Reproductive Medicine Involving Mitochondrial DNA Modification: Evolution, Legality, and Ethics

Authors: *Tetsuya Ishii

Office of Health and Safety, Hokkaido University, Sapporo, Japan
*Correspondence to tishii@general.hokudai.ac.jp

Disclosure: The author has declared no conflicts of interest.

Acknowledgements: This work was supported by the Uehiro Foundation on Ethics and Education and the JSPS KAKENHI Project Innovative Ethics (Graduate School of Humanities, Kobe University, Kobe, Japan).

Received: 23.04.18

Accepted: 06.06.18

Keywords: Egg donation, ethics, evolution, germline genetic modification, infertility, law, mitochondrial disease, mitochondrial DNA (mtDNA), mitochondrial donation.

Citation: EMJ Repro Health. 2018;4[1]:88-99.

Abstract

Human oocytes have an abundance of mitochondria that have their own genome. Mitochondrial functions are exerted through evolutionarily-developed interactions between the nucleus and mitochondria. Since 1996, fertility clinics have practiced various types of germline mitochondrial DNA (mtDNA) modification that alter the composition of mtDNA copies in oocytes or zygotes using micromanipulation. Experimental reproductive medicine has primarily intended to treat intractable infertility and has been used to prevent the maternal transmission of a pathogenic mtDNA mutation to offspring. In some cases, it has helped parents have a healthy genetically-related child; in others, it has resulted in miscarriages, aneuploid fetuses, or developmental disorders in the offspring. Adverse events have raised ethical controversy, leading to restrictive or prohibitive policies in the USA and China. Conversely, the UK recently became the first nation to explicitly permit two types of germline mtDNA modification (termed mitochondrial donation) for the sole purpose of preventing serious mitochondrial disease in offspring. The aim of this review is three-fold: first, to reshape the medical concept and evolution of germline mtDNA modification, while revisiting 14 clinical cases. Second, to analyse the legality of mtDNA modification, focussing on 16 Western countries. Finally, to consider the ethical aspects, including permissible cases, reproductive options, use of preimplantation and prenatal testing, and the humane follow-up of resultant children. The clinical use of germline mtDNA modification will likely become legal, at least for use in preventative medicine, in some countries. However, the potential clinical, ethical, and evolutionary implications mean that caution is required when considering its wider application.

INTRODUCTION

The majority of human cells have two genomes: nuclear DNA (nDNA), with approximately 24,000 protein-coding genes, and mitochondrial DNA (mtDNA), with only 13 protein-coding genes. Mitochondria are small organelles that exist in the cytoplasm and are involved in various cellular functions. The production of ATP through the respiratory chain is one of the most important functions of the organelles.

Mitochondrial functions are exerted through the co-ordinated expression of genes in mtDNA and nDNA, which have become highly specific over evolutionary time. Regarding human mtDNA, a spermatozoon has 100–1,500 copies of the organelle genome, whereas a mature oocyte has as many as 200,000–300,000 copies of mtDNA.[1] Paternal mitochondria are specifically digested after fertilisation; as a result, only maternal mtDNA is transferred to the offspring. Mutations to the 13 protein-coding mtDNA genes have been linked to various forms of human mitochondrial disease.[2] Although *POLG* in the nDNA, which encodes the catalytic subunit of mitochondrial DNA polymerase, has been suggested to be associated with infertility, mtDNA genes that only cause infertility remain elusive.[3,4]

From the 1980s to the early 2000s, rodent experiments have demonstrated the feasibility of altering the cytoplasm of oocytes (ooplasm) by cytoplasmic transfer. Soon after, it was demonstrated that the cytoplasm of embryos can be largely replaced by transferring a karyoplast (nuclei [or a nucleus] with a plasma membrane containing a small amount of cytoplasm) to a different enucleated zygote.[5-7] Such outcomes led to the development of reproductive medicine involving a cytoplasmic or karyoplast transfer that alters the composition of mtDNA copies in oocytes or zygotes. In 1996, a clinic in the USA initiated ooplasmic transfer (OT), and reported the birth of a baby in 1997; this is believed to be the first case of human germline genetic modification.[8,9] Subsequently, some OT cases have helped prospective parents have a genetically-related child, whereas others have resulted in miscarriages, aneuploid fetuses, and the onset of a developmental disorder in the offspring.[10,11] In 2003, a collaboration between a Chinese group and a team from the USA reported the first pronuclear transfer (PNT), which was performed with the intention of largely replacing the cytoplasm of a patient's zygote with that of a donor zygote.[12] The PNT performed in China led to a triplet pregnancy; however, two fetuses died after selective fetal reduction. Such adverse events have led to restrictive or prohibitive regulatory policies in the USA and China.[13] Conversely, in 2015, the UK legalised PNT and maternal spindle transfer (MST), which can largely replace ooplasm, for the sole purpose of preventing serious mitochondrial disease in offspring.[14] In 2017, the first MST procedure performed by researchers from the USA and Mexico led to the birth of a healthy baby.[15]

With the current climate concerning mtDNA modification in mind, this article first reviews the medical concept and evolution of germline mtDNA modification, while revisiting 14 clinical cases. Next, the legality of the procedures is analysed, focussing on 16 Western countries, because an international treaty in the biomedical field was established in Europe.[16] Furthermore, ethical aspects are considered regarding permissible cases, reproductive options, the use of preimplantation genetic diagnosis (PGD), and prenatal testing and humane follow-up of resultant children.

MEDICAL CONCEPT AND EVOLUTION

Table 1 shows 14 clinical cases of germline mtDNA modification that have been performed in nine countries. Eleven reports were published from 1997–2003. The remaining three reports were published within the last 3 years, after a decade-long period without relevant publications.

The Beginning of Germline mtDNA Modification

In 1996, a USA clinic initiated a clinical study of OT, in which 5–15% of ooplasm aspirated from mature oocytes donated by fertile women was injected into mature oocytes of infertile patients, along with a spermatozoon.[11] The subjects included 33 infertile women who had experienced repeated implantation failure and poor embryo development after *in vitro* fertilisation (IVF).[17] Based on a hypothesis that IVF failures could be due to cytoplasmic deficiency rather than aneuploidy in nDNA, the study intended to enhance the developmental potential of the patient's embryos. In 1997, a girl was born via OT (Table 1).[8] mtDNA typing showed sustained heteroplasmy representing both donor and recipient mtDNA in the clinical specimen, suggesting that heteroplasmic mitochondrial populations persist and may be replicated during development (Table 1).[9]

Table 1: Clinical implementations of germline mitochondrial DNA modification.

Report	Place of practice	Procedure	Origin of mitochondria for transfer	Manipulation	Patient/s	Clinical consequences	Remarks
Cohen et al.,[8] 1997	USA	OT	Donor oocytes	Cytoplasmic transfer	A 39-year-old woman with poor ovarian reserve, 6.5 years of infertility, and four IVF or ICSI failures.	A girl was born from a singleton pregnancy.	Inadequate embryo development was observed.
Cohen et al.,[18] 1998	USA	OT	Donor oocytes	Cytoplasmic transfer	Seven couples with quality-compromised oocytes and embryos.	Electrofusion of ooplasm ended in no pregnancies in three couples. Direct injection led to three pregnancies in three couples (one live birth, one miscarriage, one ongoing pregnancy at time of study publication); and no pregnancies in two couples with male infertility.	Direct injection of ooplasm into oocytes may be better than electrofusion.
Huang et al.,[20] 1999	Taiwan	OT	Donor tripronucleate zygotes	Cytoplasmic transfer	Nine women with >5 IVF or ICSI failures (32–42 years old).	Five healthy infants were born from four recipients. No pregnancies in the remaining five recipients.	<30-year-old women who underwent IVF donated tripronucleate zygotes.
Lanzendorf et al.,[19] 1999	USA	OT	Frozen-thawed donor oocytes	Cytoplasmic transfer	Three women of advanced maternal age (43, 47, and 47 years old), and one woman (35 years old) with poor embryo quality on six IVF cycles.	Three women of advanced maternal age did not achieve pregnancy. A 35-year-old woman delivered healthy male and female infants.	Live births happened using oocytes injected with cytoplasm from frozen-thawed oocytes of a 30-year-old donor.
Tzeng et al.,[22] 2001	Taiwan	AGCMT	Autologous granular cell	Mitochondrial transfer	Patients aged >38 years, recurrent implantation failure, prolonged unexplained infertility, poor fertilisation rate, or compromised embryo quality in previous cycles.	Three clinical pregnancies. One of the cases, a twin pregnancy in a 36-year-old patient with infertility of 9 years, showed normal 46XX and 46XY.	Conference paper, not peer-reviewed article. 500–5,000 mitochondria were injected in each oocyte.
Levron et al. personal communication, introduced in Barritt et al.,[11] 2001	Israel	OT	Donour oocytes	Cytoplasmic transfer	Not shown.	15 treatments led to six live births from five pregnancies.	Personal communication.
Dale et al.,[21] 2001	Italy	OT	Donor oocytes	Cytoplasmic transfer	A couple with 7 years of infertility (a 32-year-old woman and a 35-year-old man).	Healthy twins were born from a twin pregnancy.	High level of embryo fragmentation and poor development was observed.
Barritt et al.,[11] 2001	USA	OT	Donor oocytes	Cytoplasmic transfer	See remarks column.	One singleton pregnancy ended in a miscarriage (Turner syndrome: 45,X0). Another fetus (45,X0) in a twin pregnancy was selectively reduced. A male of a male–female twin was diagnosed with pervasive developmental disorder.	This paper was an analysis of the worst cases from Cohen et al.,[8] 1997 and Cohen et al.,[18] 1998.

Table 1 continued.

Report	Place of practice	Procedure	Origin of mitochondria for transfer	Manipulation	Patient/s	Clinical consequences	Remarks
Kong et al.,[23] 2003	China	AGCMT	Autologous granular cells	Mitochondrial transfer	A 37-year-old woman with two miscarriages after IVF.	A triplet pregnancy. At the 5th week of pregnancy, one fetus ceased to develop. At the 30th week, two normal infants (boy and girl) were born via caesarian section.	First live births after AGCMT in mainland China.
Kong et al.,[24] 2003	China	AGCMT	Autologous granular cells	Mitochondrial transfer	A 46-year-old infertile woman.	A singleton pregnancy ended in a miscarriage at the 9th week.	About 3,000 mitochondria were transferred into each oocyte.
Zhang et al.,[12] 2003	China	PNT	Zygotes created using donor oocytes and spermatozoa of patient's partner.	Karyoplast transfer	A 30-year-old nulligravida woman who failed two IVF cycles.	A triplet pregnancy. After selective reduction of a fetus, the other fetuses prematurely delivered and died.	First reported at a conference in 2003. Peer-reviewed article published in 2016. Electrofusion of karyoplast and enucleated zygote was performed.
Fakih et al.,[26] 2015	Canada and the United Arab Emirates	AUGMENT	Autologous 'oogonial precursor cells'	Mitochondrial transfer	93 patients with a poor prognosis after IVF (aged 20–48 years old).	11 and 18-fold increase in ongoing clinical pregnancy rates.	The presence of 'oogonial precursor cells' is controversial.
Oktay et al.,[27] 2015	Turkey	AUGMENT	Autologous 'oogonial precursor cells'	Mitochondrial transfer	10 women with >2 IVF failures (aged 27–41 years old).	Clinical pregnancy attained in four of 10 recipients. One live birth, two miscarriages, one ongoing pregnancy at time of study publication.	The presence of 'oogonial precursor cells' is controversial.
Zhang et al.,[15] 2017	USA and Mexico	MST	Donor oocytes	Karyoplast transfer	A female with a mtDNA mutation (T8993G), multiple pregnancy losses and deaths of offspring due to Leigh syndrome.	A boy was born with a mtDNA mutation load of 2.36–9.23% in tested tissues. He was healthy at 7 months of age.	Electrofusion of karyoplast and enucleated oocyte was conducted in the USA, then, an euploid embryo was shipped to Mexico for transfer.

AGCMT: autologous granular cell mitochondrial transfer; AUGMENT: autologous germline mitochondrial energy transfer; ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilisation; PNT: pronuclear transfer; OT: ooplasmic transfer.

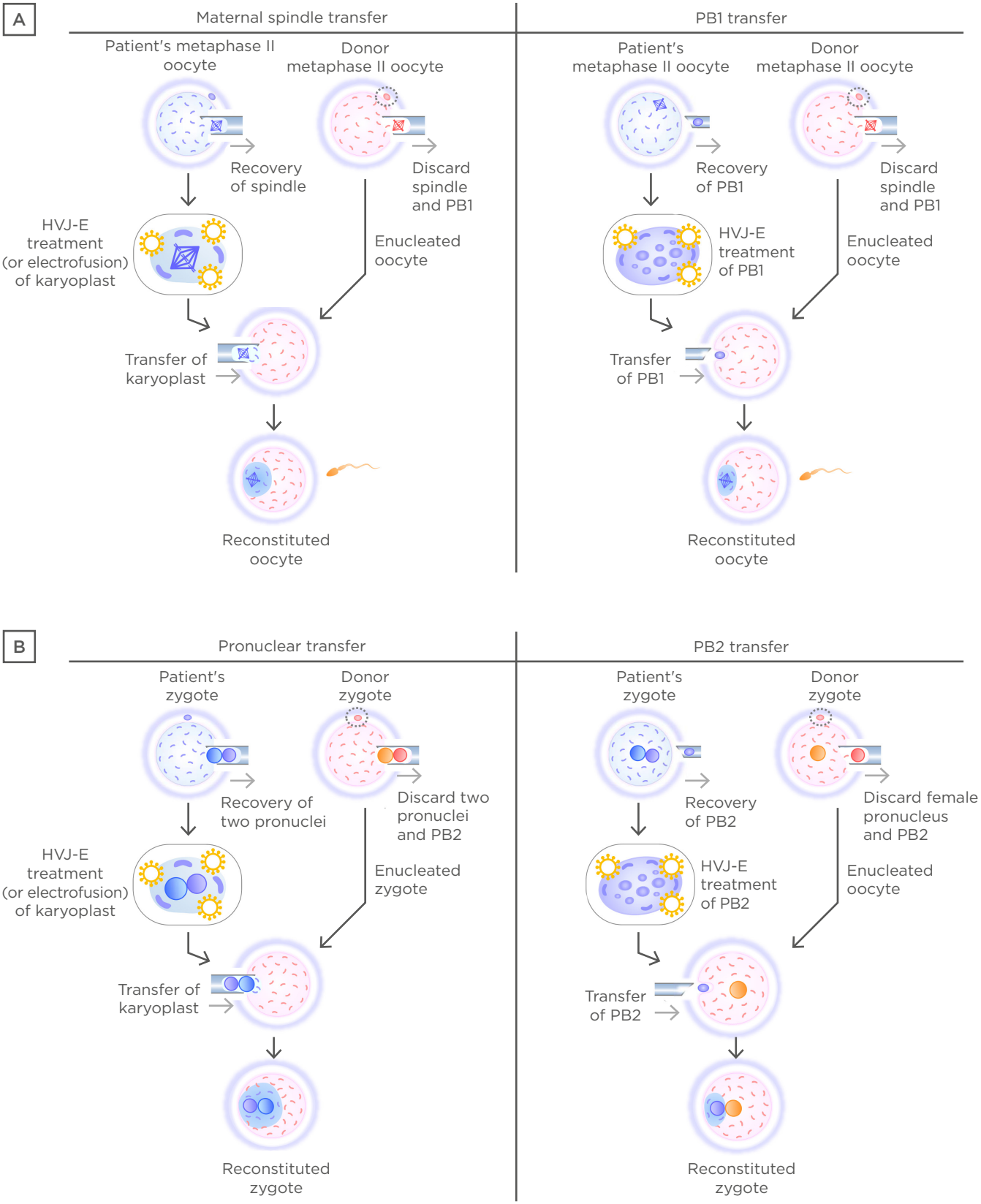

Figure 1: Procedures of maternal spindle transfer, first polar body transfer, pronuclear transfer, and second polar body transfer.

A) Procedures of maternal spindle transfer (left) and PB1 transfer (right). B: Procedures of pronuclear transfer (left) and PB2 transfer (right).

HVJ-E: haemagglutinating virus of Japan envelope; PB1: first polar body; PB2: second polar body.

Likewise, other OT cases intended as infertility treatment for women with a history of implantation failure and/or poor embryo development in women of ≥35 years of age can be found in Table 1. In typical OT, ooplasm from a fresh, mature oocyte donated from a fertile woman is transplanted into the oocytes of an infertile patient through intracytoplasmic sperm injection because electrofusion of the ooplasm and oocytes likely damages the viability of the resultant oocytes.[18] OT variants in the USA and Taiwan used frozen-thawed donor oocytes and donor tripronucleate zygotes as a source of ooplasm.[19,20] These efforts led to live births in some cases.[8,10,11,19-21] Aneuploidy, namely 45,X0 (Turner syndrome), was found in two different fetuses in the USA after OT, which resulted in a miscarriage and selective fetal reduction (Table 1). Furthermore, 1 of 17 children born via OT in the USA was diagnosed with a borderline pervasive developmental disorder (Table 1).[10]

Autologous Mitochondrial Transfer

Autologous granular cell mitochondrial transfer (AGCMT) does not depend on oocyte donation. In the three AGCMT cases from Taiwan and China, hundreds to thousands of mitochondria from the patient's own granular cells were injected into quality-compromised oocytes (Table 1).[22-24] Importantly, although AGCMT adds the patient's mitochondria to their own oocytes, it can potentially induce heteroplasmy in the injected oocytes by mixing mitochondria from somatic cells and germ cells in one individual.[25] AGCMT has led to live births as well as a fetal death and miscarriages. In 2015, two clinical reports from Canada, the United Arab Emirates, and Turkey reported the effects of autologous germline mitochondrial energy transfer (AUGMENT) on clinical pregnancy rates.[26,27] AUGMENT, which appears to be a derivative of AGCMT, uses mitochondria from the patient's oogonial precursor cells. However, the populations of the two studies included younger women of 20–27 years of age (Table 1). Furthermore, their study design, as well as the presence of oogonial precursor cells in older women, is controversial.[28-30]

Karyoplast Transfer

The first PNT implementation reported from China in 2003 intended to treat intractable infertility via karyoplast transfer using a larger micropipette[12] (30–40 µm, 5–6-times larger than the needle used in intracytoplasmic sperm injection) (Table 1, Figure 1B). The subject was a 30-year-old woman who experienced embryo arrest in infertility treatment; she had received two IVF cycles prior to PNT. PNT led to a triplet pregnancy; however, after selective fetal reduction, one of the fetuses died of respiratory distress and the other of cord prolapse. Despite a lack of detailed data, the report claimed that the karyotypes of the fetuses were normal, that the nDNA of the fetuses and the patient matched, that the mtDNA profiles of the fetuses and donor were identical, and that the patient's mtDNA was not detected in the fetuses. In PNT, electrofusion was performed to fuse the patient's karyoplast with an enucleated zygote, which differed from the technique in the USA OT study (Table 1).[18]

In 2017, a group led by the first author of the 2003 PNT report[12] published the first report on MST in a cross-border project between the USA and Mexico (Table 1, Figure 1A). MST differed from previous germline mtDNA modifications in that it used karyoplast transfer in oocytes to prevent the onset of mitochondrial disease (specifically Leigh syndrome) in offspring. The female subject had experienced miscarriages and the loss of offspring due to an ATPase gene mutation in her oocyte mtDNA. The mtDNA mutation load of the woman's oocytes was almost 100%. The mtDNA haplogroup of the patient and the oocyte donor were different (I and L2c, respectively). The heteroplasmy level in the blastocysts after MST was 5.7%, which was higher than the levels in other preclinical reports using human oocytes (undetectable or <1%).[31,32] This MST case led to the birth of a boy. However, the mtDNA mutation load of his tested tissues varied from 2.36–9.23%, and his long-term prognosis remains unclear because the reversal of a pathogenic mtDNA copy may happen.[33,34]

Other Procedures

In addition to PNT and MST, two types of karyoplast transfer have been proposed: germinal vesicle (GV) and aggregated chromosome transfer. GV transfer removes and transfers the nucleus surrounded by the

membrane in oocytes in the prophase of meiosis I.[35] Aggregated chromosome transfer is performed from the breakdown of the GV to the formation of the metaphase-I spindle, during which chromosomes are visible.[36] However, both procedures have not yet been used clinically.

More recently, newer germline mtDNA modification procedures have been proposed: first polar body transfer (PB1T) and second polar body transfer (PB2T).[37,38] In PB1T, a first polar body is transferred to an enucleated mature oocyte (Figure 1A). In PB2T, a second polar body is removed from a zygote and replaced with the female pronucleus in a donor zygote (Figure 1B). Polar body transfer may have advantages over MST and PNT in terms of mitochondrial carry-over because human polar bodies contain few mitochondria.[39] However, fusion of a polar body and karyoplast requires haemagglutinating virus of Japan-envelope treatment, the safety of which remains unknown in human reproduction. The histories of PB1T and PB2T are shorter than the histories of PNT and MST. Despite the successful production of mice using first or second polar bodies,[40] human reproduction involving polar body transfer is still a long way from clinical application; further research is required to ensure the safety of the resultant offspring.

The history of germline mtDNA modification began with the clinical use of OT in 1996. These initial techniques gave rise to variants, including autologous mitochondrial transfer in oocytes and karyoplast transfer in zygotes and oocytes. However, the characterisation of the mitochondrial functions and mtDNA profiles in patients and the resultant offspring was largely insufficient in such small-scale studies. Following the first MST procedure, the heteroplasmy levels of the patient and her baby were analysed; however, the rate of mtDNA carry-over was relatively high in the offspring. Low levels of heteroplasmy can lead to subsequent reversal of the original mitochondrial genotype in MST.[33,34] It is hypothesised that mtDNA haplotypes with specific D-loop polymorphisms are preferentially amplified, potentially causing the reversal.[34] Additionally, the need for matching between nDNA and mtDNA in MST and PNT is controversial. Some assert that mismatching between donor mtDNA and patient nDNA might cause dysfunctional respiratory chain,[41] while others disagree.[33,34,42] Thus, germline mtDNA modification that intervenes in evolutionarily-developed mitochondrial–nuclear interactions using micromanipulation remains largely experimental in human reproduction.

LEGALITY IN THE WESTERN WORLD

Although adverse events following OT and PNT for infertility treatment led to prohibition of germline mtDNA modification in the USA and China, the UK became the first nation to permit PNT and MST, for the sole purpose of preventing serious mitochondrial disease in offspring. In Europe, the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (ETS No. 164) was concluded in 1997 (the so-called Oviedo Convention).[16] This treaty, which is the only binding international law in the biomedical field, stipulates that "An intervention seeking to modify the human genome is only to be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants" (Article 13).[16] Since the Oviedo Convention appears to prohibit germline mtDNA modification for human reproduction, it is worth analysing the legality of germline mtDNA modification focussing on the Western world. Sixteen countries were selected based on observed activities, including clinical reports, trial registries, advertisements relevant to germline mtDNA modification.[13] Of the 16 countries, 10 ratified the Oviedo Convention; Germany, Italy, Northern Cyprus, Russian Federation, the UK, and Ukraine did not (Table 2).[16]

The domestic policies relevant to germline mtDNA modification in the 16 countries were further analysed (Table 2). France, Germany, and Italy legally prohibit mtDNA use in reproductive medicine. Conversely, Northern Cyprus, the Russian Federation, and Ukraine, are permissive to its use in reproductive medicine. In the remaining 10 countries, the UK maintains the legal prohibition of all germline mtDNA modifications except PNT and MST for

disease prevention (use for infertility treatment is illegal). Southern Cyprus and Turkey only permit autologous mitochondrial transfer, such as AGCMT and AUGMENT. Domestic laws in the Czech Republic, Serbia, and Spain only prohibit PNT; the legality of other procedures is ambiguous. The legality of germline mtDNA modification in Albania, Georgia, Greece, and Portugal is ambiguous because, despite their ratification of the Oviedo Convention, these countries appear to allow its use in reproductive medicine.

Thus, there is some ambiguity regarding the domestic legality of germline mtDNA modification in Southern Cyprus, Turkey, Czech Republic, Serbia, Spain, Albania, Georgia, Greece, and Portugal, which ratified the Oviedo Convention. The Oviedo Convention stipulated that "Each Party shall take in its internal law the necessary measures to give effect to the provisions of this Convention" (Article 1).[16] However, OT and AUGMENT are advertised on the internet and may be offered in those countries (Table 2). These findings suggest that these nine countries have delayed or neglected amending or enacting relevant regulations prohibiting germline mtDNA modification, as others suggest.[43] There are inherent legal issues surrounding Article 13 of the Oviedo Convention, which prohibits the introduction of "any modification in the genome of any descendants", considering the characteristics of germline mtDNA modification. For example, males who undergo germline mtDNA modification do not pass their mtDNA onto the next generation. In addition, there is no specific legal definition of the term genome.[16] Some may specifically interpret 'genome' to mean nuclear genome.[44] In contrast, 'nuclear DNA' and 'mitochondrial DNA' are used in the UK's regulations regarding mitochondrial donation. Additionally, some might narrowly interpret Article 13 as the prohibition of modifying a gene(s) in mitochondrial genome of oocytes or zygotes, although germline mtDNA modification changes the composition of the mitochondrial genome copies. Thus, it is suggested that the domestic policies in Western countries and the Oviedo Convention were never meant to regulate germline mtDNA modification.

ETHICAL ASPECTS

Although germline mtDNA modification is permitted or may not be unlawful in some countries, researchers in such countries are required to practice germline mtDNA modification with due consideration of its ethical implications.

Table 2: The policies regarding germline mitochondrial DNA modification in 16 countries.

Jurisdiction	Year of Oviedo Convention (1997) ratification	An interpretation of domestic policy	Relevant domestic legislation	Relevant provisions in legislation	Procedures indicated by a survey on relevant clinical activities*
Albania	2011	Ambiguous	Law 8876/2002 on Reproductive Health	Article 33	MST, PNT
Czech Republic	2001	Prohibitive of PNT. Ambiguous on other procedures	› Act on Research on Human Embryonic Stem Cells and Related Activities and on Amendment to Some Related Acts 227/2006 › Act on Specific Health Services 373/2011	Section 209b of Act 2006	OT
France	2011	Prohibitive	› Civil Code › Law 800/2004 on Bioethics (amended 2009, 2011)	Article 16-4 of Civil Code	OT
Georgia	2000	Ambiguous	Law on Health Protection 1997	Article 142	OT
Germany	Neither signed nor ratified	Prohibitive	Embryo Protection Law 1990 (amended 2001, 2011)	Section 5	OT
Greece	1999	Ambiguous	Law 3089/2002 on medically assisted human reproduction	Article 1455	OT
Italy	Signed but not ratified yet	Prohibitive	Law 40/2004 Rules in the Field of Medically Assisted Reproduction	Article 13	OT

Table 2 continued.

Jurisdiction	Year of Oviedo Convention (1997) ratification	An interpretation of domestic policy	Relevant domestic legislation	Relevant provisions in legislation	Procedures indicated by a survey on relevant clinical activities*
Northern Cyprus	Neither signed nor ratified	Permissive	› Law Regulating Human Cell, Tissue and Organ Transplantation Rules 57/2014 › Assisted Reproductive Treatment Centres and Assisted Reproductive Treatment Procedures Regulation 381/2016	None	OT
Portugal	2001	Ambiguous	Law on medically assisted procreation (32/2006)	Article 4, 9, 10	OT
Russian Federation	Neither signed nor ratified	Permissive	› Russian Federation Citizen's Health Protection Law (22.07.1993. Reg. No5487-I) › Order 67th of the RF Ministry for Health (Reg. No4452 24.04.03)	None	OT
Serbia	2011	Prohibitive of PNT. Ambiguous on other procedures.	No. 40/2017 and 113/2017 laws on biomedically assisted fertilisation	Article 49	OT
Southern Cyprus	2002	Permissive of autologous mitochondrial transfer. Prohibitive of other procedures.	Law 69 (I)/2015 on the application of Medically Assisted Reproduction	Article 18	OT
Spain	1999	Prohibitive of PNT. Ambiguous on other procedures.	› Law 14/2007 on Biomedical Research › Law 14/2006 on Assisted Human Reproduction Techniques	Article 33 of law 2007. Article 13 of law 2006.	OT, AUGMENT
Turkey	2011	Permissive of autologous mitochondrial transfer. Prohibitive to other procedures.	› Penal Code › Legislation Concerning Assisted Reproductive Treatment Practices and Centres 27513/2010 › Regulation on Assisted Reproduction Treatment and Assisted Reproduction Treatment Centres 29135/2014	Article 231 of penal code. Article 10 of legislation 2010.	AUGMENT
UK	Neither signed nor ratified	Permissive of PNT and MST for preventing serious mitochondrial disease in offspring. Prohibitive of other procedures.	› Human Fertilisation and Embryology Act 1990 (amended 2008) › Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015	3, 26, Part 1 of Act 2008. Part 1 of Regulation 2015.	None
Ukraine	Signed but not ratified yet	Permissive	Ministry of Health Order No. 771, Instruction on Procedures for Assisted Reproductive Technologies 2008	None	PNT

*Sixteen countries were selected based on the survey regarding germline mtDNA modification-relevant reports, trial registries, and advertisements on clinic websites or medical tourism websites.[13]

AUGMENT: autologous germline mitochondrial energy transfer; OT: ooplasmic transfer; MST: maternal spindle transfer; mtDNA: mitochondrial DNA; PNT: pronuclear transfer.

Applicable Cases

The history of PGD suggests that germline mtDNA modification will initially be used for disease prevention rather than infertility treatment.[45] Moreover, mutations in any of the 13 protein-coding mtDNA genes have been linked with various forms of mitochondrial disease.[2] However, the link between genes in mtDNA and infertility is currently controversial.[3,4] Potential targets of germline mtDNA modification to prevent mitochondrial disease in offspring include women who have lost children due to mitochondrial disease and women with an inherited mutant gene in their oocyte mtDNA.[15,46] mtDNA modification use for such women is understandable as a safeguard against genetic disease in future children.[47,48] Although PGD may be used to avoid the birth of children with mitochondrial disease, the selection of embryos or oocytes is not applicable to women who only have oocytes

with a high mtDNA mutation load. In addition, PGD that simply selects for the embryo having the lowest heteroplasmy level is unlikely to eliminate the risk of transmitting mtDNA mutations.[49] Despite these limitations, in some countries the clinical rationale and assumed welfare of the offspring might justify the use of some germline mtDNA modifications for women with a pathogenic mtDNA mutation in their oocytes who want to protect the future of their children from serious mitochondrial disease.

Reproductive Options

Excluding autologous mitochondrial transfer, the implementation of germline mtDNA modification requires oocyte donation. The direct use of donor oocytes can also help parents protect future children from life-threatening mitochondrial disease.[50] Donor oocyte availability suggests that the direct use of donor oocytes as well as germline mtDNA modification can be another reproductive option. Of course, many parents want to use PNT or MST to have a genetically-related child.[51] In contrast, some prospective mothers may be satisfied with the genetic relatedness between a resultant child and their partner. In the USA OT study, prospective parents considered the use of oocyte donation.[8] Thus, in addition to the experimental nature of germline mtDNA modification, the option of directly using donor oocytes should be explained to prospective parents.

Use of Preimplantation or Prenatal Testing

Prior to the transfer of embryos created via germline mtDNA modification, PGD can identify and exclude aneuploid embryos and embryos with an unacceptable level of heteroplasmy. Notably, PGD requires an additional intervention of cell biopsy, which can damage the viability of embryos.[45] This is particularly important when performing radical karyoplast transfer. Indeed, it was reported that physicians who plan to perform PNT in the UK were unwilling to use PGD.[52]

Instead, prenatal testing using amniotic fluid and chorionic villus sampling can confirm the genetic condition of a resultant fetus; however, invasive prenatal testing is associated with a miscarriage risk (approximately 1/300). Nevertheless, the use of prenatal testing should be carefully discussed because some parents would likely want to know whether germline mtDNA modification has been effective prior to the birth of their child. However, all treatments have risks. Prenatal testing may show that a pathogenic mtDNA mutation was not sufficiently reduced. In doing so, some women may feel distress over the decision of whether to maintain or terminate the pregnancy because they consented to experimental reproductive medicine to prevent their pathogenic mtDNA mutation from affecting their children. Due to the complicated ethics, prior sufficient counselling may be valuable for prospective women with a history of miscarriages or childbirths with mitochondrial disease.

Humane Follow-Up of Resultant Children

After the first MST, follow-up was initially planned until the resultant child reached 18 years of age.[15] However, the parents requested that no further genetic testing be undertaken, unless there was a clinical benefit for the child.[53] In 2016, Chen et al.[17] reported a survey result of 17 teenagers born from 13 couples that had used OT at a clinic in the USA between 1996 and 2001. Twelve of the 13 parents completed a questionnaire, while one parent did not respond to repeated requests. In addition, such parents did not agree to standardised clinical analysis due to a lack of disclosure to their children. Thus, the study ended in limited follow-up and possibly a high risk of bias.

It will likely be difficult to follow-up children born via germline genetic modification. However, when applying it to prevent the onset of mitochondrial disease in resultant children, the health of such children should be monitored. The period of follow-up is the most important question regarding the monitoring of such children.[54] The UK's policy on mitochondrial donation only requires physicians to prepare a follow-up plan for resultant children and parents need not consent to it.[55] Therefore, the author of this study argues that there is room for improvement in the UK's policy. Follow-up for several years, decades,

or even across generations may be necessary to confirm whether mitochondrial disease is successfully prevented and that no side effects develop. However, the lack of response from one parent in the OT survey[17] suggests that such long follow-up periods might infringe on privacy, dignity, and the welfare of the family. Thus, there may be a clash between clinical requirements and ethical considerations regarding the follow-up period of children born via germline mtDNA modification. Although this article cannot present a compelling solution, it is realistic and acceptable to perform follow-up for additional years after a primary endpoint (e.g., healthy birth) or until the resultant child becomes legally competent to refuse it.[56] Regarding the potential health risks in later life or transgenerational health risks, rigorous mouse experiments may provide meaningful evidence in advance because the generation time of mice is approximately 2 years.

CONCLUSION

The success of IVF in the UK has led to the worldwide spread of the technique since its first use in 1978. Likewise, if the first mitochondrial donation in the UK succeeds in preventing mitochondrial disease in a resultant child, the clinical use of PNT or MST will likely become legal, at least for disease prevention, in other countries. It is noteworthy that women who underwent mitochondrial donation experienced implantation failures and miscarriages.[45] Once PNT or MST for disease prevention is justified, it may be approved for treating intractable infertility in some countries. However, caution is required in its wider use from clinical, ethical, and evolutionary standpoints.

References

1. Ishii T, "Mitochondrial manipulation for infertility treatment and disease prevention," Schatten H (eds.), Human Reproduction: Updates and New Horizons (2016), Wiley Blackwell, pp.205-30.
2. Koopman WJ et al. Monogenic mitochondrial disorders. N Engl J Med. 2012;366(12):1132-41.
3. Demain LA et al. Genetics of mitochondrial dysfunction and infertility. Clin Genet. 2017;91(2):199-207.
4. Harper JC et al. Recent developments in genetics and medically assisted reproduction: From research to clinical applications. Eur J Hum Genet. 2018;26(1):12-33.
5. Pratt HP, Muggleton-Harris AL. Cycling cytoplasmic factors that promote mitosis in the cultured 2 cell mouse embryo. Development. 1988;104(1):115-20.
6. Levron J et al. Formation of male pronuclei in partitioned human oocytes. Biol Reprod. 1995;53(1):209-13.
7. Sato A et al. Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation. Proc Natl Acad Sci U S A. 2005;102(46):16765-70.
8. Cohen J et al. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. Lancet. 1997;350(9072):186-7.
9. Barritt JA et al. Mitochondria in human offspring derived from ooplasmic transplantation. Hum Reprod. 2001;16(3):513-6.
10. Barritt JA et al. Rebuttal: Interooplasmic transfers in humans. Reprod Biomed Online. 2001;3(1):47-8.
11. Barritt J et al. Cytoplasmic transfer in assisted reproduction. Hum Reprod Update. 2001;7(4):428-35.
12. Zhang J. et al. Pregnancy derived from human zygote pronuclear transfer in a patient who had arrested embryos after IVF. Reprod Biomed Online. 2016;33(4):529-33.
13. Ishii T, Hibino Y. Mitochondrial manipulation in fertility clinics: Regulation and responsibility. Reprod Biomed Soc Online. 2018;5:93-109.
14. UK Department of Health. The human fertilisation and embryology (mitochondrial donation) regulations 2015. 2015. Available at: http://www.legislation.gov.uk/ukdsi/2015/9780111125816/contents. Last accessed: 23 April 2018.
15. Zhang J et al. Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. Reprod Biomed Online. 2017;34(4):361-8. Erratum in: Reprod Biomed Online. 2017;35(1):49; Reprod Biomed Online. 2017;35(6):750.
16. Council of Europe. The Oviedo Convention: Protecting human rights in the biomedical field. Available at: http://www.coe.int/en/web/bioethics/oviedo-convention. Last accessed: 23 April 2018.
17. Chen SH et al. A limited survey-based uncontrolled follow-up study of children born after ooplasmic transplantation in a single centre. Reprod Biomed Online. 2016;33(6):737-44.
18. Cohen J et al. Ooplasmic transfer in mature human oocytes. Mol Hum Reprod. 1998;4(3):269-80.
19. Lanzendorf SE et al. Pregnancy following transfer of ooplasm from cryopreserved-thawed donor oocytes into recipient oocytes. Fertil Steril. 1999;71(3):575-7.
20. Huang CC et al. Birth after the injection of sperm and the cytoplasm of tripronucleate zygotes into metaphase II oocytes in patients with repeated implantation failure after assisted fertilization procedures. Fertil Steril. 1999;72(4):702-6.
21. Dale B et al. Pregnancy after cytoplasmic transfer in a couple suffering from idiopathic infertility: Case report. Hum Reprod. 2001;16(7):1469-72.
22. Tzeng C et al. Pregnancy derived from mitochondria transfer (MIT) into oocyte from patient's own cumulus granulosa cells (cGCs). Fertil Steril.

2001;76:S67-8.

23. Kong LH et al. [First twins born in Mainland China by autologous granular cell mitochondria transfer]. Di Yi Jun Yi Da Xue Xue Bao. 2003;23:990-1. (In Chinese).
24. Kong LH et al. Pregnancy in a 46-year-old woman after autologous granular cell mitochondria transfer. Di Yi Jun Yi Da Xue Xue Bao. 2003;23(7):743-7.
25. Wilton PR et al. A population phylogenetic view of mitochondrial heteroplasmy. Genetics. 2018;208(3):1261-74.
26. Fakih MH et al. The AUGMENT SM treatment: Physician reported outcomes of the initial global patient experience. JFIV Reprod Med Genet. 2015;3(3).
27. Oktay K et al. Oogonial precursor cell-derived autologous mitochondria injection to improve outcomes in women with multiple IVF failures due to low oocyte quality: A clinical translation. Reprod Sci. 2015;22(12):1612-7. Erratum in: Reprod Sci. 2016;23(6):NP1.
28. Woods DC, Tilly JL. Autologous germline mitochondrial energy transfer (AUGMENT) in human assisted reproduction. Semin Reprod Med. 2015;33(6):410-21.
29. Heindryckx B et al. ESHRE SIG Stem Cells statement: The use of mitochondrial transfer to improve ART outcome. 2015. Available at: https://www.eshre.eu/~/media/sitecore-files/SIGs/Stem-Cells/SIG-Stem-Cells-opinion-16102015.pdf. Last accessed: 23 April 2018.
30. Horan CJ, Williams SA. Oocyte stem cells: Fact or fantasy? Reproduction. 2017;154(1):R23-35.
31. Tachibana M et al. Towards germline gene therapy of inherited mitochondrial diseases. Nature. 2013;493(7434):627-31.
32. Paull D et al. Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. Nature. 2013;493(7434):632-7.
33. Yamada M et al. Genetic drift can compromise mitochondrial replacement by nuclear transfer in human oocytes. Cell Stem Cell. 2016;18(6):749-54.
34. Kang E et al. Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations. Nature. 2016;540(7632):270-5.
35. Zhang J. Revisiting germinal vesicle transfer as a treatment for aneuploidy in infertile women with diminished ovarian reserve. J Assist Reprod Genet. 2015;32(2):313-7.
36. Otsuki J et al. Aggregated chromosomes transfer in human oocytes. Reprod Biomed Online. 2014;28(3):401-4.
37. Ma H et al. Functional human oocytes generated by transfer of polar body genomes. Cell Stem Cell. 2017;20(1):112-9.
38. Zhang SP et al. Polar body transfer restores the developmental potential of oocytes to blastocyst stage in a case of repeated embryo fragmentation. J Assist Reprod Genet. 2017;34(5):563-71.
39. Steuerwald N et al. Quantification of mtDNA in single oocytes, polar bodies and subcellular components by real-time rapid cycle fluorescence monitored PCR. Zygote. 2000;8(3):209-15.
40. Wakayama T et al. Participation of the female pronucleus derived from the second polar body in full embryonic development of mice. J Reprod Fertil. 1997;110(2):263-6.
41. Reinhardt K et al. Medicine. Mitochondrial replacement, evolution, and the clinic. Science. 2013;341(6152):1345-6.
42. Greenfield A et al. Assisted reproductive technologies to prevent human mitochondrial disease transmission. Nat Biotechnol. 2017;35(11):1059-68.
43. Pascalev A, Vidalis T. 'Vague Oviedo': Autonomy, culture and the case of previously competent patients. Bioethics. 2010;24(3):145-52.
44. Alberts B et al., "Genetic information in eucaryotes," Alberts et al. (eds.), Molecular biology of the cell (2008) 5th edition, Garland Science, pp.26-30.
45. Stern H. Preimplantation genetic diagnosis: Prenatal testing for embryos finally achieving its potential. J Clin Med. 2014;3(1): 280-309.
46. White SL et al. Genetic counseling and prenatal diagnosis for the mitochondrial DNA mutations at nucleotide 8993. Am J Hum Genet. 1999;65(2):474-82.
47. Bredenoord AL et al. Ethics of modifying the mitochondrial genome. J Med Ethics. 2011;37(2):97-100.
48. Harris J. Germline manipulation and our future worlds. Am J Bioeth. 2015;15(12):30-4.
49. Mitalipov S et al. Limitations of preimplantation genetic diagnosis for mitochondrial DNA diseases. Cell Rep. 2014;7(4):935-7.
50. Darnovsky M. A slippery slope to human germline modification. Nature. 2013;499(7457):127.
51. Ishii T. Potential impact of human mitochondrial replacement on global policy regarding germline gene modification. Reprod Biomed Online. 2014;29(2):150-5.
52. Human Fertilisation and Embryology Authority. Licence Committee minutes: Centre 0017 (Newcastle Fertility at Life): Variation of licensed activities to include mitochondria pronuclear transfer (PNT). 2017. Available at: http://ifqtesting.blob.core.windows.net/umbraco-website/1331/2017-03-09-licence-committee-minutes-variation-of-licensed-activities-to-include-mitochondria-pronuclear-transfer-pnt-centre-0017.pdf. Last accessed: 29 March 2018.
53. Alikani M et al. First birth following spindle transfer for mitochondrial replacement therapy: Hope and trepidation. Reprod Biomed Online. 2017;34(4):333-6.
54. Cwik B. Designing ethical trials of germline gene editing. N Engl J Med. 2017;377(20):1911-3.
55. HFEA. Human Fertilisation and Embryology Authority (HFEA) Code of Practice: 33. Mitochondrial donation. 2017. Available at: https://www.hfea.gov.uk/code-of-practice/33#section-header. Last accessed: 23 April 2018.
56. Tetsuya I, César PG. Mitochondrial replacement techniques: Genetic relatedness, gender implications, and justice. Gender and the Genome. 2017;1(4):129-34.