

## Current Methods for the Prevention of Transmission of Mitochondrial DNA Diseases

Kelly A Turner and Francis YM Choy\*

Centre for Biomedical Research, Department of Biology, University of Victoria, PO Box 3020, Station CSC, Victoria, BC V8W 3N5, Canada

\*Corresponding author: Francis YM Choy, Centre for Biomedical Research, Department of Biology, University of Victoria, PO Box 3020, Station CSC, Victoria, BC V8W 3N5, Canada, Tel: 1-250-721-7107; Fax: 1-250-721-7120; E-mail: fchoy@uvic.ca

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### Abstract

It is estimated that mitochondrial diseases affect 1 in 5,000-10,000 live births. At present, there is no cure for a mitochondrial disease and current treatments are limited to reducing symptoms and slowing disease progression. The prevention of transmission of mitochondrial diseases is of vital importance to parents with a mitochondrial disease who wish to make informed reproductive decisions. This paper provides a critical evaluation of the various established and experimental techniques involved in the prevention and treatment of mtDNA disease at the germline level, including fertilization using donor oocytes, pre-implantation genetic diagnosis, chorionic villus sampling, amniocentesis, cytoplasmic transfer, germinal vesicle transfer, pronuclear transfer, and spindle-chromosomal complex transfer; the latter two of which have been publicly endorsed by the Human Fertilisation and Embryology Authority in the UK in 2014 as being potentially useful and safe methods for the prevention of transmission of severe mtDNA diseases.

**Keywords:** Mitochondrial disease, mtDNA, Treatment, Pronuclear transfer, Spindle transfer, HFEA

### Abbreviations:

OXPHOS: Oxidative Phosphorylation; ATP: Adenosine Triphosphate; mtDNA: Mitochondrial DNA; D-loop: Displacement Loop; PGD: Pre-implantation Genetic Diagnosis; CVS: Chorionic Villus Sampling; qPCR: Quantitative Polymerase Chain Reaction; HFEA: Human Fertilisation and Embryology Authority; MERRF: Myoclonic Epilepsy with Ragged Red Fibers; MELAS: Mitochondrial Encephalomyopathy Lactic Acidosis and Stroke-like Episodes; IVF: In-Vitro Fertilization; ICSI: Intracytoplasmic Sperm Injection; PNT: Pronuclear Transfer; ST: Spindle-Chromosomal Complex Transfer; RFLP: Restriction Fragment Length Polymorphism; ES: Embryonic Stem.

### Introduction

Mitochondria are ubiquitous subcellular organelles that play essential roles in energy production, metabolism, and signal transduction. Multimeric protein complexes organized within the inner membrane of the mitochondrion catalyze the reactions in the citric acid cycle and transport of electrons, resulting in the formation of a proton gradient. The energy generated in this oxidative phosphorylation (OXPHOS) process is utilized for the synthesis of adenosine triphosphate (ATP), which drives a multitude of necessitous reactions within all cells; especially those with high energy requirements such as neurons and myocytes [1-3]. Mitochondria are also involved in programmed cell death, or apoptosis; whereby upon detection of a stress signal, they will release cytochrome c into the cytosol which triggers downstream caspases to initiate apoptosis [4-6].

### Mitochondrial genome organization and inheritance

A unique feature of the mitochondria is that they contain their own mitochondrial DNA (mtDNA). This genome is a relic of their free-living bacterial origins before they were engulfed by ancient eukaryotes and co-opted for aerobic respiration [7]. Compared to the nuclear genome, replication of the mitochondrial genome is not tightly controlled and may occur at any stage of the cell cycle, instead of being confined to mitosis and meiosis [8,9]. This results in varying mtDNA copy numbers per cell [10]. Most human cells contain at least 1,000 mtDNA molecules distributed among hundreds of mitochondria, except for mature human oocytes, which have more than 100,000 mtDNA copies [11].

The human mitochondrial genome is composed of double-stranded circular DNA approximately 16.6 kbp in size and contains 37 genes. These genes encode 2 rRNA, 22 tRNA, and 13 polypeptides that are subunits of the OXPHOS system (8). All remaining components required by the mitochondria, such as DNA polymerases and other subunits of OXPHOS, are encoded by the nuclear genome. Except for a small regulatory region called the displacement loop (D-loop), the entire mitochondrial genome is comprised of coding sequences [12,13]. This characteristic, compounded with the greater potential for oxidative damage and lack of any internal DNA repair mechanisms, makes mtDNA about 10 times more likely to acquire mutations compared with nuclear DNA [14]. During mitochondrial replication, mutated and wild-type mtDNA copies segregate randomly into daughter mitochondria. If a cell contains both mutated and wild-type mtDNA copies, it is referred to as heteroplasmic; and if it contains either all mutated or all wild-type mtDNA, it is homoplasmic.

### Treatment and genetic counseling for mitochondrial diseases

Defects in mitochondrial respiratory chain function cause a wide range of diseases, often with multi-system impacts. The musculoskeletal and central nervous systems, and organs such as the

heart, liver, and kidneys are particularly affected due to their large energy demands [15]. It is estimated that mitochondrial diseases affect 1 in 5,000-10,000 live births [16] and more than 275 mutations in mtDNA have been associated with mitochondrial dysfunction [17]. Currently, there is no cure for a mitochondrial disease and current treatments are limited to reducing symptoms and slowing disease progression. In some patients, exercise training alongside vitamin therapy with Coenzyme Q10 has been shown to alleviate symptoms for patients with muscle and nerve involvement, but only transiently; other patients who experience neurosensory hearing loss benefit from cochlear implants. Seizures are one of the most common symptoms of a mitochondrial disease, and patients respond well to available anticonvulsants [18]. Though there are various established treatments of symptoms for patients with particular pathologies, none effectively cure the disease or completely arrest its progression.

For genetic counseling purposes, those with a pathogenic mutation in the nuclear DNA versus those with a pathogenic mutation in the mitochondrial DNA leading to a mitochondrial defect should be regarded as two separate groups. Pathogenic mutations in the nuclear DNA are usually transmitted as a recessive trait that follows Mendelian inheritance and counseling is more straightforward. Families can be given precise risk estimates of transmission followed by prenatal diagnosis (amniocentesis or chorionic villus sampling) or pre-implantation genetic diagnosis (PGD) for reproductive options [19]. The same is not the case for those with mtDNA diseases that are transmitted maternally, though the mechanism of exclusion of paternal mitochondria is still not very well understood [20]. Consequently, if a mother harbors a germline mtDNA mutation, it will be inherited by all of her offspring. However, due to heteroplasmy and random segregation of mutated and normal mitochondria during oogenesis, there is no way to predict how severely the offspring will be affected, if at all [21]. As well, mtDNA diseases require a specific “mutation load” or “mutation threshold”, meaning that a certain portion of the mtDNA must be mutated in order for the disease to be expressed [22]. This mutation load is disease- and tissue-specific and further compounds the problem of accurately predicting the risk that the offspring will develop the disease. For example, the mtDNA disease MERRF (myoclonic epilepsy with ragged red fibers) requires a mutant threshold of 90% in the muscle for the disease to be expressed; and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) requires a mutant load 65% or greater [22].

Due to the heterogeneity of mitochondrial disorders and the difficulty in predicting their expressivity in subsequent generations, it is of vital importance that sensitive and reliable screening methods are developed, as well as assisted reproductive techniques that prevent the transmission of mutant mtDNA. This paper describes and evaluates current methods that aim to prevent the transmission of mtDNA disease.

## Current Methods

### Donor oocytes

The easiest solution to prevent the transmission of an mtDNA disease is to use donor oocytes from an unaffected individual for *In-Vitro* Fertilizations (IVF) and, if necessary, Intracytoplasmic Sperm Injection (ICSI) procedures for fertilization and implantation. This ensures that no mutated mtDNA will be transmitted to the offspring, as the mitochondrial genome is contributed by an unaffected individual. Though this is the most straightforward approach, the

resulting child will not be the biological offspring of the patient and many families would prefer having their own biological offspring. As well, IVF and ICSI procedures are costly and efficacy is still relatively low. In a recent UK study by the Human Fertilisation and Embryology Authority (HFEA), for every 100 women who underwent assisted reproduction procedures, only 29 gave birth [23].

### Chorionic villus sampling and amniocentesis

Chorionic villus sampling (CVS) and amniocentesis are prenatal methods normally used to screen for chromosomal abnormalities, but they have also been used in the detection of mtDNA mutations, as well as predicting mutant load in the fetus. By using quantitative polymerase chain reaction-restriction fragment length polymorphism (qPCR-RFLP) analysis or real-time amplification refractory mutation system quantitative polymerase chain reaction (ARMS-qPCR) analysis, heteroplasmy levels can be detected to 3% and below 1%, respectively [24,25].

While these methods are effective tools to screen for mtDNA abnormalities, they have some shortcomings. After CVS and amniocentesis, there is a 2.4% and 1% chance of miscarriage, respectively [26,27]. As well, mtDNA diseases are extremely heterogeneous and variable mutant loads, often ranging from 60-100% depending on the disease or tissue type for the disease to be expressed [22]. Consequently, arbitrary cut-offs must be made that distinguish an unaffected fetus from one that has a high probability of developing the disease after birth. This invariably creates a grey zone where it is very difficult to accurately predict the risk of disease expression and causes undue stress on expectant parents. Furthermore, some families may be opposed to the termination of a pregnancy for social or religious reasons, which makes this method unsuitable. As well, there is some debate as to whether the resulting mutant load tested in the fetus will remain constant or increase throughout gestation and after birth, though evidence points to the former [28]. The HFEA recommends that this method is only beneficial to those women who have moderate to low levels of heteroplasmy [29].

### Pre-implantation genetic diagnosis

An alternative screening method to CVS and amniocentesis is pre-implantation genetic diagnosis (PGD). It is superior to both prenatal screening methods in that it avoids the termination of a pregnancy because screening is done prior to implantation. PGD can be performed on polar bodies, oocytes, or embryos; but for the purposes of screening for mtDNA heteroplasmy, embryos are the most reliable in a mouse model [30]. Theoretically, a group of embryos are screened for a mtDNA mutation and the mutation load is quantified. The embryo (or often embryos) with the lowest mutant load

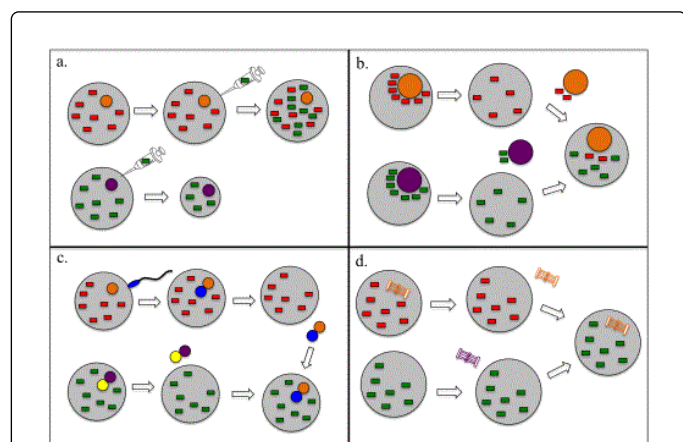
However, similar concerns as were demonstrated with CVS and amniocentesis are present with PGD. The idea of a “grey zone” of heteroplasmy makes it hard to accurately predict risk of disease expression, and there have also been reservations concerning the bioethics of testing embryos. These prenatal and pre-implantation screening methods are common practice and recommended for carriers of nuclear DNA mutations, and are useful for women with lower levels of mtDNA heteroplasmy [29]. However, it may be impossible for women with higher levels of heteroplasmy to produce an oocyte with heteroplasmy levels below the disease threshold. Therefore it is necessary to develop techniques that remove or prevent

carryover of most or all of the mother's mutant mtDNA to her offspring and subsequent generations.

### Cytoplasmic transfer

Cytoplasmic transfer was first employed to assist women who experienced continual implantation failure during IVF procedures [31]. It involved the removal of a small portion (5-15%) of cytoplasm from a healthy donor oocyte and injection into the patient's oocyte (Figure 1a). It was assumed that components of the healthy cytoplasm such as mRNAs, proteins, and mitochondria were able to compensate for the deficient oocyte. This was shown to be correct and has resulted in the birth of at least 30 babies worldwide [32]. However, no follow-up studies have been conducted, therefore the current health status of these children remains unknown.

It follows that this method could be adapted to dilute the mtDNA mutant load in an affected oocyte to below the disease threshold so that it is not expressed in the offspring. In one study, at least 30-50% of the donor cytoplasm was required to dilute heteroplasmy levels to 33% in murine embryos [19]; however, a similar study has not been done in humans. At present, it seems technically infeasible to remove enough donor cytoplasm to adequately dilute mutant mtDNA to negligible amounts and cytoplasmic transfer is, therefore, not recommended as a suitable method to prevent the transmission of mtDNA diseases in women with high levels of mutated mtDNA in oocytes or embryos.



**Figure 1:** Representation of current methods to prevent the transmission of mtDNA diseases. Mutated mitochondria are represented by red rectangles and healthy/wild-type mitochondria are represented by green rectangles. a. Cytoplasmic transfer – cytoplasm from an unaffected donor oocyte (lower left) is injected into an oocyte with mutated mitochondria resulting in a heteroplasmic oocyte. b. Germinal vesicle transfer – the germinal vesicle from an affected oocyte is transferred to an enucleated, healthy donor oocyte; mitochondria are localized in the perinuclear space, which results in carryover to the donor oocyte. c. Pronuclear transfer – the egg and sperm pronucleus from an affected embryo is transferred to an enucleated, healthy donor embryo. d. Spindle-chromosomal complex transfer – the chromosomes with attached spindle are removed from an affected metaphase II oocyte and transferred to an enucleated, healthy donor oocyte.

### Germinal vesicle transfer

The germinal vesicle refers to the nucleus during the early stages of oogenesis. During this stage, the nucleus is enlarged and easier to visualize and manipulate within the immature oocyte [33]. Germinal vesicle transfer involves the removal of the germinal vesicle from an affected patient and subsequent transfer into an enucleated donor oocyte (Figure 1b). For patients with a mtDNA disease, this ensures that the resulting offspring inherits the mother's nuclear DNA, but has the wild-type mtDNA from an unaffected/healthy donor.

This procedure has been tested in mice and showed a 12.9% birth rate, which was similar to controls [33]. This technique has not been applied to heteroplasmic mtDNA human oocytes, but studies on the *in vitro* maturation of immature oocytes have yielded lower development rates than those matured *in vivo* [34]. It has also been shown that during the germinal vesicle stage of oogenesis, mitochondria are localized in the perinuclear space [35]. This would result in some mtDNA carryover into the donor cytoplasm, making the oocyte heteroplasmic (Figure 1b). Even if the level of heteroplasmy is low, it is not acceptable for practical applications.

### Pronuclear transfer

A more recently developed method called pronuclear transfer (PNT) builds on the technique of germinal vesicle transfer, however, it is the male and female pronuclei that are removed from a zygote and transferred to an enucleated donor zygote [36] (Figure 1c). This method was tested on abnormally fertilized (uniploid and triploid) zygotes and resulted in forward development to the blastocyst stage in 8.3% of the embryos, which is 50% of the normal rate for abnormally fertilized embryos. Craven et al. [36] later quantified the amount of patient mtDNA carryover at less than 2%.

In the most recent review by the HFEA on the safety and efficacy of techniques to 'treat' mitochondrial diseases at the germline level; they endorsed PNT, stating that there was no evidence to suggest that the method was unsafe [29]. However, they recommended that follow-up experiments on normally fertilized (diploid) embryos be carried out, as well as additional studies using a non-human primate model.

### Spindle-chromosomal complex transfer

Spindle-chromosomal complex transfer (ST) is similar to PNT except that the nuclear information, in the form of chromosomes attached to the associated spindle, is removed from a metaphase II oocyte of the affected mother and transferred to an enucleated donor oocyte [37] (Figure 1d). Tachibana et al. [37] performed their earliest experiments on Rhesus macaques and the developmental potential of ST oocytes was similar to controls. The ST oocytes developed to the blastocyst stage and stable embryonic stem (ES) cell lines were derived from the blastocysts. Furthermore, healthy offspring were born; all at rates comparable to controls. Finally, using restriction fragment length polymorphism (RFLP) analysis at a sensitivity limit of 3%, they could not detect any heteroplasmy in the infants.

A follow-up study by Tachibana et al. [38] utilized healthy human oocytes for ST and the results were again similar to controls when comparing the rate of blastocyst development and establishment of ES cell lines. However, a more sensitive method to detect heteroplasmy levels below 1% was employed and the average mtDNA carryover was 0.5%. Tachibana et al. [38] also performed cryopreservation on both patient and donor metaphase II oocytes before ST to determine

viability and adaptability to IVF protocols. They reported that the transfer of vitrified spindles into fresh cytoplasts yielded the best results that were similar to controls.

In their most recent scientific review, the HFEA also endorses ST as a method to prevent the transmission of mtDNA diseases in women with high levels of heteroplasmic or homoplasmic mutant mtDNA [29].

## Conclusions

Mitochondrial diseases are quite prevalent and cause devastating impacts to patients and families. The complexity of mitochondrial genetics and disease transmission makes diagnosis, as well as treatment, extremely difficult. It is tantamount that females with a severe mtDNA disease have access to genetic screening and assisted reproductive technologies to avoid transmitting the disease to their offspring. For women who have high levels of heteroplasmy or are homoplasmic for an mtDNA mutation, current screening methods may not be adequate. The HFEA publicly endorsed PNT and ST as potentially useful techniques to prevent the transmission of severe mtDNA diseases; however, more studies need to be done on the safety and efficacy of such methods. The HFEA should also continue to engage with the public to discuss social and ethical implications of this work. It is our hope that more research on the genetics of mitochondrial diseases will lead to more reproductive options for expectant families as well as better treatment options for those currently suffering from a mitochondrial disease [39].

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## References

- Hatefi Y (1985) The mitochondrial electron transport and oxidative phosphorylation system. *Annu Rev Biochem* 54: 1015-1069.
- Cheng Z, Ristow M (2013) Mitochondria and metabolic homeostasis. *Antioxid Redox Signal* 19: 240-242.
- Saada A (2014) Mitochondria: mitochondrial OXPHOS (dys) function *in vivo*--the use of primary fibroblasts. *Int J Biochem Cell Biol* 48: 60-65.
- Gross A, McDonnell JM, Korsmeyer SJ (1999) BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 13: 1899-1911.
- Gross A, McDonnell JM, Korsmeyer SJ (1999) BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 13: 1899-1911.
- Martinou JC, Youle RJ (2011) Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Dev Cell* 21: 92-101.
- Renault TT, Chipuk JE (2014) Death upon a kiss: mitochondrial outer membrane composition and organelle communication govern sensitivity to BAK/BAX-dependent apoptosis. *Chem Biol* 21: 114-123.
- Gray MW, Burger G, Lang BF (1999) Mitochondrial evolution. *Science* 283: 1476-1481.
- Clayton DA (1982) Replication of animal mitochondrial DNA. *Cell* 28: 693-705.
- Pohjoismäki JL, Goffart S (2011) Of circles, forks and humanity: Topological organisation and replication of mammalian mitochondrial DNA. *Bioessays* 33: 290-299.
- Veltri KL, Espiritu M, Singh G (1990) Distinct genomic copy number in mitochondria of different mammalian organs. *J Cell Physiol* 143: 160-164.
- Nussbaum R, McInnes RR, Willard HF (2007) *Thompson & Thompson Genetics in Medicine*. (7th edn), Saunders-Elsevier, Philadelphia
- Anderson S, de Bruijn MH, Coulson AR, Eperon IC, Sanger F, et al. (1982) Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. *J Mol Biol* 156: 683-717.
- Spelbrink JN (2010) Functional organization of mammalian mitochondrial DNA in nucleoids: history, recent developments, and future challenges. *IUBMB Life* 62: 19-32.
- Brown WM, George M Jr, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci U S A* 76: 1967-1971.
- Kerr DS (2010) Treatment of mitochondrial electron transport chain disorders: a review of clinical trials over the past decade. *Mol Genet Metab* 99: 246-255.
- Schapira AH (2006) Mitochondrial disease. *Lancet* 368: 70-82.
- Saneto RP, Sedensky MM (2013) Mitochondrial disease in childhood: mtDNA encoded. *Neurotherapeutics* 10: 199-211.
- DiMauro S, Hirano M, Schon EA (2006) Approaches to the treatment of mitochondrial diseases. *Muscle Nerve* 34: 265-283.
- Thorburn DR, Dahl HH (2001) Mitochondrial disorders: genetics, counseling, prenatal diagnosis and reproductive options. *Am J Med Genet* 106: 102-114.
- Sato M, Sato K (2013) Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. *Biochim Biophys Acta* 1833: 1979-1984.
- Carling PJ, Cree LM, Chinnery PF (2011) The implications of mitochondrial DNA copy number regulation during embryogenesis. *Mitochondrion* 11: 686-692.
- Rosignol R, Faustin B, Rocher C, Malgat M, Mazat JP, et al. (2003) Mitochondrial threshold effects. *Biochem J* 370: 751-762.
- Human Fertilisation and Embryology Authority (2012) UK birth rates following IVF or ICSI fertility treatment. Retrieved June 20, 2014 from <http://www.hfea.gov.uk/2588.html>
- Harding AE, Holt IJ, Sweeney MG, Brockington M, Davis MB (1992) Prenatal diagnosis of mitochondrial DNA8993 T---G disease. *Am J Hum Genet* 50: 629-633.
- Bai RK, Wong LJ (2004) Detection and quantification of heteroplasmic mutant mitochondrial DNA by real-time amplification refractory mutation system quantitative PCR analysis: a single-step approach. *Clin Chem* 50: 996-1001.
- Jackson LG, Zachary JM, Fowler SE, Desnick RJ, Golbus MS, et al. (1992) A randomized comparison of transcervical and transabdominal chorionic-villus sampling. The U.S. National Institute of Child Health and Human Development Chorionic-Villus Sampling and Amniocentesis Study Group. *N Engl J Med* 327: 594-598.
- Eddleman KA, Malone FD, Sullivan L, Dukes K, Berkowitz RL, et al. (2006) Pregnancy loss rates after midtrimester amniocentesis. *Obstet Gynecol* 108: 1067-1072.
- White SL, Shanske S, McGill JJ, Mountain H, Geraghty MT, et al. (1999) Mitochondrial DNA mutations at nucleotide 8993 show a lack of tissue- or age-related variation. *J Inher Metab Dis* 22: 899-914.
- Human Fertilisation and Embryology Authority (2014) Third scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: Update, 2014. Retrieved June 20, 2014 from [http://www.hfea.gov.uk/docs/Third\\_Mitochondrial\\_replacement\\_scientific\\_revie\\_w.pdf](http://www.hfea.gov.uk/docs/Third_Mitochondrial_replacement_scientific_revie_w.pdf)
- Neupane J, Vandewoestyne M, Heindryckx B, Ghimire S, Lu Y, et al. (2014) A systematic analysis of the suitability of preimplantation genetic diagnosis for mitochondrial diseases in a heteroplasmic mitochondrial mouse model. *Hum Reprod*. 29: 852-859. doi: 10.1093/humrep/deu016
- Cohen J, Scott R, Schimmel T, Levron J, Willadsen S (1997) Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. *Lancet* 350: 186-187.
- Barritt J, Willadsen S, Brenner C, Cohen J (2001) Cytoplasmic transfer in assisted reproduction. *Hum Reprod Update* 7: 428-435.

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34. Takeuchi T, Neri QV, Katagiri Y, Rosenwaks Z, Palermo GD (2005) Effect of treating induced mitochondrial damage on embryonic development and epigenesis. *Biol Reprod* 72: 584-592.
  35. Hwang JL, Lin YH, Tsai YL (2000) In vitro maturation and fertilization of immature oocytes: a comparative study of fertilization techniques. *J Assist Reprod Genet* 17: 39-43.
  36. Wilding M, Dale B, Marino M, di Matteo L, Alviggi C, et al. (2001) Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. *Hum Reprod* 16: 909-917.
  37. Craven L, Tuppen, HA, Greggains GD, Harbottle SJ, Murphy JL, et al. (2010) Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature* 465: 82-85. doi: [10.1038/nature08958](https://doi.org/10.1038/nature08958)
  38. Tachibana M, Sparman M, Sritanaudomchai H, Ma H, Clepper L, et al. (2009) Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature* 461: 367-372. doi:[10.1038/nature08368](https://doi.org/10.1038/nature08368)
  39. Tachibana M, Amato P, Sparman M, Woodward J, Sanchis DM, et al. (2013) Towards germline gene therapy of inherited mitochondrial diseases. *Nature* 493: 627-631.