International Research Journal of Natural and Applied Sciences
ISSN: (2349-4077)
Impact Factor 5.46 Volume 7, Issue 06, June 2020
Website- www.aarf.asia, Email : editoraarf@gmail.com

REVIEW: PREVENTION OF TRANSMISSION OF MITOCHONDRIAL DNA DISEASE THROUGH PRONUCLEAR TRANSFER

Hettiarachchige Dona Hasindri[1], Udesh Nilanga Perera[1*]
[1]Undergraduate Department of Biomedical Science, International College of Business and Technology, No. 36, De Krester Place, Bambalapitiya, Sri Lanka.

Abstract

Mitochondrial DNA disease is a maternally inherited disease caused due to the presence of mutant mtDNA (mitochondrial DNA). Research has been conducted to prevent the transmission of the mtDNA disease by preventing the transfer of mutant mtDNA. Pronuclear Transfer (PNT) is one such therapy to prevent the transmission of mtDNA. At first, the technique was tested on abnormally fertilized human zygotes. Following its success, the technique was tested on normally fertilized human zygotes. PNT showed a minimal mtDNA carryover of <2%. Two successful births have been reported following PNT. This review article provides an understanding of the mtDNA disease, unpredictable nature of its inheritance and the need to opt for prevention methods in transmitting the disease. An integrated knowledge on the prevention of mtDNA through PNT, its effectiveness and future research are provided.

Keywords - Mitochondrial DNA Disease, Pronuclear Transfer, Polar Body Transfer, Mitotic Spindle Transfer.

1. INTRODUCTION

Mitochondria is the primary source of intracellular energy produced in the form of ATP[1]. It is the only location where extra chromosomal DNA is present in the cell which is the mitochondrial DNA. Mitochondrial DNA is strictly maternally inherited. The mitochondria is under the control of both the nuclear genome and the mitochondrial genome. The mitochondrial genome consists of multiple copies of dsDNA (double stranded Deoxy Ribose Nucleic Acid) molecules[15]. For a healthy mitochondrial function 1500 proteins are needed in which 13 proteins are encoded by mtDNA and the rest by nDNA (nuclear Deoxy Ribose Nucleic Acid)[5]

The mutations in the mitochondrial DNA leads to a broad spectrum of life-threatening disorders which are collectively called as 'mitochondrial DNA mutations'. ATP is the key molecule produced by mitochondria via oxidative phosphorylation. It is an essential energy source for the body and drives all the biological functions that allow cells to function. Moreover, ATP is a critical signalling molecule that allows cells throughout the body to communicate with one another. Therefore the mtDNA mutations largely affect organs highly dependent on oxidative phosphorylation such as nervous system, heart and skeleton muscles[7]. Mitochondrial impairment thus resulted also play a key role in the pathogenesis of many neurodegenerative disorders such as Alzheimer's, Parkinson's disease [13]

Earlier, mtDNA disorders were considered as a rare group of disorders, where only one in a million individuals got affected. However, the recent epidemiological studies show that approximately 1 in 5000 individuals gets affected [9]. Mitochondrial disorders still have no curative treatment. It often leads to severe disability and death [7]. Currently, women carrying mitochondrial DNA who wish to have healthy children without the risk of developing mtDNA disease can consider options such as Adoption, In Vitro Fertilization using healthy donor eggs, Preimplantation Genetic Diagnosis and Prenatal Diagnosis. However, these options are not always ideal due to various limitations.

Mitochondrial DNA disease is not a single disease but a wide spectrum of diseases. Even a classification of the diseases remain difficult as there are numerous types of symptoms some of which remain yet to be identified [13]

Even though certain therapies and treatments such as medications, special diets or avoidance of triggers are available, they are mainly aimed at preventing or slowing down the complications of the condition. Moreover, such treatments are mostly ineffective as they do not meet with the wide variety of symptoms associated with the disease.

Therefore it is of paramount importance to consider methods to prevent the transmission of the mtDNA, from mother to offspring from the birth itself. Currently, the scientists have approached this methodology through Mitochondrial Genome DNA Replacement Therapy (MGRT) which replace abnormal mitochondria with normal mitochondria. MGRT includes three techniques namely Maternal Spindle Transfer (MST), Pronuclear Transfer (PNT) and Polar Body Transfer (PBT).

In this review article, the use of Pronuclear Transfer Technique in the prevention of mtDNA disease is discussed.

2. INHERITANCE OF MITOCHONDRIAL DNA DISEASE.

Mitochondrial DNA (mtDNA) is strictly maternally inherited. Therefore human mitochondrial diseases caused by mutated mitochondrial DNA is maternally inherited.

Either before or after fertilization, the mixing of maternal and paternal mtDNA is averted by preventing transmission of paternal mtDNA through various mechanisms. Such mechanisms are, dilution of paternal mtDNA which is present in low number by the excess of oocyte mtDNA and by the selective degradation of paternal mtDNA or mitochondria themselves. Thus only the maternal mitochondrial DNA will be transmitted to the offspring [12].

In a cell, there are multiple copies of mitochondrial DNA. Mitochondrial DNA diseases are resulted due to the mutations in the copies of mitochondrial DNA in a cell. The mutations can occur in two ways, either as heteroplasmic; where both wild type and mutant mtDNA co-exist or as homoplasmic; where all the mitochondria in the cell are mutated [6]

An important factor which determines the severity of the disease condition is the level of heteroplasmy in the patient. Presence of higher levels of mutated mtDNA causes severe conditions whereas the presence of low levels of mutated mtDNA (<30%) would not cause any clinical symptoms[6]. Heteroplasmic individuals are healthy if the cells have sufficient numbers of normal mitochondria to provide adequate respiratory functions[10].

The transmission of the mutated mitochondrial DNA from a heteroplasmic mother to the offspring follows a phenomenon called ‘genetic bottleneck’ which occurs during the early stages of Oogenesis. The transmission is a random process which generates a variability between offsprings. Accordingly, an unaffected female can give birth to either unaffected, mildly or severely affected offspring[9].

© Association of Academic Researchers and Faculties (AARF)

3. OPTIONS FOR WOMEN CARRYING MUTATED MITOCHONDRIAL DNA TO HAVE A HEALTHY OFFSPRING.

Currently, a woman carrying mutated mtDNA can consider the following options to have a healthy offspring. However, these options have certain difficulties and limitations which are outlined in the table below.

Option	**Limitations**
Adoption	An adopted child will not be genetically related to the parents
In Vitro fertilization (IVF) using donor egg	Donor oocyte from an unaffected individual is used. This method ensures that no mutated mtDNA will be transmitted to the offspring. The resultant child will not be genetically related to the mother[16]
Preimplantation Genetic Diagnosis (PGD)	Embryos are created using IVF. Cells from the early stages of the embryo are removed and tested for the presence of mutated mtDNA. Healthy embryos or embryos with lower levels of mutated DNA are selected and implanted. The technique is suitable only for women carrying lower levels of heteroplasmy and not for women with higher levels of mutated genes or homoplasmy[6].
Prenatal Diagnosis (PND)	Via chronic villus sampling, the fetus is tested during the late first trimester. The couple can choose to terminate the pregnancy if the fetus carries the mutation. This method helps to reduce the risk of transmission of the mutation but it doesn't eliminate mutation.

Table1: Options for women carrying mutated mitochondrial DNA to have healthy offspring

Considering the limitations of the above options, researches have proposed 'Mitochondrial Genome Replacement Therapy' (MGRT) to prevent transmission of mtDNA disease. MGRT includes three methods of prevention namely, Mitotic Spindle Transfer (MST), Polar Body Transfer (PBT) and Pronuclear Transfer (PNT)

3.1 Mitotic Spindle Transfer (MST)

In this method, the spindle chromosome complex of the affected mother is removed and transferred into the donor's oocyte from which the chromosome complex was previously removed. Next, the reconstructed egg is fertilized with the father's sperm. Mature metaphase II oocytes are used here [14]

3.2 Polar Body Transfer

In this method, both the mother's oocyte and the donor's oocyte will be fertilized by the father's sperm separately. Next, the second polar body of both oocytes are removed. Next, the second polar body of the donor will be inserted to mother's oocyte.

The most recent discovery in MGRT, the Pronuclear Transfer Method (PNT) is discussed in detail in this review.

4. METHODOLOGY FOLLOWED IN PRONUCLEAR TRANSFER (PNT).

After receiving the research license from the Human Fertilization and Embryology Authority (HFEA), the research on the pronuclear transfer technique was conducted.

Firstly the technique was tested on abnormally fertilized zygotes. Abnormally fertilized zygotes are unipronuclear or tripronuclear zygotes generated by human IVF. They are resulted when an oocyte fertilizes with more than one sperm.

The maternal and paternal pronuclei from the abnormally fertilized zygote were removed. Next, an enucleated healthy donor oocyte was taken and the above two pronuclei removed were transferred to the enucleated donor. The pronuclei will be removed along with a small amount of cytoplasm called as the karyoplast. The karyoplast can contain minute amounts of mtDNA.

The pronuclei thus removed were placed under the zona pellucida of the healthy donor oocyte. Later the two pronuclei were fused and reconstituted zygote was formed. It was cultured for 6-8 days in vitro.

Next, the microsatellite markers of resultant embryos were analyzed. The analysis confirmed that the pronuclei did not result in any change in the nuclear genotype of the embryo and that the embryos contained only the nuclear genotype of the pronuclear donor.

Following PNT, 10 out of 44 (22.7%) of one pronuclear transfer zygotes and 8 out of 36 (22.2%) of two pronuclear transfer zygotes developed to 8> cell stage. Furthermore, no difference in embryo development at any stage was seen irrespective of one or two pronuclei nature. Following two pronuclei transfer, 8.3 % of embryos developed to the blastocyst stage. This amount is approximately 50% of the blastocyst formation rate for unmanipulated abnormally fertilized embryos[2]. Accordingly, the inability of the remaining 50% to reach the blastocyst stage could be due to the reduced developmental capacity and the considerable variations in the chromosomal constitution in the abnormally fertilized zygotes.[3]

Ensuring that the onward development of pronuclear transferred zygotes is possible, next the carryover of the mtDNA of the pronuclei donor in the reconstituted embryo was tested [2]. Since the karyoplast is also transferred along with the pronuclei, a small fraction of the mtDNA, termed carryover will get into the recipient oocyte. Therefore it is inevitable to transfer pronuclei without any mutant mitochondria [17]

Next, the non-coding mtDNA control region from both the pronuclei donor and recipient were sequenced. It showed that the presence of polymorphic mtDNA variants unique to donor and recipient [2].

Next, Hot last cycle PCR RFLP assays were developed for the specific mtDNA variants observed form the previous sequencing step. The results of the assays depicted the variations in the amount of mtDNA genotype in pronuclear recipient zygote. These variations are due to the mutated mtDNA transferred from the pronuclear donor. Therefore these findings confirm the presence of mtDNA carryover during PNT [2].
Researchers then tried to see whether the proportion of mtDNA genotype of the pronuclei recipient varies between blastomeres formed from the reconstituted embryo. Accordingly, they found that 1/8 embryos had no detectable mtDNA from pronuclei donor zygote. The remaining seven embryos contained various levels of donor mtDNA[2].
The researchers next took measures to reduce the mtDNA carryover. This was done by carefully manipulating the pronuclear karyoplast and removing the pronuclei with a minimum amount of cytoplasm[2].
Afterwards, Hot last cycle PCR – RFLP assay was conducted. The assay results showed that 4/9 embryos contained undetectable levels of mtDNA carryover. Also, the average mtDNA carryover in all the remaining embryos were <2%. These embryos also showed lesser variations in mtDNA carryover between individual blastomeres. Furthermore, these levels of mtDNA variations were equivalent to those seen in unaffected individuals in epidemiological studies[2].
Having succeeded in PNT with minimum mtDNA carryover in abnormally fertilized zygotes, the next step was to test for PNT in normally fertilized zygotes.
Surprisingly the normally fertilized zygote did not tolerate the PNT as in abnormally fertilized zygotes. To overcome this situation, the researchers followed an alternative strategy where the pronucleus was transferred shortly after they first appeared. Such PNT is termed as early PNT (ePNT)
Accordingly, ePNT showed 92% of survival in the normally fertilized egg, while the late PNT (LtPNT) showed only 59%. Moreover, these zygotes showed normal division to 2 cell stage [8]
Following the success of PNT in normally fertilized zygotes, this technique was practised on women carrying mtDNA disease.
In 5th January 2017, a child conceived using PNT was successfully delivered. Another such successful delivery was reported on 19th February 2017 [11]

5. CHALLENGES FACED IN PNT

Four major challenges were faced in terms of ethically, legally, safeness and efficiency when accessing license to perform PNT [6]. Ethical issues regarding PNT was mainly on the fact that PNT results in a genetic modification in the germline which would pass to future generations.

Another issue which arose along with this is that the child born following PNT would have a genetic link to three people (Three parent IVF)

However, the use of the term 'Three parent IVF' is misleading since the pronuclei recipient only transfers the mitochondrial genome to the zygote, not the nuclear genome. The mitochondrial genome will not encode for any unique genetic identity of the zygote. It will only affect the metabolic fitness of the child. Moreover, according to UK law, the donor will have no legal responsibility to the child [6]

6. CONCLUSIONS

Mitochondrial DNA disease is a broad spectrum of diseases which occurs due to the presence of various levels of mutant mtDNA. The disease is strictly maternally inherited. These disorders often lead to severe complications and death.

The mtDNA mutations can occur in two ways namely heteroplasmic and homoplasmic nature. The inheritance of mtDNA disease is unpredictable as it follows a genetic bottleneck effect. The options currently available for women carrying mutant mtDNA have limitations. Therefore, MGRT is used in which PNT is one technique.

At first, PNT was tested on abnormally fertilized human zygotes. Following its success, the normally fertilized human zygotes were tested for PNT. The failure in the tolerance of the PNT in normally fertilized zygotes was overcome by the use of ePNT. To date, two successful births upon the use of PNT has been reported.

Research on the prevention of transfer of mtDNA disease has shown a remarkable advance over the past years. Even though it is inevitable to prevent mtDNA carryover, PNT has the potential to minimize the mtDNA carryover to <2%, thus preventing the transmission of mtDNA disease. Further research has to be done to minimize the mtDNA carryover to less than the current 2%[4].

Another technology emerged to prevent the transmission of mutant mtDNA is the use of mitochondria targetted nucleases namely mtZFNs (Mitochondrial targetted zinc finger nucleases) and mitoTALENS (mitochondrially targeted transcription activator-like effector nucleases). Both these nucleases remove mutant mtDNA and reduce the heteroplasmy level[4]. This technique should be further developed to allow the use of it in clinical trials [11].

CRISPR/Cas 9 system is of widespread use in genome editing. There have been concerns using this approach in mtDNA to prevent transmission of mutated mtDNA. However, further research has to be done in this regard to confirm the safety of this technique[4]

ACKNOWLEDGEMENT
The authors wish to thank the International College of Business and Technology, Sri Lanka for the financial support.

REFERENCES

[1]. Chinnery PF, Elliott HR, Hudson G, Samuels DC, Relton CL.(2012) Epigenetics, epidemiology and mitochondrial DNA diseases. *Int J Epidemiol*, 41(1),177–187.

[2]. Craven, L., Tuppen, H. A., Greggains, G. D., Harbottle, S. J., Murphy, J. L., Cree, L. M., Murdoch, A. P., Chinnery, P. F., Taylor, R. W., Lightowlers, R. N., Herbert, M., & Turnbull, D. M. (2010). Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature*, *465*(7294), 82–85.

[3]. Craven, L., Elson, J. L., Irving, L., Tuppen, H. A., Lister, L. M., Greggains, G. D., Byerley, S., Murdoch, A. P., Herbert, M., & Turnbull, D. (2011). Mitochondrial DNA disease: new options for prevention. *Human molecular genetics*, *20*(R2), R168–R174.

[4]. Craven, L., Alston, C.L., Taylor, R.W. and Turnbull, D.M. (2017). Recent Advances in Mitochondrial Disease. *Annual Review of Genomics and Human Genetics*, 18(1), pp.257–275.

[5]. Gorman, G.S., Chinnery, P.F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., Suomalainen, A., Thorburn, D.R., Zeviani, M. and Turnbull, D.M. (2016). Mitochondrial diseases. *Nature Reviews Disease Primers*, 2(1). 1-22

[6]. Herbert, M & Turnbull, H (2015) .Mitochondrial replacement to prevent transmission of mitochondrial DNA disease. *EMBO reports*, 16 (5), 539 -540

[7]. Herbert, M. and Turnbull, D. (2018). Progress in mitochondrial replacement therapies. *Nature Reviews. Molecular Cell Biology*, 19(2),71–72.

[8]. Hyslop, L.A., Blakeley, P., Craven, L., Richardson, J., Fogarty, N.M.E., Fragouli, E., Lamb, M., Wamaitha, S.E., Prathalingam, N., Zhang, Q., O'Keefe, H., Takeda, Y., Arizzi, L., Alfarawati, S., Tuppen, H.A., Irving, L., Kalleas, D., Choudhary, M., Wells, D., Murdoch, A.P., Turnbull, D.M., Niakan, K.K. and Herbert, M. (2016). Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. *Nature*, 534(7607),383–386.

[9]. Khan, N.A., Govindaraj, P., Meena, A.K. and Thangaraj, K. (2015). Mitochondrial disorders: challenges in diagnosis & treatment. *The Indian journal of medical research*, 141(1), 13–26.

© Association of Academic Researchers and Faculties (AARF)

[10]. Marlow, F. (2017). Mitochondrial matters: mitochondrial bottlenecks, self-assembling structures and entrapment in female germ line. *Stem cell research*. 21,178-186

[11] Rai PK, Craven L, Hoogewijs K, Russell OM, Lightowlers RN. (2018) Advances in methods for reducing mitochondrial DNA disease by replacing or manipulating the mitochondrial genome. *Essays Biochem*. 62(3),455–465.

[12]. Sato, M & Sato, K. (2013). Maternal Inheritance Mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. *Biochimica et Biophysica Acta*. 1979 -1984

[13].Scarpelli, M., Todeschini, A., Volonghi, I., Padovani, A. and Filosto, M. (2017). Mitochondrial diseases: advances and issues. *The Application of Clinical Genetics*, 10, 21–26

[14]. Saxena, N., Taneja, N., Shome, P., & Mani, S. (2018). Mitochondrial Donation: A Boon or Curse for the Treatment of Incurable Mitochondrial Diseases. *Journal of human reproductive sciences*, *11*(1), 3–9

[15]. Taylor, R. W., & Turnbull, D. M. (2005). Mitochondrial DNA mutations in human disease. *Nature reviews. Genetics*, *6*(5), 389–402.

[16]. Turner, K. (2014). Current Methods for the Prevention of Transmission of Mitochondrial DNA Diseases. *Journal of Molecular and Genetic Medicine*, 08(03).

[17]. Wolf, D. P., Hayama, T., & Mitalipov, S. (2017). Mitochondrial genome inheritance and replacement in the human germline. *The EMBO journal*, *36*(15), 2177–2181.