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Expert Statement

Mitochondrial Donation Expert Working Committee

NHMRC

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Professor Anne Kelso AO
Chief Executive Officer
National Health and Medical Research Council

Dear Professor Kelso

As Chair of the Mitochondrial Donation Expert Working Committee (the Committee), I am pleased to present to you the *Mitochondrial Donation Expert Working Committee Statement to the NHMRC CEO on the science of mitochondrial donation* (the Expert Statement).

The Expert Statement has been prepared following the Government's response to the Senate Community Affairs References Committee 2018 *Inquiry into the science of mitochondrial donation and related matters*. It builds on the outcomes of the Senate Inquiry by addressing the three specific questions from Recommendation 2 of the Inquiry relating to the science of mitochondrial donation.

The Committee engaged in robust discussion in meetings held between March 2019 and March 2020. The Committee was respectful in its interactions and members recognised and appreciated each other's different perspectives and expertise. The range of expertise and the diversity of viewpoints brought a richness to the debate and enabled a meaningful engagement with the questions posed.

The Committee agreed that, given the complexity of the issues raised, some disagreement was both to be expected and acceptable. As such, the Expert Statement reflects the consensus view of the Committee on each question as far as was possible.

The Expert Statement provides four responses to Question One, one response to Question Two and four responses to Question Three, and the derivation of these responses is described in Section 6–8 of the Expert Statement. Given the considerable expertise and knowledge of the Committee, members also agreed to provide three general responses for your consideration, as discussed in Section 9.

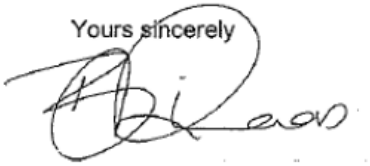
The Committee strongly supports the publication of the Expert Statement since it has been informed by some of Australia's leading experts in this field and will be of interest to a variety of stakeholders both nationally and internationally.

In parallel to developing the Expert Statement, the Committee also provided advice and support for the NHMRC consultation on the social and ethical issues related to mitochondrial donation. The Committee noted that there will be significant interest from the community in the outcomes of the consultation. The Committee also strongly encourages the publication of the consultation report.

I would like to acknowledge and thank the Committee members for their contribution and commitment in preparing this Expert Statement. It has been a privilege to serve as Chair and I am grateful for the professional contributions of my fellow members, which was integral to finalising this work. On behalf of the Committee, I would also like to thank the NHMRC team for their support and exemplary quality of work during this process.

Finally, on behalf of the Committee, we appreciate the opportunity to contribute to this important conversation about the possible introduction of mitochondrial donation into clinical practice in Australia. We look forward with interest to the evolution of this discussion.

Yours sincerely



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11 March 2020

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Executive Summary

Mitochondrial DNA disease refers to a group of inherited conditions that can cause serious health issues and, in severe cases, reduced life expectancy. Currently, there is no known cure, and treatment options are limited largely to management of symptoms. Between one in 5,000 and one in 10,000 Australians are estimated to develop severe mitochondrial DNA disease during their lifetime. The average lifespan of children with mitochondrial DNA disease is estimated to be between three and 12 years of age. However, mitochondrial DNA disease can affect people at any age – some individuals do not develop symptoms until their adult years.

Mitochondrial donation is a new assisted reproductive technology that seeks to reduce the risk of a child inheriting mitochondrial DNA disease from a woman carrying the condition. Mitochondrial donation involves combining the nuclear DNA from a male and female with healthy mitochondrial DNA from a donor egg. Current Australian legislation prohibits the use of mitochondrial donation in clinical practice.

As with all new medical technologies, any consideration of introduction into Australian clinical practice must be informed by a consideration of their underlying science, including whether the technologies are effective and safe.

The Office of the National Health and Medical Research Council (ONHMRC) established the Mitochondrial Donation Expert Working Committee to provide expert advice to NHMRC's Chief Executive Officer (CEO) on the science of mitochondrial donation. The *Mitochondrial Donation Expert Working Committee Statement to the NHMRC CEO on the science of mitochondrial donation* (the Statement) provides the Committee's advice on the science of mitochondrial donation and was formulated through a series of meetings, workshops, and out-of-session advice.

The Statement has been prepared following the Government's response to the Senate Community Affairs References Committee 2018 *Inquiry into the science of mitochondrial donation and related matters*. It builds on the outcomes of the Senate Inquiry by addressing three specific questions relating to the science of mitochondrial donation. The Statement reflects the consensus view of the Committee on each question as far as possible.

The Statement does not make a recommendation on whether or not mitochondrial donation should be introduced into Australian clinical practice. There were differing views within the Committee as to whether the current risks and scientific unknowns are such that it would be appropriate at this time to consider mitochondrial donation for introduction into Australian clinical practice.

A summary of the approach taken and the Committee response to each question is below.

1. **Whether mitochondrial donation is distinct from germline genetic modification**

   To address this question, the Committee considered important terms and concepts, international considerations of this issue, and the outcomes of the Australian Senate Inquiry.

   The Committee advises that it is essential to recognise the potential heritability of changes to the genome introduced by mitochondrial donation, regardless of whether

or not the term germline genetic modification applies. While there is scope to prevent the transmission of changes resulting from mitochondrial donation beyond the first generation by restricting the clinical procedure to produce male offspring only, there are ethical, scientific and practical considerations that make this practice problematic. The term "germline genetic modification" also has conceptual drawbacks and therefore would not be appropriate for classifying mitochondrial donation. Furthermore, the techniques that collectively constitute mitochondrial donation may warrant separate legislative considerations.

2. **Is there any new information to indicate that research findings from the United Kingdom, that the science of mitochondrial donation is safe for introduction into controlled clinical practice, cannot be applied in an Australian context?**

   To address this question, the Committee identified key research publications that were not considered by the UK Human Fertilisation and Embryology Authority (HFEA) scientific review process because they had not been published or were not within scope of the review. A list of 35 publications was compiled covering five broad research themes: developments in mitochondrial donation techniques; developments in carryover and reversion; mitochondrial-nuclear interactions; clinical use of mitochondrial transfer techniques; and analysis of patterns of mitochondrial DNA transmission and inheritance. The Committee considered the evidence presented in the publications, the relevance and significance of the findings and remaining evidence and knowledge gaps related to the science of mitochondrial donation. The Committee did not review the findings of the 2016 HFEA scientific review.

   The Committee advises that incremental developments have been made on some aspects of the science of mitochondrial donation since the 2016 HFEA scientific review. However, there is no significant new evidence, since the 2016 HFEA scientific review, about the safety and efficacy of mitochondrial donation.

3. **Whether other approaches to inheriting mitochondrial disease should also be the focus of Australian research.**

   To address this question, the Committee considered all the reproductive options currently available to prospective parent/s, including non-medical options, currently available clinical options, mitochondrial donation options and emerging gene editing options. In this context, the Committee provided advice about whether further research should be undertaken for any of these options.

   The Committee advises that further in vitro, animal and clinical research into the safety and efficacy of two techniques for mitochondrial donation (maternal spindle transfer and pronuclear transfer) would enable the techniques to be better understood and refined. The Committee did not reach a consensus about whether this research should precede introduction of mitochondrial donation into clinical use in Australia, or be undertaken during or after its introduction.

   Emerging mitochondrial donation techniques, including polar body transfer and germinal vesicle transfer, would require further research to refine the techniques and evaluate whether the level of safety and efficacy would make these techniques appropriate for introduction into clinical practice.

Currently, mitochondrial donation techniques are not the focus of significant Australian research. This may be due to lack of opportunities, a decline in skills-based expertise or legislation prohibiting clinical use of mitochondrial donation. The Committee was divided about whether it is essential for research into mitochondrial donation techniques to be carried out in Australia or by Australian researchers.

However, the Committee advises that further research into gene editing techniques for the purpose of preventing the transmission of mitochondrial disease should not be a priority at this time in Australia.

In considering the three questions, the Committee also discussed several broader issues related to the possible introduction of mitochondrial donation into Australian clinical practice.

The Committee advises that, if mitochondrial donation is introduced into Australian clinical practice, people at risk of transmitting mitochondrial DNA disease need to be provided with comprehensive information on all available reproductive options. The Committee further advises that, if mitochondrial donation is introduced, the legal framework should allow for the use of all techniques that demonstrate acceptable levels of safety and efficacy and should also be able to be responsive to new developments in the field.

The Committee further acknowledges that, when considering the appropriateness of introducing mitochondrial donation into Australian clinical practice, the responses provided here need to be considered alongside the outcomes of the wider NHMRC consultation on the social and ethical issues associated with mitochondrial donation.

Summary of Committee responses

Question 1	Whether mitochondrial donation is distinct from germline genetic modification
	A. It is essential to recognise the potential heritability of changes to the genome introduced by mitochondrial donation, regardless of whether or not the term germline genetic modification applies.
	B. While there is scope to prevent the transmission of changes resulting from mitochondrial donation beyond the first generation by restricting the clinical procedure to male offspring only, there are ethical, scientific and practical considerations that make this practice problematic.
	C. The term “germline genetic modification” has conceptual drawbacks and therefore would not be appropriate for classifying mitochondrial donation.
	D. The techniques that collectively constitute mitochondrial donation may warrant separate legislative considerations.

Question 2	**Is there any new information to indicate that research findings from the United Kingdom, that the science of mitochondrial donation is safe for introduction into controlled clinical practice, cannot be applied in an Australian context?**
	E. There is no significant new evidence, since the 2016 HFEA scientific review, about the safety and efficacy of mitochondrial donation.

Question 3	**Whether other approaches to inheriting mitochondrial disease should also be the focus of Australian research.**
	F. Further in vitro, animal and laboratory research into the safety and efficacy of MST and PNT would enable the techniques to be better understood and refined. The Committee did not reach a consensus about whether this research should precede introduction of mitochondrial donation into clinical use in Australia, or be undertaken during or after its introduction.
	G. Emerging mitochondrial donation techniques, including PBT and GVT, could benefit from further research to refine the techniques and evaluate whether the level of safety and efficacy would make these techniques appropriate for introduction into clinical practice in Australia.
	H. Currently, mitochondrial donation techniques are not the focus of significant Australian research. This may be due to lack of opportunities, a decline in skills-based expertise or legislation prohibiting clinical use of mitochondrial donation. The Committee was divided about whether it is essential for research into mitochondrial donation techniques to be carried out in Australia or by Australian researchers.
	I. Further research into gene editing techniques for the purpose of preventing the transmission of mitochondrial disease should not be a priority at this time in Australia.

General responses	J. If mitochondrial donation is introduced into Australian clinical practice, people at risk of transmitting mitochondrial DNA disease will need to be provided with comprehensive information on all available reproductive options, including advantages and disadvantages (for example, see table in Appendix C).
	K. If mitochondrial donation is introduced into clinical practice in Australia, the legal framework should allow for the use of all techniques that demonstrate acceptable levels of safety and efficacy.
	L. These recommendations must be considered alongside the outcomes of the consultation on the social and ethical issues associated with mitochondrial donation.

1. Abbreviations

ART	Assisted reproductive technology
CRISPR or CRISPR-Cas 9	Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). CRISPR/Cas-9 stands for CRISPR-associated protein-9 nuclease (Cas-9).
ESC	Embryonic Stem Cell
GMO	Genetically modified organism
GVT	Germinal vesicle transfer
HFEA	Human Fertilisation and Embryology Authority
iPSC	Induced Pluripotent Stem Cells
IVF	In vitro fertilisation
MRT	Mitochondrial Replacement Therapy
MST	Maternal spindle transfer, also known as Metaphase II spindle transfer
mtDNA	Mitochondrial DNA
NHMRC	National Health and Medical Research Council
NuMTs	Nuclear mitochondrial DNA segments
PBT	Polar body transfer
PGT	Preimplantation genetic testing (previously known as Preimplantation genetic diagnosis; PGD)
PHCR Act	*Prohibition of Human Cloning for Reproduction Act 2002*
PNT	Pronuclear transfer
RIHE Act	*Research Involving Human Embryos Act 2002*
RNA	Ribonucleic acid
SCNT	Somatic cell nuclear transfer
TALENS	Transcription Activator-Like Effector Nucleases
ZFNs	Zinc finger nucleases

2. Glossary

assisted reproductive technology (ART)	The application of laboratory or clinical techniques, such as IVF, to gametes (oocytes and sperm) and/or embryos for the purposes of reproduction.
blastocyst	A five to seven-day old embryo, containing approximately 150 cells. It is the first time that the embryo divides into two compartments (one which will give rise to the placenta and the other to the embryo). This is an embryo that has the potential to implant.
CRISPR or CRISPR-Cas9	A technique of gene editing. Also known as the CRISPR-Cas9 technique.
Deoxyribonucleic acid (DNA)	A molecule which is composed of four different types of chemical compounds (called nucleotides). The sequence of these nucleotides encodes the genetic instructions for the development, functioning, growth and reproduction of all known organisms. Changes (or 'mutations') to the sequence can introduce errors into the genetic instructions.
gamete	A sperm or an oocyte.
genetically modified organism (GMO)	A plant, animal or other organism which had been changed using gene technology.
genome	The nuclear and mitochondrial DNA composition that represents the complete set of genes/genetic material present in the organism's nucleus and the cellular organelles (i.e. the mitochondria), respectively.
germinal vesicle transfer (GVT)	A mitochondrial donation technique similar to MST. However, it involves extracting and transferring chromosomes from an oocyte at an earlier stage of maturation.
haplogroup	Corresponds to the common maternal origins of a species as defined by the haplotypes.
haplotype	A set of markers (identified DNA sequences) on the mtDNA or the nuclear DNA that tend to be inherited together trans-generationally.
heritable changes	Changes that can be inherited by future generations.
heteroplasmy	Heteroplasmy occurs where a cell, tissue or person contains more than one mitochondrial DNA genotype, which may include a mix of healthy and mutated mitochondrial DNA. This may lead to mitochondrial DNA disease of varying severity.

homoplasmy	Homoplasmy occurs where all copies of mitochondrial DNA in a cell, tissue or a person are identical. As a consequence, all the mitochondrial DNA may be affected by mutations, which could lead to mitochondrial DNA disease, or completely unaffected.
human embryo	A discrete entity that has arisen from either the first mitotic division when fertilisation of a human oocyte by sperm is complete, or any other process that initiates organised development of a biological entity with a human genome that has the potential to develop to the stage at which the primitive streak appears, which has not yet reached eight weeks of development since the first mitotic division.
Human Fertilisation and Embryology Authority (HFEA)	The UK's independent regulator overseeing the use of gametes and embryos in fertility treatment and research. The HFEA ensures that fertility clinics and research centres comply with the Human Fertilisation and Embryology Act 1990 and the Human Fertilisation and Embryology Act 2008 and provides guidance through the HFEA Code of Practice.
in vitro	Research or process performed outside a living organism.
in vivo	Research or process performed within an entire living organism.
in vitro fertilisation (IVF)	A technology using human oocytes and sperm to create embryos in the laboratory.
karyoplast	A nucleus surrounded by a nuclear membrane and adhering cytoplasm.
mitochondria	Organelles (small structures within a cell) for energy generation and other cell functions. Mitochondria contain a small amount of DNA.
mitochondrial DNA	The DNA that resides in the mitochondria rather than the nucleus of a cell. Unlike nuclear DNA, mitochondrial DNA is only inherited from the mother.
mitochondrial donation	A technique that involves the transfer of nuclear DNA into an oocyte or zygote that has a native population of mitochondria but has had its nuclear DNA removed. Also known as mitochondrial replacement therapy (MRT) or mitochondrial transfer.
maternal spindle transfer (MST)	A mitochondrial donation technique that involves transfer of the nuclear DNA, in the form of the maternal spindle, from an unfertilised oocyte into an enucleated oocyte from a donor who has healthy mitochondrial DNA. Also known as Metaphase II spindle transfer.
mutation	A change in the DNA sequence that can result in biological effects or disease.

nuclear DNA	The genetic material in the nucleus of a cell. DNA is assembled into chromosomes. A human cell usually has 46 chromosomes, 23 from each parent. Sperm cells and oocyte cells each have 23 chromosomes.
NUMTs	Mitochondrial DNA-like sequences in the nucleus segments or nuclear DNA of mitochondrial origin
oocyte	A female reproductive cell, i.e. an egg.
polar body transfer (PBT)	A mitochondrial donation technique that involves transfer of the nuclear DNA, in the form of a polar body, from an oocyte or zygote into an enucleated oocyte from a donor who has healthy mitochondrial DNA.
preimplantation genetic testing (PGT) or PGD (as used by HFEA)	A procedure used in assisted reproductive technology to screen for embryos affected by a genetic condition prior to transfer of the embryo to the woman's uterus.
prenatal testing	A form of genetic testing during pregnancy (such as chorionic villus sampling or amniocentesis) that can be used to identify whether a fetus is likely to develop a genetic disease.
pronuclear transfer (PNT)	A mitochondrial donation technique that involves transfer of nuclear DNA, in the form of female and male pronuclei, from a zygote into an enucleated zygote constructed from the oocyte of a donor who has healthy mitochondrial DNA.
ribonucleic acid (RNA)	A polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes.
somatic cells	Any cell in the body other than a reproductive (germline) cell.
somatic cell nuclear transfer (SCNT)	An assisted reproductive technology that involves introduction of a nucleus from a somatic cell into an oocyte with the chromosomal DNA removed.
transmission	The passing of genetic material from parents to their offspring.
zygote	A fertilised oocyte before the completion of first cell division. It contains genetic material from the male and the female.

3. Background

3.1 Mitochondrial DNA disease and mitochondrial donation

Mitochondrial DNA disease is an inherited condition that can cause serious health issues and, in severe cases, reduced life expectancy. Currently, there are no known cures, and treatment options are limited largely to management of symptoms.

Due to the role of mitochondria in energy production within cells, mitochondrial DNA disease particularly affects organs that use the most energy, such as the heart, muscles and brain. The symptoms and prognosis for people with mitochondrial DNA disease depend on the type and number of mutations and how the affected mitochondria are distributed among the person's tissues and organs.

Mitochondrial donation is a new assisted reproductive technology (ART) that seeks to reduce the risk of a child inheriting mitochondrial DNA disease from a woman carrying the condition. It allows for an embryo to be produced using the nuclear DNA from a man and a woman, and the mitochondrial DNA from an oocyte donated by another woman. It does not treat existing mitochondrial DNA disease, but may allow a mother who carries mutations in her mitochondrial DNA to have a genetically related child with a reduced risk of a mitochondrial DNA disease occurring. The long-term consequences of this new technology are not yet known.

3.2 Mitochondrial donation techniques

Mitochondrial donation involves the transfer of nuclear DNA from the oocyte or zygote of an affected mother into an enucleated donor oocyte or zygote containing healthy mitochondrial DNA. The resultant embryo is implanted into the uterus through standard in vitro fertilisation (IVF) processes. Techniques for mitochondrial donation include:

- Maternal spindle transfer (MST)

In MST, the spindle shaped group of chromosomes containing the mother's nuclear DNA, known as the 'maternal spindle', is extracted from one of the mother's oocytes and transferred to an unfertilised donor oocyte containing healthy mitochondrial DNA from which the maternal spindle has been removed. The oocyte is then fertilised with the father's sperm and implanted into the uterus. This technique is also known as metaphase II spindle transfer.

- Pronuclear transfer (PNT)

PNT involves the fertilisation of a mother's oocyte and a donor oocyte before the transfer of nuclear DNA. In this process, an oocyte is fertilised with sperm and becomes a zygote, including two pronuclei which contain the mother's and father's nuclear DNA. A donor oocyte is also fertilised and then the pronuclei from the donor zygote are removed and replaced by the pronuclei containing the mother's and father's nuclear DNA.

- Polar body transfer (PBT)

PBT involves the transfer of nuclear DNA from the polar body of an oocyte or zygote into an enucleated oocyte or zygote from a donor who has healthy mitochondrial DNA. In the case of an oocyte, it is then fertilised with the father's sperm.

- Germinal vesicle transfer (GVT).

GVT is a mitochondrial donation technique similar to MST; however it involves extracting chromosomes from oocytes that are at an earlier stage of development.

Mitochondrial donation techniques are discussed in Section 8.3.

3.3 Mitochondrial donation and the law

Mitochondrial donation for human reproduction is currently illegal in Australia (see also Section 6.5.2). NHMRC is responsible for administering the two relevant pieces of legislation through the Embryo Research Licensing Committee: the *Research Involving Human Embryos Act 2002* (RIHE Act) and the *Prohibition of Human Cloning for Reproduction Act 2002* (PHCR Act). Among other things, the PHCR Act prohibits:

- the creation of embryos with genetic material from more than two people
- heritable alterations to the genome of human embryos for reproductive purposes.

Mitochondrial donation is legal in the United Kingdom under a licensing regime for limited use, to prevent the transmission of serious mitochondrial DNA disease. Other countries such as the United States of America, Canada and many EU countries do not allow the technology at present. Mitochondrial donation has been reported as having occurred in other countries where there is no specific legal regime.

3.4 Outcomes of the Senate Inquiry

On 20 February 2019, the Government released its response to the 27 June 2018 report of the Senate Community Affairs References Committee Inquiry into *The science of mitochondrial donation and related matters* (Senate Inquiry report).

The Senate Inquiry report made five recommendations (see Senate Inquiry report paragraphs 5.99, 5.100, 5.101, 5.103 and 5.104).

Of relevance to this Statement is **Recommendation 2**, namely:

> 5.100 The committee recommends that the Australian Government task the National Health and Medical Research Council with advising on the following questions:
>
> - Whether mitochondrial donation is distinct from germline genetic modification. [hereafter referred to as Question 1]
> - Is there any new information to indicate that research findings from the United Kingdom, that the science of mitochondrial donation is safe for introduction into controlled clinical practice, cannot be applied in an Australian context? [hereafter referred to as Question 2]
> - Whether other approaches to inheriting mitochondrial disease should also be the focus of Australian research. [hereafter referred to as Question 3]

The Government response to this recommendation was:

> The Government supports the Senate Committee recommendation to seek expert advice on key questions. The Government notes the Report recommends that an assessment of the UK scientific findings should be

made by a panel of Australian experts with relevant scientific and consumer knowledge, to be appropriately constituted and overseen by NHMRC. NHMRC is well equipped to undertake this task and provide advice in line with recommendation 2.

The Government understands that currently experts in Australia and elsewhere agree that the clinical use of mitochondrial donation, where permitted, should proceed cautiously with appropriate oversight and follow-up, and that research in Australia and overseas should continue to confirm which techniques are the most clinically efficacious.

The advice sought may include broader information than that set out by the Senate Committee and it may also inform the consultation process, and any consideration of any possible legislative change, including necessary regulation.

On 13 March 2019, the Minster for Health tasked NHMRC with establishing an expert panel to provide advice on the three questions.

4. Purpose of this Statement

The purpose of this Statement is to advise the NHMRC CEO on the three scientific questions about mitochondrial donation arising from the Senate Inquiry.

5. Development of the Statement

The Mitochondrial Donation Expert Working Committee (the Committee) was established (terms of reference and membership are at Appendix A) to provide advice on this Statement. The Committee undertook this work in parallel with providing advice on a public consultation about the social and ethical issues associated with mitochondrial donation. Committee members were also involved significantly in public engagement activities, which were undertaken as part of the public consultation.

The recommendations presented in this Statement are the result of extensive discussion in Committee meetings and workshops, and additional advice provided out of session. Formal meetings were held on 26 March 2019, 30 August 2019, 29 October 2019, 19 December 2019 and 3 March 2020. Workshops on the specific questions addressed in this Statement were held on 24 September 2019 and 14 October 2019.

In considering the responses to the three questions in this Statement, the Committee only considered the use of mitochondrial donation for reducing the risk of mitochondrial disease being transmitted to offspring. Other possible uses of mitochondrial donation were not considered.

Throughout the process, disclosures of interests and management of conflicts of interest were undertaken in accordance with the requirements of the NHMRC Act and NHMRC's *Policy on the disclosure of interest requirements for prospective and appointed NHMRC committee members*. Members were required to disclose their interests prior to appointment to the Committee and had an ongoing responsibility to disclose new

interests. A record of Committee members' interests was maintained by NHMRC, and an overview of the disclosed interests was published on the NHMRC website.

The Committee as a whole determined if any interest could affect a member's capacity to bring an independent mind to bear on the matters being considered by the Committee. Members determined to be conflicted were excluded from discussion of the specific issues and did not contribute to any decisions on the issue.

In particular, Committee members with authored publications of relevance to Question Two did not participate in discussion and decision-making related to their own publications.

6. Response to Question One

Whether mitochondrial donation is distinct from germline genetic modification.

The Senate Inquiry identified the matter of whether mitochondrial donation is distinct from germline genetic modification as a question that must be resolved before potentially considering legislative change to allow mitochondrial donation. There is scientific and ethical uncertainty about how mitochondrial donation should be classified, and this question is important to resolve because such classifications can have ethical and legal implications.

6.1 Committee approach

To address this question, the Committee has considered important terms and concepts, international approaches to this issue, and outcomes of the Senate Inquiry.

Based on these considerations, the Committee provides advice and a series of recommendations on:

- a comparison of mitochondrial donation and germline genetic modification
- legislative implications if mitochondrial donation is classified as germline genetic modification, and
- whether mitochondrial donation is distinct from germline genetic modification.

6.2 Initial consideration of important terms and concepts

The following ideas and concepts are important in considering whether mitochondrial donation constitutes germline genetic modification.

6.2.1 Difference between "germline" and "somatic"

The "germline" refers to cells through which DNA is inherited by offspring. In humans, these include the reproductive cells, that is, sperm and oocytes. Somatic cells include all other types of cells and are not inherited by future generations. The main distinction between somatic cells and reproductive cells is that somatic cells contain 23 pairs of chromosomes within their nuclear DNA, whereas sperm and oocytes possess only 23 single chromosomes within their nuclear DNA.

When gene(s) in the somatic cells are intentionally altered through somatic gene therapy, these changes are only intended to affect the person being treated and are not in general heritable. In contrast, germline gene therapy involves intentional changes to the germline cells, which introduces changes that are passed on to future generations.

Currently, only somatic gene therapy is legal in Australia and is carefully regulated. Any intentional alteration of the DNA in germline cells is currently prohibited.

6.2.2 Difference between the use of "genetics" and "genomics"

The World Health Organization (WHO) contrasts genetics and genomics, with genetics being considered as exploring the functioning and composition of individual genes, and genomics being concerned with all genes and their combined influence on the growth and development of the organism (World Health Organization, 2019).

As such, a 'genetic modification' could be used to refer to changes that have been intentionally made to individual gene(s) in the DNA sequence, or the genetic code of a given individual. A 'genomic modification' could refer to intentional changes at the level of the genome, such as changes across multiple genes, or to components such as whole chromosomes. However, these words are sometimes used interchangeably.

6.2.3 Differences in how mitochondrial DNA and nuclear DNA are inherited

Heritability is the characteristic of being capable of transmission from one generation to the next. Mitochondrial DNA and nuclear DNA are inherited differently through the germline. Mitochondrial DNA is transmitted through the maternal line, via female offspring, to subsequent generations. In contrast, nuclear DNA is passed through both maternal and paternal germ cells to future generations. In other words, nuclear DNA is inherited from both biological (i.e. genetic) parents, but mitochondrial DNA is inherited from the biological mother.

When changes occur to the nuclear DNA in sperm and oocytes, this will likely result in these changes also being present in any offspring produced from these cell types. However, when changes occur to the mitochondrial DNA of germ cells, they can only be transmitted to future generations through female offspring. Changes to mitochondrial DNA in sperm will not usually be passed on to subsequent generations because the transmission of mitochondrial DNA is typically via the maternal line. Inheritance of mitochondrial DNA mutations also differs from nuclear DNA due to heteroplasmy and the bottleneck effect. Heteroplasmy refers to the fact that human cells typically have thousands of copies of mitochondrial DNA, so mutations can be present in a cell at any level ranging from 0% to 100% of the mitochondrial DNA population and typically only cause disease when present at high levels. The bottleneck effect describes a process during oocyte development whereby individual oocytes from the same female can end up with vastly different amounts of mutant mitochondrial DNA.

6.2.4 Framework for approaching the classification of mitochondrial donation

To decide whether mitochondrial donation is distinct from germline genetic modification, consideration could be given to:

- The target of the change, for instance, whether the intervention is applied to the nucleus or organelle of a cell.

- The method used to achieve the change, i.e. the characteristics of the particular techniques being used.
- How the introduced change is inherited (refer to Section 6.2.3).

All of these considerations can impact on the classification of mitochondrial donation when compared with other technologies that may involve gene editing or gene therapy of human embryos or germ cells.

6.3 International approaches

The Committee considered the reviews undertaken in the United Kingdom (UK) and the United States of America (US), which resulted in different classifications of mitochondrial donation.

6.3.1 United Kingdom

In 2014, the UK Government consulted with the British public on draft regulations to enable mitochondrial donation to be used in clinical practice in the UK. This followed a series of reviews by the UK's national fertility regulator, the HFEA, into the safety and efficacy of two mitochondrial donation techniques: maternal spindle transfer (MST) and pro-nuclear transfer (PNT). These techniques are explained in Section 3.2. Subsequently, the draft regulations were passed in 2015, thereby allowing the use of these mitochondrial donation techniques in the UK.

The UK Government asserted that MST and PNT resulted in germline modification, because their effects will be passed to future generations. However, they decided these mitochondrial donation techniques did not constitute *genetic* modification, since they defined genetic modification as requiring "germline modification of *nuclear* DNA…that can be passed on to future generations".[1] As such, because mitochondrial donation does not involve the modification of nuclear DNA, the UK Government concluded that it does not constitute genetic modification. This conclusion has been the subject of some conceptual criticism in bioethics and philosophy of science literature.[2,3,4]

6.3.2 United States of America

The US Food and Drug Administration (FDA) requested the National Academies of Sciences, Engineering, and Medicine (NASEM) to make recommendations as to whether the US should proceed with mitochondrial donation, referred to in the US as mitochondrial replacement therapy. This independent body convened a committee of experts and developed a consensus report which was published in 2016.[5] This committee considered

[1] Public Health Directorate/Health Science and Bioethics Division. Mitochondrial Donation: Government response to the consultation on draft regulations to permit the use of new treatment techniques to prevent the transmission of a serious mitochondrial disease from mother to child. London: Department of Health; 2014.

[2] Lewens T. Blurring the germline: Genome editing and transgenerational epigenetic inheritance. Bioethics. 2020 Jan;34(1):7-15. doi: 10.1111/bioe.12606.

[3] Newson AJ, Wrigley A. Is Mitochondrial Donation Germ-Line Gene Therapy? Classifications and Ethical Implications. Bioethics. 2017 Jan;31(1):55-67. doi: 10.1111/bioe.12312.

[4] Scott R, Wilkinson S. Germline Genetic Modification and Identity: the Mitochondrial and Nuclear Genomes. Version 2. Oxf J Leg Stud. 2017 Dec;37(4):886-915. doi: 10.1093/ojls/gqx012.

[5] National Academies of Sciences, Engineering, and Medicine. Mitochondrial replacement techniques: Ethical, social and policy considerations. Washington, DC: The National Academies Press; 2016.

the ethical, social and policy issues raised by mitochondrial donation, including how it should be classified.

In contrast to the UK, NASEM defined genetic modification as "changes to the genetic material within a cell", and as such concluded that mitochondrial donation constituted genetic modification because the mitochondrial DNA from one woman would be replaced by that of another woman within an oocyte or zygote (a fertilised oocyte). However, since NASEM used the definition of germline modification as "human inheritable genetic modification", and since changes to mitochondrial DNA are *not* always heritable (i.e. changes will not be passed from a male to the next generation), NASEM recommended limiting the use of mitochondrial donation to male embryos to avoid the technique being germline modification.

No action has yet been taken in response to the NASEM report.

6.4 Outcomes of the Australian Senate Inquiry

The Senate Inquiry considered whether mitochondrial donation might constitute germline genetic modification, since it was recognised that this would have implications for how mitochondrial donation can be legalised in Australia (see also Section 6.5.2).

The Senate Inquiry received several submissions on this issue, and the report stated that the majority of the evidence suggested that "mitochondrial donation is not considered a form of germline genetic modification as envisioned by Australian laws which prohibit cloning and other similar forms of genetic modification" (Senate Inquiry report, paragraph 5.25, p81). The Senate Inquiry report asserted that there is an ethical difference between the manipulation of nuclear and mitochondrial DNA, since each provide different contributions to heritable characteristics.

However, the Senate Inquiry report acknowledged that mitochondrial donation does result in changes to mitochondrial DNA that can affect future generations.

As such, the Senate Inquiry report suggested referring this issue to an expert panel to make a recommendation about whether mitochondrial donation constitutes germline genetic modification.

6.5 Committee's advice

6.5.1 Comparison of mitochondrial donation and germline genetic modification

The Committee considered the consistencies and distinctions between mitochondrial donation and other technologies that could be regarded as germline genetic modification.

The Committee agrees that the most important consistency is that mitochondrial donation can result in changes to the total genetic composition of a cell that can be inherited by future generations. This is the central issue, regardless of how mitochondrial donation is classified.

If germline genetic modification is to be contrasted with germline genomic modification then mitochondrial donation will fall in the latter grouping. However, the historical usage and precedence is that germline genetic modification refers to all forms of introduced

heritable alterations. Mitochondrial donation is a form of genome replacement, which could be deemed a heritable alteration.

The Committee advises that there are several potential differences between mitochondrial donation and germline genetic modification. These are:

- Mitochondrial donation is a form of ART. Germline gene editing techniques may not necessarily be used in conjunction with ARTs, for example, gene therapy could be targeted to egg and sperm cells which may then be fertilised naturally.
- Mitochondrial donation involves replacement of whole organelles, while germline genetic modification techniques generally involve direct editing of nuclear genes or the expression of artificial gene constructs.
- The target of modification in mitochondrial donation is the whole mitochondrial genome rather than specific genes, as in some other technologies such as gene therapy.
- Mitochondrial donation for the purpose of preventing the transmission of mitochondrial DNA disease from a mother to her offspring is in contrast to some germline gene editing techniques that could potentially be used for the purpose of enhancement.

6.5.2 Legislative implications if mitochondrial donation is classified as germline genetic modification

If mitochondrial donation was to be introduced into clinical practice in Australia, legislative change would be necessary. The Committee advises that the manner in which mitochondrial donation is classified has important consequences for how legislation and regulations would need to change, and could impact the introduction and regulation of other emerging technologies.

Commonwealth legislation that is potentially relevant to whether mitochondrial donation is classified as germline genetic modification includes the PHCR Act and the *Gene Technology Act 2000.*

It should be noted that the term “germline genetic modification” does not appear in either of these Acts, and mitochondrial donation is not specifically referred to in these Acts. However, the terms “genetic” and “modification” do appear in the *Gene Technology Act 2000,* including in the definition of gene technology.

Clinical use of mitochondrial donation for reproductive purposes is currently prohibited in Australia. The PHCR Act prohibits the creation of an embryo containing “genetic material provided by more than 2 persons” (Section 13), and placing that prohibited embryo into a woman (Section 20). It also prohibits altering the genome if the alteration is heritable or “intended to be heritable by descendants of the human whose cell was altered” (Section 15). As such, these sections prohibit mitochondrial donation.

The *Gene Technology Act 2000* is relevant because it defines genetically modified organisms (GMOs), and currently only excludes humans where modification is through somatic gene therapy. This means if mitochondrial donation is not classified as somatic gene therapy all biological entities, including gametes, zygotes, embryos and humans resulting from this technology could be regulated as GMOs, unless this legislation is amended.

The Committee also noted that Australia is a member of the United Nations Educational, Scientific and Cultural Organization (UNESCO), which set out guidance for how human germline interventions should be approached. The UNESCO *Universal Declaration on the*

Human Genome and Human Rights (1997) suggests that human germline intervention is not appropriate.[6]

6.5.3 Is mitochondrial donation distinct from "germline genetic modification"?

The Committee advises that the term "germline genetic modification" has conceptual drawbacks in light of emerging technologies because:

- The term was developed to apply to other sorts of technologies, and interpreting this term by comparing mitochondrial donation with these other technologies is complex.
- Australian legislation does not specifically use the term "germline genetic modification".
- There is a range of terms and sub-terms that are being regularly used to classify genetic/genomic technologies with greater specificity. For instance, these expressions include "germ-line intervention", "intentional modification of the human genome", "germ-line gene therapy on humans", "the genetic manipulation of human germ cells", "gene alteration (including 'gene therapy') that involves human germline cells", and "genetic manipulation of human germ cells".[7]
- Several international organisations use terminology other than germline genetic modification to describe new and emerging technologies. For instance, both the International Commission on the Clinical Use of Human Germline Genome Editing and the WHO Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing use the term "human germline genome editing" and the Second International Summit on Human Genome Editing refers to "germline genome editing".[8]
- It would be preferable to use more specific or appropriate terms than the over-arching term "germline genetic modification".

As such, the Committee advises that, rather than focussing on whether mitochondrial donation is distinct from "germline genetic modification," it is more relevant to advise on the underlying concepts of this definition, since these have practical implications for the possible introduction of mitochondrial donation into Australia.

The underlying concepts of this definition are:

1. mitochondrial donation results in changes to the mitochondrial genome, and
2. modifications to the mitochondrial genome can be passed on to future generations as a result of mitochondrial donation.

Consequently, the Committee advises that the term germline genetic modification is not necessarily useful in considering whether to proceed with mitochondrial donation in Australia.

The Committee agrees that the key feature of mitochondrial donation is that it does result in potentially heritable changes to the genome. As such, the Committee concludes that

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[6] UN Educational, Scientific and Cultural Organization (UNESCO). The Universal Declaration on the Human Genome and Human Rights; 1997. Retrieved from United Nations Human Rights Office of the High Commissioner: https://www.ohchr.org/EN/ProfessionalInterest/Pages/HumanGenomeAndHumanRights.aspx

[7] International Bioethics Committee (IBC). *Report of the IBC on pre-implantation genetic diagnosis and germ-line intervention.* Paris: United National Educational, Scientific and Cultural Organisation; 2003.

[8] World Health Organization. Human genomics in global health; 2019. Retrieved from World Health Organization: https://www.who.int/genomics/en/

mitochondrial donation can be a form of germline modification, since the modified mitochondrial genome can be inherited by future generations.

The Committee is divided on whether mitochondrial donation is also a form of genetic modification, with some members preferring mitochondrial donation to be described as a form of *genomic* modification, given that the intention is to replace the mitochondrial genome rather than edit specific genes.

While there is scope to prevent the transmission of changes resulting from mitochondrial donation beyond the first generation, by restricting resultant offspring to males (given the transmission of mitochondrial DNA occurs through the maternal line and only very rarely through the paternal germ line), there are ethical, scientific and practical considerations that may make this problematic.

6.6 Committee responses to Question One

The Committee makes the following responses to Question 1 (Whether mitochondrial donation is distinct from germline genetic modification):

A. It is essential to recognise the potential heritability of changes to the genome introduced by mitochondrial donation, regardless of whether the term germline genetic modification applies.

B. While there is scope to prevent the transmission of changes resulting from mitochondrial donation beyond the first generation by restricting the clinical procedure to male offspring only, there are ethical, scientific and practical considerations that may make this practice problematic.

C. The term "germline genetic modification" has conceptual drawbacks and therefore would not be appropriate for classifying mitochondrial donation.

D. The techniques that collectively constitute mitochondrial donation may warrant separate legislative considerations.

7. Response to Question Two

Is there any new information to indicate that research findings from the United Kingdom, that the science of mitochondrial donation is safe for introduction into controlled clinical practice, cannot be applied in an Australian context?

7.1 Committee approach

To address this question, the Committee identified key research findings that were not considered by the HFEA scientific review process because they had not been published or were not within scope of the review. Publications are considered according to five broad research themes, based on the themes of the 2016 HFEA scientific review.

The themes are: developments in mitochondrial donation techniques; developments in carryover and reversion; mitochondrial-nuclear interactions; clinical use of mitochondrial transfer techniques; and, analysis of patterns of mitochondrial DNA (hereafter mtDNA) transmission and inheritance.

Following an overview of the HFEA scientific review process (Section 7.2) and the outcomes of the Australian Senate Inquiry (Section 7.3), the Committee advises for each theme on:

- the "state-of-play" based on the conclusions of the 2016 HFEA scientific review
- a detailed analysis of the new evidence about the safety and efficacy of mitochondrial donation, and
- a summary of the new evidence and recommendations on the relevance and significance of the findings, in an Australian context, and remaining evidence and knowledge gaps related to the science of mitochondrial donation.

The Committee did not review the findings of the 2016 HFEA scientific review.

7.2 Overview of HFEA scientific review process

In February 2015, the UK Parliament approved regulations permitting the use of two assisted reproduction technologies, maternal spindle transfer (MST) and pronuclear transfer (PNT), to prevent the inheritance of severe mitochondrial disease. The decision by the UK Parliament was informed in part by three scientific reviews (2011, 2013 and 2014) by HFEA on the safety and efficacy of mitochondrial donation techniques.

The 2014 review incorporated the findings from the two previous reviews. The main conclusions were that:

- MST and PNT are likely to be effective in avoiding mitochondrial disease caused by mutations in mtDNA
- no evidence was found to suggest that the techniques would be unsafe in humans, and
- the direction of travel of current research is consistent with both these findings.

An addendum to the review dealing specifically with the safety and efficacy of polar body transfer (PBT) was provided by HFEA in October 2014. As with MST and PNT, no evidence was identified to indicate PBT is unsafe; however, it was noted that research into PBT was at an early stage and required further monitoring.

In November 2016, following the passage of mitochondrial donation legislation, the HFEA provided the UK Government with a further scientific review (*Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2016 update*) of the safety and efficacy of mitochondrial donation techniques. This review was part of an assessment of whether mitochondrial donation was ready for use in clinical practice. The HFEA's review specifically considered the effectiveness and safety of MST and PNT, as well as clinical considerations for mitochondrial donation. The review did not explicitly consider other emerging mitochondrial donation techniques, such as PBT or Germinal Vesicle Transfer (GVT). The panel undertaking the review continued to see clinical value in the use of MST and PNT to mitigate or prevent the inheritance of mitochondrial disease. The review recommended the cautious adoption of MST and PNT in clinical practice, only in situations where the inheritance of mitochondrial disease is likely to cause death or serious disease and where there are no acceptable alternatives.

The 2016 HFEA scientific review included a number of recommendations to make sure the technology was adopted with caution. The recommendations covered topics including patient selection, the need to establish acceptable levels of mtDNA heteroplasmy to

indicate when transfer of an embryo is advised, and consideration of matching mtDNA haplotype/haplogroup.

7.3 Outcomes of the Australian Senate Inquiry

The Senate Inquiry heard evidence on mitochondrial donation techniques that could be used to reduce the risk of women transmitting mitochondrial disease caused by mutated mtDNA to their children. The Inquiry also considered potential risks of the techniques and whether mitochondrial donation techniques are considered to be safe to perform on human embryos that will develop into live babies. The Senate Inquiry report acknowledged that its role was not to make definitive scientific findings and recognised the importance of seeking further advice on the safety and efficacy of mitochondrial donation since its introduction into the UK in 2016. The Senate Inquiry recommended that formal endorsement of the UK scientific findings should be made by a panel of Australian experts with relevant scientific knowledge, and that further scientific consideration should be given to emerging issues such as mtDNA carryover during mitochondrial donation and haplogroup matching. This Statement focusses on the new information about the safety and efficacy of mitochondrial donation since the 2016 HFEA scientific review and does not seek to make a formal endorsement of the UK scientific findings.

7.4 Key research findings since the 2016 HFEA scientific review

Key publications relevant to the safety and efficacy of mitochondrial donation that have been published since the 2016 HFEA scientific review are presented in Appendices B1 and B2. Publications related to the safety and efficacy of PBT, which were not considered in the 2016 HFEA scientific review, are also included.

Appendix B1 contains information on the 35 publications (ref #1–ref #35) identified, such as publication title, journal, keywords, year of publication, location of research and method used. The publications are grouped into primary research articles (ref #1–ref #22) or other articles (ref #23–ref #35). The publications discussed in Section 7.5–7.9 are referred to by the reference number in Appendix B1.

Appendix B2 contains full publication details and abstract (where available) for each publication.

The majority of new research findings since 2016 have originated from the UK, elsewhere in Europe, the US and China. Three primary research publications have come from Australian researchers. None of the research from Australia has been specifically performed using any of the mitochondrial donation techniques.

The research conducted has involved a range of different methods to investigate the safety and efficacy of mitochondrial donation techniques, including animal studies (mouse and pig), human studies and studies using oocytes, embryos, and cell lines such as human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). It is important to note that each of these methods has a range of advantages and limitations, but are necessary due to the limited opportunities for research on mitochondrial donation using human embryos. The use of data from these methods requires judgement and interpretation to understand their relevance to the clinical use of mitochondrial donation in humans. The limitations and advantages of each method are discussed below.

The Committee recognises that the use of animal models often is a prerequisite for human studies into new medical interventions. Animal models, especially mice, have been used in eight of the publications listed in Appendix B1. Animal models generally have a number of advantages for understanding the safety and efficacy of the mitochondrial donation. Animal models allow studies to be undertaken in vivo and allow multigenerational studies to be undertaken in a much shorter time period than the human reproductive cycle. However, the applicability of animal model findings to human mitochondrial donation in a clinical setting is limited by differences between the animal model and humans in terms of the reproductive system, cells or cellular processes, and characteristics of mtDNA genomes. Despite this, such studies can still highlight areas that should be considered in approval or monitoring of mitochondrial donation.

Results from other mitochondrial manipulation technologies (besides MST, PNT, GVT or PBT) in animal or human models can also inform our understanding of mitochondrial donation safety and efficacy. These methods have been used in four of the publications in Appendix B1 and include cytoplasmic transfer and mitochondrial supplementation. The former has been used clinically in humans in a limited number of cases since 1996. Although these technologies allow examination of in vivo instances where different mtDNA populations have been mixed, consideration needs to be given to the differences between these technologies and mitochondrial donation. Results from these technologies, which involve the introduction of a small amount of mtDNA into a cell, may inform our understanding of the consequences of mtDNA carryover during mitochondrial donation.

Research using cell lines such as human ESCs has the advantage of being an accessible method of investigating aspects of the safety and efficacy of mitochondrial donation in human cells. This method has been used in three of the publications. There are a number of considerations about the applicability of experimental results from human ESCs to the understanding of mitochondrial donation safety and efficacy. There are a number of differences between ESC lines (often called “primed” ESCs) and in vivo ESCs (often called “naive” ESCs) or embryonic tissues, including differences in developmental and cellular processes, energy production and tissue homeostasis.

Recognising that research on mitochondrial donation in humans is limited, it remains that results of research using human ESC lines are valuable and such lines provide a model for investigating mitochondrial donation safety and efficacy in humans, without the need for fetal intervention.

One publication used somatic cell nuclear transfer (SCNT). SCNT is an accessible method of investigating factors such as the influence of different mtDNA haplotypes on the nuclear genome by using primary cells which all possess the same nuclear genotype. It is similar to mitochondrial donation techniques, since most of the mtDNA is from the donor oocyte. As with mitochondrial donation techniques, SCNT also suffers from a small amount of mtDNA carryover introduced with the transfer of nuclear DNA. Although SCNT provides an interesting model to study segregation of carried over mtDNA, the major difference is that it uses somatic rather than germ cells and this needs to be considered when applying the results to clinical use of mitochondrial donation.

7.5 Developments in mitochondrial donation techniques

7.5.1 HFEA’s conclusion in 2016 review

The 2016 HFEA scientific review only examined the safety and efficacy of MST and PNT. Due to legislative restrictions in the UK, other mitochondrial donation techniques were not

addressed. It was concluded that the success rates of both MST and PNT were suitable for clinical implementation. In particular, PNT had been optimised since the 2014 review by altering the timing of pronuclear transplantation and changing the media used in the process, which appeared to lead to reduced carryover and maintained blastocyst formation rates and quality, while not resulting in changes to gene expression profiles or aneuploidy rates.

7.5.2 New evidence on mitochondrial donation techniques

Since the 2016 HFEA scientific review, Zhang et al. reported in 2017 (ref #15) that MST was used clinically in attempting to prevent the transmission of mtDNA disease in a human. The oocyte manipulations were carried out in the US but implantation was performed in Mexico as the technology is currently banned in the US. The intending mother carried a mtDNA mutation associated with Leigh syndrome and was at risk of transmitting it to her offspring. A male blastocyst (a five- to seven-day old embryo containing approximately 150 cells) with a mitochondrial mutation load of 5.7% was produced from reconstituted oocytes. The embryo was implanted into the mother, leading to the birth of a boy with mutation loads of 2.36–9.23% in the tested tissues. The boy was reported to be healthy at seven months of age. There appear to have been no further follow-ups. However, there are concerns about some aspects of this case, including some uncertainties concerning the necessity of using MST, methodologies and results (see ref #35).

In 2017, Wu et al. (ref #18) described a modified PNT protocol (pre-pronuclear transfer) which involved the transfer of just the maternal pre-pronucleus approximately four hours after fertilisation rather than both maternal and paternal pronuclei 6–8 hours after fertilisation, as is usual in PNT procedures. This modified procedure has the potential advantage of avoiding the need for compounds (e.g. cytochalasin B or nocodazole) used in the MST and PNT procedures, as their safety in human embryo procedures has not been rigorously evaluated. The results of this experiment revealed that developmental potential of pre-PNT embryos was comparable to unmanipulated embryos and carryover of donor mtDNA was comparable to MST and PNT at about 1% or less. The authors noted the need for further studies in animal models to prove safety and efficacy.

Research on PBT from Wu et al. in 2017 (ref #17) has shown that PBT techniques involving either the transfer of a first polar body to an unfertilised human oocyte (PB1T) or a second polar body to a fertilised human oocyte (PB2T) can lead to a substantial proportion of the manipulated cells developing into embryos. These embryos appear to have normal chromosomes and low levels of mtDNA carryover. This research further supported the potential utility of PBT as a mitochondrial donation technique as did earlier research such as Ma, O'Neil, Gutierrez et al. (2017)[9]. [Although published after 2016, this article was considered by the HFEA and is therefore not included in Appendix B1 and B2].

In 2019, Tang et al. published findings aimed at comparing the efficiency of MST, PNT, PB1T and PB2T techniques (ref #4). Using a mouse model, they found that all techniques could result in a similar rate of blastocyst development. It was also shown that MST and PNT resulted in a similar level of carryover, and that this level was higher than that resulting from PB1T and PB2T techniques.

[9] Ma H, O'Neil RC, Marti Gutierrez N, Hariharan M, Zhang ZZ, He Y, Cinnioglu C, Kayali R, Kang E, Lee Y, Hayama T, Koski A, Nery J, Castanon R, Tippner-Hedges R, Ahmed R, Van Dyken C, Li Y, Olson S, Battaglia D, Lee DM, Wu DH, Amato P, Wolf DP, Ecker JR, Mitalipov S. Functional Human Oocytes Generated by Transfer of Polar Body Genomes. Cell Stem Cell. 2017 Jan 5;20(1):112-119. doi: 10.1016/j.stem.2016.10.001.

Research into whether GVT is a viable option for mitochondrial donation is underway but no peer-reviewed publications were identified for inclusion in Appendix B1 and B2.

7.5.3 Summary

Since the 2016 HFEA scientific review, research into mitochondrial donation techniques has continued. Research into MST and PNT has neither reported significant safety or efficacy concerns, nor demonstrated clearly that it is safe. Research has also progressed on other mitochondrial donation techniques such as PBT and GVT, though research suggests these techniques are not currently as refined as MST and PNT.

7.6 Developments in Carryover and Reversion

In the mitochondrial donation process, carryover arises when mutated mtDNA is transferred from the mother's affected oocyte along with the nuclear DNA. This is of concern because it is not yet known what level of carryover may lead to a risk of mtDNA disease in the future child. Reversion is where a child born following mitochondrial donation develops mtDNA disease later in life, perhaps as a result of the carried-over mtDNA replicating more efficiently than donor mtDNA.

7.6.1 HFEA's conclusion in 2016 review

The HFEA concluded that MST and PNT had been sufficiently refined (in expert laboratories) to reduce carryover to a level that was not expected to cause disease, i.e. less than 2% in most embryos tested. These refinements had in part come from developments in techniques of karyoplast extraction. The HFEA asserted that preimplantation genetic diagnosis (referred to here as preimplantation genetic testing, or PGT) following mitochondrial donation could enable embryos with low levels of mutant mtDNA to be selected and implanted, thus potentially reducing the risk of reversion. The HFEA also stated that prenatal genetic testing during a pregnancy following mitochondrial donation could provide reassurance about having reduced the risk of mitochondrial disease resulting from reversion for subsequent generations.

However, the HFEA review noted that even low levels of mutant mtDNA could lead to reversion if it is preferentially amplified (perhaps by effects on replication) or segregated differentially during cell division. The HFEA review also noted that this possibility was based on experiments with ESC lines cultured in vitro and stated that results from these experiments should be interpreted with caution when considering their implications for reversion following clinical use of mitochondrial donation.

The HFEA panel asserted that further research was required on whether different mtDNA sequences conferred a replicative advantage to one haplotype over another in specific combinations and emphasised the importance of systematic, long-term follow-up of people born as a result of MST or PNT to identify potential risks of the technology.

7.6.2 New evidence on carryover and reversion

There have been a number of studies since 2016 that provide further information about carryover rates for mitochondrial donation techniques. The clinical use of MST reported by Zhang's group (ref #15) reported a mtDNA carryover rate of 5.7% in the resulting blastocyst and 2.36–9.23% in tissues tested from the resulting boy. Tang et al. (ref #4) reported that carryover levels for MST and PNT were similar in a mouse model (specifically mean levels of 3.57% and 4.13%, respectively). Both techniques had

significantly higher levels of carryover than PBT, with PB1T blastocysts having 0.62% carryover and PB2T blastocysts having 0.91%.

Tsai et al. (ref #14) and Cagnone et al. (ref #21) examined the effect of mitochondrial supplementation in pig oocytes with abnormally low amounts of mtDNA that were preventing normal embryonic development. Supplementation of relatively small amounts of mitochondria (representing <1% of their existing mtDNA content) from sister oocytes was found to improve the developmental potential of the oocytes and resulted in epigenetic changes to a nuclear gene responsible for mtDNA replication, suggesting that carryover could be biologically relevant. Similarly, mtDNA supplementation in mouse oocytes using mitochondria from egg precursor cells by St John et al. (ref #5) was found to lead to several changes across generations, including increases in litter size and the number of primordial follicles in the ovary, and abnormal heart structure.

In 2019, a study was published by Hudson et al. (ref #26) that challenged the conclusion of previous studies that reversion to the original maternal mtDNA in embryos was only seen when the amount of mtDNA carryover was greater than 2%. This group reanalysed reversion data from MST experiments and reported that reversion could occur in prolonged culture of ESCs derived from an embryos with less than 2% carryover. The group also explored the previous recommendation of matching donor and recipients according to the similarity of a specific region of mtDNA to assist in avoiding reversion and concluded that this was not supported. In a reply, Kang et al. (ref #27) disputed this and provided further analysis of the sequence of this region and suggested that pairing this region between maternal and donor mtDNA may be important for reducing the incidence of reversion.

Yin et al. (ref #6) used a pig model to study the introduction, segregation and heritability of mtDNA transfer during the SCNT process. They reported that nuclear donor mtDNA was less than 5% or undetectable in ear biopsies and blood samples in most of the SCNT-derived pigs, but did identify 14 tissues in the pigs where levels of nuclear donor mDNA were as high as 95%. The group also reported that mtDNA haplotypes influenced mitochondrial respiration capacity in fibroblasts from offspring.

A recent review article suggests that techniques based on gene editing tools such as transcription activator-like effector nucleases (TALENs) or zinc-finger nucleases (ZFNs) (Section 8.4) could be used in conjunction with mitochondrial donation to eliminate carried over mutant mtDNA and prevent reversion (ref #23).

7.6.3 Summary

Carryover of mtDNA from mitochondrial donation and the potentially resulting reversion are the focus of ongoing research. Results from some studies using ESCs have identified the possibility that mtDNA carryover could lead to reversion to significant levels of maternal mtDNA.

Likewise, animal experiments involving mitochondrial supplementation have suggested that small amounts of carried-over mtDNA could have biological impact. However, whether these findings apply to mitochondrial donation for humans is not yet established.

7.7 Mitochondrial-nuclear interactions

Mitochondrial DNA is maternally inherited. People from different haplotypes have different mtDNA. A haplogroup corresponds to the common maternal origins of a species. In

humans, there are about 25 different major variations of the mtDNA sequence and they largely correspond to continental population groups. Each haplogroup contains sub-categories, called haplotypes.

Mitochondrial-nuclear interactions refer to the potential ways that components of the cell nucleus could affect the functioning of the mitochondria within that cell, or vice-versa.

Mitochondrial donation will result in the intending mother's cell nucleus being paired with the donor mtDNA haplogroup, which may be different to the intending mother's haplogroup. There has been some concern that this mismatch could have negative consequences for the child born following mitochondrial donation.

7.7.1 HFEA's conclusion in 2016 review

In 2014 and 2016, the HFEA panel concluded that consideration should be given to mtDNA haplogroup matching when considering donors, but believed the risks of not doing so would be very low. The panel also recommended that follow-up of children born following mitochondrial donation should include assessment of whether the degree of haplotype mismatch is correlated with any preferential replication of mutated mtDNA over time, or the general state of health of the child, to inform future decision making. It was acknowledged that further research was required to understand the clinical requirement for haplotype matching.

7.7.2 New evidence for mitochondrial-nuclear interactions

Several recent publications have reported findings about the importance of considering haplotypes for mitochondrial donation, including a number of large scale studies. However, these findings do not provide a consensus on the importance of haplogroup matching for mitochondrial donation.

In a study of 2,504 individuals across 26 populations, Rishiwar and Jordan (ref #16) observed the extent of naturally occurring nuclear-mitochondrial mismatch seen in individuals with parents from different mitochondrial haplotypes. They concluded that mitochondrial and nuclear genomes from divergent human populations can co-exist within healthy individuals, suggesting that human nuclear-mitochondrial mismatches are not likely to jeopardise the safety of mitochondrial donation. Similarly, Eyre-Walker (ref #34) provided a population genetics perspective on evidence of whether mitochondrial-nuclear interactions are likely to pose a problem for mitochondrial donation. Eyre-Walker undertook a meta-analysis of 231 cases, from a variety of animals, in which mtDNA from one strain was placed into the nuclear background of another strain of the same species. Eyre-Walker predicted that deleterious mitochondrial-nuclear interactions are unlikely to be much more prevalent in individuals born following mitochondrial donation than for normal reproduction.

In contrast, a systematic review of 116 publications on mitochondrial-nuclear interactions across species by Dobler et al. (ref #28) predicted that mitochondrial donation would have adverse health implications and affect phenotypic features such as gene expression, anatomy, metabolism and life history. Humans were predicted to be the most affected and they "conservatively estimate negative effects in at least one in every 130 resulting offspring born to the therapy [MRT]". They asserted that further research into the molecular nature of mitochondrial-nuclear interactions would be needed to refine the clinical application of mitochondrial donation and establish what level of mtDNA haplotype variation between patient and donor would be acceptable to ensure a match. Further, Royrvik et al. (ref #22) undertook a study of human mtDNA sequences and

modelled random haplotype pairings. The model predicts that haplogroup matching in mitochondrial donation would help to reduce the transmission of mtDNA mutations/deletions. They provide a chart to assist with matching haplotypes for mitochondrial donation.

Recent studies, including Latorre-Pellicer et al. (ref #2), Wei et al. (ref #3) and Pickett et al. (ref #10) have also reported that mitochondrial-nuclear DNA interactions have an effect on the resulting level of heteroplasmy and offspring health and well-being.

Burgstaller et al. (ref #13) reported in 2018 that there is a divergence in heteroplasmy between germline and somatic precursor cells early in development, with a haplotype specific direction of segregation. This suggests that the choice of oocyte donor may require both consideration of haplotype matching as well as whether reversion would be predicted to take place against that particular haplotype.

While not specifically relating to mitochondrial donation, a follow-up report was published in 2016 by Chen et al. (ref #20) about the children born after cytoplasmic transfer performed originally by the Cohen group over 1996–2001[10,11]. The cytoplasmic transfer involved taking a small amount of cytoplasm (including mtDNA) from a donor oocyte and transferring it to a patient oocyte in an attempt to address recurrent implantation failure from IVF. Although the report only provided anecdotal evidence (rather than conducting medical assessment or tests to report on features such as heteroplasmy) and did not refer to abnormalities cited in previous work by the same group, it was noted that parents had reported their children to be in good health.

7.7.3 Summary

A number of new studies have examined aspects of mitochondrial-nuclear interactions resulting from mitochondrial donation. These publications have not provided a consensus on the importance of haplogroup matching or the risks of mis-matching. However, there is evidence from some studies to suggest that haplogroup matching may be important and that this area warrants further consideration.

7.8 Clinical alternatives to mitochondrial donation

This section considers whether there has been new evidence about the clinical aspects of reducing the transmission of mtDNA disease, including the use of preimplantation genetic testing (PGT) and prenatal testing. PGT is a procedure sometimes used in assisted reproductive technology to identify and screen for embryos affected by a genetic condition prior to transfer of the embryo to the women's uterus.

7.8.1 HFEA's conclusion in 2016 review

The 2016 HFEA report did not contain an in-depth review of PGT to avoid mitochondrial disease as this had been provided in the 2014 report. In the 2014 report, the HFEA asserted that PGT may be effective in reducing the risk of a child being born with mitochondrial disease for some women, though it is not suitable if there is a high proportion of abnormal mtDNA or the mtDNA is homoplasmic. Further, they considered girls born following PGT may themselves be at risk of having affected children as they

[10] Brenner CA, Barritt JA, Willadsen S, Cohen J. Mitochondrial DNA heteroplasmy after human ooplasmic transplantation. Fertil Steril. 2000;74(3):573–578. doi:10.1016/s0015-0282(00)00681-6

[11] Barritt JA, Brenner CA, Malter HE, Cohen J. Mitochondria in human offspring derived from ooplasmic transplantation. Hum Reprod. 2001 Mar;16(3):513-6. doi: 10.1093/humrep/16.3.513.

may have mutant mtDNA present in their oocytes. The HFEA report also identified that research into whether PGT provides a reliable indicator of heteroplasmy level was mixed, with some research suggesting it was and other research suggesting significant variability.

7.8.2 New evidence for clinical alternatives to mitochondrial donation

A number of recent studies have looked at the appropriateness of genetic testing as an alternate clinical option for people at risk of transmitting mtDNA disease.

Pickett et al. (ref #10) reviewed data on 238 adults with a common mtDNA mutation and could not accurately predict clinical outcomes based on an individual's mutation load. This work suggests that reproductive options such as prenatal testing and PGT may not accurately predict the likelihood of an embryo or pregnancy resulting in a child who will develop mtDNA disease.

This group also attempted to estimate the likely success of PGT in women at risk of passing on mtDNA disease (ref #25). They found that PGT is unlikely to be able to prevent the transmission of mitochondrial disease in women with mutation loads above 50%.

Sallevelt et al. (ref #19) reviewed 105 families with mtDNA disease and concluded that approximately a quarter of affected children appeared to be de novo cases, meaning the mother lacked detectable mutation and there was no other indication of mitochondrial disease in the family. This suggested that the majority of the mother's oocytes may have mostly or entirely healthy mtDNA. They identified 137 similar cases by literature review, and concluded that the risk of having a second affected child was low in such families, meaning they could potentially be offered confirmatory testing by prenatal testing or choose to use PGT, accompanied by careful genetic counselling.

7.8.3 Summary

There has been new research on the adequacy of genetic testing to accurately predict health outcomes for the children of those at risk of transmitting mtDNA disease. This research indicates there may be limitations in its utility in many cases. However, in a minority of families with mtDNA disease, the risk of having another affected child appears to be sufficiently low that techniques like prenatal testing or PGT can have clinical utility and be acceptable options for couples.

7.9 Analysis of patterns of mitochondrial DNA transmission and inheritance

Research on mtDNA transmission and inheritance is directly applicable to consideration of mitochondrial donation as it potentially impacts the efficacy of the techniques in preventing mitochondrial disease.

7.9.1 HFEA's conclusion in 2016 review

While aspects of this body of research were considered by the HFEA, this was not the focus of specific conclusions about the safety and efficacy of mitochondrial donation.

7.9.2 New evidence about mitochondrial DNA transmission and inheritance

A number of recent studies have looked at mtDNA heteroplasmy transmission and changes. Burgstaller et al. (ref #13) looked at the population dynamics of mtDNA heteroplasmy throughout the lifetime and generations in two genetically distinct mouse

models. They showed that mtDNA heteroplasmy is dynamic in oocytes and somatic tissues, with increases during aging. They also report that there is a divergence in heteroplasmy between germline and somatic precursor cells early in development, with a haplotype specific direction of segregation.

Luo et al. (ref #9) described three families in which paternal as well as maternal mtDNA appear to be inherited over multiple generations. If this finding is robust, it suggests that males as well as females could potentially transmit mtDNA disease, which would have many implications for preventing transmission of mtDNA disease. However, a number of commentators have cast doubt on the findings (e.g. Lutz-Bonegel and Parson, 2019)[12], suggesting they could be due to technical error. Santibanez-Koref et al. (ref #7) also highlight the potential confounding effect of "NuMTs" (copies of mtDNA sequence in the nuclear genome) in sequencing studies of mtDNA using new Next Generation Sequencing technologies, because of the potential for such studies to confuse "NuMTs" with bona fide mtDNA results. Recent whole genome sequencing of cohorts of parent-child trios from Australia and the UK found no clear evidence for paternal transmission, suggesting that, if it does occur, it is extremely rare (Rius et al., 2019[13]; Wei et al., ref #3).

Otten et al. (ref #12) determined the mtDNA mutation loads of 160 oocytes, zygotes and blastomeres of carriers with mtDNA mutations and then analysed mtDNA segregation patterns. The data showed that non-random mechanisms were involved during mtDNA segregation. It was concluded that mutation-specific selection mechanisms occur during mtDNA inheritance.

The timing of heteroplasmy shift provides further information on the underlying mechanism of transmission. A recent study by Ma et al. (ref #1) used a human ESC model to investigate the timing of selection of mutated, or non-mutated, mtDNA during oocyte or embryonic development. Noting that mutated mtDNA is common in mature oocytes, the researchers developed ESCs with oocyte mtDNA mutations. They subsequently reintroduced these stem cells into chimeras and showed that mutant mtDNA is selected against during post-implantation development. In contrast, Floros et al. (ref #8) argued that the selection against deleterious mutations in mtDNA occurs during early germ cell development. With reference to the genetic bottleneck, Floros et al. found that the metabolic shift from glycolytic to oxidative metabolism in primordial germ cells exposed deleterious mutations to selection during early germ cell development, preventing the relentless accumulation of mtDNA mutations in the human population and explaining the phenotypic variation in humans with inherited disorders.

The HFEA review committee discussed the relevance of using ESC lines to study reversion in #ref 33.

The type of mtDNA mutation may also affect the dynamics of DNA heteroplasmy and mutation transmission. Russell et al. (ref #11) used induced pluripotent stem cells to show that mtDNA genomes with large-scale deletion mutations appear to be preferentially replicated, potentially resulting in higher levels of heteroplasmy and disease. The research team noted that further work is needed to understand the mechanism behind the preferential amplification.

[12] Lutz-Bonengel S, Parson W. No further evidence for paternal leakage of mitochondrial DNA in humans yet. Proc Natl Acad Sci U S A. 2019 Feb 5;116(6):1821-1822. doi: 10.1073/pnas.1820533116.

[13] Rius R, Cowley MJ, Riley L, Puttick C, Thorburn DR, Christodoulou J. Biparental inheritance of mitochondrial DNA in humans is not a common phenomenon. Genet Med. 2019;21(12):2823–2826. doi:10.1038/s41436-019-0568-0

7.9.3 Summary

Mitochondrial DNA transmission and inheritance are complex and dynamic. While not yet fully understood, the available knowledge is being used to provide existing reproductive options (prenatal testing and PGT) to couples at risk of transmitting mtDNA disease. Unless addressed, an ongoing lack of knowledge may affect our ability to develop and refine mitochondrial donation techniques.

7.10 Committee responses to Question Two

The Committee advises that the increase in understanding of the safety and efficacy of mitochondrial donation has been incremental since the 2016 HFEA scientific review. While there has been some progress in particular areas, such as the impact of mitochondrial-nuclear interactions, and some increased understanding of potential risks, overall the new evidence since 2016 does not greatly strengthen, or raise doubt about, the findings of the 2016 HFEA scientific review.

As such, the Committee makes the following recommendation:

E. There is no significant new evidence, since the 2016 HFEA scientific review, about the safety and efficacy of mitochondrial donation.

8. Response to Question Three

Whether other approaches to inheriting mitochondrial disease should also be the focus of Australian research.

The Senate Inquiry recommended that NHMRC advise on whether other approaches to inheriting mitochondrial disease should also be the focus of Australian research. The Senate Inquiry report notes that a preferred method for mitochondrial donation does not yet appear to have been identified and that it is important to ensure that the safest and most up-to-date scientific techniques are used in Australia (Senate Inquiry report, para 3.105).

8.1 Committee approach

The Committee's approach to determining whether other ways of preventing mtDNA disease should also be the focus of Australian research, was to consider a wide range of reproductive options, those currently available to prospective parent/s (including non-medical options as well as currently available clinical options), mitochondrial donation options and emerging gene editing options.

Based on consideration of these options, the Committee provides advice and a series of recommendations on:

- the advantages and disadvantages of the options to prevent transmission of mtDNA disease
- the likelihood of significant advances for these options and whether any of these options should be the focus of Australian research, and
- considerations for possible future implementation.

No options were excluded from this consideration, including different mitochondrial donation techniques.

8.2 Non-medical and currently available clinical options

The Committee's advice on the advantages, disadvantages and considerations for implementation of non-medical and currently available clinical options is at Appendix C.

Non-medical options include deciding not to have children, conceiving naturally and adoption. Currently available clinical options include prenatal testing of fetal or placental tissue, PGT and use of a donor oocyte. The Committee advises that these options do not require further research to be used clinically. However, there may be areas where further social or other types of research would improve or refine their application.

The Committee notes that, if mitochondrial donation is introduced into Australian clinical practice, people at risk of transmitting mtDNA disease will need to be provided with comprehensive information on all their available options, including advantages and disadvantages for each, to support informed decision making.

8.3 Mitochondrial donation techniques

Mitochondrial donation involves the transfer of nuclear DNA from the oocyte or zygote of an affected mother into an enucleated donor oocyte or zygote containing healthy mtDNA.

A summary of four mitochondrial donation techniques is provided at Section 3.2. MST and PNT are currently allowed in UK clinical practice to prevent the inheritance of severe mtDNA disease, whereas PBT and GVT are emerging mitochondrial donation techniques. Currently, none of these options are legal in Australian clinical practice.

The Committee's advice on the advantages and disadvantages of each technique is at Appendix C, and is summarised below.

The main advantage of MST and PNT is that these techniques are more refined and have a significantly larger evidence base than PBT and GVT. This status quo may continue as only MST and PNT are allowed in the UK.

In contrast, the HFEA concluded in 2014 that additional experimentation was required to examine the safety and efficacy of PBT.

PBT and GVT both require significant further research to demonstrate adequate levels of safety and efficacy before they could be considered for introduction into Australian clinical practice. The potential advantages of these techniques mean that it is worthwhile for PBT and GVT to be the focus of more research. These advantages include:

- PBT may result in less carryover of mutated mtDNA into the reconstructed oocyte/zygote and could be used for the reconstruction of two oocytes per manipulation.
- GVT may have a reduced chance of damaging the chromosomes in the process of transferring them to the reconstructed oocyte, as the chromosomes are extracted at an earlier stage when they are packaged in an intact membrane. It may also avoid the requirement for the oocyte donor and mother to undergo more severe ovarian hyperstimulation and allow more time for the reconstructed oocyte to establish normal cellular functions.

However, further in vitro, animal and laboratory research into the safety and efficacy of all four mitochondrial donation techniques (MST, PNT, PBT and GVT) would be expected to lead to:

- improved safety and efficacy of all techniques
- direct comparison of the safety and efficacy of all four techniques, and
- a better understanding of the current 'unknowns' and risks of the techniques.

It would be appropriate for this research to be carried out using animal models. This research could be undertaken in Australia or internationally. However, the Committee notes that, to its knowledge, animal models for mtDNA disease are not currently established in Australia. Based on the evidence gaps described in Section 7, research using animal models could include full pathological analysis from multiple generations of animals born following mitochondrial donation to identify tissue or organ dysfunction, and epigenetic analysis of DNA from all organs and tissues of multiple generations of animals born following mitochondrial donation to identify any significant changes.

The Committee did not reach a consensus about whether this research should precede introduction of mitochondrial donation into clinical use in Australia, or be undertaken during or after its introduction.

8.4 Emerging gene editing options

Several emerging gene editing technologies have been put forward as potential options for preventing the inheritance of mtDNA disease. They can be divided into techniques to shift mtDNA heteroplasmy and CRISPR technology.

8.4.1 Techniques to shift mitochondrial DNA heteroplasmy

Mitochondrial DNA heteroplasmy arises where a cell, tissue or person contains more than one mtDNA genotype, which may include a mix of healthy and mutated mtDNA. This may lead to mtDNA disease of varying severity, given that the proportion of mutated mtDNA must exceed a threshold for mtDNA disease to manifest. Techniques to shift heteroplasmy aim to lower the ratio of mutated mtDNA below the threshold for disease symptoms, for example by selectively inhibiting the replication of mutant mtDNA or by selectively cleaving mutant mtDNA. The techniques include use of ZFNs or TALENs. It has been suggested that these techniques could be used in an oocyte to shift heteroplasmy and help prevent the transmission of mtDNA disease.

8.4.2 CRISPR-Cas9 gene editing system

CRISPR technology based on the CRISPR-Cas9 gene editing system has been used extensively in recent years to target pathogenic nuclear mutations in cells. It has been suggested that using it to edit the mitochondrial genome could lead to advances in mitochondrial genetics and therapeutics. There has been one report of the use of CRISPR-Cas9 to shift mtDNA heteroplasmy in mammalian cells; however this finding remains controversial as CRISPR technology relies on a guide ribonucleic acid (RNA) for targeting the desired genome location for editing and there is no clear evidence that a mechanism for importing RNA into mitochondria exists. Mitochondria also appear to lack the mechanisms for repairing DNA breaks induced by CRISPR-Cas9. Hence even if guide RNAs could be imported, CRISPR-Cas9 could not be used for correction of mtDNA mutations but only for destroying mutant genomes. Most researchers believe that CRISPR

will not work on mtDNA and so should not be considered an appropriate field for further Australian research at the expense of more promising options.

8.4.3 Committee advice on emerging gene editing options

The Committee advises that research on emerging gene technologies is at a very early stage, and that these technologies will not be clinically relevant as options for addressing mtDNA disease within the next few years. In addition, these technologies are technically challenging as they require changes to be made in the oocyte, which can have up to 500,000 copies of mtDNA, compared to somatic cells which have significantly fewer copies of mtDNA. For this reason, it is likely that gene editing techniques will be more beneficial for treatment of existing mtDNA disease through somatic cell therapy directly targeting specific tissues, rather than preventing transmission of mtDNA disease during reproduction.

There are also additional ethical and legal considerations associated with gene editing options, and international considerations such as the call for a global moratorium on heritable gene editing technologies (if indeed such a moratorium were to extend to DNA contained within mitochondria).

8.5 Summary

There are currently a number of options for prospective parents at risk of transmitting mtDNA disease to their children. These options are broadly grouped into non-medical options and currently available clinical options. New approaches aimed at preventing the inheritance of mtDNA disease, such as mitochondrial donation, have the potential to provide prospective parents with additional options when making reproductive choices.

Given the range of options, and the advantages and disadvantages associated with each, there is not a single right choice for prospective parents faced with the risk of transmitting mtDNA disease. Different options will appeal to different people for a variety of reasons. Even if mitochondrial donation was available for clinical use, some people who may be eligible to use it might choose to use other options, such as using a donor oocyte. In giving people choice, clear information about the risks and benefits of all available options needs to be provided.

MST and PNT are the two mitochondrial donation techniques currently permitted under UK legislation. However, the Committee advises that it would be appropriate to direct further Australian research into the safety and efficacy of all mitochondrial donation techniques. Enabling such research could position Australia as a leader in mitochondrial donation research and allow practitioners to develop the skills that would be required if mitochondrial donation was to be introduced into Australian clinical practice. To the Committee's knowledge, this research is not currently being undertaken in Australia.

While this additional safety and efficacy research could potentially assist in clarifying the long-term health impacts and risks of mitochondrial donation, the Committee notes that a significant amount of research is required before the implications of mitochondrial donation technologies are fully understood. There is a variety of views, in the Committee and in the broader scientific community, on the level of scientific certainty that is needed before mitochondrial donation could be implemented in clinical practice in Australia. Calculating the level of acceptable risk is not purely a scientific decision but also involves significant social and ethical considerations.

8.6 Committee responses to Question Three

The Committee makes the following responses:

F. Further in vitro, animal and laboratory research into the safety and efficacy of MST and PNT would enable the techniques to be better understood and refined. The Committee did not reach a consensus about whether this research should precede introduction of mitochondrial donation into clinical use in Australia, or be undertaken during or after its introduction.

G. Emerging mitochondrial donation techniques, including PBT and GVT, could benefit from further research to refine the techniques and evaluate whether the level of safety and efficacy would make these techniques appropriate for introduction into clinical practice in Australia.

H. Currently, mitochondrial donation techniques are not the focus of significant Australian research. This may be due to lack of opportunities, a decline in skills-based expertise or legislation prohibiting clinical use of mitochondrial donation. The Committee was divided about whether it is essential for research into mitochondrial donation techniques to be carried out in Australia or by Australian researchers.

I. Further research into gene editing techniques for the purpose of preventing the transmission of mitochondrial disease should not be a priority at this time in Australia.

9. General advice

In preparing the responses to the three questions, the Committee discussed several broader issues related to the possible introduction of mitochondrial donation into Australian clinical practice.

The first is the importance of noting that there is currently a range of reproductive options available to people who want to avoid or minimise the risk of transmitting mitochondrial DNA. If mitochondrial donation is introduced, it will add to the range of reproductive options available. Discussion of the importance and ethical aspects of increasing the available reproductive options should take into consideration outcomes from the public consultation on the social and ethical issues related to mitochondrial donation. The Committee strongly advises the need to provide thorough, balanced and accurate information to prospective parents with mitochondrial DNA disease. As such, the Committee advises that information based on the table presented in Appendix C could inform such advice.

The second issue relates to the development of legislation to allow the introduction of mitochondrial donation into Australian clinical practice, if the Government decided to proceed with this approach. The Committee noted the following challenges to legislative change:

- The PHCR and RIHE Acts would need revision and amendment especially in relation to the offence provisions.
- Other legislation and guidelines concerning ART would need to be amended.

- The legislative changes required to allow mitochondrial donation are extremely complex due to Australia's federated system. Any proposed changes will require further consultation with relevant stakeholders.
- It may be difficult to develop a suitable regulatory framework given:
    - the different regulatory pathways for embryo research and assisted reproductive technology (ART) currently operating in Australia, and
    - The complexity and pace of development of scientific advances regarding mitochondrial donation.

Due to these challenges, if mitochondrial donation is to be permitted in Australia, the Committee advises that the legal framework should be neutral to technique. That is, it should facilitate the introduction and the use of all techniques that demonstrate acceptable levels of safety and efficacy. This will enable new or emerging mitochondrial donation techniques that may have demonstrated sufficient safety and efficacy to be introduced without needing to further amend legislation. Consideration could be given, for example, to regulating outcomes rather than techniques.

The Committee agreed that all new medical technologies contain an element of risk when first used in humans. However, any decisions about the possible introduction of mitochondrial donation into Australian clinical practice must take into consideration the advice in this Statement about the scientific and technical aspects of the technology, as well as the views of the community about the social and ethical issues. Different people will require different levels of certainty regarding the risk before they consider mitochondrial donation is "safe enough" for introduction into clinical practice. There is a variety of views, both in the Committee and in the broader community, about the level of scientific certainty that is needed before mitochondrial donation could be implemented safely in clinical practice in Australia.

In summary, the Committee makes the follow general responses:

J. If mitochondrial donation is introduced into Australian clinical practice, people at risk of transmitting mitochondrial DNA disease need to be provided with comprehensive information on all available reproductive options, including advantages and disadvantages (for example, see table in Appendix C).

K. If mitochondrial donation is introduced into clinical practice in Australia, the legal framework should allow for the use of all techniques that demonstrate acceptable levels of safety and efficacy.

L. These recommendations must be considered alongside the outcomes of the consultation on the social and ethical issues associated with mitochondrial donation.

Appendix A—Mitochondrial Donation Expert Working Committee

Terms of Reference

The Mitochondrial Donation Expert Working Committee ('the Committee') will provide advice to the NHMRC Chief Executive Officer on the legal, regulatory, scientific and ethical issues identified by the Senate Community Affairs References Committee Inquiry into*: The Science of Mitochondrial Donation and Related Matters* ('the Inquiry').

The Committee will:

a. advise on key questions to underpin community-wide consultation and increase community literacy on issues raised by mitochondrial donation to be delivered by April 2019

b. consider relevant literature and advise on questions posed within Recommendation 2 of the Inquiry Report, specifically:

   i. whether mitochondrial donation is distinct from germline genetic modification

   ii. is there any new information to indicate that research findings from the United Kingdom, that the science of mitochondrial donation is safe for introduction into controlled clinical practice, cannot be applied in an Australian context, and

   iii. whether other approaches to inheriting mitochondrial disease should also be the focus of Australian research

c. advise on any other relevant issues as requested by the NHMRC Chief Executive Officer.

The Committee will be established under section 39 of the *National Health and Medical Research Council Act 1992.* Its membership shall comprise a Chair, and members with expertise and experience in the following areas:

- The genetics of mitochondrial disease and/or genetic modification.
- The science of embryology and developmental biology.
- Consumer health issues relating to mitochondrial disease.
- The clinical application of assisted reproductive technologies or gene therapies.
- Ethical and theological considerations relating to mitochondrial donation.
- The legislative and regulatory framework relevant to mitochondrial donation.
- A representative from an NHMRC Principal Committee (e.g. Embryo Research Licensing Committee and/or Australian Health Ethics Committee).

Membership

Associate Professor Bernadette Richards	Chairperson
Professor Justin St. John	Member with expertise in the genetics of mitochondrial disease and/or genetic modification and assisted reproductive technologies.
Professor David Thorburn	Member with expertise in the genetics of mitochondrial disease and/or genetic modification.
Professor Patrick Tam	Member with expertise in the science of embryology and developmental biology.
The Hon Judi Moylan AO	Member with expertise in consumer health issues relating to mitochondrial disease.
Mr Sean Murray	Member with expertise in the consumer health issues relating to mitochondrial disease.
Dr Clare Boothroyd	Member with expertise in the clinical application of assisted reproductive technologies or gene therapies.
Professor John Rasko AO	Member with expertise in the clinical application of assisted reproductive technologies or gene therapies.
Reverend Kevin McGovern	Member with expertise in the ethical and theological considerations relating to mitochondrial donation.
Professor Sheryl de Lacey	Member with expertise in the ethical and theological considerations relating to mitochondrial donation.
Professor Ainsley Newson	Member with expertise in the ethical and theological considerations relating to mitochondrial donation.
Professor Dianne Nicol	Member with expertise in legislative and regulatory framework relevant to mitochondrial donation.

Appendix B—Scientific publications since 2016 HFEA scientific review

B1: Publications table

Table B1 provides the compiled list of articles identified by the Committee as providing information about the safety and efficacy of mitochondrial donation and published since the HFEA 2016 review. Keywords were obtained from PubMed. Year is the year of publication. Location is based on the home institutions of authors. Methods identify whether mitochondrial donation (MD) or other techniques were used in primary research. Lineage identifies whether human, animal or cell line models were used in the research. Abstract provides the reference number, with the complete bibliographic details and abstracts presented at B2.

Reference in Statement	Title	Journal	DOI	Keywords	Year	Location	Methods	Lineage
PRIMARY RESEARCH ARTICLES								
Ref #1	Deleterious mtDNA Mutations are Common in Mature Oocytes	Biol Reprod	10.1093/biolre/ioz202	mitochondria; mtDNA; oocyte	2019	USA, Republic of Korea	unclear	Mouse, ESCs
Ref #2	Regulation of Mother-to-Offspring Transmission of mtDNA Heteroplasmy	Cell Metab	10.1016/j.cmet.2019.09.007	embryo; germline selection; heteroplasmy; mitochondria; mitochondrial replacement; mtDNA competition; mtDNA inheritance	2019	Spain, Finland, UK	No MD - analysis of mtDNA heteroplasmy	Mouse, iPSCs
Ref #3	Germline selection shapes human mitochondrial DNA diversity	Science	10.1126/science.aau6520	None available/ provided	2019	UK	No MD - analysis of mtDNA heteroplasmy	Human

Ref #4	Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants	Mol Hum Reprod	10.1093/molehr/gaz062	germline nuclear transfer; mitochondrial DNA; mouse model; mtDNA disease; mtDNA heteroplasmy	2019	Belgium	PBT, PNT, MST	Mouse
Ref #5	The transgenerational effects of oocyte mitochondrial supplementation	Sci Rep	10.1038/s41598-019-43135-4	None available/ provided	2019	Australia	Mitochondrial transfer (autologous mt)	Mouse
Ref #6	Dynamic Characteristics of the Mitochondrial Genome in SCNT Pigs	Biol Chem	10.1515/hsz-2018-0273	SCNT pigs; heteroplasmy; mitochondria; mtDNA	2019	China, USA	No MD - SCNT	Pig
Ref #7	Assessing mitochondrial heteroplasmy using next generation sequencing: A note of caution	Mitochondrion	10.1016/j.mito.2018.08.003	Bioinformatic analysis; Heteroplasmy; Mitochondrial DNA; Next-generation sequencing	2018	UK	No MD - analysis of mtDNA heteroplasmy	Human? (not clearly identified
Ref #8	Segregation of mitochondrial DNA heteroplasmy through a developmental genetic bottleneck in human embryos	Nat Cell Biol.	10.1038/s41556-017-0017-8 Note following correction: 10.1038/s41556-018-0064-9	None available/ provided	2018	UK	No MD - analysis of cell mito content in embryos	Human

Ref #9	Biparental Inheritance of Mitochondrial DNA in Humans	Proceedings of the National Academy of Sciences	10.1073/pnas.1810946115	biparental inheritance; human genetics; mitochondria; mtDNA; paternal transmission	2018	USA, Taiwan, China	No MD - analysis of mtDNA heteroplasmy in cell samples from patients	Human
Ref #10	Phenotypic heterogeneity in m.3243A>G mitochondrial disease: The role of nuclear factors	Ann Clin Transl Neurol	10.1002/acn3.532	heritability; m.3243A>G; mitochondrial disease	2018	UK	No MD - analysis of specific mtDNA mutation using cell samples from patients	Human
Ref #11	Preferential amplification of a human mitochondrial DNA deletion in vitro and in vivo	Sci Rep	10.1038/s41598-018-20064-2	None available/ provided	2018	UK, Switzerland	No MD - looking at mtDNA loads during cell replication/division	Human iPSCs
Ref #12	Mutation-specific effects in germline transmission of pathogenic mtDNA variants	Hum Reprod	10.1093/humrep/dey114	None available/ provided	2018	Netherlands, UK, USA	No MD - looking at transmission of mtDNA mutations	Human (oocytes, zygotes and blastomeres)
Ref #13	Large-scale genetic analysis reveals mammalian mtDNA heteroplasmy dynamics and variance increase through lifetimes and generations	Nat Commun	10.1038/s41467-018-04797-2	None available/ provided	2018	UK, Austria	No MD - analysis of mtDNA heteroplasmy	Mouse

Ref #14	The molecular characterisation of mitochondrial DNA deficient oocytes using a pig model	Hum Reprod	0.1093/humrep/dey052	None available/ provided	2018	Australia	Mitochondrial transfer (autologous mt)	Pig
Ref #15	Live birth derived from oocyte spindle transfer to prevent mitochondrial disease	Reprod Biomed Online	10.1016/j.rbmo.2017.01.013 Note following corrections: 1. 10.1016/j.rbmo.2017.04.002 2. 10.1016/j.rbmo.2017.07.008	Cytoplasm; Leigh syndrome; Meiotic spindle; Mitochondria; Nuclear transfer; Oocyte	2017	USA, Mexico	MST	Human
Ref #16	Implications of human evolution and admixture for mitochondrial replacement therapy	BMC Genomics	10.1186/s12864-017-3539-3	Population genomics; Three-person baby; mtDNA	2017	USA	No MD - mtDNA sequencing	Human
Ref #17	Polar bodies are efficient donors for reconstruction of human embryos for potential mitochondrial replacement therapy	Cell Res	10.1038/cr.2017.67	None available/ provided	2017	China	PBT	Human, ESCs
Ref #18	Mitochondrial replacement by pre-pronuclear transfer in human embryos	Cell Res	10.1038/cr.2017.48	None available/ provided	2017	China	PNT	Human, ESCs

Ref #19	De novo mtDNA point mutations are common and have a low recurrence risk	J Med Genet	10.1136/jmedgenet-2016-103876	de novo; genetic counselling; mDNA mutations; prenatal diagnosis (PND)	2017	The Netherlands UK	No MD	
Ref #20	A limited survey-based uncontrolled follow-up study of children born after ooplasmic transplantation in a single centre	Reprod Biomed Online	10.1016/j.rbmo.2016.10.003	cytoplasmic transfer; disclosure to children; limited follow-up; repeated implantation failure; survey	2016	USA	No MD - survey/follow-up study on children born after ooplasmic transfer as part of IVF	Human
Ref #21	Restoration of normal embryogenesis by mitochondrial supplementation in pig oocytes exhibiting mitochondrial DNA deficiency	Sci Rep	10.1038/srep23229	None available/ provided	2016	Australia, USA	No MD – mitochondrial transfer (autologous mt)	Pig
Ref #22	mtDNA diversity in human populations highlights the merit of haplotype matching in gene therapies	Mol Hum Reprod	10.1093/molehr/gaw062	None available/ provided	2016	Austria	No MD - simulation study of mtDNA haplotyping	Human
OTHER ARTICLES								
Ref #23	Mitochondrial DNA heteroplasmy in disease and targeted nuclease-based therapeutic approaches	EMBO reports	10.15252/embr.201949612	gene editing; heteroplasmy; mitochondrial DNA	2020	US	N/A	N/A

Ref #24	243rd ENMC international workshop: Developing guidelines for management of reproductive options for families with maternally inherited mtDNA disease, Amsterdam, the Netherlands, 22-24 March 2019	Neuromuscul Disord	10.1016/j.nmd.2019.08.004	None available/ provided	2019	Multiple countries	N/A	N/A
Ref #25	Mitochondrial Donation - Which Women Could Benefit?	N Engl J Med	10.1056/NEJMc1808565	None available/ provided	2019	UK	N/A	N/A
Ref #26	Reversion after replacement of mitochondrial DNA	Nature	10.1038/s41586-019-1623-3	None available/ provided	2019	UK	N/A	N/A
Ref #27	Reply to: Reversion after replacement of mitochondrial DNA	Nature	10.1038/s41586-019-1624-2	None available/ provided	2019	Republic of Korea, US	N/A	N/A
Ref #28	A systematic review and meta-analysis reveals pervasive effects of germline mitochondrial replacement on components of health	Hum Reprod Update	10.1093/humupd/dmy018	biological, biomedical, epistasis, hybrid breakdown, maternal spindle transfer, mitochondrial disease, mito-nuclear mismatch, offspring, pronuclear transfer, three-parent baby	2018	UK, Australia, Germany	N/A	Various

Ref #29	Scientific and Ethical Issues in Mitochondrial Donation	New Bioeth	10.1080/20502877.2018.1440725	ethics; human embryo; maternal spindle transfer; mitochondrial donation; oocytes; pronuclear transfer	2018	UK	MST, PNT	Human
Ref #30	Recent Advances in Mitochondrial Disease	Annual Review of Genomics and Human Genetics	10.1146/annurev-genom-091416-035426	genetic diagnosis; mitochondrial disease; mitochondrial function; mitochondrial therapy; reproductive options	2017	UK	N/A	N/A
Ref #31	Novel reproductive technologies to prevent mitochondrial disease	Hum Reprod Update	10.1093/humupd/dmx018	fertility; heteroplasmy; mitochondria; mtDNA; mutation; nuclear transfer; oocyte; preimplantation; reproductive; spindle transfer	2017	UK	N/A	N/A
Ref #32	Mitochondrial genome inheritance and replacement in the human germline	EMBO J	10.15252/embj.201797606 Note following correction: 10.15252/embj.201797843	None available/ provided	2017	USA	N/A	N/A

Ref #33	Assisted reproductive technologies to prevent human mitochondrial disease transmission	Nat Biotechnol	10.1038/nbt.3997 Note following corrections: 1. 10.1038/nbt0218-196b 2. 10.1038/nbt0718-660b	None available/ provided	2017	UK	N/A	N/A
Ref #34	Mitochondrial Replacement Therapy: Are Mito-nuclear Interactions Likely To Be a Problem?	Genetics	10.1534/genetics.116.196436	coadaptation; mito-nuclear incompatibility; mito-nuclear interaction; mitochondrial replacement therapy	2017	UK	N/A	N/A
Ref #35	Editorial: First birth following spindle transfer for mitochondrial replacement therapy: hope and trepidation	Reprod Biomed Online	10.1016/j.rbmo.2017.02.004	None available/ provided	2017	N/A	N/A	N/A

B2: Publication list and abstracts

Reference in Statement	*Citation and abstract*
Ref #1	*Ma H, Hayama T, Van Dyken C, Darby H, Koski A, Lee Y, Gutierrez NM, Yamada S, Li Y, Andrews M, Ahmed R, Liang D, Gonmanee T, Kang E, Nasser M, Kempton B, Brigande J, McGill TJ, Terzic A, Amato P, Mitalipov S. Deleterious mtDNA Mutations are Common in Mature Oocytes. Biol Reprod. 2019 Oct 17:ioz202. doi: 10.1093/biolre/ioz202. Epub ahead of print.* Heritable mitochondrial DNA (mtDNA) mutations are common, yet only a few recurring pathogenic mtDNA variants account for the majority of known familial cases in humans. Purifying selection in the female germline is thought to be responsible for the elimination of most harmful mtDNA mutations during oogenesis. Here, we show that deleterious mtDNA mutations are abundant in ovulated mature mouse oocytes and preimplantation embryos recovered from PolG mutator females but not in their live offspring. This implies that purifying selection acts not in the maternal germline per se, but during post-implantation development. We further show that oocyte mtDNA mutations can be captured and stably maintained in embryonic stem cells (ESCs) and then reintroduced into chimeras, thereby allowing examination of the effects of specific mutations on fetal and postnatal development.
Ref #2	*Latorre-Pellicer A, Lechuga-Vieco AV, Johnston IG, Hämäläinen RH, Pellico J, Justo-Méndez R, Fernández-Toro JM, Clavería C, Guaras A, Sierra R, Llop J, Torres M, Criado LM, Suomalainen A, Jones NS, Ruíz-Cabello J, Enríquez JA. Regulation of Mother-to-Offspring Transmission of mtDNA Heteroplasmy. Cell Metab. 2019 Dec 3;30(6):1120-1130.e5. doi: 10.1016/j.cmet.2019.09.007.* mtDNA is present in multiple copies in each cell derived from the expansions of those in the oocyte. Heteroplasmy, more than one mtDNA variant, may be generated by mutagenesis, paternal mtDNA leakage, and novel medical technologies aiming to prevent inheritance of mtDNA-linked diseases. Heteroplasmy phenotypic impact remains poorly understood. Mouse studies led to contradictory models of random drift or haplotype selection for mother-to-offspring transmission of mtDNA heteroplasmy. Here, we show that mtDNA heteroplasmy affects embryo metabolism, cell fitness, and induced pluripotent stem cell (iPSC) generation. Thus, genetic and pharmacological interventions affecting oxidative phosphorylation (OXPHOS) modify competition among mtDNA haplotypes during oocyte development and/or at early embryonic stages. We show that heteroplasmy behavior can fall on a spectrum from random drift to strong selection, depending on mito-nuclear interactions and metabolic factors. Understanding heteroplasmy dynamics and its mechanisms provide novel knowledge of a fundamental biological process and enhance our ability to mitigate risks in clinical applications affecting mtDNA transmission.
Ref #3	*Wei W, Tuna S, Keogh MJ, Smith KR, Aitman TJ, Beales PL, Bennett DL, Gale DP, Bitner-Glindzicz MAK, Black GC, Brennan P, Elliott P, Flinter FA, Floto RA, Houlden H, Irving M, Koziell A, Maher ER, Markus HS, Morrell NW, Newman WG, Roberts I, Sayer JA, Smith KGC, Taylor JC, Watkins H, Webster AR, Wilkie AOM, Williamson C; NIHR BioResource–Rare Diseases; 100,000 Genomes Project–Rare Diseases Pilot, Ashford S, Penkett CJ, Stirrups KE, Rendon A, Ouwehand WH, Bradley JR, Raymond FL, Caulfield M, Turro E, Chinnery*

	PF. Germline selection shapes human mitochondrial DNA diversity. Science. 2019 May 24;364(6442):eaau6520. doi: 10.1126/science.aau6520. Approximately 2.4% of the human mitochondrial DNA (mtDNA) genome exhibits common homoplasmic genetic variation. We analyzed 12,975 whole-genome sequences to show that 45.1% of individuals from 1526 mother-offspring pairs harbor a mixed population of mtDNA (heteroplasmy), but the propensity for maternal transmission differs across the mitochondrial genome. Over one generation, we observed selection both for and against variants in specific genomic regions; known variants were more likely to be transmitted than previously unknown variants. However, new heteroplasmies were more likely to match the nuclear genetic ancestry as opposed to the ancestry of the mitochondrial genome on which the mutations occurred, validating our findings in 40,325 individuals. Thus, human mtDNA at the population level is shaped by selective forces within the female germ line under nuclear genetic control, which ensures consistency between the two independent genetic lineages.
Ref #4	*Tang M, Guggilla RR, Gansemans Y, Van der Jeught M, Boel A, Popovic M, Stamatiadis P, Ferrer-Buitrago M, Thys V, Van Coster R, Deforce D, De Sutter P, Van Nieuwerburgh F, Heindryckx B. Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants. Mol Hum Reprod. 2019 Dec 1;25(12):797-810. doi: 10.1093/molehr/gaz062.* Prevention of mitochondrial DNA (mtDNA) diseases may currently be possible using germline nuclear transfer (NT). However, scientific evidence to compare efficiency of different NT techniques to overcome mtDNA diseases is lacking. Here, we performed four types of NT, including first or second polar body transfer (PB1/2T), maternal spindle transfer (ST) and pronuclear transfer (PNT), using NZB/OlaHsd and B6D2F1 mouse models. Embryo development was assessed following NT and mtDNA carry-over levels were measured by next generation sequencing (NGS). Moreover, we explored two novel protocols (PB2T-a and PB2T-b) to optimize PB2T using mouse and human oocytes. Chromosomal profiles of NT-generated blastocysts were evaluated using NGS. In mouse, our findings reveal that only PB2T-b successfully leads to blastocysts. There were comparable blastocyst rates amongst PB1T, PB2T-b, ST and PNT embryos. Furthermore, PB1T and PB2T-b had lower mtDNA carry-over levels than ST and PNT. After extrapolation of novel PB2T-b to human in vitro matured (IVM) oocytes and in vivo matured oocytes with smooth endoplasmic reticulum aggregates (SERa) oocytes, the reconstituted embryos successfully developed to blastocysts at a comparable rate to ICSI controls. PB2T-b embryos generated from IVM oocytes showed a similar euploidy rate to ICSI controls. Nevertheless, our mouse model with non-mutated mtDNAs is different from a mixture of pathogenic and non-pathogenic mtDNAs in a human scenario. Novel PB2T-b requires further optimization to improve blastocyst rates in human. Although more work is required to elucidate efficiency and safety of NT, our study suggests that PBT may have the potential to prevent mtDNA disease transmission.
Ref #5	*St John JC, Makanji Y, Johnson JL, Tsai TS, Lagondar S, Rodda F, Sun X, Pangestu M, Chen P, Temple-Smith P. The transgenerational effects of oocyte mitochondrial supplementation. Sci Rep. 2019 Apr 30;9(1):6694. doi: 10.1038/s41598-019-43135-4.* Many women suffer from either failed fertilisation or their embryos arrest early during development. Autologous mitochondrial supplementation has been proposed as an assisted reproductive technology to overcome these problems. However, its safety remains to be tested in an animal model to determine if there are transgenerational effects. We have supplemented oocytes with autologous populations of mitochondria to generate founders. We mated the female founders and their offspring to produce three generations. We assessed litter size, the ovarian reserve, and weight gain and conducted a full histopathological analysis from each of the three generations. Across the generations, we observed significant increases in litter size and in the number of primordial follicles in the ovary matched by

	changes in global gene expression patterns for these early-stage oocytes. However, full histopathological analysis revealed that cardiac structure was compromised in first and second generation offspring, which could seriously affect the health of the offspring. Furthermore, the offspring were prone to increased weight gain during early life. Mitochondrial supplementation appears to perturb the regulation of the chromosomal genome resulting in transgenerational phenotypic gains and losses. These data highlight the need for caution when using autologous mitochondrial supplementation to treat female factor infertility.
Ref #6	*Yin T, Wang J, Xiang H, Pinkert CA, Li Q, Zhao X. Dynamic characteristics of the mitochondrial genome in SCNT pigs. Biol Chem. 2019;400(5):613–623. doi:10.1515/hsz-2018-0273.* Most animals generated by somatic cell nuclear transfer (SCNT) are heteroplasmic; inheriting mitochondrial genetics from both donor cells and recipient oocytes. However, the mitochondrial genome and functional mitochondrial gene expression in SCNT animals are rarely studied. Here, we report the production of SCNT pigs to study introduction, segregation, persistence and heritability of mitochondrial DNA transfer during the SCNT process. Porcine embryonic fibroblast cells from male and female Xiang pigs were transferred into enucleated oocytes from Yorkshire or Landrace pigs. Ear biopsies and blood samples from SCNT-derived pigs were analyzed to characterize the mitochondrial genome haplotypes and the degree of mtDNA heteroplasmy. Presence of nuclear donor mtDNA was less than 5% or undetectable in ear biopsies and blood samples in the majority of SCNT-derived pigs. Yet, nuclear donor mtDNA abundance in 14 tissues in F0 boars was as high as 95%. Additionally, mtDNA haplotypes influenced mitochondrial respiration capacity in F0 fibroblast cells. Our results indicate that the haplotypes of recipient oocyte mtDNA can influence mitochondrial function. This leads us to hypothesize that subtle developmental influences from SCNT-derived heteroplasmy can be targeted when using donor and recipient mitochondrial populations from breeds of swine with limited evolutionary divergence.
Ref #7	*Santibanez-Koref M, Griffin H, Turnbull DM, Chinnery PF, Herbert M, Hudson G. Assessing mitochondrial heteroplasmy using next generation sequencing: A note of caution. Mitochondrion. 2019 May;46:302-306. doi: 10.1016/j.mito.2018.08.003.* The mitochondrial genome has recently become the focus of several high-impact next-generation sequencing studies investigating the effect of mutations in disease and assessing the efficacy of mitochondrial replacement therapies. However, these studies have failed to take into consideration the capture of recurring translocations of mitochondrial DNA to the nuclear genome, known as nuclear mitochondrial sequences (NUMTs), continuing to align sequence data to the revised Cambridge reference sequence alone. Here, using different mtDNA enrichment techniques and a variety of tissues, we demonstrate that NUMTs are present in sequence data and that, dependent upon downstream analysis, are at a level which affects variant calling.
Ref #8	*Floros VI, Pyle A, Dietmann S, Wei W, Tang WCW, Irie N, Payne B, Capalbo A, Noli L, Coxhead J, Hudson G, Crosier M, Strahl H, Khalaf Y, Saitou M, Ilic D, Surani MA, Chinnery PF. Segregation of mitochondrial DNA heteroplasmy through a developmental genetic bottleneck in human embryos. Nat Cell Biol. 2018 Feb;20(2):144-151. doi: 10.1038/s41556-017-0017-8.* Mitochondrial DNA (mtDNA) mutations cause inherited diseases and are implicated in the pathogenesis of common late-onset disorders, but how they arise is not clear1,2. Here we show mtDNA mutations are present in primordial germ cells (PGCs) within healthy female human embryos. Isolated PGCs which have a profound reduction in mtDNA content, with discrete mitochondria containing ~5 mtDNA molecules. Single cell deep mtDNA sequencing of in vivo human female PGCs showed rare variants reaching higher heteroplasmy levels in later PGCs,

	consistent with the observed genetic bottleneck. We also saw the signature of selection against non-synonymous protein-coding, tRNA gene and D-loop variants, concomitant with a progressive upregulation of genes involving mtDNA replication and transcription, and linked to a transition from glycolytic to oxidative metabolism. The associated metabolic shift would expose deleterious mutations to selection during early germ cell development, preventing the relentless accumulation of mtDNA mutations in the human population predicted by Muller's ratchet. Mutations escaping this mechanism will show massive shifts in heteroplasmy levels within one human generation, explaining the extreme phenotypic variation seen in human pedigrees with inherited mtDNA disorders.
Ref #9	*Luo S, Valencia CA, Zhang J, Lee NC, Slone J, Gui B, Wang X, Li Z, Dell S, Brown J, Chen SM, Chien YH, Hwu WL, Fan PC, Wong LJ, Atwal PS, Huang T. Biparental Inheritance of Mitochondrial DNA in Humans. Proc Natl Acad Sci U S A. 2018 Dec 18;115(51):13039-13044. doi: 10.1073/pnas.1810946115.* Although there has been considerable debate about whether paternal mitochondrial DNA (mtDNA) transmission may coexist with maternal transmission of mtDNA, it is generally believed that mitochondria and mtDNA are exclusively maternally inherited in humans. Here, we identified three unrelated multigeneration families with a high level of mtDNA heteroplasmy (ranging from 24 to 76%) in a total of 17 individuals. Heteroplasmy of mtDNA was independently examined by high-depth whole mtDNA sequencing analysis in our research laboratory and in two Clinical Laboratory Improvement Amendments and College of American Pathologists-accredited laboratories using multiple approaches. A comprehensive exploration of mtDNA segregation in these families shows biparental mtDNA transmission with an autosomal dominantlike inheritance mode. Our results suggest that, although the central dogma of maternal inheritance of mtDNA remains valid, there are some exceptional cases where paternal mtDNA could be passed to the offspring. Elucidating the molecular mechanism for this unusual mode of inheritance will provide new insights into how mtDNA is passed on from parent to offspring and may even lead to the development of new avenues for the therapeutic treatment for pathogenic mtDNA transmission.
Ref #10	*Pickett SJ, Grady JP, Ng YS, Gorman GS, Schaefer AM, Wilson IJ, Cordell HJ, Turnbull DM, Taylor RW, McFarland R. Phenotypic heterogeneity in m.3243A>G mitochondrial disease: The role of nuclear factors. Ann Clin Transl Neurol. 2018 Feb 7;5(3):333-345. doi: 10.1002/acn3.532.* OBJECTIVE: The pathogenic mitochondrial DNA m.3243A>G mutation is associated with a wide range of clinical features, making disease prognosis extremely difficult to predict. We aimed to understand the cause of this heterogeneity. METHODS: We examined the phenotypic profile of 238 adult m.3243A>G carriers (patients and asymptomatic carriers) from the UK MRC Mitochondrial Disease Patient Cohort using the Newcastle Mitochondrial Disease Adult Scale. We modeled the role of risk factors for the development of specific phenotypes using proportional odds logistic regression. As mitochondria are under the dual control of their own and the nuclear genome, we examined the role of additive nuclear genetic factors in the development of these phenotypes within 46 pedigrees from the cohort. RESULTS:

	Seizures and stroke-like episodes affect 25% and 17% of patients, respectively; more common features include hearing impairment, gastrointestinal disturbance, psychiatric involvement, and ataxia. Age, age-adjusted blood heteroplasmy levels, and sex are poor predictors of phenotypic severity. Hearing impairment, diabetes, and encephalopathy show the strongest associations, but pseudo-R2 values are low (0.14-0.17). We found a high heritability estimate for psychiatric involvement (h2=0.76, P = 0.0003) and moderate estimates for cognition (h2=0.46, P = 0.0021), ataxia (h2 = 0.45, P = 0.0011), migraine (h2 = 0.41, P = 0.0138), and hearing impairment (h2 = 0.40, P = 0.0050). INTERPRETATION: Our results provide good evidence for the presence of nuclear genetic factors influencing clinical outcomes in m.3234A>G-related disease, paving the way for future work identifying these through large-scale genetic linkage and association studies, increasing our understanding of the pathogenicity of m.3243A>G and providing improved estimates of prognosis.
Ref #11	*Russell OM, Fruh I, Rai PK, Marcellin D, Doll T, Reeve A, Germain M, Bastien J, Rygiel KA, Cerino R, Sailer AW, Lako M, Taylor RW, Mueller M, Lightowlers RN, Turnbull DM, Helliwell SB. Preferential amplification of a human mitochondrial DNA deletion in vitro and in vivo. Sci Rep. 2018 Jan 29;8(1):1799. doi: 10.1038/s41598-018-20064-2.* We generated induced pluripotent stem cells (iPSCs) from patient fibroblasts to yield cell lines containing varying degrees of heteroplasmy for a m.13514 A > G mtDNA point mutation (2 lines) and for a ~6 kb single, large scale mtDNA deletion (3 lines). Long term culture of the iPSCs containing a single, large-scale mtDNA deletion showed consistent increase in mtDNA deletion levels with time. Higher levels of mtDNA heteroplasmy correlated with increased respiratory deficiency. To determine what changes occurred in deletion level during differentiation, teratomas comprising all three embryonic germ layers were generated from low (20%) and intermediate heteroplasmy (55%) mtDNA deletion clones. Regardless of whether iPSCs harbouring low or intermediate mtDNA heteroplasmy were used, the final levels of heteroplasmy in all teratoma germ layers increased to a similar high level (>60%). Thus, during human stem cell division, cells not only tolerate high mtDNA deletion loads but seem to preferentially replicate deleted mtDNA genomes. This has implications for the involvement of mtDNA deletions in both disease and ageing.
Ref #12	*Otten ABC, Sallevelt SCEH, Carling PJ, Dreesen JCFM, Drüsedau M, Spierts S, Paulussen ADC, de Die-Smulders CEM, Herbert M, Chinnery PF, Samuels DC, Lindsey P, Smeets HJM. Mutation-specific effects in germline transmission of pathogenic mtDNA variants. Hum Reprod. 2018 Jul 1;33(7):1331-1341. doi: 10.1093/humrep/dey114.* STUDY QUESTION: Does germline selection (besides random genetic drift) play a role during the transmission of heteroplasmic pathogenic mitochondrial DNA (mtDNA) mutations in humans? SUMMARY ANSWER: We conclude that inheritance of mtDNA is mutation-specific and governed by a combination of random genetic drift and negative and/or positive selection. WHAT IS KNOWN ALREADY:

	mtDNA inherits maternally through a genetic bottleneck, but the underlying mechanisms are largely unknown. Although random genetic drift is recognized as an important mechanism, selection mechanisms are thought to play a role as well. STUDY DESIGN, SIZE, DURATION: We determined the mtDNA mutation loads in 160 available oocytes, zygotes, and blastomeres of five carriers of the m.3243A>G mutation, one carrier of the m.8993T>G mutation, and one carrier of the m.14487T>C mutation. PARTICIPANTS/MATERIALS, SETTING, METHODS: Mutation loads were determined in PGD samples using PCR assays and analysed mathematically to test for random sampling effects. In addition, a meta-analysis has been performed on mutation load transmission data in the literature to confirm the results of the PGD samples. MAIN RESULTS AND THE ROLE OF CHANCE: By applying the Kimura distribution, which assumes random mechanisms, we found that mtDNA segregations patterns could be explained by variable bottleneck sizes among all our carriers (moment estimates ranging from 10 to 145). Marked differences in the bottleneck size would determine the probability that a carrier produces offspring with mutations markedly different than her own. We investigated whether bottleneck sizes might also be influenced by non-random mechanisms. We noted a consistent absence of high mutation loads in all our m.3243A>G carriers, indicating non-random events. To test this, we fitted a standard and a truncated Kimura distribution to the m.3243A>G segregation data. A Kimura distribution truncated at 76.5% heteroplasmy has a significantly better fit (P-value = 0.005) than the standard Kimura distribution. For the m.8993T>G mutation, we suspect a skewed mutation load distribution in the offspring. To test this hypothesis, we performed a meta-analysis on published blood mutation levels of offspring-mother (O-M) transmission for the m.3243A>G and m.8993T>G mutations. This analysis revealed some evidence that the O-M ratios for the m.8993T>G mutation are different from zero (P-value <0.001), while for the m.3243A>G mutation there was little evidence that the O-M ratios are non-zero. Lastly, for the m.14487T>G mutation, where the whole range of mutation loads was represented, we found no indications for selective events during its transmission. LARGE SCALE DATA: All data are included in the Results section of this article. LIMITATIONS, REASON FOR CAUTION: The availability of human material for the mutations is scarce, requiring additional samples to confirm our findings. WIDER IMPLICATIONS OF THE FINDINGS: Our data show that non-random mechanisms are involved during mtDNA segregation. We aimed to provide the mechanisms underlying these selection events. One explanation for selection against high m.3243A>G mutation loads could be, as previously reported, a pronounced oxidative phosphorylation (OXPHOS) deficiency at high mutation loads, which prohibits oogenesis (e.g. progression through meiosis). No maximum mutation loads of the m.8993T>G mutation seem to exist, as the OXPHOS deficiency is less severe, even at levels close to 100%. In contrast, high mutation loads seem to be favoured, probably because they lead to an increased mitochondrial membrane

	potential (MMP), a hallmark on which healthy mitochondria are being selected. This hypothesis could provide a possible explanation for the skewed segregation pattern observed. Our findings are corroborated by the segregation pattern of the m.14487T>C mutation, which does not affect OXPHOS and MMP significantly, and its transmission is therefore predominantly determined by random genetic drift. Our conclusion is that mutation-specific selection mechanisms occur during mtDNA inheritance, which has implications for PGD and mitochondrial replacement therapy.
Ref #13	*Burgstaller JP, Kolbe T, Havlicek V, Hembach S, Poulton J, Piálek J, Steinborn R, Rülicke T, Brem G, Jones NS, Johnston IG. Large-scale genetic analysis reveals mammalian mtDNA heteroplasmy dynamics and variance increase through lifetimes and generations. Nat Commun. 2018 Jun 27;9(1):2488. doi: 10.1038/s41467-018-04797-2.* Vital mitochondrial DNA (mtDNA) populations exist in cells and may consist of heteroplasmic mixtures of mtDNA types. The evolution of these heteroplasmic populations through development, ageing, and generations is central to genetic diseases, but is poorly understood in mammals. Here we dissect these population dynamics using a dataset of unprecedented size and temporal span, comprising 1947 single-cell oocyte and 899 somatic measurements of heteroplasmy change throughout lifetimes and generations in two genetically distinct mouse models. We provide a novel and detailed quantitative characterisation of the linear increase in heteroplasmy variance throughout mammalian life courses in oocytes and pups. We find that differences in mean heteroplasmy are induced between generations, and the heteroplasmy of germline and somatic precursors diverge early in development, with a haplotype-specific direction of segregation. We develop stochastic theory predicting the implications of these dynamics for ageing and disease manifestation and discuss its application to human mtDNA dynamics.
Ref #14	*Tsai TS, Tyagi S, St John JC. The molecular characterisation of mitochondrial DNA deficient oocytes using a pig model. Hum Reprod. 2018 May 1;33(5):942-953. doi: 10.1093/humrep/dey052.* STUDY QUESTION: What are the molecular differences between mitochondrial DNA (mtDNA)-deficient and mtDNA-normal oocytes and how does mitochondrial supplementation alter these? SUMMARY ANSWER: Changes to DNA methylation in a 5′ cytosine-phosphate-guanine 3′ (CpG) island in the mtDNA-specific replication factor (DNA polymerase gamma (POLG)) of mtDNA-deficient oocytes mediates an increase in mtDNA copy number by the 2-cell stage that positively modulates the expression of nuclear genes, which affect cellular and metabolic processes, following autologous mitochondrial supplementation. WHAT IS KNOWN ALREADY: Too few copies of mtDNA in mature oocytes can lead to fertilisation failure or preimplantation embryo arrest. mtDNA-deficient oocytes that progress to blastocyst express genes associated with poor cellular and metabolic processes, transcriptional activation and mitochondrial biogenesis. STUDY DESIGN, SIZE, DURATION:

	Using a pig oocyte model, we assessed mtDNA-deficient and mtDNA-normal oocytes during in vitro maturation for mtDNA variants and levels of DNA methylation in POLG. We supplemented mtDNA-deficient oocytes with autologous populations of mitochondria to determine if there were changes to DNA methylation in POLG that coincided with increases in mtDNA copy number. We assessed metaphase II mtDNA-deficient and mtDNA-normal oocytes by RNA sequencing to identify differentially expressed genes and compared their profiles to blastocysts derived from mtDNA-normal, mtDNA-deficient and supplemented mtDNA-deficient oocytes. PARTICIPANTS/MATERIALS, SETTING, METHODS: mtDNA variant analysis (n = 24), mtDNA copy number (n = 60), POLG gene expression (n = 24), and RNA sequencing (n = 32 single; and 12 pooled cohorts of n = 5) were performed on oocytes and embryos. DNA methylation of a CpG island in POLG was determined quantitatively by pyrosequencing on oocytes to 2-cell embryos (n = 408). Bioinformatics tools were used to assess differences between mtDNA-normal and mtDNA-deficient oocytes and between mtDNA-normal and mtDNA-deficient oocytes and supplemented oocytes and their blastocyst stage equivalents. MAIN RESULTS AND THE ROLE OF CHANCE: Whilst mtDNA-deficient oocytes regulated variants less stringently during maturation ($P < 0.05$), there were no differences in the ratio of variants in mature-stage oocytes. However, mtDNA-normal mature oocytes had significantly more molecules affected due to their higher copy number ($P < 0.0001$). Normal mature oocytes differently DNA methylated a CpG island in POLG compared with mtDNA-deficient oocytes ($P < 0.01$). Supplementation of mtDNA-deficient oocytes modulated DNA methylation at this CpG island leading to a mtDNA replication event prior to embryonic genome activation inducing significant increases in mtDNA copy number. RNA-Seq identified 57 differentially expressed genes (false discovery rate (FDR) < 0.05) between the two cohorts of oocytes with blastocyst stage gene expression altered by supplementation of mtDNA-deficient oocytes ($P < 0.05$) including genes associated with metabolic disorders. One key factor was branched chain amino acid transaminase 2 (BCAT2), a regulator of amino acid metabolism and associated with diabetes. LARGE SCALE DATA: Sequence data are available on the NCBI Sequence Read Archive under the project number PRJNA422295. RNA sequencing data were deposited into NCBI Gene Expression Omnibus, under the accession number GSE108900. LIMITATIONS, REASONS FOR CAUTION: Whilst this work was conducted in a species that is highly relevant to human reproduction, the outcomes need to be tested in human oocytes and blastocysts prior to clinical application. WIDER IMPLICATIONS OF THE FINDINGS: The outcomes demonstrate a mechanism of action following mtDNA supplementation of mtDNA-deficient oocytes that results in improved gene expression at the blastocyst stage of development.

Ref #15	*Zhang J, Liu H, Luo S, Lu Z, Chávez-Badiola A, Liu Z, Yang M, Merhi Z, Silber SJ, Munné S, Konstantinidis M, Wells D, Tang JJ, Huang T. Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. Reprod Biomed Online. 2017 Apr;34(4):361-368. doi: 10.1016/j.rbmo.2017.01.013.* Mutations in mitochondrial DNA (mtDNA) are maternally inherited and can cause fatal or debilitating mitochondrial disorders. The severity of clinical symptoms is often associated with the level of mtDNA mutation load or degree of heteroplasmy. Current clinical options to prevent transmission of mtDNA mutations to offspring are limited. Experimental spindle transfer in metaphase II oocytes, also called mitochondrial replacement therapy, is a novel technology for preventing mtDNA transmission from oocytes to pre-implantation embryos. Here, we report a female carrier of Leigh syndrome (mtDNA mutation 8993T > G), with a long history of multiple undiagnosed pregnancy losses and deaths of offspring as a result of this disease, who underwent IVF after reconstitution of her oocytes by spindle transfer into the cytoplasm of enucleated donor oocytes. A male euploid blastocyst wasobtained from the reconstituted oocytes, which had only a 5.7% mtDNA mutation load. Transfer of the embryo resulted in a pregnancy with delivery of a boy with neonatal mtDNA mutation load of 2.36-9.23% in his tested tissues. The boy is currently healthy at 7 months of age, although long-term follow-up of the child's longitudinal development remains crucial.
Ref #16	*Rishishwar L, Jordan IK. Implications of human evolution and admixture for mitochondrial replacement therapy. BMC Genomics. 2017 Feb 8;18(1):140. doi: 10.1186/s12864-017-3539-3.* BACKGROUND: Mitochondrial replacement (MR) therapy is a new assisted reproductive technology that allows women with mitochondrial disorders to give birth to healthy children by combining their nuclei with mitochondria from unaffected egg donors. Evolutionary biologists have raised concerns about the safety of MR therapy based on the extent to which nuclear and mitochondrial genomes are observed to co-evolve within natural populations, i.e. the nuclear-mitochondrial mismatch hypothesis. In support of this hypothesis, a number of previous studies on model organisms have provided evidence for incompatibility between nuclear and mitochondrial genomes from divergent populations of the same species. RESULTS: We tested the nuclear-mitochondrial mismatch hypothesis for humans by observing the extent of naturally occurring nuclear-mitochondrial mismatch seen for 2,504 individuals across 26 populations, from 5 continental populations groups, characterized as part of the 1000 Genomes Project (1KGP). We also performed a replication analysis on mitochondrial DNA (mtDNA) haplotypes for 1,043 individuals from 58 populations, characterized as part of the Human Genome Diversity Project (HGDP). Nuclear DNA (nDNA) and mtDNA sequences from the 1KGP were directly compared within and between populations, and the population distributions of mtDNA haplotypes derived from both sequence (1KGP) and genotype (HGDP) data were evaluated. Levels of nDNA and mtDNA pairwise sequence divergence are highly correlated, consistent with their co-evolution among human populations. However, there are numerous cases of co-occurrence of nuclear and mitochondrial genomes from divergent populations within individual humans. Furthermore, pairs of individuals with closely related nuclear genomes can have highly divergent mtDNA haplotypes. Supposedly mismatched nuclear-mitochondrial genome combinations are

	found not only within individuals from populations known to be admixed, where they may be expected, but also from populations with low overall levels of observed admixture. CONCLUSIONS: These results show that mitochondrial and nuclear genomes from divergent human populations can co-exist within healthy individuals, indicating that mismatched nDNA-mtDNA combinations are not deleterious or subject to purifying selection. Accordingly, human nuclear-mitochondrial mismatches are not likely to jeopardize the safety of MR therapy.
Ref #17	*Wu K, Zhong C, Chen T, Zhang X, Tao W, Zhang J, Li H, Zhao H, Li J, Chen ZJ. Polar bodies are efficient donors for reconstruction of human embryos for potential mitochondrial replacement therapy. Cell Res. 2017 Aug;27(8):1069-1072. doi: 10.1038/cr.2017.67.*
Ref #18	*Wu K, Chen T, Huang S, Zhong C, Yan J, Zhang X, Li J, Gao Y, Zhao H, Chen ZJ. Mitochondrial replacement by pre-pronuclear transfer in human embryos. Cell Res. 2017 Jun;27(6):834-837. doi: 10.1038/cr.2017.48.*
Ref #19	*Sallevelt SC, de Die-Smulders CE, Hendrickx AT, Hellebrekers DM, de Coo IF, Alston CL, Knowles C, Taylor RW, McFarland R, Smeets HJ. De novo mtDNA point mutations are common and have a low recurrence risk. J Med Genet. 2017 Feb;54(2):73-83. doi: 10.1136/jmedgenet-2016-103876.* Background: Severe, disease-causing germline mitochondrial (mt)DNA mutations are maternally inherited or arise de novo. Strategies to prevent transmission are generally available, but depend on recurrence risks, ranging from high/unpredictable for many familial mtDNA point mutations to very low for sporadic, large-scale single mtDNA deletions. Comprehensive data are lacking for de novo mtDNA point mutations, often leading to misconceptions and incorrect counselling regarding recurrence risk and reproductive options. We aim to study the relevance and recurrence risk of apparently de novo mtDNA point mutations. Methods: Systematic study of prenatal diagnosis (PND) and recurrence of mtDNA point mutations in families with de novo cases, including new and published data. 'De novo' based on the absence of the mutation in multiple (postmitotic) maternal tissues is preferred, but mutations absent in maternal blood only were also included. Results: In our series of 105 index patients (33 children and 72 adults) with (likely) pathogenic mtDNA point mutations, the de novo frequency was 24.6%, the majority being paediatric. PND was performed in subsequent pregnancies of mothers of four de novo cases. A fifth mother opted for preimplantation genetic diagnosis because of a coexisting Mendelian genetic disorder. The mtDNA mutation was absent in all four prenatal samples and all 11 oocytes/embryos tested. A literature survey revealed 137 de novo cases, but PND was only performed for 9 (including 1 unpublished) mothers. In one, recurrence occurred in two subsequent pregnancies, presumably due to germline mosaicism. Conclusions: De novo mtDNA point mutations are a common cause of mtDNA disease. Recurrence risk is low. This is relevant for genetic counselling, particularly for reproductive options. PND can be offered for reassurance.

Ref #20	*Chen SH, Pascale C, Jackson M, Szvetecz MA, Cohen J. A limited survey-based uncontrolled follow-up study of children born after ooplasmic transplantation in a single centre. Reprod Biomed Online. 2016 Dec;33(6):737-744. doi: 10.1016/j.rbmo.2016.10.003.* Experimental ooplasmic transplantation from donor to recipient oocyte took place between 1996 and 2001 at Saint Barnabas Medical Center, USA. Indication for 33 patients was repeated implantation failure. Thirteen couples had 17 babies. One patient delivered twins from mixed ooplasmic and donor egg embryos. A limited survey-based follow-up study on the children is reported: 12 out of 13 parents completed a questionnaire on pregnancy, birth, health, academic performance and disclosure. Parents of a quadruplet did not participate. Prenatal development and delivery were uneventful. School grades ranged from good to excellent. Children were of good health. Body mass index (BMI) was normal in 12 out of 13 children. One child had chronic migraine headaches, two mild asthma, three minor vision and three minor skin problems. One boy from a boy/girl twin was diagnosed with borderline pervasive developmental disorder - not otherwise specified at age 18 months, but with no later symptoms. One couple disclosed the use of egg donor to their child. One reported intention to disclose; six were undecided and four reported they would not disclose. This limited follow-up strategy presents a high risk of bias. Parents may not assent to standardized clinical analysis owing to lack of disclosure to their children.
Ref #21	*Cagnone GL, Tsai TS, Makanji Y, Matthews P, Gould J, Bonkowski MS, Elgass KD, Wong AS, Wu LE, McKenzie M, Sinclair DA, St John JC. Restoration of normal embryogenesis by mitochondrial supplementation in pig oocytes exhibiting mitochondrial DNA deficiency. Sci Rep. 2016 Mar 18;6:23229. doi: 10.1038/srep23229.* An increasing number of women fail to achieve pregnancy due to either failed fertilization or embryo arrest during preimplantation development. This often results from decreased oocyte quality. Indeed, reduced mitochondrial DNA copy number (mitochondrial DNA deficiency) may disrupt oocyte quality in some women. To overcome mitochondrial DNA deficiency, whilst maintaining genetic identity, we supplemented pig oocytes selected for mitochondrial DNA deficiency, reduced cytoplasmic maturation and lower developmental competence, with autologous populations of mitochondrial isolate at fertilization. Supplementation increased development to blastocyst, the final stage of preimplantation development, and promoted mitochondrial DNA replication prior to embryonic genome activation in mitochondrial DNA deficient oocytes but not in oocytes with normal levels of mitochondrial DNA. Blastocysts exhibited transcriptome profiles more closely resembling those of blastocysts from developmentally competent oocytes. Furthermore, mitochondrial supplementation reduced gene expression patterns associated with metabolic disorders that were identified in blastocysts from mitochondrial DNA deficient oocytes. These results demonstrate the importance of the oocyte's mitochondrial DNA investment in fertilization outcome and subsequent embryo development to mitochondrial DNA deficient oocytes.
Ref #22	*Røyrvik EC, Burgstaller JP, Johnston IG. mtDNA diversity in human populations highlights the merit of haplotype matching in gene therapies. Mol Hum Reprod. 2016 Nov;22(11):809-817. doi: 10.1093/molehr/gaw062.* STUDY QUESTION: Does mitochondrial DNA (mtDNA) diversity in modern human populations potentially pose a challenge, via mtDNA segregation, to mitochondrial replacement therapies? SUMMARY ANSWER:

	The magnitude of mtDNA diversity in modern human populations is as high as in mammalian model systems where strong mtDNA segregation is observed; consideration of haplotype pairs and/or haplotype matching can help avoid these potentially deleterious effects. WHAT IS KNOWN ALREADY: In mammalian models, substantial proliferative differences are observed between different mtDNA haplotypes in cellular admixtures, with larger proliferative differences arising from more diverse haplotype pairings. If maternal mtDNA is 'carried over' in human gene therapies, these proliferative differences could lead to its amplification in the resulting offspring, potentially leading to manifestation of the disease that the therapy was designed to avoid-but existing studies have not investigated whether mtDNA diversity in modern human populations is sufficient to permit significant amplification. STUDY DESIGN, SIZE, DURATION: This theoretical study used over 7500 human mtDNA sequences from The National Center for Biotechnology Information (NCBI), a range of international and British mtDNA surveys, and 2011 census data. PARTICIPANTS/MATERIALS, SETTING, METHODS: A stochastic simulation approach was used to model random haplotype pairings from within different regions. In total, 1000 simulated pairings were analysed using the basic local alignment search tool (BLAST) for each region. Previous data from mouse models were used to estimate proliferative differences. MAIN RESULTS AND THE ROLE OF CHANCE: Even within the same haplogroup, differences of around 20-80 single-nucleotide polymorphisms (SNPs) are common between mtDNAs admixed in random pairings. These values are sufficient to lead to substantial segregation in mouse models over an organismal lifetime, even given low starting heteroplasmy, inducing increases from 5% to 35% over 1 year. Substantial population mixing in modern UK cities increases the expected genetic differences. Hence, the likely genetic differences between humans randomly sampled from a population may well allow substantial amplification of a disease-carrying mtDNA haplotype over the timescale of a human lifetime. We report ranges and mean differences for all statistics to quantify uncertainty in our results. LIMITATIONS/REASONS FOR CAUTION: The mapping from mouse and other mammalian models to the human system is challenging, as timescales and mechanisms may differ. Reporting biases in NCBI mtDNA data, if present, may affect the statistics we compute. We discuss the robustness of our findings in the light of these concerns. WIDER IMPLICATIONS OF THE FINDINGS: Matching the mtDNA haplotypes of the mother and third-party donor in mitochondrial replacement therapies is supported as a means of ameliorating the potentially deleterious results of human mtDNA diversity. We present a chart of expected SNP differences between mtDNA haplogroups, allowing the selection of optimal partners for therapies.

	LARGE SCALE DATA: N/A
Ref #23	*Nissanka N, Moraes CT. Mitochondrial DNA heteroplasmy in disease and targeted nuclease-based therapeutic approaches. EMBO Rep. 2020 Mar 4;21(3):e49612. doi: 10.15252/embr.201949612.* Mitochondrial DNA (mtDNA) encodes a subset of the genes which are responsible for oxidative phosphorylation. Pathogenic mutations in the human mtDNA are often heteroplasmic, where wildtype mtDNA species co-exist with the pathogenic mtDNA and a bioenergetic defect is only seen when the pathogenic mtDNA percentage surpasses a threshold for biochemical manifestations. mtDNA segregation during germline development can explain some of the extreme variation in heteroplasmy from one generation to the next. Patients with high heteroplasmy for deleterious mtDNA species will likely suffer from bona-fide mitochondrial diseases, which currently have no cure. Shifting mtDNA heteroplasmy toward the wild-type mtDNA species could provide a therapeutic option to patients. Mitochondrially targeted engineered nucleases, such as mitoTALENs and mitoZFNs, have been used in vitro in human cells harboring pathogenic patient-derived mtDNA mutations and more recently in vivo in a mouse model of a pathogenic mtDNA point mutation. These gene therapy tools for shifting mtDNA heteroplasmy can also be used in conjunction with other therapies aimed at eliminating and/or preventing the transfer of pathogenic mtDNA from mother to child.
Ref #24	*Poulton J, Steffann J, Burgstaller J, McFarland R; workshop participants. 243rd ENMC international workshop: Developing guidelines for management of reproductive options for families with maternally inherited mtDNA disease, Amsterdam, the Netherlands, 22-24 March 2019. Neuromuscul Disord. 2019 Sep;29(9):725-733. doi: 10.1016/j.nmd.2019.08.004.* The 243rd ENMC workshop met in Amsterdam, The Netherlands in March 2019 to discuss current perspectives and knowledge in reproductive options in patients with mtDNA-related mitochondrial disease. The 29 participants came from The Netherlands, UK, France, Germany, Spain, Austria, Belgium, Australia, USA and Brazil, and was multi-disciplinary, including patients, clinicians, basic scientists, ethicists, a sociologist, and representatives of industry and patient organizations (including the Lily Foundation, the Dutch Muscular Disease Association, International Mito Patients (IMP) and the LHON group of the Dutch Eye Association). Genetic counselling is uniquely complicated in mitochondrial diseases, and the ENMC has played an important role in developing consensus guidelines for reproductive options. As molecular characterisation has become routine, more options have become available. Pre-implantation genetic diagnosis (PGD, where routinely 1–5 cells are sampled from a pre-implantation embryo) is now robust and safety is established for maternally inherited mtDNA disease, albeit that data remains relatively limited. Furthermore, great strides have been made in mitochondrial replacement therapy (MRT). In MRT the nucleus is removed from either a zygote (pronuclear transfer, PNT) or an oocyte (maternal spindle transfer, MST) and placed into a corresponding enucleated cell at the same stage, but from a donor with normal mitochondria. These techniques are being applied to a range of disorders beyond the purely mitochondrial, in which some investigators believe that cytoplasmic transfer is useful for regeneration of poor quality oocytes. Patients are enthusiastic for these new options, but need appropriate, informed counselling regarding the risks and benefits of novel techniques such as MRT where clinical experience is limited. In the UK the HFEA have established a rigorous regulatory framework, with a detailed case-by-case review process for each MRT

	application. This consensus document is a response to the pressing need for internationally agreed guidelines on referral and counselling of couples seeking advice on assisted reproductive options for mtDNA disease.
Ref #25	*Pickett SJ, Blain A, Ng YS, Wilson IJ, Taylor RW, McFarland R, Turnbull DM, Gorman GS. Mitochondrial Donation - Which Women Could Benefit? N Engl J Med. 2019 May 16;380(20):1971-1972. doi: 10.1056/NEJMc1808565.*
Ref #26	*Hudson G, Takeda Y, Herbert M. Reversion after replacement of mitochondrial DNA. Nature. 2019 Oct;574(7778):E8-E11. doi: 10.1038/s41586-019-1623-3.*
Ref #27	*Kang E, Koski A, Amato P, Temiakov D, Mitalipov S. Reply to: Reversion after replacement of mitochondrial DNA. Nature. 2019 Oct;574(7778):E12-E13. doi: 10.1038/s41586-019-1624-2.*
Ref #28	*Dobler R, Dowling DK, Morrow EH, Reinhardt K. A systematic review and meta-analysis reveals pervasive effects of germline mitochondrial replacement on components of health. Hum Reprod Update. 2018 Sep 1;24(5):519-534. doi: 10.1093/humupd/dmy018.* BACKGROUND Mitochondrial replacement, a form of nuclear transfer, has been proposed as a germline therapy to prevent the transmission of mitochondrial diseases. Mitochondrial replacement therapy has been licensed for clinical application in the UK, and already carried out in other countries, but little is known about negative or unintended effects on the health of offspring born using this technique. OBJECTIVE AND RATIONALE Studies in invertebrate models have used techniques that achieve mitochondrial replacement to create offspring with novel combinations of mitochondrial and nuclear genotype. These have demonstrated that the creation of novel mitochondrial–nuclear interactions can lead to alterations in offspring characteristics, such as development rates, fertility and longevity. However, it is currently unclear whether such interactions could similarly affect the outcomes of vertebrate biomedical studies, which have sought to assess the efficacy of the replacement therapy. SEARCH METHODS This systematic review addresses whether the effects of mitochondrial replacement on offspring characteristics differ in magnitude between biological (conducted on invertebrate models, with an ecological or evolutionary focus) and biomedical studies (conducted on vertebrate models, with a clinical focus). Studies were selected based on a key-word search in 'Web of Science', complemented by backward searches of reviews on the topic of mitochondrial–nuclear (mito-nuclear) interactions. In total, 43 of the resulting 116 publications identified in the search contained reliable data to estimate effect sizes of mitochondrial replacement. We found no evidence of publication bias when examining effect-size estimates across sample sizes. OUTCOMES

	Mitochondrial replacement consistently altered the phenotype, with significant effects at several levels of organismal performance and health, including gene expression, anatomy, metabolism and life-history. Biomedical and biological studies, while differing in the methods used to achieve mitochondrial replacement, showed only marginally significant differences in effect-size estimates (−0.233 [CI: −0.495 to −0.011]), with larger effect-size estimates in biomedical studies (0.697 [CI: 0.450–0.956]) than biological studies (0.462 [CI: 0.287–0.688]). Humans showed stronger effects than other species. Effects of mitochondrial replacement were also stronger in species with a higher basal metabolic rate. Based on our results, we conducted the first formal risk analysis of mitochondrial replacement, and conservatively estimate negative effects in at least one in every 130 resulting offspring born to the therapy. WIDER IMPLICATIONS Our findings suggest that mitochondrial replacement may routinely affect offspring characteristics across a wide array of animal species, and that such effects are likely to extend to humans. Studies in invertebrate models have confirmed mito-nuclear interactions as the underpinning cause of organismal effects following mitochondrial replacement. This therefore suggests that mito-nuclear interactions are also likely to be contributing to effects seen in biomedical studies, on vertebrate models, whose effect sizes exceeded those of biological studies. Our results advocate the use of safeguards that could offset any negative effects (defining any unintended effect as being negative) mediated by mito-nuclear interactions following mitochondrial replacement in humans, such as mitochondrial genetic matching between donor and recipient. Our results also suggest that further research into the molecular nature of mito-nuclear interactions would be beneficial in refining the clinical application of mitochondrial replacement, and in establishing what degree of variation between donor and patient mitochondrial DNA haplotypes is acceptable to ensure ‘haplotype matching’.
Ref #29	*Craven L, Murphy J, Turnbull DM, Taylor RW, Gorman GS, McFarland R. Scientific and Ethical Issues in Mitochondrial Donation. New Bioeth. 2018 Apr;24(1):57-73. doi: 10.1080/20502877.2018.1440725.* The development of any novel reproductive technology involving manipulation of human embryos is almost inevitably going to be controversial and evoke sincerely held, but diametrically opposing views. The plethora of scientific, ethical and legal issues that surround the clinical use of such techniques fuels this divergence of opinion. During the policy change that was required to allow the use of mitochondrial donation in the UK, many of these issues were intensely scrutinised by a variety of people and in multiple contexts. This extensive process resulted in the publication of several reports that informed the recommendations made to government. We have been intrinsically involved in the development of mitochondrial donation, from refining the basic technique for use in human embryos through to clinical service delivery, and have taken the opportunity in this article to offer our own perspective on the issues it raises.
Ref #30	*Craven L, Alston CL, Taylor RW, Turnbull DM. Recent Advances in Mitochondrial Disease. Annu Rev Genomics Hum Genet. 2017 Aug 31;18:257-275. doi: 10.1146/annurev-genom-091416-035426.* Mitochondrial disease is a challenging area of genetics because two distinct genomes can contribute to disease pathogenesis. It is also challenging clinically because of the myriad of different symptoms and, until recently, a lack of a genetic diagnosis in many patients. The last five years has brought remarkable progress in this area. We provide a brief overview of mitochondrial origin, function, and biology, which are key to understanding the genetic basis of mitochondrial disease. However, the primary purpose of this review is to describe the

	recent advances related to the diagnosis, genetic basis, and prevention of mitochondrial disease, highlighting the newly described disease genes and the evolving methodologies aimed at preventing mitochondrial DNA disease transmission.
Ref #31	*Craven L, Tang MX, Gorman GS, De Sutter P, Heindryckx B. Novel reproductive technologies to prevent mitochondrial disease. Hum Reprod Update. 2017 Sep 1;23(5):501-519. doi: 10.1093/humupd/dmx018* BACKGROUND The use of nuclear transfer (NT) has been proposed as a novel reproductive treatment to overcome the transmission of maternally-inherited mitochondrial DNA (mtDNA) mutations. Pathogenic mutations in mtDNA can cause a wide-spectrum of life-limiting disorders, collectively known as mtDNA disease, for which there are currently few effective treatments and no known cures. The many unique features of mtDNA make genetic counselling challenging for women harbouring pathogenic mtDNA mutations but reproductive options that involve medical intervention are available that will minimize the risk of mtDNA disease in their offspring. This includes PGD, which is currently offered as a clinical treatment but will not be suitable for all. The potential for NT to reduce transmission of mtDNA mutations has been demonstrated in both animal and human models, and has recently been clinically applied not only to prevent mtDNA disease but also for some infertility cases. In this review, we will interrogate the different NT techniques, including a discussion on the available safety and efficacy data of these technologies for mtDNA disease prevention. In addition, we appraise the evidence for the translational use of NT technologies in infertility. OBJECTIVE AND RATIONALE We propose to review the current scientific evidence regarding the clinical use of NT to prevent mitochondrial disease. SEARCH METHODS The scientific literature was investigated by searching PubMed database until Jan 2017. Relevant documents from Human Fertilisation and Embryology Authority as well as reports from both the scientific and popular media were also implemented. The above searches were based on the following key words: 'mitochondria', 'mitochondrial DNA'; 'mitochondrial DNA disease', 'fertility'; 'preimplantation genetic diagnosis', 'nuclear transfer', 'mitochondrial replacement' and 'mitochondrial donation'. OUTCOMES While NT techniques have been shown to effectively reduce the transmission of heteroplasmic mtDNA variants in animal models, and increasing evidence supports their use to prevent the transmission of human mtDNA disease, the need for robust, long-term evaluation is still warranted. Moreover, prenatal screening would still be strongly advocated in combination with the use of these IVF-based technologies. Scientific evidence to support the use of NT and other novel reproductive techniques for infertility is currently lacking. WIDER IMPLICATIONS It is mandatory that any new ART treatments are first adequately assessed in both animal and human models before the cautious implementation of these new therapeutic approaches is clinically undertaken. There is growing evidence to suggest that the translation of these innovative technologies into clinical practice should be cautiously adopted only in highly selected patients. Indeed, given the limited safety and efficacy data, close monitoring of any offspring remains paramount.

Ref #32	*Wolf DP, Hayama T, Mitalipov S. Mitochondrial genome inheritance and replacement in the human germline. EMBO J. 2017 Aug 1;36(15):2177-2181. doi: 10.15252/embj.201797606.* Mitochondria, the ubiquitous power packs in nearly every eukaryotic cell, contain their own DNA, known as mtDNA, which is inherited exclusively from the mother. The number of mitochondrial genomes varies depending on the cell's energy needs. The mature oocyte contains the highest number of mitochondria of any cell type, although there is little if any mtDNA replication after fertilization until the embryo implants. This has potential repercussions for mitochondrial replacement therapy (MRT; see description of currently employed methods below) used to prevent the transmission of mtDNA-based disorders. If only a few mitochondria with defective mtDNA are left in the embryo and undergo extensive replication, it might therefore thwart the purpose of MRT. In order to improve the safety and efficacy of this experimental therapy, we need a better understanding of how and which mtDNA is tagged for replication versus transcription after fertilization of the oocyte.
Ref #33	*Greenfield A, Braude P, Flinter F, Lovell-Badge R, Ogilvie C, Perry ACF. Assisted reproductive technologies to prevent human mitochondrial disease transmission. Nat Biotechnol. 2017 Nov 9;35(11):1059-1068. doi: 10.1038/nbt.3997.* Mitochondria are essential cytoplasmic organelles that generate energy (ATP) by oxidative phosphorylation and mediate key cellular processes such as apoptosis. They are maternally inherited and in humans contain a 16,569-base-pair circular genome (mtDNA) encoding 37 genes required for oxidative phosphorylation. Mutations in mtDNA cause a range of pathologies, commonly affecting energy-demanding tissues such as muscle and brain. Because mitochondrial diseases are incurable, attention has focused on limiting the inheritance of pathogenic mtDNA by mitochondrial replacement therapy (MRT). MRT aims to avoid pathogenic mtDNA transmission between generations by maternal spindle transfer, pronuclear transfer or polar body transfer: all involve the transfer of nuclear DNA from an egg or zygote containing defective mitochondria to a corresponding egg or zygote with normal mitochondria. Here we review recent developments in animal and human models of MRT and the underlying biology. These have led to potential clinical applications; we identify challenges to their technical refinement.
Ref #34	*Eyre-Walker A. Mitochondrial Replacement Therapy: Are Mito-nuclear Interactions Likely To Be a Problem? Genetics. 2017 Apr;205(4):1365-1372. doi: 10.1534/genetics.116.196436.* It has been suggested that deleterious interactions between the mitochondrial and nuclear genomes could pose a problem for mitochondrial replacement therapy (MRT). This is because the mitochondrial genome is placed in a novel nuclear environment using this technique. In contrast, it is inherited with half the mother's genome during normal reproduction, a genome that it is relatively compatible with, since the mother is alive. Here, I review the evidence of whether mito-nuclear interactions are likely to pose a problem for MRT. The majority of the available experimental evidence, both in humans and other species, suggests that MRT is not harmful. These results are consistent with population genetic theory, which predicts that deleterious mito-nuclear interactions are unlikely to be much more prevalent in individuals born to MRT than normal reproduction, particularly in a species such as humans with low population differentiation. This is because selection is unlikely to be strong enough to establish significant linkage disequilibrium between the mitochondrial and nuclear genomes. These results are supported by a meta-analysis of 231 cases, from a variety of animals, in which the mitochondrial DNA (mtDNA)

	from one strain has been introgressed into the nuclear background of another strain of the same species. Overall, there is little tendency for introgression of mtDNA to be harmful.
Ref #35	*Alikani M, Fauser BCJ, García-Valesco JA, Simpson JL, Johnson MH. First birth following spindle transfer for mitochondrial replacement therapy: hope and trepidation. Reprod Biomed Online. 2017 Apr;34(4):333-336. doi: 10.1016/j.rbmo.2017.02.004.*

Appendix C— Current and potential reproductive options for prospective parent/s where there is a risk of transmitting mtDNA disease to offspring

	Options	Description	Advantages	Disadvantages	Implementation considerations
Non-medical options	**Not have children**	Not have children.	• No chance of transmitting mitochondrial disease.	• No child.	• Currently available. Counselling may be beneficial.
	Conceive naturally	Conceive naturally.	• Child has nuclear DNA from mother and father.	• Risk that child will develop mitochondrial DNA disease.	• Currently available. Counselling may be beneficial.
	Adoption	Adoption.	• No transmission of mitochondrial disease.	• Child is generally not genetically related to social parents. • Subject to screening and assessment processes and parental age limits, which might preclude some prospective parents.	• Currently available but there are limited opportunities for adoption in Australia.

	Options	Description	Advantages	Disadvantages	Implementation considerations
Currently available clinical options	**Prenatal testing of fetal or placental tissues**	Prenatal genetic testing (such as chorionic villus sampling or amniocentesis) can be used to identify whether a fetus is likely to develop mitochondrial disease.	• Potentially natural/low-intervention conception. • Child has nuclear DNA from mother and father. • Can be used in conjunction with conceiving naturally or preimplantation genetic testing.	• Only suitable for women with a low level of mitochondrial DNA mutation. • May not accurately predict the risk of the child developing mitochondrial DNA disease. • Only useful for prevention of disease if parent/s are willing to consider termination of pregnancy.	• Currently available in clinical practice in Australia.
	Preimplantation genetic testing (PGT)	PGT can be used following IVF to select embryos with a lower risk of mitochondrial DNA disease.	• Can allow selection of an embryo with a suitably low level of mitochondrial DNA mutation. • Child has nuclear DNA from mother and father.	• Not appropriate for all women, especially those with a high mutation load. • May not accurately predict the risk of developing mitochondrial DNA disease.	• Currently available in clinical practice in Australia.
	Use donor egg	Undertake standard ART using an oocyte from a donor who does not have mitochondrial disease.	• No transmission of mitochondrial disease.	• Child is not genetically related to the mother. • Limited availability of donor eggs.	• Currently available in clinical practice in Australia.

	Options	Description	Advantages	Disadvantages	Implementation considerations
Mitochondrial Donation options	**Maternal Spindle Transfer**	MST is a mitochondrial donation technique that uses an oocyte reconstructed from a donor oocyte and nuclear DNA from the oocyte of an affected mother.	• Animal model data available. • Child has nuclear DNA from mother and father.	• Safety and efficacy of the technique has not been fully resolved. • Limited availability of donor eggs.	• Currently prohibited in Australian clinical practice. • Approved for use in a clinical trial in the.
	Pronuclear Transfer	PNT is a mitochondrial donation technique that uses a fertilised oocyte reconstructed from a fertilised donor oocyte that has had its pronuclei removed and the pronuclei from a fertilised oocyte from the mother and father.	• Animal model data available. • Child has nuclear DNA from mother and father.	• Requires the fertilisation of two oocytes. • Safety and efficacy of the technique has not been fully resolved. • Limited availability of donor eggs.	• Currently prohibited in Australian clinical practice. • Approved for use in a clinical trial in the UK.
	Polar Body Transfer	PBT is a mitochondrial donation technique that transfers a polar body from the oocyte or zygote of an affected woman into a donor oocyte that has had its nuclear DNA removed.	• Child has nuclear DNA from mother and father. • A maternal spindle and a polar body could both be removed from the mother's oocyte, with the potential to fertilise two donor oocytes and perhaps lead to two children. • Possibly less carryover than MST and PNT.	• Polar bodies are in the process of degrading so use of them might increase the chance of chromosomal abnormalities. • Limited experimental evidence. • Limited availability of donor eggs.	• Currently prohibited in Australian clinical practice. • Requires further experimentation in animal models. Would need to demonstrate a high level of safety and efficacy.
	Germinal Vesicle Transfer	GVT is a mitochondrial donation technique that uses an oocyte at an earlier stage (the germinal vesicle) than other mitochondrial donation techniques.	• Technique performed prior to breakdown of germinal vesicle, which may allow nucleus additional time to adjust to the new environment. • Child has nuclear DNA from mother and father. • Could potentially be used without the need for ovarian hyperstimulation and thereby avoid the risk of that process.	• Requires maturation of the germinal vesicle to a mature oocyte in the laboratory. • Limited experimental evidence.	• Currently prohibited in Australian clinical practice. • Requires further experimentation in animal models. Would need to demonstrate a high level of safety and efficacy.

	Options	Description	Advantages	Disadvantages	Implementation considerations
Emerging gene editing options	**Techniques to shift mtDNA heteroplasmy**	Techniques that aim to shift mtDNA heteroplasmy are based on the 'threshold effect', i.e. that a threshold for the ratio of mutated to healthy mtDNA must be exceeded for mitochondrial disease to manifest. In general, these techniques aim to selectively inhibit the replication of mutant mtDNA or target and cleave mutant mtDNA to shift heteroplasmy below the threshold for mitochondrial disease. Examples of techniques that are currently being investigated are antigenomic mtDNA therapy, targeted restriction endonucleases, Zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENS). If these techniques can be applied in the oocyte, they present alternate options to mitochondrial donation for women who want to have a genetically related child with a suitably low risk of developing mitochondrial disease. They could also be used in combination with mitochondrial donation to prevent the transmission of carried over mtDNA.	• Does not introduce new mtDNA and thus avoids haplotype matching concerns. • Preserves endogenous mtDNA to nuclear DNA interactions.	• Unsuitable for use in women with a homoplasmic mitochondrial DNA mutation, where there is no healthy mtDNA. • If developed to be appropriate for use on oocytes, may require development of a technique to replenish with healthy mitochondrial DNA. • Technically challenging, e.g. delivery of these agents to the egg and the very high number of mitochondrial DNA copies in the egg. • Uncertainty of the consequence of the leftover of "uninhibited" mutant mtDNA	• Research is at a very early stage. • More likely to prove effective as a treatment for existing mitochondrial disease than preventing transmission of mitochondrial disease.
	CRISPR technology	The CRISPR-Cas9 gene editing system has been used extensively in recent years to target pathogenic nuclear mutations in cells. The possibility of using it to edit the mitochondrial genome could lead to advances within mitochondrial genetics and therapeutics.	• If it worked with mitochondrial DNA it would allow targeting of specific mitochondrial DNA mutations.	• Unlikely to work on mitochondrial DNA as there is no known mechanism for importing the required RNA into mitochondria.	• Consideration of the global call for a moratorium on heritable gene editing.

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