	The genetic material contained within the mitochondria
Mitochondrial haplogroup	A group of similar mitochondrial DNA types as defined by DNA sequence
Mosaicism	When cells within the same individual have a different genetic makeup
Mutation	A permanent, heritable change in the DNA sequence
Nuclear DNA	The vast majority of the cell's genetic information, which is contained in the nucleus of the cell
Oocyte	An egg

Ooplasm	Cytoplasm contained in an egg
Organelle	Small structure within a cell
Oxidative phosphorylation (OxPhos)	The reaction that produces ATP to generate energy in the cell
Phenotype	Any observable characteristic in an organism, including a disease
Preimplantation genetic diagnosis (PGD)	A technique that removes and examines one or more cells from an early embryo to identify those embryos that are unlikely to develop a genetic disease
Prenatal diagnosis	Testing for diseases or conditions in a fetus before it is born
Primordial germ cell	A specialised cell, formed at early stages of development, that is destined to give rise to egg or sperm cells
Pronuclei	The independent nuclei derived from the sperm and egg during the process of fertilisation, as distinct from the nucleus formed from the combination of the maternal and paternal genomes that is present in every cell type from the 2-cell stage onwards
Pronuclear transfer (PNT)	Technique to transfer a couple's nuclear genetic material from a fertilised egg (zygote) into an donor zygote with its nuclear genetic material removed
Reagents	Chemicals used in experiments

Reproductive cloning	Using somatic cell nuclear transfer (see below) to create a genetically identical organism
Segregation	The process that determines the genetic information present in different tissues or organisms
Selection	The process by which particular entities, such as genes, or cells, are favoured
Sequencing	Method for determining the order of chemicals that make up DNA in an individual
Sex selection	Selecting an embryo of a particular sex to avoid passing on a serious sex-linked condition
Single embryo transfer	Transferring just one embryo into the uterus of a woman during a cycle of treatment
Somatic cell	Any cell of an embryo or adult that is not a germline cell
Somatic cell nuclear transfer (SCNT)	Fusion of an adult (somatic) cell with an egg that has had its nucleus removed
Spindle	A structure in a cell, made up of microtubules, that move the chromosomes when the cell divides
Syngamy	The fusion of an egg and sperm

Transcription	The first part of the process of gene expression, whereby a complementary RNA-copy of a sequence of DNA is made. This can then be translated to make the protein encoded by the DNA
Transfer RNA (tRNA)	RNA molecule involved in the creation of proteins encoded by DNA
Trophoblast	The outer layer of cells of an embryo that will develop into the placenta
Vitrify	To freeze (an embryo) by plunging into liquid nitrogen
Zygote	A fertilised egg