Should Mitochondrial Replacement Therapy be Legalised in Hong Kong?

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Mitochondrial replacement techniques (MRTs) of pronuclear transfer, maternal spindle transfer, polar body transfer, and mitochondrial gene editing can be used to prevent mitochondrial diseases. This study reviews the ethical principles for MRTs in terms of autonomy, beneficence, non-maleficence, and justification. MRTs appear to be compatible with existing norms and standards of reproductive medicine.

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Introduction

Human beings are eukaryotic organisms. All cells possess a double membrane-bound structure within the cell cytoplasm known as the mitochondria. This organelle serves as the energy warehouse that enables cells to function properly. Mitochondrial DNA (mtDNA) is located outside the cell nucleus and is passed on solely by maternal inheritance. mtDNA comprises 37 genes and accounts for only 0.1% of all human DNA materials[1]. mtDNA diseases occur when a sufficient proportion of mitochondria with deleterious DNA mutations affects cellular energy production to the extent that cell physiology is impaired. Such deleterious mutations can occur spontaneously during cell division and mtDNA replication or can be inherited from the maternal side. Homoplasmy is mutation in all mitochondrial genomes and affected women always pass this condition to their children. Heteroplasmy is mutation in some mitochondrial genomes and affected women pass a mix of normal and mutated mitochondria to their children. Manifestations of mitochondrial diseases depend on the type of DNA mutation and the proportion of deleterious mutated DNA.[2] Mitochondrial inheritance does not follow the simple Mendelian pattern. Instead, during production of primary oocytes, a variable number of mtDNA molecules are transferred to each oocyte followed by rapid replication of this mtDNA population. This sophisticated restriction-amplification mechanism results in a random shift of mutational mtDNA load between generations known as the mtDNA bottleneck effect[3,4]. In women with a heteroplasmic mutation, the phenotypical expression is likely to vary widely so the outcome is unpredictable. Deleterious mutation in mtDNA has been documented to cause various heritable diseases including Leigh syndrome, Leber hereditary optic neuropathy, and other conditions and syndromes that can lead to dementia, stroke, blindness, deafness, cardiac failure, and major organ failure[5,6].

Mitochondrial Replacement Techniques

Pronuclear Transfer

During in-vitro fertilisation, two zygotes are produced, one using the intended parents' gametes and the other using an oocyte donated from a healthy woman and the intended spouse's sperm. Within the first 24 hours of fertilisation, the male and female pronuclei are manually removed from the zygotes before fusion to form an embryo. The pronucleus produced from the donor oocyte's nuclear material is disposed of, and the intended parents' pronuclei are enucleated from the original zygote and transferred to this enucleated donor zygote. The intended parents' nuclear material continues to develop in a zygote that comprises healthy mitochondrial DNA. The zygote is then transferred back to the woman as an embryo[4,6,7] (Figure 1).

Maternal Spindle Transfer

Using standard IVF techniques, oocytes are obtained from the woman with mitochondrial mutations and from a healthy donor. During metaphase II of cellular division, the chromosomes are aligned to one side of the oocyte in a spindle shape group, and the chromosomes of both oocytes are removed. The donor's chromosomes and the woman's enucleated oocyte are disposed of, and the woman's chromosomes are transferred to the donor's enucleated oocyte. The reconstructed oocyte carries healthy mitochondria of the donor and the chromosomes

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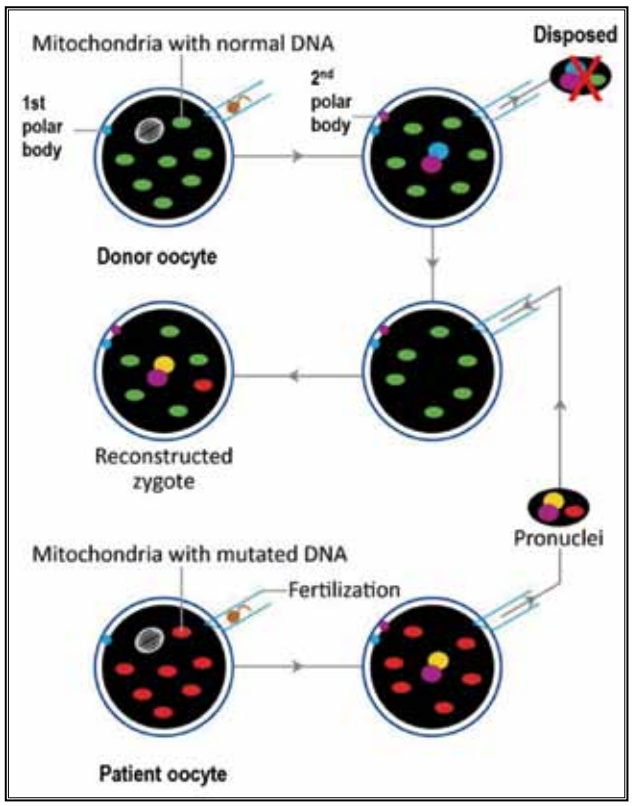

Figure 1. Pronuclear transfer

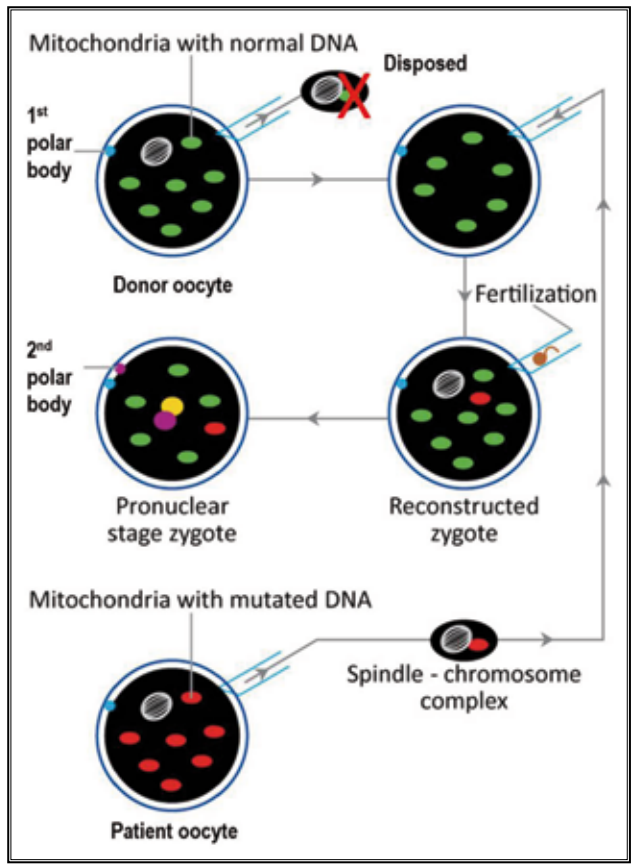

Figure 2. Maternal spindle transfer

of the woman. This oocyte is fertilised with the spouse's sperm using standard IVF techniques and then transferred to the woman for conception[5,6,8,9] (Figure 2).

Polar Body Transfer

During oocyte maturation, the first polar body is formed as DNA duplication occurs, so that the oocyte contains four sets of chromosomes. Of these, two remain within the oocyte, and the other two are packaged into the polar body and subsequently extruded and not present in the embryo. Similarly, when the second polar body is formed during fertilisation, one set of the remaining chromosomes is packaged into the second polar body, and the remaining set joins the sperm DNA of the male to become the nuclear DNA of the embryo. Polar bodies have very few mitochondria and may avoid mitochondrial carry-over. Polar body transfer is the transfer of the first polar body to an unfertilised enucleated donor egg (which is stringently preventive) or transfer of the second polar body to a half-enucleated zygote (not stringently preventive)[5,7,10] (Figure 3).

Mitochondrial Gene Editing

CRISPR/Cas9 is a natural system that enables bacteria with an adaptive response against viruses[11,12]. TALENs are engineered nucleases that comprise a transcription activator-like effector DNA-binding domain from Xanthomonas fused to a FokI nuclease domain. Mito-TALENs are TALENs that are directed specifically at the mitochondrial DNA. These mitochondrial gene editing techniques are undergoing animal phase studies[5,7,9] (Figure 4).

Comparison of the different techniques

Mitochondrial replacement of DNA has specific relevance to law and regulation as well as to ethical considerations. Pronuclear transfer involves zygotes, and destruction of an early embryo to reconstitute another selected embryo is controversial as the law considers all human embryos to have the same legal or moral status as human being and forbids experimenting with and selection of embryos. Maternal spindle transfer involves oocytes, with the donor oocyte being discarded. Similarly, transfer of the first polar body involves the oocyte, whereas transfer of the second polar body after fertilisation involves destruction of one embryo for every healthy embryo produced. From an ethical point of view, procedures that involve oocytes alone are more acceptable[8,9,12]. Gene editing techniques do not involve any donor and hence evade the legal and ethical problem of the genetic linkage of three persons[7,9].

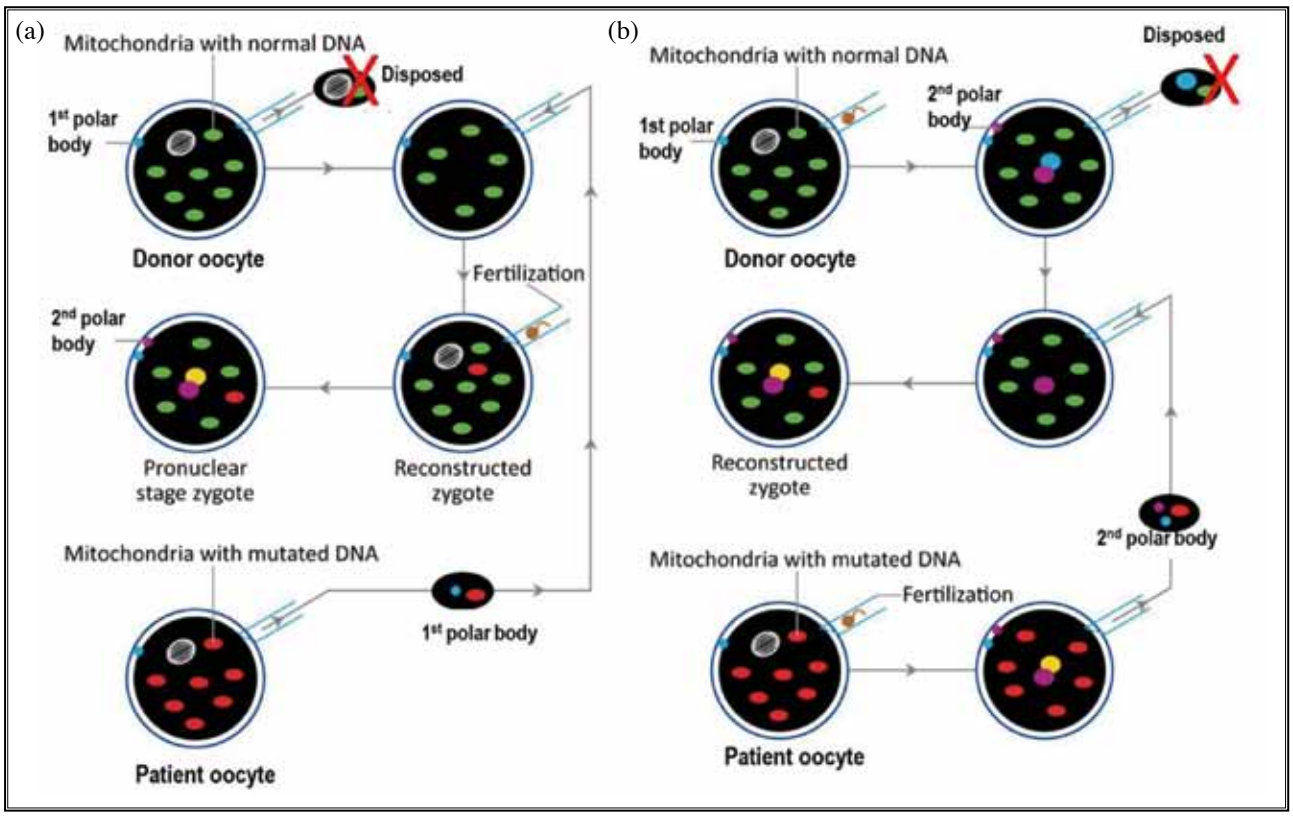

Figure 3. (a) First and (b) second polar body transfer

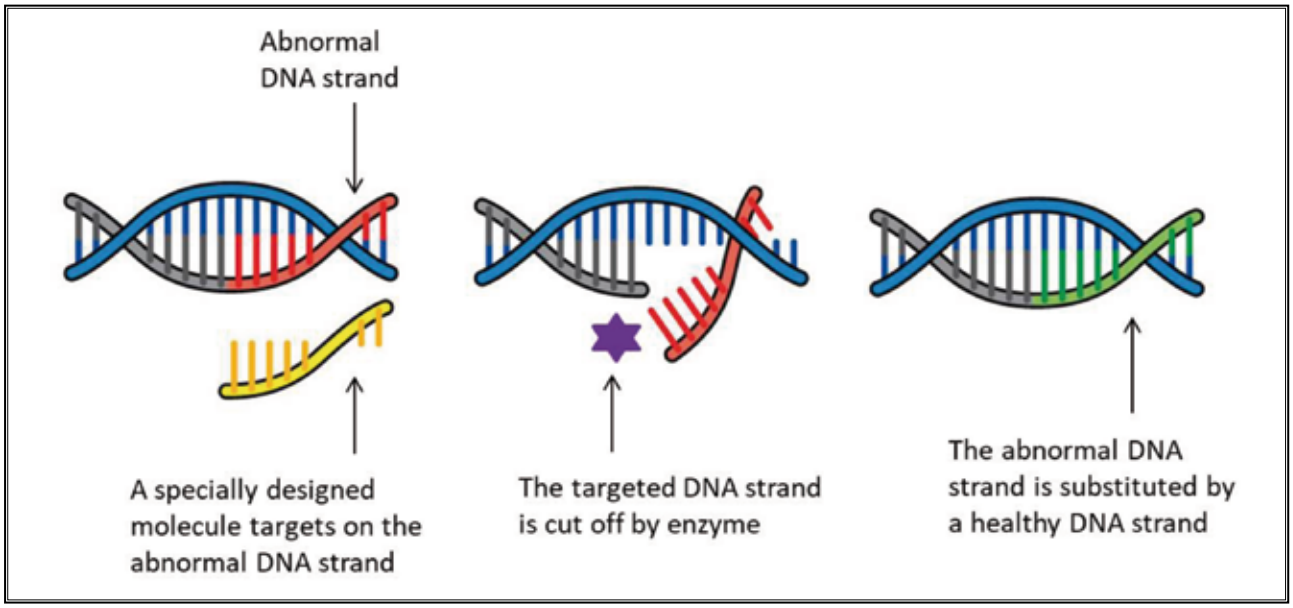

Figure 4. Mitochrondrial gene editing

Ethical Principles

Autonomy

Prior to the availability of mitochondrial replacement techniques (MRTs), the possible option for a woman with mitochondrial disorders was prenatal genetic diagnosis after normal conception with termination of the pregnancy if the fetus was affected, or preimplantation genetic diagnosis that involves selection of embryos with

the lowest proportion of abnormal mtDNA for implantation so as to reduce (rather than eliminate) the risk of having a baby severely affected by mitochondrial disease[7]. These options are relevant only to heteroplasmic women. The only choice for homoplastic women was oocyte or embryo donation from a healthy woman, with consequent children having no genetic linkage to the mother.

The 'slippery slope' argument is the main argument against the principle of autonomy. There is a fear of playing God by changing mtDNA or any DNA and eventually producing 'designer' babies. By tampering with the germline genetic constitution that will be inherited by future generations, altering genetics intentionally to enhance humans and to produce mutants deprive future generations of their right to receive an un-manipulated gene pool[13]. This is an example of a weak 'slippery slope' argument[14]. It is unreasonable to argue that MRTs inevitably lead to the pursuit of germline modifications to enhance healthy embryos and lead to human mutants. Such projection is speculative and can be safely put aside if internal and external monitoring systems are established under legislation to ensure technology is used appropriately and with proper restrictions[15]. Thus, women should have the autonomy to choose MRTs if they are fully counselled about the implications of all the options available.

Beneficence

Although vitamin supplements, drugs, and physical exercise have been used to treat mitochondrial diseases in isolated cases and clinical trials, evidence for their effectiveness is lacking[16]. Preventing a child from being born with a severely handicapping and non-curable mitochondrial disorder appeals to both affected families and the general public. The conventional management of pregnancy termination is unacceptable to many families and religions. Pregnancy termination seems to be the greater evil compared with manipulating oocytes or sacrificing donor embryos. In addition, MRTs enable healthy mtDNA to be passed on and terminate the family history of mitochondrial disease.

Non-maleficence

Safety Issues

Germline modification involves ooplasmic transfer (injection of donor ooplasm with normal mitochondria into an oocyte with mutant mtDNA) and has been developed as a fertility technique for women with repeat embryonic development failure. Its first applications resulted in a relatively high number of children with chromosomal abnormalities (two of 16 pregnancies); there were concerns about mitochondrial heteroplasmy (two of 15 born children carried mtDNA from the donor and recipient) and the possible epigenetic effects of ooplasmic transfer[17]. Results of animal experiments and the first human case indicate that MRTs are free of such problems.

Evolutionary biologists have raised concerns about the safety of MRTs based on the extent to which nuclear and mitochondrial DNA co-evolve within natural populations, i.e. the nuclear-mitochondrial mismatch hypothesis. Animal models have provided evidence of incompatibility between nuclear and mitochondrial genomes from divergent populations of the same species. Nonetheless, a study of a naturally occurring nuclear-mitochondrial mismatch across 26 populations revealed that mitochondrial and nuclear genomes from divergent human populations could co-exist in healthy humans, indicating that mismatched nuclear DNA-mtDNA combinations are not deleterious, and are unlikely to challenge the safety of MRT[18].

The UK Human Fertilization and Embryology Act (HFEA) expert panel has reviewed the safety and effectiveness of MRTs and concluded that there is no evidence to show that such techniques are unsafe or one method is superior to the other[11]. The Nuffield Council on Bioethics also concluded that if the treatments were acceptably safe and effective, it would be ethical for families to use[13]. A public consultation exercise conducted by HFEA concluded that "there is general support for permitting mitochondria replacement in the UK, so long as it is sufficiently safe to be offered in a treatment setting and done so within a regulatory framework".

In pregnancies conceived after MRTs, polar body biopsy, preimplantation genetic diagnosis, ultrasonography, and prenatal diagnosis can be used to determine whether the embryo is developing normally and whether any affected mitochondria have been transferred. Follow-up studies of children conceived by MRTs are also necessary to determine long-term safety issues.

Donor Status and Parenthood

Theoretically, any embryo created by pronuclear transfer or maternal spindle transfer contains DNA of three people, the so-called three-parent in-vitro fertilisation. Genetically, the woman and her spouse contribute 99.9% of the genetic materials and the oocyte donor contributes

only the 0.1% mitochondrial DNA, unlike conventional oocyte donation that contributes 50% of the DNA. The Nuffield ethics review suggested that mitochondria donors should have the same status as women who donate eggs or embryos for conventional in-vitro fertilisation[11]. Mitochondria donors should receive compensation and be safeguarded, as they undergo the same invasive procedures of ovarian stimulation and oocyte retrieval as those who undergo conventional in-vitro fertilisation. While the panel saw no reason why they should be identifiable to the adults born as a result of their donation, a person conceived by MRTs can have a legitimate interest in knowing who contributed to his or her genetic make-up. Following public consultation, the HFEA expert panel advised that mitochondrial donors should be awarded similar status to tissue donors. Under common law, the legal mother is the woman who carried and gave birth to the child and the father is the man who provided the sperm. The expectation of the oocyte donor in MRTs to claim parenthood is much lower than the conventional oocyte donor or surrogate mother[19].

Impact on the Child

Having three genetic 'parents' may cause a person to suffer[13]. Nonetheless, there is no reason for any particular parenting arrangement to be followed[19]. Concerns that children with genetic ties to three persons will experience psychosocial problems are likely unfounded. Evidence from families created by gamete donation can provide valuable insight into the psychosocial development of children who share genetic ties with an individual who may not play any role in their daily living.

Justification

The UK Department of Health estimated that around one in every 6500 children born in the country has a mtDNA disease; it has been estimated that about 10 to 150 children per year would have benefited from mitochondrial therapy[14,20]. Similar figures are not available in Hong Kong. When considering the bioethical tenet of beneficence, there is a consensus that MRTs are worth pursuing if the quality of life of those affected can be improved, even if the number is small[16]. Nonetheless, MRTs are expensive. Whether the government should fund such services remains controversial. In Hong Kong public hospitals, in-vitro fertilisation is partially self-financed; government subsidy of MRTs may motivate academic institutions to invest in the development of the technology.

Legislation

Although the US National Academy of Sciences Panel considered MRTs to be ethically acceptable, the Congress blocked the technology through a federal spending bill by prohibiting the Food and Drug Administration from considering applications to carry out MRTs[6,21]. In the UK, the HFEA prohibits implantation in a woman of eggs or embryos with altered DNA. However, the HFEA makes provision, subject to parliamentary consent, to permit this for a single specific purpose of "preventing the transmission of serious mitochondrial disease". In 2015, both houses of parliament approved regulations put forward by the Department of Health, and the UK became the first country in which MRTs are explicitly legal and yet under stringent control of the authorities. Centres must apply for and be granted a license from the HFEA for each proposed procedure. Nonetheless, a petition brought forward by the European Union parliament aimed to stop the legalisation of MRTs in the UK on the basis of the risks of eugenics and the harm to human dignity. Nonetheless, the arguments were weak and did not address the issues at stake in a convincing manner[22,23].

In Hong Kong, the Human Reproductive Technology Council was established with reference to the HFEA. To legalise such practices, clear indications for carrying out each proposed procedure should be documented and strictly confined to patients with mitochondrial diseases that have a significant health impact. As the assisted reproductive procedures are highly sophisticated, confining the licensee to one or two institutions with academic background enables more stringent monitoring. Donor information should be kept confidentially in a central registry, with a similar legal handling of semen donors.

Conclusion

MRTs can be used to prevent mitochondrial diseases. The ethical principles for MRTs appear to be compatible with existing norms and standards of reproductive medicine. Legislation of MRTs in Hong Kong can be based on the existing Human Reproductive Technology Ordinance with stringent surveillance by the Human Reproductive Technology Council.

Declaration

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