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# Mitochondrial transfer: Implications for assisted reproductive technologies

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ABSTRACT

The use of mitochondrial transfer as a clinic procedure is drawing closer to reality. Here we provide a detailed overview of mitochondrial transfer techniques – both established and recent – including pronuclear, spindle, ooplasmic and blastomere transfer. Reasons as to why some techniques are more suitable for the prevention of mitochondrial DNA disease than others, as well as the advantages and disadvantages of each methodology, are discussed. The possible clinical introduction of these techniques has raised concerns about the adverse effects they may have on resultant embryos and offspring. Success rates of each technique, embryo viability and developmental consequences post mitochondrial transfer are addressed through analysis of evidence obtained from both animal and human studies. Counterarguments against potential mitochondrial-nuclear genome incompatibility are also provided. Additional clinical applications of mitochondrial transfer techniques are discussed. These include the rescue or enhancement of fertility in women of advanced maternal age or those suffering from diabetes. An alternative to using mitochondrial DNA transfer for germ line therapies is the therapeutic use of somatic cell nuclear transfer for the generation of personalised stem cells. Although ethically challenging, this method could offer patients already suffering from mitochondrial DNA diseases a novel treatment option.



**Contents**



## 1. Introduction

Mitochondria are the energy-producing powerhouses of the cell. Their function is dependent on proteins transcribed from nuclear and mitochondrial DNA (mtDNA). Mitochondrial DNA is circular in

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structure and contains 37 genes. mtDNA diseases are inherited by all offspring irrespective of gender, as all mitochondria in the embryo originate from oocyte cytoplasm (Giles et al., 1980). With progressive genetic technologies in detecting disease-associated mtDNA mutations, over 300 rRNA/tRNA mutations (http://www.mitomap.org/foswiki/bin/view/MITOMAP/MutationsRNA) and over 300 coding/control region point mutations have now been identified (http://www.mitomap.org/foswiki/bin/view/MITOMAP/MutationsCodingControl).

Disruption of essential metabolic pathways in persons suffering from mtDNA disease, especially in high energy-demanding organs, leads to severe disability and early death. At present, no preventative treatments are available to prospective parents who are afflicted and wish to conceive (Pfeffer et al., 2012). Clinical diagnosis is challenging due to varying symptoms and phenotypes, even within a single family. This is due to mitochondrial heteroplasmy, where mutational load at the cellular level determines phenotype: low mutational load will result in asymptomatic phenotypes or low severity, but once the number of affected mitochondria exceed a certain threshold, patients become increasingly symptomatic. There may also be unequal distribution of mutated mtDNA between organs depending on mitochondrial segregation patterns during fetal development, giving rise to various phenotypes depending on the levels of mutated mtDNA present and the organs involved (Larsson and Clayton, 1995).

This review focuses on “mtDNA disease”, not “mitochondrial disease”, the latter referring to disorders that are caused by mutations in both mtDNA and nuclear DNA. In the case of nuclear DNA mutations, Mendelian genetics are applicable (Angelini et al., 2009), whereas mtDNA mutations are inherited maternally (Giles et al., 1980). Only around 20% of disorders involving defective mitochondrial oxidative phosphorylation (OXPHOS) are accounted for by mutations in mtDNA (Darin et al., 2001).

Research in both animals and humans has offered hope for the prevention of mtDNA disease via assisted reproductive technology (ART) techniques involving micromanipulation. This review explores techniques that could be used for the prevention of mtDNA disease transmission, their additional potential uses in the clinical setting and the concern of mitochondrial-nuclear genome mismatch.

## 2. Mitochondrial transfer techniques

### 2.1. Pronuclear transfer

Pronuclear transfer (Fig. 1A) involves the transfer of pronuclei from one zygote to another (Craven et al., 2010). This technique first requires fertilisation of healthy donated egg/s (provided by the mitochondrial donor) with the intending male parent sperm. Simultaneously, the intending mother's affected oocytes are fertilised with the intending father's sperm. Both sets of fertilised oocytes are allowed to develop to the early zygote stage where the pronuclei are visible. Using micromanipulation equipment, the pronuclei of zygotes formed from donated oocytes are removed within a karyoplast, and discarded. Therapeutic pronuclear transfer involves the movement of two pronuclei from the affected zygotes (also in the form of a karyoplast), into the enucleated healthy zygotes. The resulting zygotes contain nuclear DNA from each of the intending parents and a donor's mtDNA.

Using this method, Craven et al. (2010) have demonstrated less than 2.0% carryover of mtDNA between abnormally fertilised zygotes that possessed either one or three pronuclei. They also demonstrated that pronuclear transfer was compatible with onward in vitro embryo development. Blastocyst development rate was approximately half (17%) of that found amongst normally fertilised embryos (32%). This can be explained by the lack of a reliable morphological factor that can be used to differentiate between male and female pronuclei in zygotes. When pronuclei were transferred from the abnormally fertilised zygotes in this study, the authors could not be certain whether they were reconstructing zygotes with one female and one male pronucleus. If the resultant zygote is deficient in one or the other genome, development will be impaired.

A disadvantage from an ethical point of view is that half of the embryos (affected zygotes which will be enucleated and have their nuclear genetic material transferred) will be destroyed. However, unlike spindle transfer, in vitro fertilisation (IVF) can be performed if the sperm parameters so permit, and intracytoplasmic sperm injection (ICSI) is not necessarily required. Another advantage of pronuclear over spindle transfer is that pronuclei can be more easily visualised than spindles, using a conventional inverted microscope. During the process of nuclear transfer, the karyoplast is placed within the perivitelline space and then allowed to fuse to the donor cytoplast using the inactivated viral vector SeV (Sendai virus, also known as HVJ-E) (Tachibana et al., 2009; Craven et al., 2010). This raises concern about the possible effects of a viral vector on an embryo or its development. Tachibana et al. (2009) demonstrated the lack of SeV genetic material in reconstructed embryos by reverse transcription (RT)-PCR analysis, indicating that the viral vector does not pose any potential threats in the offspring or further generations. As this is a novel technique, pronuclear transfer has only been applied to abnormally fertilised zygotes, which possessed either one or three pronuclei (Craven et al., 2010). This method needs to be assessed in a primate animal model, as has been done with spindle transfer, in order to prove its efficacy and safety (Tachibana et al., 2009).

### 2.2. Spindle transfer

Spindle transfer (ST) (Fig. 1B) aims to achieve the same results as pronuclear transfer – inheritance of parental nuclear DNA and donor mtDNA in the offspring. In this method, the transfer of parental nuclear DNA occurs before fertilisation (Tachibana et al., 2009). This technique involves excision of the metaphase II spindle from the donor oocyte, which will provide the cytoplasmic constituent (including mitochondria) to the embryo. Again, contained within a karyoplast, the donor nuclear DNA will be discarded. The chromosome-spindle complex will then be removed from the oocyte of the intending mother, and transferred to the enucleated healthy donor oocyte (Tachibana et al., 2009).

A rhesus macaque study using this technique resulted in the birth of three healthy offspring (Tachibana et al., 2009). The presence of nuclear DNA originating from spindle donors and mtDNA from recipient oocytes was established though genetic analysis. No spindle donor mtDNA was observed in the offspring, demonstrating efficient mitochondrial replacement. Postnatal follow up analysis into adulthood (from birth to 3 years old) has shown that routine blood and body weight measurements were comparable to age-matched controls (Tachibana et al., 2013).

The same research group has also implemented this technique in human oocytes to study the feasibility of fertilisation, embryo development and embryonic stem cell (ESC) yield (Tachibana et al., 2013). Fertilisation rate in ST oocytes was comparable to controls (73% versus 75%, respectively). Although a substantial number of ST zygotes (52%) contained abnormal numbers of pronuclei, showing abnormal fertilisation, blastocyst development (62%) in normally fertilised zygotes was similar to controls. ESC isolation rates from ST blastocysts (38%) were also similar to controls, and all cell lines exhibited the presence of donor mtDNA only and normal karyotypes. The relatively high incidence of abnormal fertilisation was surprising, as it was not observed previously in the 2009 primate study. Possibly, human meiotic spindles were prematurely activated during the ST procedure resulting in impaired continuation of meiosis post fertilisation. Analysis after immunolabeling with α- and β-tubulins revealed that some ST oocyte spindles had prematurely progressed to late anaphase II (Tachibana et al., 2013). ESCs derived from the abnormally fertilised zygotes exhibited triploidy, stemming from the presence of two sets of the maternal genome. This finding showed that some oocytes failed to complete meiosis and the chromosomes failed to segregate into the second polar body, probably resulting from premature activation. This study highlights the efficacy and potential use of ST for the prevention of mtDNA disease in

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