Australian Academy of Science

SUBMISSION TO THE

SENATE INQUIRY INTO MITOCHONDRIAL DONATION

FROM THE AUSTRALIAN ACADEMY OF SCIENCE / MAY 2018

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AUSTRALIAN ACADEMY OF SCIENCE SUBMISSION TO THE SENATE COMMUNITY AFFAIRS REFERENCES COMMITTEE INQUIRY ON THE SCIENCE OF MITOCHONDRIAL DONATION AND RELATED MATTERS

The Australian Academy of Science (the Academy) welcomes the opportunity to provide input a submission to the Senate Community Affairs References Committee Inquiry into the Science of Mitochondrial Donation. This submission was prepared by the Academy's National Committee on Cellular and Developmental Biology, the National Committee on Biomedical Sciences, and the National Committee on Medicine and Public Health. The Academy's National Committees were formed to develop their scientific field in Australia and to serve as links between Australian and overseas science organisations in the same field. They provide advice to the Academy on scientific matters and related policy areas in their respective disciplines.

To discuss or clarify any aspect of this submission or to arrange an appearance before the Committee, please contact Dr Stuart Barrow, Senior Policy Analyst at stuart.barrow@science.org.au **or 02 6201 9464.**

Background

On 21 March 2018, the Australian Senate referred the following matters to the Senate Community Affairs References Committee for inquiry and report:

(a) the science of mitochondrial donation and its ability to prevent transmission of mitochondrial disease
(b) the safety and efficacy of these techniques, as well as ethical considerations
(c) the status of these techniques elsewhere in the world and their relevance to Australian families
(d) the current impact of mitochondrial disease on Australian families and the healthcare sector
(e) consideration of changes to legal and ethical frameworks that would be required if mitochondrial donation was to be introduced in Australia
(f) the value and impact of introducing mitochondrial donation in Australia
(g) other related matters.

Mitochondrial DNA, mitochondrial disease and mitochondrial donation

Most of the DNA we inherit from our parents is located in the 23 pairs of chromosomes in the nucleus of our cells. In addition, we inherit from our mothers a small amount of DNA that resides within the mitochondria, particles (organelles) in the cell responsible for producing most of the cell's energy. Human mitochondrial DNA (or mtDNA for short) contains 37 genes involved in the production of 13 critical inner mitochondrial membrane proteins that cannot be made elsewhere. This is a very small fraction of the more than 20,000 genes encoded in the human genome.

Mitochondrial diseases are a group of relatively rare disorders resulting from defects in the mtDNA. People with mitochondrial diseases experience a range of symptoms including

developmental delays, poor growth and coordination, muscle weakness, sensory disorders, cardiovascular and metabolic disorders, dementia, and reduced life-expectancy. There are a number of treatments that can help to manage or alleviate symptoms, but no cures.

Mitochondrial donation offers a possible way to reduce the risk of inherited mitochondrial disease by allowing pre-conception transplantation of non-defective mtDNA from someone with shared ancestry (such as a sibling of the mother) as part of an in vitro fertilisation (IVF) process. The amount of new DNA introduced in such cases is trivial and is closely related to that which would have been inherited from the mother. This approach offers a possible way to reduce the risk of inherited mitochondrial disease for some couples seeking to have a genetically-related child.

For these reasons, following a community consultation process, the UK Government recently approved, with caution, human mtDNA transplantation (HFEA, 2016a).

Summary of recommendations

The Academy recognises the scientific importance and strong community interest in mitochondrial donation. In the interests of the health and happiness of people affected by mitochondrial disease, the Academy of Science and the National Committees named in this submission urge the Australian government to consider similar approval. Specifically, the Academy recommends:

- Noting the 2016 recommendation of the UK Human Fertilisation and Embryology Authority (HFEA), the mitochondrial donation techniques of maternal spindle transfer (MST) and pronuclear transfer (PNT) should be introduced into clinical practice in Australia, under due diligence of regulation and oversight.
- Research into these techniques should be allowed under certain circumstances, under appropriate regulation and oversight.
- Commonwealth and state legislation will need to be amended to allow both clinical use and research into mitochondrial donation.
- Research, training and clinical use of these techniques should be reviewed and approved on a case-by-case basis adopting a two-phase approach as has been implemented by HFEA, involving licensing of *in-vitro* fertilisation (IVF) clinics with specialist skills in mitochondrial donation and relevant assisted reproductive treatment (ART) techniques, and full review of each application.
- The National Health and Medical Research Council's Human Embryo Research Licensing Committee (ERLC) is well placed to regulate research applications of mitochondrial donation on a case-by-case basis. Any amendment to the Australian legislation should consider an extension to the remit of this body.
- Research is required to better understand mitochondrial function during development, and how to reduce or limit the risk of carryover of maternal mitochondrial DNA in mitochondrial donation.
- It is imperative for necessary regulatory changes to allow limited use of mitochondrial donation to be communicated clearly and accurately, without sensationalism.

Response to Terms of Reference
The following section provides a response to each of the terms of reference.

1) Science of mitochondrial donation and its ability to prevent transmission of mitochondrial disease

Mitochondrial disease results from dysfunction of mitochondria, the specialised entities within almost every cell of the body responsible for providing energy needed to support organ function and sustain life. Mitochondrial diseases are the most common group of inherited metabolic disorders and are among the most common forms of inherited neurological disorders (Gorman *et al*., 2016). Dysfunction can arise from mutations in genes either within the nuclear DNA, or within the mitochondrial DNA that encode for proteins with specific structural and functional roles within the mitochondria. Mitochondrial disease caused by mutations in the mitochondrial DNA is inherited only from the mother, as paternal mitochondria are not passed with the sperm during reproduction.

Mitochondrial donation offers a possible way to reduce the risk of inherited mitochondrial disease for some couples seeking to have a genetically related child (Craven et al., 2017; Richardson et al., 2015). This involves the transfer of nuclear DNA from the oocyte of the affected woman to a donor oocyte containing 'healthy' mitochondrial but devoid of its own nuclear DNA. Assisted reproductive treatment (ART) techniques are then employed to create an embryo, by in vitro fertilisation (IVF), that contains nuclear DNA from the parents and the mitochondria (including the constituent DNA) from a healthy donor.

Currently there are two different micromanipulation approaches being implemented. These approaches are illustrated in the Attachment.

One approach that involves *unfertilised oocytes* is maternal spindle transfer (MST; Attachment Figure 1). Hereby the nuclear DNA from the oocytes of the affected woman is transferred to the oocytes of a healthy donor from which the nuclear DNA has been removed. The reconstructed oocyte is composed of nuclear DNA from the affected woman and cytoplasm containing mitochondria from the donor. The reconstructed oocyte is then fertilised *in vitro* by sperm from the affected woman's partner, or sperm donor, and the developing embryo used to achieve a pregnancy through ART.

The second approach involves the transfer of pronuclei (the genetic material of the oocyte and the sperm that are visible as discrete nuclei in the hours *after fertilisation*) between *one-cell embryos* (also referred to as zygotes). Known as pronuclear transfer (PNT; Attachment Figure 2), this technique involves fertilising both the donor oocyte and the affected woman's oocyte using sperm from the affected woman's partner, or from a donor. The pronuclei of the donor oocyte are then replaced by the pronuclei from the affected woman's oocyte, and the resulting embryo used to achieve a pregnancy through ART.

In both approaches the DNA encoding heritable traits like eye and skin colour, height and other physical attributes are sourced from the affected woman and her partner or the sperm donor. The oocyte donor contributes the mitochondria and the DNA contained within the mitochondria (mtDNA) but does not contribute any nuclear DNA.

A child resulting from this technique would derive the vast majority of its genetic make-up from two parents, encoded by the nuclear DNA of the mother and father, with less than 0.1% of the child's DNA (mtDNA) derived from the donor of the healthy mitochondria. The phrase "three-parent baby" has been used to refer to the outcome of clinical application of mitochondrial donation, but this is inaccurate.

2) Safety and efficacy of these techniques, as well as ethical considerations

Mitochondrial donation is at an early stage of development. Neither micromanipulation technique – MST or PNT – can provide complete reassurance to intended parents that a resulting child will be free of affected mitochondria. These are risk reduction strategies rather than guarantees of health. These approaches also rely on 'traditional' ART and the limitations and challenges of these approaches.

However, after a comprehensive evaluation of risks and efficacy of these techniques, the Human Fertilisation and Embryology Authority (HFEA) in the UK recommended that MST and PNT are sufficiently safe to be "cautiously introduced into clinical practice in specific circumstances" (HFEA, 2016b).

Contributing to this decision were methodological developments that provided greater assurance around safety and efficacy. For example, by altering the timing associated with PNT, performing the micromanipulation soon after fertilisation, and making some other adjustments researchers were able to enhance the proportion of reconstituted zygotes that developed into embryos (Hyslop et al., 2016). These embryos also exhibited a low rate of carryover of affected mtDNA. This study illustrated the importance of optimising the methodology and highlighted the need for extensive training of staff conducting the microsurgical procedures.

This study also explored the stability of the mitochondrial contribution over time by isolating human embryonic stem cell (hESC) lines from PNT-derived embryos (Hyslop et al., 2016). With one exception, the hESC lines showed consistent levels of heteroplasmy (the proportion of mutant and unaffected mtDNA). However, the level of heteroplasmy of one hESC line, with a high starting level of mutant mtDNA, increased or 'drifted up' overtime. On the basis of this study, it has been recommended that the carryover of no more than 2% of the maternal mtDNA from the affected mother would be clinically acceptable.

With respect to MST, earlier studies raised concerns about the potential to introduce karyotypic (i.e. chromosomal) abnormalities with approximately 50% of blastocysts shown to have an abnormal karyotype (Tachibana et al., 2013).

An additional consideration is whether it is necessary to source donor oocytes from a woman with a shared ancestry (i.e. matching of haplogroup). It has been speculated that mtDNA haplogroups can affect susceptibility to neurodegenerative disease, infection resistance and other phenotypes (Kenney et al., 2014). A recent study showed that mutant mtDNA heteroplasmy in MST-derived human embryos was less than 1% and that this was stable for most embryos and in hESC lines created from them (Kang et al., 2016). However, some hESC lines displayed a reversal over time with loss of donor mtDNA, which may be a result of sub-optimal donor-maternal mtDNA interactions resulting in preferential

replication of specific mtDNA haplotypes. While it remains controversial exactly how hESC lines represent mtDNA heteroplasmy *in vivo*, it has been suggested that it would be reasonable to recommend haplogroup matching as part of oocyte donor selection (HFEA, 2016b; NAS, 2016). However, it also needs to be recognised that haplogroup matching may decrease the number of potential oocyte donors.

In the US, the Institute of Medicine of the National Academies of Science, Engineering and Medicine conducted an extensive review of the ethical, social, and policy considerations of mitochondrial donation (NAS, 2016). They concluded that given the potential benefits, in terms of increasing the reproductive choices for women carrying defective mtDNA, "cautious" clinical application of mitochondrial donation could be considered ethically justifiable under specific circumstances. These included a similar provision to the HFEA recommendation: future clinical trials should be limited to women with serious risk of transmitting defective mtDNA likely to have severe clinical impact, and application of mitochondrial transfer should be limited to male embryos to limit the risk of inheritance of faulty mitochondria. The HFEA considered but rejected this latter point in their most recent deliberations due to concerns associated with applying a further ART technique, in this case preimplantation genetic diagnosis (PGD) to select the sex of already heavily manipulated embryos. It would also halve the number of suitable embryos and thus undermine the chance of achieving a pregnancy (HFEA, 2016b).

Given the many questions raised by mitochondrial donation, clinical application should require long-term follow-up of children born to ascertain greater insight into safety and efficacy.

Both the HFEA and NAS note that participants need to be informed of the experimental nature and unknowns associated with mitochondrial donation, which may reduce risk but not guarantee a healthy child.

Those seeking to access mitochondrial donation should be fully informed of the lack of evidence around risks and efficacy, and other options that they could pursue – including prenatal diagnosis and PGD, as well as the option of having non-genetically related children.

3) Status of these techniques elsewhere in the world and their relevance to Australian families

In 2016 the Human Fertilisation and Embryology Authority (HFEA) in the UK recommended that MST and PNT are sufficiently safe to be cautiously introduced into clinical practice in specific circumstances (HFEA, 2016b). To be eligible those seeking mitochondrial donation there must be a likelihood that inheritance of mitochondrial disease without intervention will cause death or serious disease, and there must be no acceptable alternative way to prevent inheritance of the condition. The clinic conducting the treatment must also be licensed by HFEA, and each case must additionally be reviewed and approved by HFEA. This decision followed four reviews, a public dialogue on the social and ethical impact of making these techniques available to patients and amendments to the UK legislation (HFEA 2011, 2013, 2014, 2016; Nuffield Council on Bioethics, 2012).

Currently in the UK, two approved cases using PNT are underway but no pregnancies have been confirmed (Hamzelou, 2018).

Mitochondrial donation remains unavailable in the USA (Adashi and Cohen, 2017). Although a 2016 report by the Institute of Medicine of the National Academies of Science, Engineering and Medicine recommended that mitochondrial donation could be ethically justified subject to specific limitations, US federal legislation currently bans the intentional creation of human embryos that include a heritable genetic modification (NAS, 2016). However, in 2016 a baby boy was born following the use of MST in Mexico by a US-based team. The mother risked passing on Leigh syndrome, a fatal neurological disorder (Zhang et al., 2017). Although he remained healthy at seven months of age, some of the boy's cells contained diseased mitochondrial DNA from the mother. This example illustrates the challenge of eradicating all risk of the disease and raises questions about the long-term impact for children born using this approach.

There are also reports of some unregulated clinics claiming to offer commercial mitochondrial donation services to patients at risk of mitochondrial disease, as well as a derivation of the techniques to enhance fertility (Coghlan, 2017). These interventions remain unproven and the legitimacy of their claims is highly questionable.

4) Current impact of mitochondrial disease on Australian families and the healthcare sector

According to the Australian Mitochondrial Disease Foundation, one in 200 people, i.e. more than 120,000 Australians, may develop mitochondrial disease during their lifetime (AMDF, 2018). Mitochondrial disease can be inherited or result from a spontaneous mutation. In about half of those afflicted, the disease is caused by a mutation in the mitochondrial genes (mtDNA). Mutations in mtDNA are passed on from mother to child. One in every 5,000 children will develop a severe form of the disease and half will die in childhood (AMDF, 2018).

Some couples at risk of passing on mitochondrial disease may be able to resort to prenatal diagnosis or ART using PGD (Thorburn et al., 2003; Nesbitt et al., 2014). However, where the mutation is yet to be fully described, or where the proportion of dysfunctional mitochondria is very high, these options are of limited value. In the UK, the HFEA licensing process seeks to ensure that mitochondrial donation will only be offered to couples where these alternatives are unlikely to be appropriate.

5) Consideration of changes to legal and ethical frameworks that would be required if mitochondrial donation was to be introduced in Australia

In Australia, current Commonwealth legislation prohibits mitochondrial transfer for clinical use and limits research applications (RIHEA, 2002; PHCR, 2002). It remains an offence to create or develop a human embryo by fertilisation that contains genetic material provided by more than two people. There is also a prohibition related to the creation of an embryo from an oocyte and a sperm outside the body of a woman, unless intended for a pregnancy. While it is possible to undertake research and/or training involving fertilisation of a human oocyte by human sperm outside the body of a woman in a licensed research project, this is

restricted to development up to but not including the first mitotic division. Additional state legislation may also restrict applications.

If legislative change was considered in Australia, research, training and clinical use must be reviewed and approved on a case-by-case basis adopting a similar two-phase approach as has been implemented by HFEA. This should involve licensing of IVF clinics with specialist skills in mitochondrial donation and relevant ART techniques, and full review of each application. Mitochondrial donation will not be suitable for all couples at risk of mitochondrial disease. Those contemplating mitochondrial donation will need to be fully informed of the experimental nature of these interventions, including risks involved. The clinic must also conduct long-term follow-up of children born from the mitochondrial donation techniques to determine safety and efficacy. Best practice should also include collecting haplotype information on the donor (as well as the affected mother).

In Australia, accreditation by the Reproductive Technology Accreditation Committee (RTAC) is required for use of any ART application. Accreditation from RTAC requires ART clinics to comply with ART laws and the related guidelines. RTAC was established by the Fertility Society of Australia. Any clinical application of mitochondrial donation will need to be accredited by RTAC.

The National Health and Medical Research Council's Human Embryo Research Licensing Committee (ERLC), which has been responsible for the oversight of research involving the use of human embryos since 2002, is well placed to regulate research of mitochondrial donation on a case-by-case basis. Any amendment to the Australian legislation should consider an extension to the remit of this body. The ERLC could also oversee a publicly available database containing information about licences issued and outcomes, as well as regularly report to the Parliament of Australia.

Any application in Australia will require a change in legislation. Australian regulation currently prohibits/restricts clinical application of mitochondrial donation for reproductive purposes as well as for research or training use primarily due to clauses within *Prohibition of Human Cloning for Reproduction Act 2002* and *Research Involving Human Embryos Act 2002* specifically around creation and use of human embryos that contain the "genetic material provided by more than two persons". State-based legislation may also restrict application of the technology. If regulatory change is contemplated to allow limited clinical use under licence (similar to the UK model) then further amendments to allow basic research to assess technique optimisation should also be enabled so Australian researchers can contribute to the development and implementation of this emerging technology.

6) Value and impact of introducing mitochondrial donation in Australia

Introducing mitochondrial donation into Australia will provide some at-risk couples (or single affected women) with the option of having a genetically related child with a reduced risk of being affected by mitochondrial disease. For parents who may have already had and lost a child to this condition, this option would be invaluable.

However, mitochondrial donation is at an early stage of development and further research is required. In addition to introducing changes to Australian legislation to allow access for

development of clinical applications, further legislative amendments should be enabled to allow Australian researchers to contribute to improvements in safety and efficacy of mitochondrial donation. Historically, Australian researchers have been instrumental in developing reproductive options for couples at risk of mtDNA disease and other inherited diseases. For example, Australian researchers were the first to better understand the transmission of a pathogenic mtDNA mutation in human oocytes (Block et al., 1997); the first to provide a structured approach to offering mtDNA prenatal diagnosis (White et al., 1999) and among the first to define a structured approach to suitability of reproductive options including PGD (Thorburn and Dahl, 2001). Australian researchers and clinicians should be provided the opportunity to participate in advancing this important area of research.

7) Other related matters

Additional research is required to better understand mitochondrial function during development and how to reduce or limit the risk of mtDNA carryover. It is possible future technical approaches will enhance efficacy (see Section 7, HFEA 2016). Case-by-case review and approval of suitable applications will ensure Australian patients are offered the most suitable technical approach available at the time.

It is unclear how the broader Australian community would view necessary regulatory changes to allow targeted use of mitochondrial donation. A key challenge will be ensuring scientifically inaccurate and emotive terms such as “three-parent baby” do not distort public perception of mitochondrial donation. A child resulting from this technique would derive its genetic make-up (from nuclear DNA) contributed by the mother and father, with less than 0.1% of the child’s DNA (mtDNA) derived from the donor of the healthy mitochondria.

The Australian Mitochondrial Disease Foundation, the peak body representing Australians affected by mitochondrial disease, is strongly calling for legislative change to allow families access to this technique in the wake of developments in the UK. This issue was last contemplated in Australia in 2010 by an independent Legislation Review Committee. At that time, techniques enabling mitochondrial donation were considered not sufficiently advanced to be permitted. As a result of recent scientific developments, it is now time to review current restrictions and fully evaluate the scientific, ethical, social and policy considerations in an Australian context.

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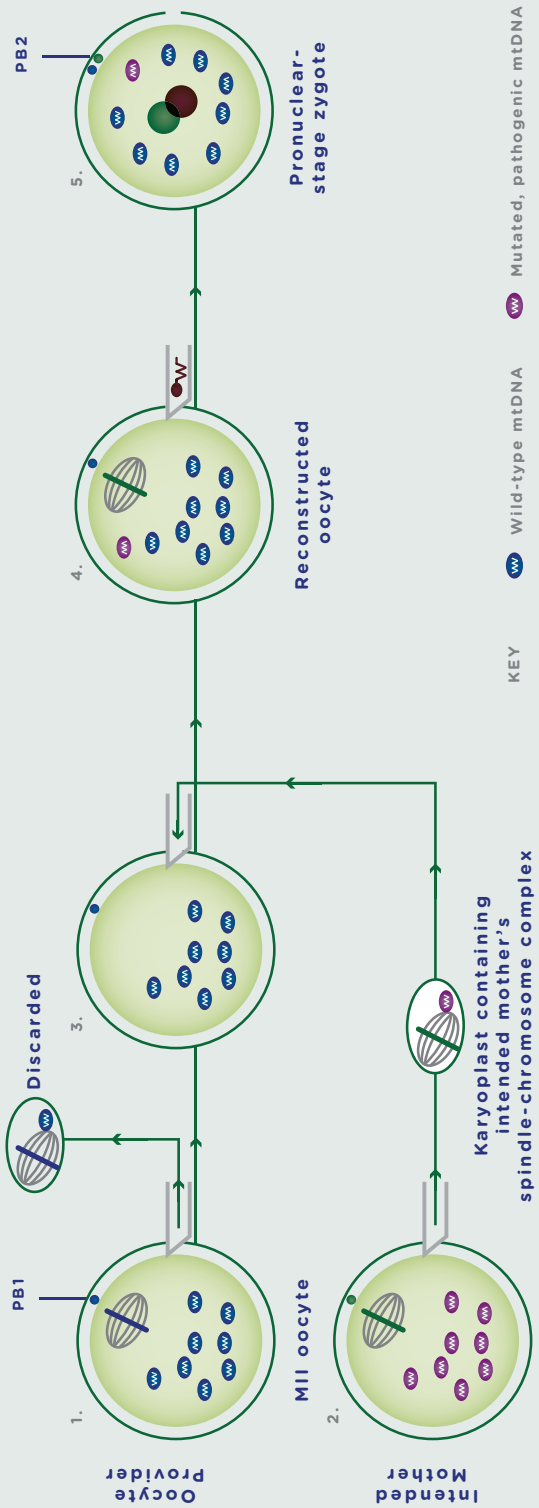
http://www.nationalacademies.org/hmd/~/media/Files/Report%20Files/2016/Mitochondrial%20Replacement%20Techniques/MitoEthics-RIB.pdf

FIGURE 1 *Maternal Spindle Transfer.*



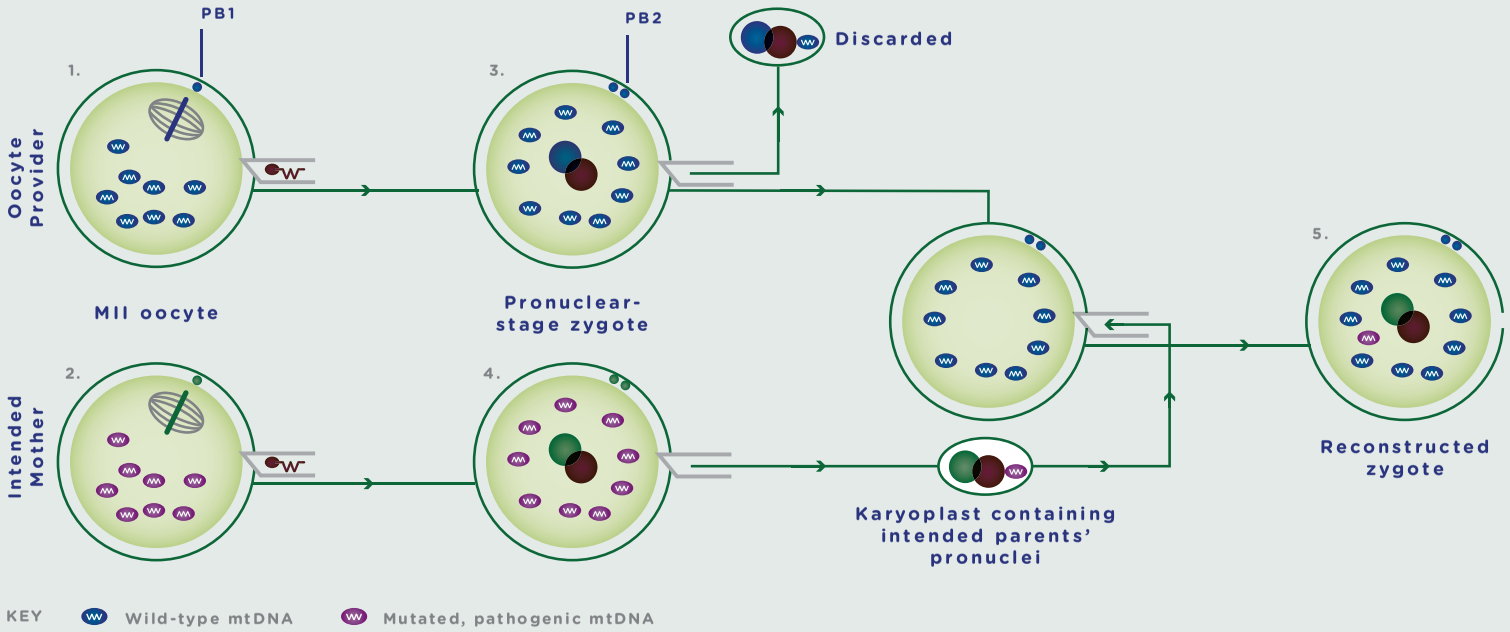
1. The spindle-chromosome complex is removed as a karyoplast from the provider oocyte and discarded.
2. The spindle-chromosome complex is removed as a karyoplast from the intended mother's oocyte and fused to the provider oocyte from which the nuclear DNA (nDNA) material has been removed; the intended mother's oocyte is discarded.
3. The reconstructed oocyte contains the intended mother's nDNA and provider's nonpathogenic mtDNA.
4. The reconstructed oocyte is fertilized by intracytoplasmic sperm injection (ICSI) with the sperm provider's sperm.
5. The fertilized oocyte is cultured in vitro and transferred at the blastocyst stage to the woman who will carry the pregnancy.

NOTES: Cells and cellular contents not drawn to scale; MII oocyte=metaphase II oocyte; PB1 and PB2=1st and 2nd polar body

SOURCE: Richardson, J., L. Irving, L. A. Hyslop, M. Choudhary, A. Murdoch, D. M. Turnbull, and M. Herbert. 2015. Concise reviews: Assisted reproductive technologies to prevent transmission of mitochondrial DNA disease. Stem Cells 33(3):639-645.

FIGURE 1 Maternal spindle transfer (MST) would entail removal of nDNA from the intended mother's oocyte and its subsequent fusion to an oocyte provided by another woman that contained nonpathogenic mtDNA and from which the nDNA had been removed.

FIGURE 2 *Pronuclear Transfer.*



1. The provider oocyte is fertilized by intracytoplasmic sperm injection (ICSI) with the sperm provider's sperm.
2. The intended mother's oocyte is fertilized by ICSI with the sperm provider's sperm.
3. The male and female pronuclei are removed from the provider zygote and discarded.
4. The male and female pronuclei are removed from the intended mother's zygote and fused to the enucleated provider zygote. The enucleated zygote of the intended mother is discarded.
5. The reconstructed zygote contains male and female nDNA from the intended mother and sperm provider and nonpathogenic mtDNA from the oocyte provider. The zygote is cultured in vitro and transferred at the blastocyst stage to the woman who would carry the pregnancy.

NOTES: Cells and cellular contents not drawn to scale; MII oocyte=metaphase II oocyte; PB1 and PB2=1st and 2nd polar body

SOURCE: Richardson, J., L. Irving, L. A. Hyslop, M. Choudhary, A. Murdoch, D. M. Turnbull, and M. Herbert. 2015. Concise reviews: Assisted reproductive technologies to prevent transmission of mitochondrial DNA disease. Stem Cells 33(3):639-645.

FIGURE 2 Pronuclear transfer (PNT) would entail the transfer of nDNA between fertilized oocytes, or zygotes, prior to fusion of the pronuclei (syngamy). The reconstructed zygote would contain nDNA from the intended parents and mtDNA from a provider.