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Enhancing Ivf Techniques To Prevent Mitochondrial Diseases Transmissin In India

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ABSTRACT
Mitochondria, the energy-producing organelles in cells, have their own maternally inherited DNA, and mutations in this mitochondrial DNA (mtDNA) can lead to severe genetic disorders. Mitochondrial Replacement Therapy (MRT), an advanced form of in vitro fertilization (IVF), offers a way to prevent the transmission of these mutations. Techniques like spindle transfer (ST), pronuclear transfer (PNT), and polar body transfer (PBT) involve replacing the nucleus of a mother's egg containing faulty mitochondria with that from a donor egg containing healthy mitochondria. These methods can be applied either before or after fertilization. One such method, ooplasmic spindle transfer (OST), pioneered by Dr. John Zhang, led to the birth of the first healthy baby using this technique. The UK and Australia have established clear regulatory frameworks for MRT, while countries like India are still navigating the cultural and ethical implications. Despite rapid technological growth, societal resistance in India poses challenges to adopting such reproductive innovations. This article focuses on the potential of MRT in India, its techniques, ethical concerns, and global experiences in implementing this promising therapy.

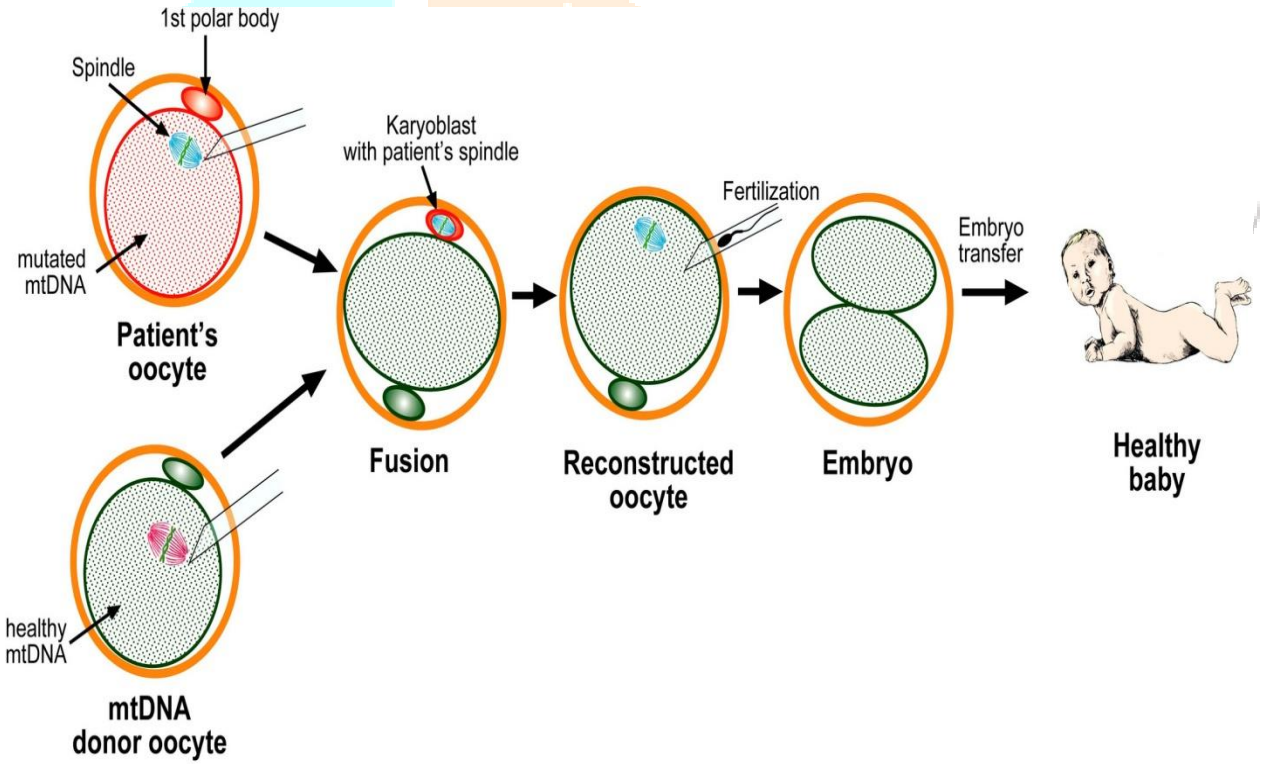
KEY WORDS: Mitochondrial Replacement Therapy (MRT), mtDNA Mutations, Pronuclear Transfer (PNT), Maternal Spindle Transfer (MST), Polar Body Transfer (PBT), Heteroplasmy, Three-Parent Baby, Assisted Reproductive Technology (ART)

INTODUCTION

A person's ability to express their genotype and phenotype can be significantly compromised by metabolic disorders, many of which are linked to mutations in mitochondrial DNA (mtDNA). These mutations commonly result in mitochondrial diseases, particularly affecting the respiratory chain, which is vital for cellular energy production. Unlike nuclear DNA (nDNA), mtDNA exists in multiple copies within each cell, and this heteroplasmy—where both normal and mutated mtDNA coexist—plays a critical role in determining the severity and onset of mitochondrial disorders. The complexity of mtDNA-related conditions makes diagnosis challenging, as not all individuals carrying mtDNA mutations exhibit clinical symptoms. Furthermore, although both parents may harbor or even suffer from mitochondrial-related mutations, only mothers transmit mtDNA to their offspring, making maternal inheritance a key concern in disease transmission.

Numerous human diseases have been associated with mtDNA mutations, and yet, therapeutic options remain scarce and largely ineffective. In response, advanced reproductive technologies have emerged—most notably, Mitochondrial Replacement Therapy (MRT). MRT involves transferring the nuclear genetic material from a woman's egg containing defective mitochondria into a donor egg that has healthy mitochondria but has had its nucleus removed. Techniques such as Maternal Spindle Transfer (MST), Germinal Vesicle Transfer (GVT), and Ooplasmic Spindle Transfer (OST) are at the forefront of this intervention. Once the reconstructed egg is fertilized with the father's sperm, the resulting embryo is implanted into the mother. Notably, OST was pioneered by Dr. John Zhang and his team at the New Hope Fertility Center in Mexico and the U.S., resulting in the birth of a healthy child—the first successful application of this technology.

MRT, also known as mitochondrial donation or nuclear genome transfer, offers hope to women at risk of passing on mitochondrial diseases to their children. The United Kingdom became the first country to formally regulate and approve MRT in 2015, followed by Australia in 2021. As the clinical application of MRT evolves rapidly, it is crucial to establish standardized guidelines and ethical frameworks to ensure safe and responsible use. Particularly in countries with limited or lenient reproductive laws, the ethical implications of germline modification, embryo manipulation, and donor involvement must be carefully addressed. While MRT holds immense potential for preventing the inheritance of mitochondrial disorders, its application must be guided by scientific evidence, robust regulation, and respect for ethical boundaries.



The commonly used term "three genetic parents" to describe children born through mitochondrial replacement therapy (MRT) is a mischaracterization and lacks scientific accuracy. This phrase has gained traction due to the involvement of a third party—the mitochondrial donor—in the reproductive process. However, the donor's contribution is limited solely to the mitochondrial DNA (mtDNA), which comprises less than 1% of the total genomic content in the resulting offspring. Unlike nuclear DNA (nDNA), which encodes the genetic instructions for traits such as physical appearance, intelligence, and personality, mtDNA is exclusively responsible for regulating cellular energy production through oxidative phosphorylation. Therefore, the donor's mtDNA does not influence inherited traits or familial characteristics, and the child's identity remains genetically determined by the nuclear DNA from the biological mother and father.

Mitochondrial replacement therapy is a revolutionary assisted reproductive technique developed to prevent the maternal transmission of mitochondrial diseases, which result from mutations in mtDNA. These mutations can severely impair the function of mitochondria—organelles known as the "powerhouses" of the cell—leading to a broad spectrum of metabolic disorders that often affect high-energy-demand organs such as the brain, heart, and muscles. Since mtDNA is exclusively maternally inherited, women with pathogenic mitochondrial mutations face the risk of passing these debilitating conditions to their children.To address this challenge, several MRT techniques have been developed, primarily including Cytoplasmic Transfer (CT), Maternal Spindle Transfer (MST), and Pronuclear Transfer (PNT).

Cytoplasmic Transfer (CT) involves injecting a small portion of cytoplasm, which contains healthy mitochondria, from a donor egg into the recipient's egg. This method has been explored for improving egg quality in women with infertility but has raised concerns about genetic heteroplasmy due to the mixing of mitochondrial populations.

Maternal Spindle Transfer (MST) is performed before fertilization and involves transferring the meiotic spindle apparatus, containing the mother's chromosomes, into a donor egg that has had its own spindle removed. The reconstructed egg is then fertilized with the father's sperm, and the embryo is implanted into the mother's uterus.

Pronuclear Transfer (PNT), in contrast, is carried out after fertilization. The pronuclei (containing nuclear DNA from the mother and father) are removed from a fertilized egg with defective mitochondria and inserted into a donor zygote that has healthy mitochondria and has had its own pronuclei removed.

These MRT techniques are designed to ensure that the resulting child inherits healthy mitochondria while maintaining genetic continuity with the intended parents. This advancement holds immense promise for families affected by mitochondrial disorders and is reshaping the future of reproductive medicine. However, it also raises important ethical, legal, and social considerations, particularly in terms of donor anonymity, long-term health monitoring, and regulatory oversight.

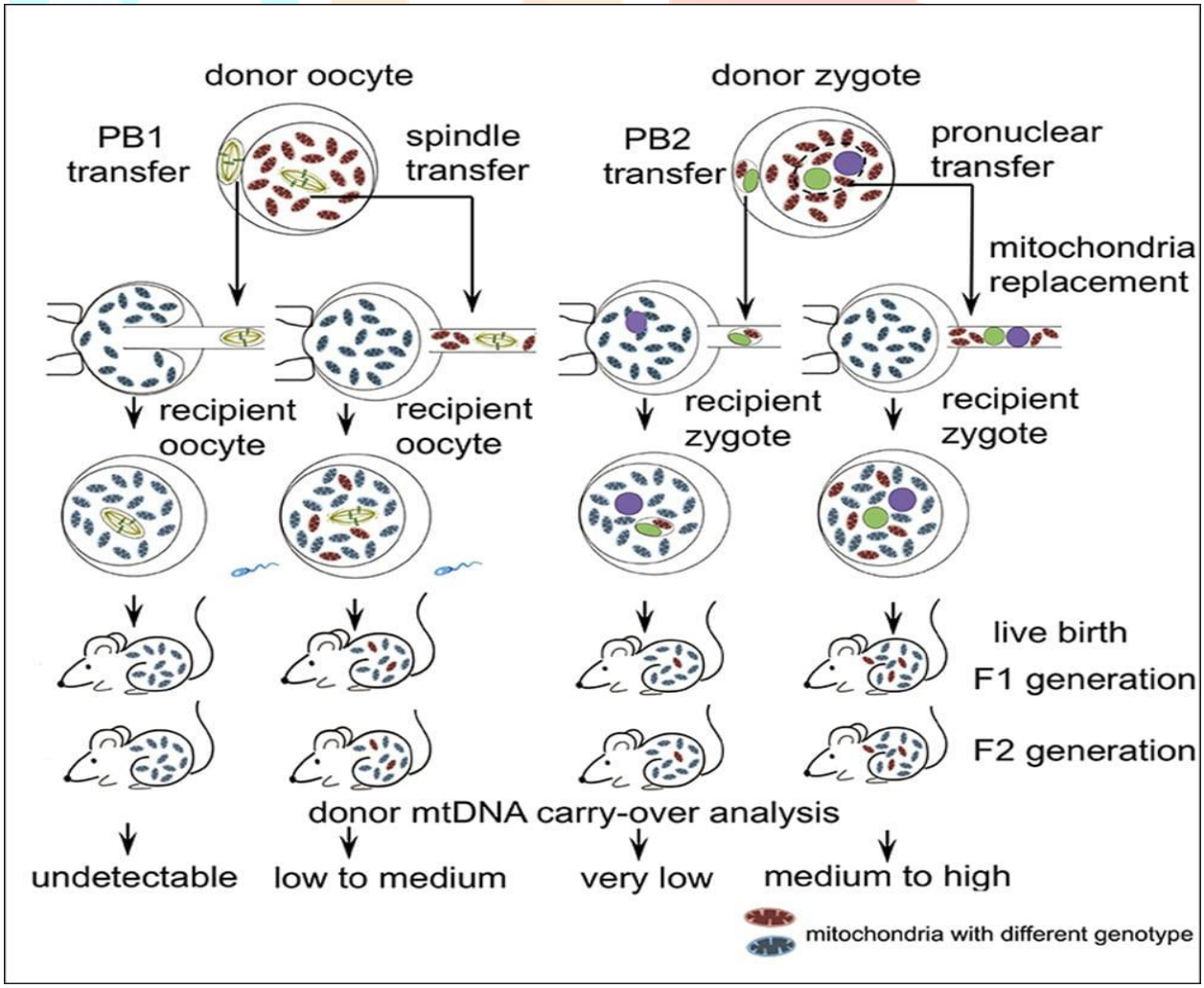
METHODOLOGY

Several approaches that are employed in the treatment of microchondrial replacement.Techniques including pronuclear transfer (PNT), spindle transfer (ST), polar body transfer (PBT), and germinal vesicle transfer (GVT) can be used to transfer the nuclear genome from oocytes or zygotes.[6]Pro nuclear transfer, or PNT, promotes efficient development up to the blastocyst stage without having any appreciable effect on aneuploidy or gene expression. Following optimization, the majority of PNT blastocysts (79%) had mtDNA carryover reduced to less than 2%, and a stem cell line generated from a PNT blastocyst with 4% mtDNA carryover exhibits a progressive rise in heteroplasmy, underscoring the importance of reducing carryover to the lowest possible levels.[7]The zygote's continuous development in vitro until the blastocyst stage is consistent with the low donor zygote mtDNA carry-over observed when pronuclei from incorrectly formed human zygotes are transferred.[8]The pro nuclear transfer process is used to remove the nuclear material following fertilization. The two poles of a patient's zygote, which are enclosed in the karyoplast, are removed and moved to a corresponding enucleated stage of the zygote cytoplast. This stage is derived from donors who have mitochondria that are intact, and the birth of living offspring in mice shows that this procedure is successful.[9] Still, because technique involves the disposal of zygotes, pronuclear transfer may cause ethical and theological issues in some cultures.[10]

Maternal spindle transfer, or MST, has been proposed as a way to prevent the spread of some diseases, and in Mexico, a disease-free baby was born in 2017. Potential problems in research governance and the accompanying criticism arose from the extension of MST to provide a potentially novel assisted reproductive technique to address infertility issues characterized by recurrent in vitro embryo development arrest caused by mitochondrial dysfunction and cytoplasm deficiencies of the oocyte. This applied technique is a great example of the need to strike "a balance between taking appropriate precautions and hampering innovation."[11]After

being separated in a tiny quantity of cytoplasm, the meiotic spindle of the patient's oocytes was transplanted into a donor oocyte that had already undergone enucleation. For up to six days following ICSI insemination, MST oocytes were cultured in a time-lapse incubator. Aneuploidy testing, mtDNA carryover level analysis, and biopsy were performed on blastocysts with good shape. The origin of the nuclear genome and mtDNA in the amniotic fluid, biopsied samples, and somatic tissues of the resulting newborns was verified using DNA fingerprinting and SNP analysis.[12]"To solve sterility problems that could not be otherwise solved" is not a very logical interpretation of the SNT research, which was carried out to prevent women from passing on mitochondrial disease to their children, as stated in the Regulations [13]MST-derived embryos have the ability to implant and successfully carry a pregnancy to term.[14]

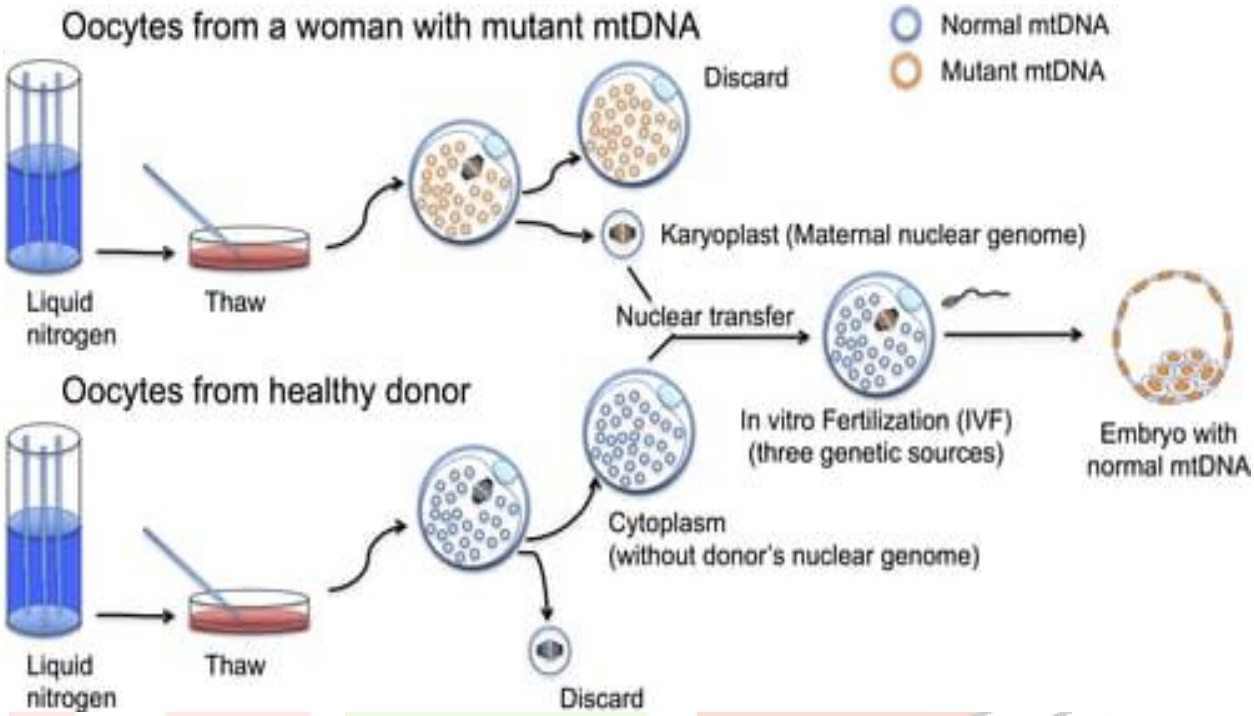
Polar body biopsy was initially used to detect anomalies that were caused by a single mother gene. The discovery of single gene anomalies in polar bodies was the subject of one of the most comprehensive studies reported by Rechitsky et al.[15] First polar bodies (PB1s) were transferred to cryopreserved enucleated metaphase II oocytes (PB1T), and fresh PB2s were taken from fertilized oocytes and used to replace the female pronucleus in donor zygotes. The reconstructed oocytes were then cultured to blastocyst after receiving an intracytoplasmic sperm injection (ICSI). A woman who had previously undergone several cycles of severe embryo fragmentation underwent cryopreserved PB1T; the blastocysts derived from PB1T were checked for aneuploidy but were not administered to the patient. Next-generation sequencing (NGS) was used to examine the chromosomes in biopsied trophectoderm cells of PBT-derived blastocysts[16] Hereditary examination of the first polar body enables the identification of oocytes that carry the mother unaffected gene in women who are heterozygous for a hereditary disorder and It is not possible to become pregnant with an embryo that has genetic defects by fertilizing and transferring these eggs to the mother[17]



GRAPHICAL REPRESENTATION OF POLAR GENOME BODY TRANSFER

ADVANTAGES:

The kind of tissue determines how many copies of mitochondrial DNA (mtDNA) are present in each cell. Mutations in the mtDNA can cause a variety of illnesses. The phenomenon known as heteroplasmy occurs when mutant mtDNA is commonly found as a subset of the total mtDNA population in a cell or tissue. There have been attempts to purposefully reduce or replace the mutant species because a certain level of heteroplasmy must be obtained before mitochondrial Dysfunction appears. **(19)**



Mitochondrial replacements by genome transfer in human oocytes

A woman with an mtDNA mutation may want to think about mitochondrial replacement therapy if she wants to have genetically related children who are healthy.

CONCLUSION:

In this review study, we therefore draw the conclusion that, in the present world, where kids are born to three parents, the mechanical replacement therapy procedure is highly beneficial. Despite its many benefits, scientists continue to consider this treatment to be among the most advanced assisted reproductive methods currently in use, despite the fact that there are many arguments in favor of it that have been made all over the world.Countries like India, in particular, are in dire need of this mitochondrial replacement therapy.

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Dr.D.Nivedita: Head of the department of pharm.d and assistant professor with eight years of experience in training the students in Srinivasa Rao College of Pharmacy accredited with A grade by NAAC affiliated by Andhra University and approved by PCI. Authorized various journals for the benefit of the pharmacy department and published many papers in various international journals.

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