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Review Article

Obstetrics and Gynaecology Section

The Intersection of Mitochondrial Replacement Therapy and In-vitro Fertilisation: A Review of Emerging Therapeutic Approaches

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ABSTRACT

Mitochondrial Replacement Therapy (MRT) is a development in the area of reproductive health, as it provides new possibilities for women who are at risk of transmitting mitochondrial diseases to their offspring. A cell's powerhouse, known as mitochondria, contains inherited Deoxyribonucleic Acid (DNA) called mitochondrial DNA (mtDNA) and is inherited only through the mother. Changes in specific areas of the mtDNA can lead to severe mitochondrial disorders affecting major bodily processes and might involve metabolic or neurological problems. New In-Vitro Fertilisation (IVF) procedures, incorporated into Pronuclear Transfer (PNT) and Maternal Spindle Transfer (MST), use MRT to transplant mtDNA along with a donor's mitochondria, thereby reducing the chances of passing on mitochondrial diseases. The advantage of MRT is the ability to minimise mitochondrial diseases, which is achievable through key techniques. However, MRT poses specific technical issues and raises ethical concerns, particularly concerning germline modifications. The following paper examines the clinical, ethical and technical aspects of MRT with regard to IVF compatibility, success rates and resulting health impacts.

Keywords: Artificial reproductive technology, Embryo development, Maternal spindle, Powerhouse, Pronuclear transfer, Transfer

INTRODUCTION

Cells require energy to function and mitochondria are organelles that supply it [1]. Two features of mitochondria are that they possess their own DNA and can only be inherited through the maternal line. A group of hereditary conditions known as mitochondrial illnesses is defined by abnormalities in oxidative phosphorylation (OXPHOS), which are brought on by mutations in the nuclear DNA (nDNA) or mtDNA genes that code for structural or functional mitochondrial proteins. In addition to being the most prevalent group of inherited metabolic illnesses, mitochondrial diseases also account for many inherited neurological problems [2]. One of the challenges in managing mitochondrial illnesses is the significant variation in clinical presentations among patients, which frequently causes delays in diagnosis. However, the development of next-generation sequencing techniques has contributed significantly to the improvement of diagnosis, especially for children [3]. With recent advancements in genetic testing and novel reproductive techniques, there is hope for reducing the inheritance of mitochondrial diseases. Although most patients with mitochondrial diseases cannot undergo effective treatment, guidelines have been established for managing the complications of the disease [4].

The first IVF baby was born in 1978 and since then, 0.1% of the world's population has been conceived using Assisted Reproductive Technology (ART) [5]. The MRT which is a recent addition to the IVF procedure, holds the promise of preventing mitochondrial diseases. However, it also raises significant ethical questions that need to be addressed. The main aim of MRT is to prevent the next generation from inheriting mitochondrial disorders that are transmitted through maternal DNA. There is currently no known treatment for these illnesses, which have the potential to result in severe physical impairment or even mortality. Hence, MRT offers an intervention in which a set of healthy mitochondria from a donor is used to replace the dysfunctional mitochondria in the embryo or zygote. The UK and Australia have implemented special laws permitting the use of MRTs in clinical trials only in cases where patients are at high-risk of developing severe mitochondrial abnormalities [6].

This review aims to discuss MRT's involvement and contribution to IVF, its application, use, ethical implications and potential for the future.

MITOCHONDRIAL DYSFUNCTION AND INHERITANCE IN REPRODUCTIVE HEALTH

Mitochondria contain their own genome, which replicates separately from the nuclear genome. The human mtDNA is 16.6 kbp long and encodes 13 peptides that, with the exception of complex II, are involved in all the complexes necessary for OXPHOS [7]. The nuclear genome codes for the remaining mitochondrial proteins, which are then transported into the mitochondria. Mitochondria are primarily responsible for producing the Adenosine Triphosphate (ATP) that cells require. This mechanism depends on OXPHOS, which produces Reactive Oxygen Species (ROS) as a byproduct [8]. Furthermore, mitochondria can sequester and release Ca^{2+}, thus regulating calcium responses. They also mediate apoptosis, differentiation and proliferation of cells [9]. Preimplantation development and implantation of embryos are energy-intensive processes that entail a variety of active cellular processes and require large amounts of ATP [7,10]. Therefore, mitochondria are of paramount significance for normal fertilisation and development.

mtDNA Mutations Leading to Inherited Disorders

Metabolic defects can severely disrupt the typical genotypic and phenotypic traits in individuals, often caused by mutations in mtDNA, which impair the function of the respiratory chain and lead to mitochondrial disorders. A key distinction of mtDNA from nDNA is its existence in multiple copies within cells, with this variation strongly influencing the type and impact of mtDNA mutations, as well as the manifestation of mitochondrial disorders [11]. The timing and severity of these disorders are closely tied to the proportion of defective mtDNA, which must exceed a specific 'threshold level' for symptoms to appear [12]. A significant challenge with mtDNA is its instability, which makes mutations difficult to identify [13]. Each year

in the United Kingdom (UK), around 100 babies are born with severe mitochondrial disorders, the majority of whom do not survive infancy [14]. Research by Set KK et al., estimates that mtDNA mutations account for 10-15% of mitochondrial diseases [15]. It is important to note that mitochondrial dysfunction can result from mutations in both mtDNA and nDNA, adding complexity to these disorders.

Impact of these Mutations on Fertility and Embryo Development

Many aspects of embryogenesis and fertilisation depend on mitochondrial function. ATP, which is produced by the mitochondria, is required by the developing embryo for energy [16]. Consequently, insufficient ATP levels have been linked to aberrant embryo growth and ineffective fertilisation. Oocytes differ in their actual number of mitochondria, but a sufficient number are needed to provide the burst of activity necessary until the blastocyst stage. Therefore, when the number falls below the required level, mitochondrial dysfunction becomes apparent. However, as mitochondrial activity is tightly controlled by nuclear signals, intracellular ion concentrations and substrate availability, it should not be viewed solely through the lens of copy number [17].

Maternal Inheritance of mtDNA

Most organisms exhibit maternal uniparental inheritance, as the mitochondrial genome is passed down solely from the egg [18]. This occurs due to two main events: first, during spermatogenesis, there is a significant reduction in the number of mitochondria and during early embryogenesis, there is an aggressive breakdown of mitochondria and paternal mtDNA. These mechanisms effectively destroy paternal mtDNA [19]. However, during oogenesis, the mitochondrial pool is amplified globally, resulting in the production of several hundred thousand copies of mtDNA. As a result, the mature oocyte contains the highest number of mitochondria of any cell type in the organism. The typical number of mtDNA copies in a human oocyte is believed to be approximately 250,000 [20].

OVERVIEW OF MITOCHONDRIAL REPLACEMENT THERAPY (MRT)

MRT, also known as the 'Mitochondrial Donation Technique' [21], encompasses a set of procedures in which an embryo containing the nDNA of the biological parents undergoes IVF to incorporate the mtDNA from a donor female [22]. In this era of advanced biomedical technologies, MRT offers a solution to women with mitochondrial diseases, enabling them to have healthy, biologically related offspring [23]. Mitochondrial disorders, often caused by point mutations, can have life-threatening consequences for the offspring, especially considering the mitochondria's role in essential organ function. While some mitochondrial disorders respond to treatment, the MRT procedure explicitly targets the elimination of mutated mtDNA from the mother, thereby safeguarding the offspring from the detrimental effects of these conditions by providing healthy mtDNA. Although point mutations in mtDNA are relatively rare, they are linked to numerous mitochondrial diseases affecting vital organs like the retina, brain, optic nerve, muscles, endocrine system, heart and liver [24,25].

Types of MRT

PNT and MST are two fundamental techniques used in MRT

Pronuclear Transfer (PNT) technique: MRT is a pivotal post-fertilisation technique in which two zygotes are developed in-vitro. One zygote is derived from the biological parents, containing pronuclei and defective mitochondria, while the second zygote is from a donor with healthy mitochondria and pronuclei [23]. The pronuclei from the parents are extracted and transplanted into the donor's enucleated zygote, which contains healthy mitochondria. This transfer is facilitated either through the application of electric pulses or by using the inactivated haemagglutinating virus of Japan [14]. The reconstructed zygote is then implanted into the mother's uterus.

Maternal Spindle Transfer (MST) technique: In this method, the maternal spindle complex, extracted during the metaphase stage, is removed from the mother's defective egg and transferred to the perivitelline space of the donor's enucleated egg, which contains healthy mitochondria [26]. The reconstituted embryo is then transferred into the mother's womb. This method is preferred as the maternal spindle contains minimal cytoplasm, reducing the likelihood of mtDNA carryover and the potential for mutations [21].

Application of MRT in IVF

This technology is integrated with IVF for individuals with a higher chance of passing on mitochondrial diseases. After the nuclear transfer, the altered egg or embryo undergoes the same set of IVF processes as any other treatment course; the sperm is introduced to the egg in the laboratory and the resulting zygote is implanted in the mother's uterus [23]. Combining MRT with IVF offers a significant benefit: the reduction in the incidence of mitochondrial diseases among the children born. Juvenile-onset disorders can, therefore, be decreased as defective mitochondria are shuttled out and harmonised with healthy mitochondria sourced from a donor. This not only increases the likelihood of having a healthy pregnancy but also decreases the chances of conditions that may render the child's lifetime disabled [12,23,26].

It is noteworthy, however, that despite the potential benefits and prospects associated with the use of MRT, the technique is accompanied by controversy and ethical concerns. Intrauterine manipulation is currently legal only in the UK and is still primarily conducted on an experimental basis in many other nations [11]. However, for families with children suffering from mitochondrial diseases, MRT represents a ray of hope that could enable them to have healthy offspring. It necessitates close integration with IVF, which is complex, but it has the potential to minimise the spread of these genetic conditions. In the course of systematic study, it is necessary to evaluate the positive effects compared to the negative ones and to fine-tune the methodology of research to avoid adverse consequences and achieve the maximum positive outcome [23,27].

CLINICAL APPLICATIONS OF MRT IN IVF

With an increase in maternal age, particularly beyond 35, there is a significant risk of birth defects, miscarriages and infertility [28]. Research indicates that the main issue stems from a decline in oocyte quality as women age. Impaired cytoplasmic maturation has been associated with an increase in meiotic and mitotic irregularities, leading to higher rates of aneuploidy. Deficiencies in the cytoplasm, particularly in essential transcripts and proteins, further exacerbate this decline [27]. Mitochondrial dysfunction appears to play a significant role in this process. A research study demonstrated a strong correlation between advanced maternal age and an increase in mtDNA mutations within eggs, reduced energy production in embryos and disruptions in mitochondrial calcium regulation [28]. These mitochondrial dysfunctions can destabilise the nuclear genome of the ageing oocyte, often resulting in aneuploid embryos. The spindle abnormalities that arise are thought to occur due to insufficient ATP production or oxidative stress [26,28,29].

MRT is an efficient way to bypass these abnormalities while retaining the DNA of the female. Even with the ongoing challenges surrounding MRT, a team led by Dr. Zhang J from New Hope Fertility Centre in New York made a significant breakthrough by briefly reporting successful translational research using MST in a woman with a mtDNA mutation linked to Leigh syndrome (8993 T>G), resulting in the birth of a child [29]. This milestone was presented at the

2016 annual meeting of the American Society for Reproductive Medicine (ASRM). However, as highlighted in an ethical statement from the UK, families utilising such advanced reproductive techniques must commit to the long-term follow-up of their children and future generations [30]. In 2015, the UK became the first country to allow mitochondrial donation treatment in a well-regulated environment [11,31]. As of May 2023, about 32 women have been granted approval for mitochondrial donation treatment following case-based evaluation by the Human Fertilisation and Embryology Authority (HFEA) [32]. Consequently, this has led to the successful delivery of the first MRT baby, which was born in accordance with all regulations set by the HFEA. However, the article detailing this achievement has not yet been published.

Success Rates and Outcomes

Being a novel procedure, the exact success rates of MRT cannot be determined. Due to the ethical and legal considerations involved, the cases in which MRT has been performed are extremely low. Furthermore, with the scarcity of documented evidence, long-term implications have not been studied.

Long-term Health and Development

The evaluation of long-term health and development of children born through ART, especially with the advent of novel procedures like MRT, is of immense importance. A study evaluated the perinatal complications associated with ART and reported various complications, like premature rupture of membranes, placental abruption, polyhydramnios, birth defects and low birth weight [33]. Over eight million children have been conceived using ART in the past four decades globally. The majority of these pregnancies were uncomplicated and resulted in the birth of healthy children [34]. However, with MRT, due to the scarcity of data, the trials conducted for children born through this procedure have not yet reached a stage where long-term health outcomes can be evaluated.

POTENTIAL CHALLENGES AND LIMITATIONS

Technical Challenges

One of the biggest technical challenges of MRT is the complete removal of maternal mtDNA. Essentially, it involves transferring the nDNA from the mother's egg or embryo into a donor egg that contains healthy mitochondria, with the aim of eliminating the mutations. However, during the procedure, there are risks associated with the possibility that some mutated DNA may be carried over during the transfer, potentially causing heteroplasmy, which is the mixing of healthy and diseased mitochondria [30].

Ethical Challenges

The prospect of genetically modified children has sparked intense ethical debate, particularly concerning genome editing technologies. Advocates argue that these technologies hold immense potential to combat disease and alleviate human suffering, framing the development of such innovations as a moral obligation. They emphasise the possibility of eradicating genetic disorders and enhancing overall human health [35]. Critics, however, raise profound concerns, contending that gene editing may introduce significant harm on multiple levels. They argue that it risks commodifying children, reducing them to products of genetic engineering [36]. This could, in turn, damage parent-child relationships by shifting the focus from unconditional acceptance to an emphasis on genetic 'perfection'. Beyond individual families, it is important to highlight the potential for societal harm, warning that gene editing could exacerbate existing social inequalities, granting the wealthy access to genetic enhancements while leaving others behind. On a broader scale, it is possible that such technologies might disrupt the natural course of human evolution, fundamentally altering the genetic makeup of future generations [37].

Regulatory Challenges in Approving MRT for Clinical Use in IVF

Regulatory challenges in approving MRT for IVF involve consideration of multiple factors and must grant permission following a thorough case-based evaluation of individual cases. This should also incorporate a balance between ethical safety and long-term outcomes. There is a pressing need for comprehensive preclinical studies and long-term follow-up of children born through MRT to ensure that the benefits outweigh the risks [35-37].

CONCLUSION(S)

MRT represents a remarkable advance in reproductive medicine, offering hope to families affected by mitochondrial diseases. However, the technical challenges, such as incomplete mtDNA removal and embryo viability, coupled with safety concerns regarding the genetic integrity and health of offspring, must be addressed through rigorous research and clinical trials. The adoption of MRT must be approached with caution, ensuring that patient selection is based on a clear understanding of the risks and benefits. Economic considerations also play a crucial role, as the high cost of the procedure may limit its accessibility. As we move forward, it is essential that the ethical, technical and economic aspects of MRT are carefully balanced to ensure the safety and wellbeing of patients and their future children.

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