Chapter 3

Science of mitochondrial donation

3.1 Chapters one and two of this report briefly covered what mitochondria are and how mutations in mitochondrial DNA[1] (mtDNA) can cause mitochondrial disease.

3.2 Mitochondrial donation techniques allow the mother's mutated mtDNA, which will lead to the potential formation of a mitochondrial disease, to be substituted for a donor's healthy mtDNA.

3.3 This chapter will cover the mitochondrial donation techniques that could be used to prevent the transmission of this mutated mtDNA to the children of women living with a mitochondrial disease and will consider some of the scientific risks associated with these techniques.

Mitochondrial donation techniques

3.4 Throughout the course of this inquiry, the committee was advised there were four possible methods of mitochondrial donation: maternal spindle transfer, pronuclear transfer, polar body transfer and germinal vesicle transfer. These techniques are outlined below.

Maternal spindle transfer

3.5 Maternal spindle transfer is a technique in which 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 eggs (oocytes) and transferred to an unfertilised donor egg from which the maternal spindle has been removed and that contains healthy mtDNA.[2]

3.6 Once the maternal spindle has been transferred to the donated egg with the healthy mtDNA, the egg is fertilised with the father's sperm and then implanted into the uterus in a manner similar to other in vitro fertilisation (IVF) techniques.[3]

3.7 By removing the maternal spindle and inserting it into an egg with healthy mtDNA, the resulting offspring will receive the 22 000 base pairs of nuclear DNA from the parents, but will have the 37 base pairs of healthy mtDNA from the oocyte donor.[4]

3.8 A visual diagram of a maternal spindle transfer is included below.

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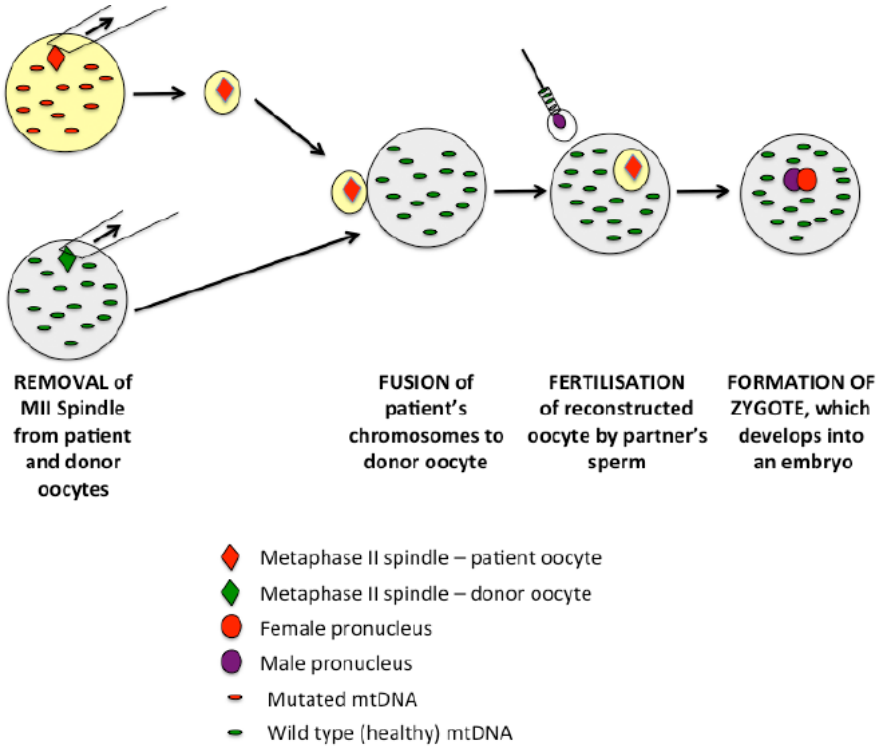
1 Deoxyribonucleic acid.

2 Wellcome Trust, *Submission 1—Attachment 3*, [p. 3]; National Health and Medical Research Council (NHMRC), *Submission 4*, p. 3; Dr Peter McCullagh, *Submission 46*, [p. 6].

3 Wellcome Trust, *Submission 1—Attachment 3*, [p. 3].

4 Mr Sean Murray, Chief Executive Officer, Australian Mitochondrial Disease Foundation (AMDF), *Committee Hansard*, 17 May 2018, pp. 3–4.

Figure 3.1—Maternal spindle transfer



Source: Professor Justin St John, *Submission 31*, [p. 7].

Current status

3.9 In the United Kingdom (UK), maternal spindle transfer is one of two methods that have been legalised by The Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015 (UK).[5]

3.10 As it is not the method that is preferred by the clinic that is currently licensed to conduct mitochondrial donation, less research has been conducted using this method. Professors David Thorburn, John Christodoulou, Carolyn Sue, John Carroll, Mike Ryan and Aleksandra Filipovska advised the committee that this technique has been used successfully in Macque monkeys by a research group in Oregon in the United States of America (USA) and has led to one live birth.[6] However, at this stage, maternal spindle transfer has not yet 'been fully optimised for human eggs'.[7]

5 The Human Fertilisation and Embryology (Mitochondrial Donation) Regulation 2015 (UK), reg. 4 (UK Regulations).

6 Professors David Thorburn, John Christodoulou, Carolyn Sue, John Carroll, Mike Ryan, Aleksandra Filipovska, *Submission 59*, p. 5. See also J Zhang, H Liu, S Luo, Z Lu, A Chavez-Badiola, Z Liu, M Yang, Z Merhi, SJ Silber, S Munne, M Konstantinidis, D Wells, JJ Tang, T Huang, 'Live birth derived from oocyte spindle transfer to prevent mitochondrial disease', *Reproductive Biomedicine Online*, vol. 34, no. 4, pp. 361–368.

7 Professors David Thorburn, John Christodoulou, Carolyn Sue, John Carroll, Mike Ryan, Aleksandra Filipovska, *Submission 59*, p. 5.

3.11 In Australia, the National Health and Medical Research Council (NHMRC) has advised the committee that research on maternal spindle transfer is currently prohibited by section 13 of the *Prohibition of Human Cloning for Reproduction Act 2002* (Cloning Act) which provides:

> A person commits an offence if:
>
> (a) the person intentionally creates or develops a human embryo by a process of the fertilisation of a human egg by a human sperm outside the body of a woman; and
>
> (b) the human embryo contains genetic material provided by more than 2 persons.
>
> Penalty: Imprisonment for 15 years.

3.12 The maternal spindle transfer method is prohibited because the process requires fertilising a human egg, creating an embryo with genetic material from more than two persons.

Pronuclear transfer

3.13 The same difficulty is not experienced with the pronuclear transfer technique because the egg is fertilised, prior to the nuclear DNA transfer occurring.[8] When an egg is fertilised and becomes a zygote, two pronuclei are formed (one from the mother and one from the father) containing the parents' nuclear DNA. For pronuclear transfer, a second zygote must be created from a donor egg and the father's sperm. The two pronuclei from the first zygote are removed and transferred to the donor zygote with healthy mtDNA.[9] The donor zygote, which needs to be at the same stage of development, has had its pronuclei removed to facilitate the transfer.[10]

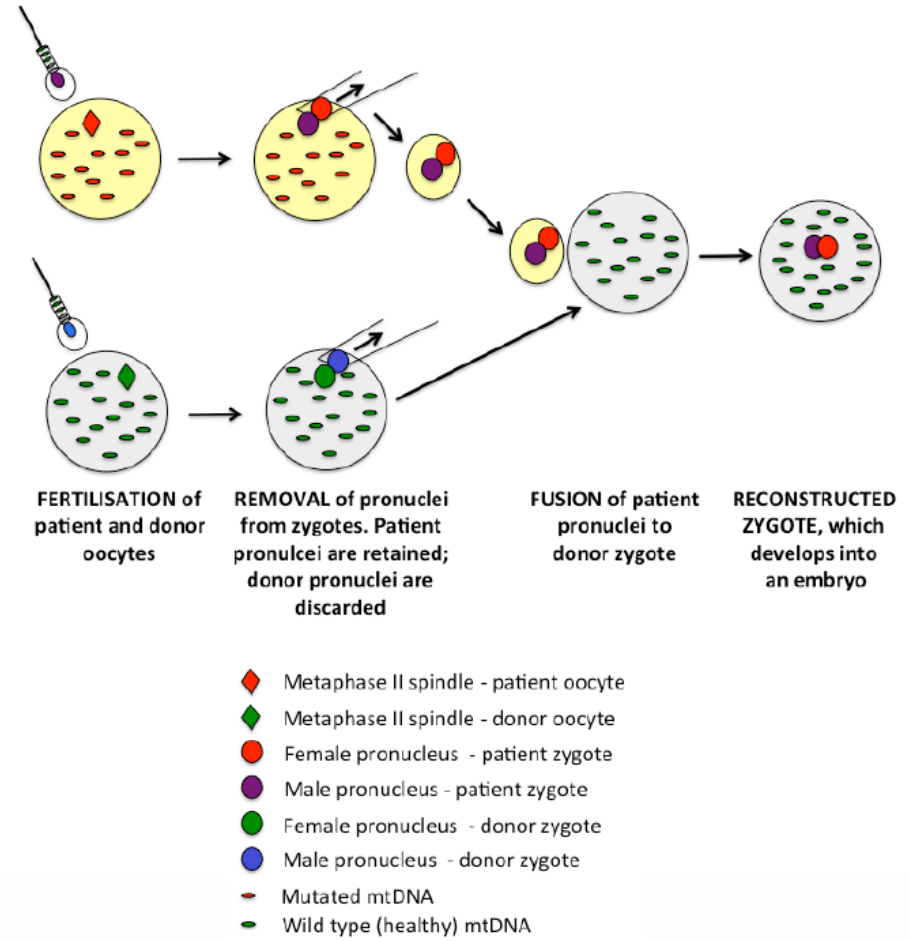
3.14 A visual diagram of pronuclear transfer is included below.

8 A zygote is a fertilised egg which may then develop into an embryo.

9 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 4.

10 Professor Justin St John, Professor and Head, Mitochondrial Genetics Group, Hudson Institute of Medical Research, *Committee Hansard*, 17 May 2018, p. 44.

Figure 3.2—Pronuclear transfer



Source: Professor Justin St John, *Submission 31*, [p. 7].

Current status

3.15 Pronuclear transfer is the second method that has been legalised in the UK.[11]

3.16 Pronuclear transfer is the technique that has been investigated in greater depth by the clinic at the University of Newcastle-upon-Thyne in the UK which holds the licence from the Human Fertilisation Embryology Authority (HFEA) to perform mitochondrial donation.[12]

3.17 In Australia, the NHMRC has advised the committee that a licence to research the pronuclear transfer technique can be granted under current legislation because the egg has already been fertilised prior to its transfer to the donor egg.

11 UK Regulations, reg. 7.

12 Mr Murray, *Committee Hansard*, 17 May 2018, p. 4; Professor John Carroll, Director, Monash Biomedicine Discovery Institute, Monash University, *Committee Hansard*, 17 May 2018, p. 25.

3.18 It is, however, subject to two other restrictions: first, the Cloning Act restricts the development of any embryo outside the body of a woman to a period of 14 days.[13] The NHMRC explained that it was possible for some research to be conducted:

> The creation of the reconstructed embryo could also be licensed under paragraph 20(1)(c) and it could be maintained in culture to assess the success of the procedure provided it was discarded before 14 days had elapsed.[14]

3.19 The second restriction precludes pronuclear transfer, or any other form of mitochondrial donation technique, from being used for reproduction. This restriction arises because any embryo containing the genetic material of more than two persons is considered to be a prohibited embryo for the purposes of the Cloning Act and cannot be implanted into a uterus for development into a foetus.[15]

Polar body transfer

3.20 A third possible method for mitochondrial donation is known as polar body transfer. There are two different techniques for polar body transfer.

3.21 During each menstrual cycle, some eggs are 'selected' for maturation and growth. As part of this process, the cell divides and leads to the formation of a secondary egg that contains mostly nuclear DNA and very little cytoplasm, which is the surrounding material in which the mitochondria are found. This is known as the first polar body.

3.22 The first polar body transfer technique extracts the first polar body, which sits outside of the main egg, and fuses it to an unfertilised egg that has had its maternal spindle removed. The reconstituted egg is then fertilised by the patient's partner's sperm.

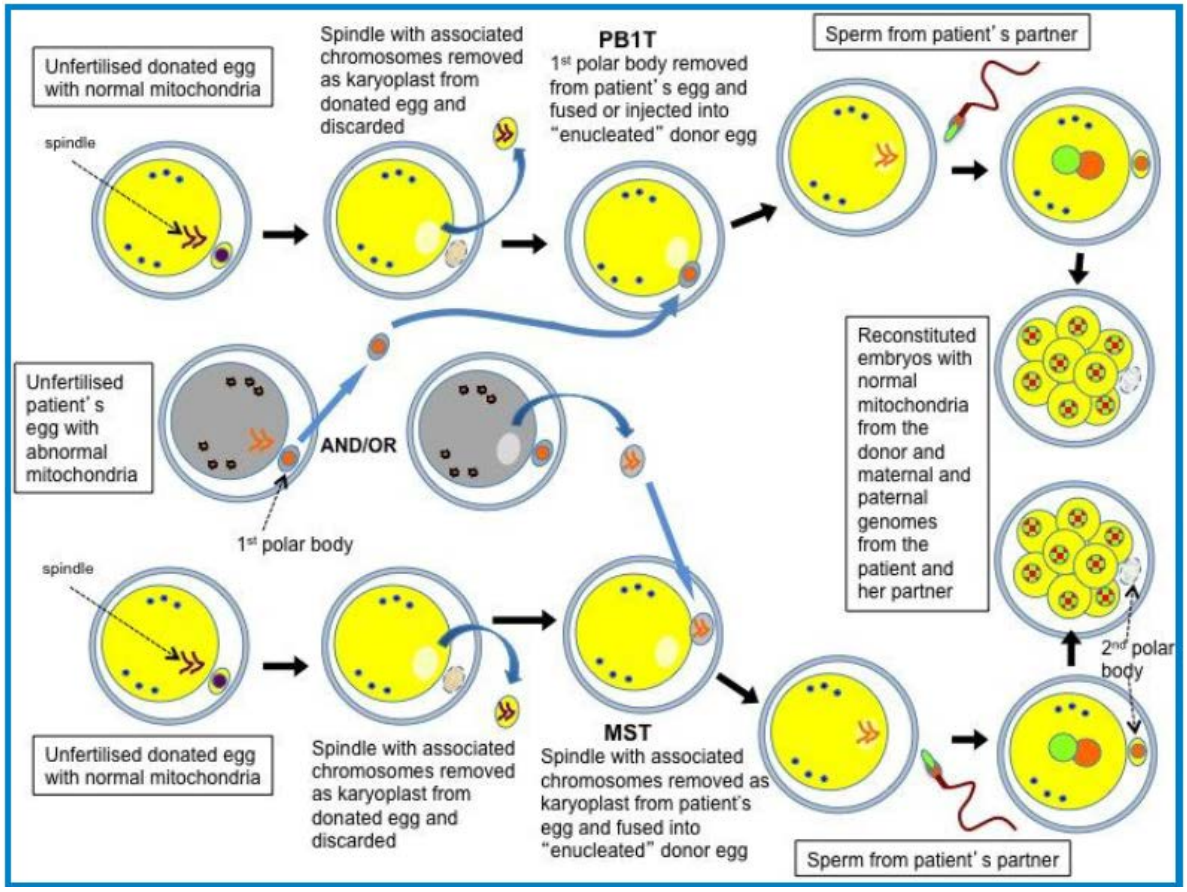
3.23 A visual representation of the first polar body technique in comparison to the maternal spindle transfer technique is included below.

13 *Prohibition of Human Cloning for Reproduction Act 2002* (Cloning Act), s. 14; NHMRC, *Submission 4*, p. 7.

14 NHMRC, *Submission 4*, p. 7.

15 Cloning Act, s. 20; NHMRC, *Submission 4*, p. 7.

Figure 3.3—First polar body transfer



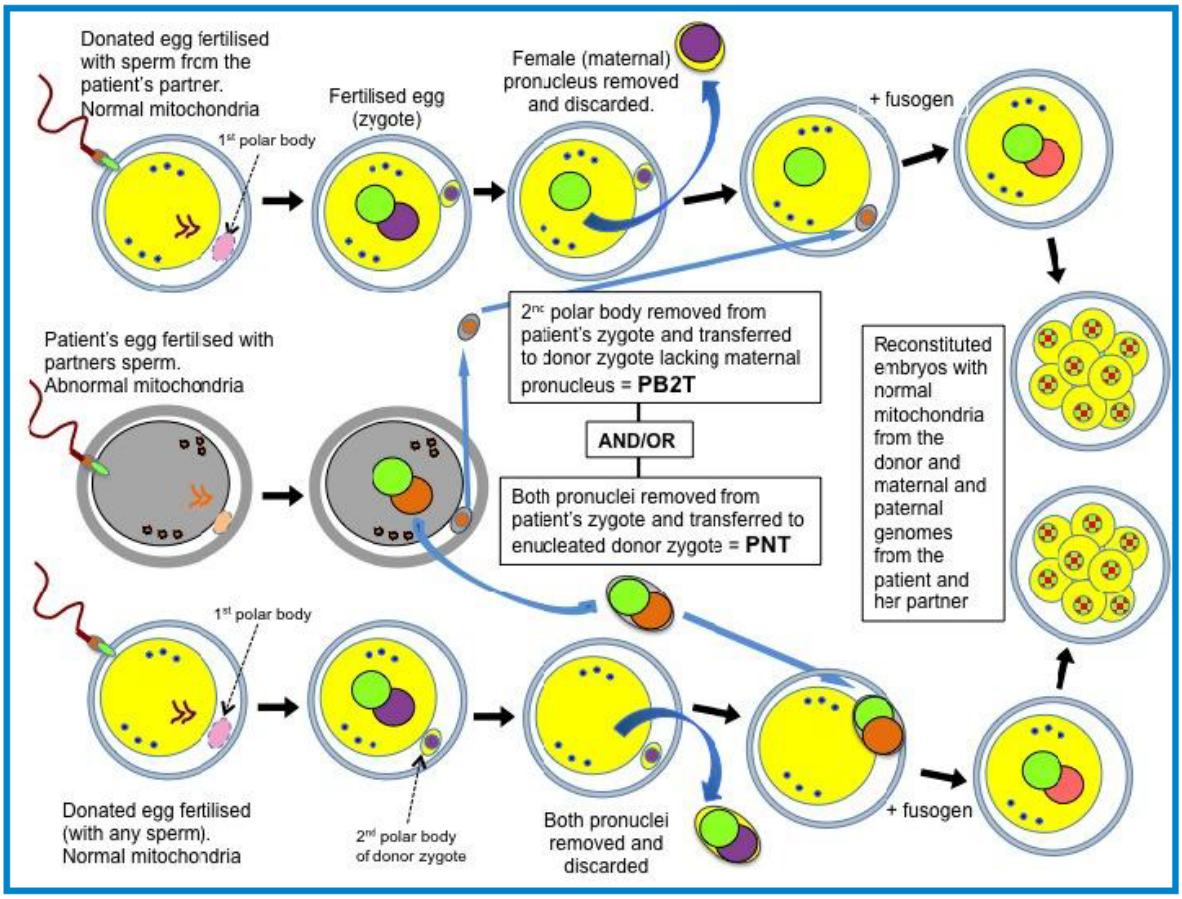
Source: HFEA, *Review of the safety and efficacy of polar body transfer to avoid mitochondrial disease*, October 2014, additional information received 30 May 2018, p. 17.

3.24 The second polar body is formed during fertilisation when the egg splits again. The second polar body transfer technique involves extracting the second polar body after fertilisation and transferring it to a newly fertilised egg that has had its maternal nuclear DNA removed.[16] The second polar body is then fused into the reconstituted egg.

3.25 A visual representation of second polar body transfer and how it compares to pronuclear transfer is included below.

16 Human Fertilisation and Embryology Authority (HFEA), *Review of the safety and efficacy of polar body transfer to avoid mitochondrial disease*, October 2014, additional information received 30 May 2018, p. 4.

Figure 3.4—Second polar body transfer



Source: HFEA, *Review of the safety and efficacy of polar body transfer to avoid mitochondrial disease*, October 2014, additional information received 30 May 2018, p. 18.

3.26 There may be advantages to using polar body transfer over maternal spindle transfer or pronuclear transfer because it may:

- reduce mtDNA carryover;
- reduce the risk of leaving chromosomes behind in maternal spindle transfer; and
- be possible to carry out both polar body transfer and either maternal spindle transfer or pronuclear transfer.[17]

3.27 However, at this stage it does not appear that polar body transfer techniques have been as advanced as some of the other methods.

Current status

3.28 In the UK, polar body transfer cannot legally be used in clinical practice. A safety and efficacy review of polar body transfer conducted by the HFEA found that

17 HFEA, *Review of the safety and efficacy of polar body transfer to avoid mitochondrial disease*, October 2014, additional information received 30 May 2018, p. 6.

while polar body transfer techniques were developing quickly, they were still at an early stage.[18]

3.29 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan and Filipovska advised the committee that they understand that the technique is still at the preclinical study stage and further work is still required to understand and optimise the procedure.[19]

3.30 The committee is not aware of polar body transfer research being conducted in Australia.

Germinal vesicle transfer

3.31 Another possible technique pioneered by Professor Justin St John is called germinal vesicle transfer. This method, which was not well-known by many of the submitters, is similar to maternal spindle transfer except that it uses an egg that is at an earlier stage of development.

3.32 In germinal vesicle transfer, the germinal vesicle (which will develop into the maternal spindle) is extracted from an egg that is at an earlier stage of development and the germinal vesicle is allowed to develop in vitro.[20]

3.33 Professor St John explained that there may be benefits to using this technique because it would not require the woman to undergo superovulation protocols and may give the chromosomes 'a bit longer to readjust to the new environment they are in'.[21]

3.34 Currently, there is little data of germinal vesicle transfer. [22]

3.35 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan and Filipovska advised the committee that success rates using this technique are currently low that 'the need to retain the egg's supporting cells will create technical challenges'.[23]

3.36 A visual diagram of the germinal vesicle transfer technique is included below.

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18 HFEA, *Review of the safety and efficacy of polar body transfer to avoid mitochondrial disease*, October 2014, additional information received 30 May 2018, pp. 5–6.

19 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 7.

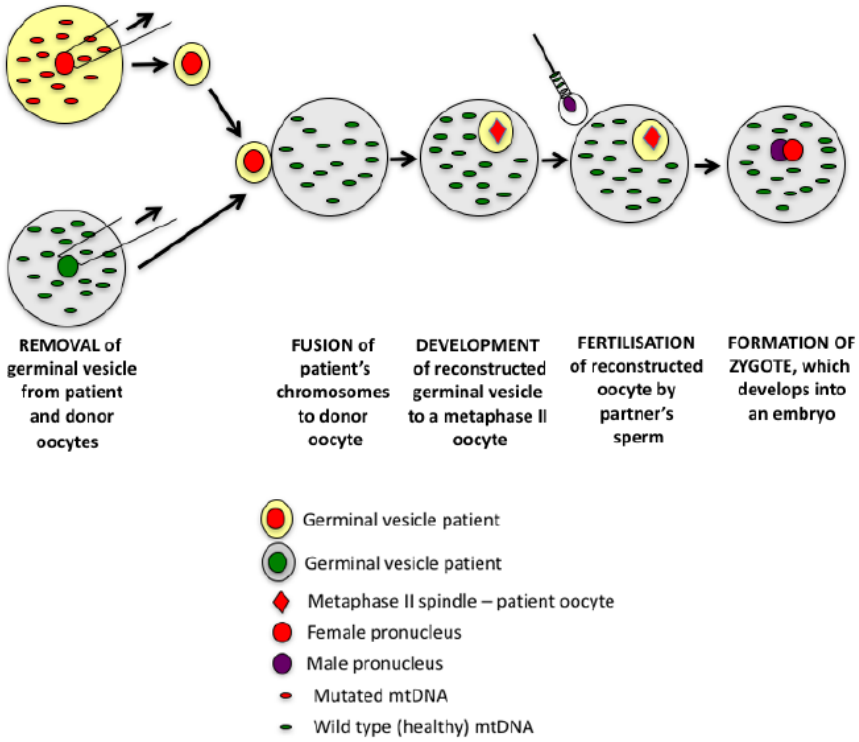
20 Professor Justin St John, *Submission 31*, [p. 2].

21 Professor St John, *Committee Hansard*, 17 May 2018, p. 43.

22 Professor St John, *Committee Hansard*, 17 May 2018, p. 43.

23 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 5.

Figure 3.5—Germinal vesicle transfer



Source: Professor St John, *Submission 31*, [p. 9].

Current status

3.37 Germinal vesicle transfer has not been legalised in the UK for clinical implementation. In Australia, the same restrictions are likely to apply as currently apply to maternal spindle transfer.

Potential risks

3.38 Any emerging reproductive technology includes a degree of risk.[24] Witnesses and submitters to the inquiry explained the risks that may exist with mitochondrial donation techniques.

3.39 The threshold question for the committee's consideration of the matter was whether mitochondrial donation techniques are considered to be safe to perform on human embryos that will develop into live babies.

3.40 Submitters to the inquiry were largely of the opinion that mitochondrial donation is now safe to perform.[25]

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24 Professor David Thorburn, Head of Mitochondrial Research and Diagnostic Laboratories, Murdoch Children's Research Institute and Victorian Clincial Genetics Services, *Committee Hansard*, 17 May 2018, p. 12; Associate Professors Catherine Mills and Karinne Ludlow, Professor Robert Sparrow, Dr Narelle Warren, *Submission 20*, p. 3; Murdoch Children's Research Institute and Victorian Clinical Genetics Services (Murdoch Children's Research Institute), *Submission 23*, p. 4; AMDF, *Submission 26*, [p. 10]; Biomedical Ethics Group, Murdoch Children's Research Institute, *Submission 34*, [p. 2].

3.41 Before mitochondrial donation was legalised in the UK, these techniques were subject to four scientific reviews. The Wellcome Trust, a UK based charitable foundation that funds mitochondrial disease research, told the committee that scientific reviews conducted prior to legalisation concluded that the techniques were safe:

> Safety of the techniques is, and will always be, of paramount importance and has received unprecedented scrutiny. On three separate occasions the HFEA's specially convened independent Expert Scientific Review panel examined the safety and efficacy of mitochondrial donation. The panel reported that they found no evidence to suggest that the techniques are unsafe for clinical use, and concluded that both techniques have the potential to be used in patients with mitochondrial disease.[26]

Box 3.1—UK Scientific Reviews

Before mitochondrial donation was legalised in the UK three scientific reviews were undertaken by an expert panel convened by the regulator, the HFEA, to assess the safety and efficacy of the techniques.

The third scientific review was completed in 2014. The 2014 review recommended that:

a) additional experiments needed to be conducted to corroborate and improve the efficiency of the maternal spindle transfer technique;

b) additional experiments needed to be conducted to compare pronuclear transfer ooyctes with intracytoplasmic sperm injection oocytes; and

c) consideration should be given to mtDNA haplogroup matching.[27]

The fourth scientific review was completed in 2016. The 2016 review was conducted to update the 2014 scientific review and to consider whether the recommendations made in that report had been met. The review considered that good progress had been made on each recommendation. In addition, it recommended that clinicians carefully select patients, conduct prenatal testing and follow up and maintained the recommendation to use haplogroup matching as a precautionary step.[28]

25 Wellcome Trust, *Submission 1—Attachment 1*, p. 2; The Human Genetics Society of Australia, *Submission 2*, [p. 1]; Monash Biomedicine Discovery Institute, *Submission 19*, [p. 2]; Murdoch Children's Research Institute, *Submission 23*, p. 2; AMDF, *Submission 26*, [p. 10]; Fertility Society of Australia, *Submission 27*, p. 2 (Fertility Society); Dr Nigel Turner, *Submission 37*, [p. 1]; Nuffield Council on Bioethics, *Submission 43*, [p. 1]; Wellcome Centre for Mitochondrial Research, *Submission 45*, [p. 3]; Dr Shanti Balasubramaniam, *Submission 52*, [p. 3].

26 Wellcome Trust, *Submission 1—Attachment 1*, p. 2.

27 HFEA, *Review of the safety and efficacy of methods to avoid mitochondrial disease*, June 2014, additional information received 30 May 2018, p. 5.

28 HFEA, *Review of the safety and efficacy of methods to avoid mitochondrial disease*, November 2016, additional information received 30 May 2018, p. 5.

3.42 A similar conclusion has been reached by Australian experts. Professor John Carroll, Director of the Monash Biomedicine Discovery Institute at Monash University told the committee that the evidence did not indicate that there were any serious safety concerns:

> …a good deal of research has been done, and to date there's really very little evidence for serious safety concerns and certainly nothing that comes anywhere near close to the impact that genetic disease has. Being able to assess the risks associated with the procedure with the alternative outcome, I think there's very little doubt in my mind, at least, that they're very well balanced, and we should be able to proceed with investigating the treatment.[29]

3.43 Professor John Christodoulou, Chair of Genomic Medicine in the Department of Paediatrics at the University of Melbourne told the committee that he was unaware of any evidence that pointed to there being significant risks to a child born of a mitochondrial donation:

> There has been some theorizing that mitochondrial donation through proposed epigenetic mechanisms, or as a consequence of not using mtDNA haplogroup matched donor egg cells for the procedure, could lead to untoward effects on the health of the embryo or the child after birth. However, I am aware of no such evidence supporting the notion that there would be any significant risks to children born following mitochondrial donation.[30]

3.44 Some submitters though were more cautious about declaring the techniques as being safe to use. The submission from the NHMRC noted that although one child is known to have been born in Mexico using the maternal spindle transfer technique, his mutation load is currently unknown because:

> …his parents have requested no more testing for mtDNA unless there is a clinical benefit. Consequently, it may be difficult to assess the long-term success of the procedure.[31]

3.45 Professor St John considered that additional studies were required on large animal models to test the consequences of mtDNA carryover and test the effects of using eggs with different haplotypes.[32] These issues are considered in turn below.

Carryover of mutated mtDNA

3.46 Some submitters and witnesses to the inquiry expressed concerns about the potential effect of carrying over mutated mtDNA to the reconstituted donor egg during the transfer process.[33]

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29 Professor Carroll, *Committee Hansard*, 17 May 2018, p. 22.

30 Professor Christodoulou, *Submission 12*, p. 2.

31 NHMRC, *Submission 4*, p. 4.

32 Professor St John, *Submission 31*, [p. 4].

3.47 Professor Christodoulou explained that when the nucleus of the cell is transferred from one egg to another, a certain amount of the mutated mtDNA may be carried over:

> The process involves removing a nucleus and then implanting that nucleus into the egg cell that's had the nucleus removed and has the mitochondria. In the early days, the process of removing the nucleus, as part of it, took a number, or a proportion, of mitochondria—and therefore mitochondrial DNA—along with it in that process. That's that sort of carryover phenomenon.[34]

3.48 Some submitters expressed concern that if mutated mtDNA is transferred to the donor egg, the child may still end up with a mitochondrial disease.

3.49 According to Professor Christodoulou, the expert committee for the HFEA recommended that mtDNA carryover rates should not exceed two per cent and should be no greater than 10 per cent per embryo.[35] A number of witnesses who provided evidence to the committee, including Professor Christodoulou, endorsed the two per cent figure as representing a safe level below which a child was unlikely to develop a mitochondrial disease.[36]

3.50 This figure is considered to be a safe level because, as Associate Professor Damian Dowling from the School of Biological Sciences at Monash University explained in his submission, mutations in mtDNA do not generally cause mitochondrial disease until the mutated mtDNA comprises 70–80 per cent of the pool of mtDNA.[37] However, Associate Professor Dowling suggested that even a small amount of carryover may present a risk to the child. The risk may exist because mtDNA cell numbers are not static across a person's life and experimental studies have shown that the unhealthy mtDNA cells can 'outcompete' the healthy mtDNA cells:

> Experimental studies in flies, yeast, worms, and human cell lines have shown that defective mtDNA molecules often proliferate more rapidly than healthy molecules, and can thus, somewhat ironically, outcompete their healthy mtDNA counterparts…This means that mitochondrial disease could plausibly reemerge in children born to the technique, or in the children of daughters born to this technique. [38]

3.51 Professor St John and Dr Ian Trounce both noted that there have been some studies in which the original mtDNA outcompeted the donor's mtDNA to become the

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33 Associate Professor Damian Dowling, School of Biological Sciences, Monash University, *Committee Hansard*, 17 May 2018, p. 52; Associate Professor Dowling, *Submission 25*, p. 2.

34 Professor John Christodoulou, Chair, Genomic Medicine, Department of Paediatrics, University of Melbourne, *Committee Hansard*, 17 May 2018, p. 17.

35 Professor Christodoulou, *Submission 12*, p. 3.

36 Professor Christodoulou, *Submission 12*, p. 3.

37 Associate Professor Dowling, *Submission 25*, p. 2.

38 Associate Professor Dowling, *Submission 25*, p. 2.

dominant mtDNA in the population.[39] Murdoch Children's Research Institute and Victorian Clinical Genetic Services and Professor Mary Herbert also noted that in 15–20 per cent of cases, stem cells tested after mitochondrial donation showed that the mtDNA had reverted to the maternal mtDNA.[40]

3.52 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan and Filipovska doubted whether reversion to the maternal mtDNA would be seen in live babies, noting significant differences between the long-term culture of stem cells and live births:

> …embryonic stems cells are considered a poor proxy for normal development in the womb…The state of pluripotency, which allows stem cells to proliferate indefinitely in cell culture, lasts for only a few days during normal development.[41]

3.53 Furthermore, international evidence suggests that there is not significant drift overtime and the amount of mutated mtDNA present at the eight-cell stage and the prenatal diagnosis stage is consistent with the 'level found in multiple tissues at birth'.[42]

3.54 Many submitters were of the opinion that mtDNA carryover could be managed and minimised. In its submission, the Wellcome Centre for Mitochondrial Research, the clinic that currently holds the licence to conduct trials from the UK's HFEA, told the committee that in its initial studies the level of carryover was minimal:

> The study revealed that human PNT embryos had the potential for onward development and importantly, that the level of mtDNA co-transferred with the nuclear DNA during the procedure was minimal (<2% on average). This is well below the level of mutant mtDNA associated with clinical symptoms and led us to conclude that PNT had the potential to prevent transmission of mitochondrial disease.[43]

3.55 Since then, additional studies have been done to examine whether there was the potential for the mutated mtDNA to increase to substantial levels. In a joint submission, the Murdoch Children's Research Institute and Victorian Clinical Genetic Services advised the committee that additional research had been conducted in the UK which found that the levels of mtDNA did not increase provided the original transfer was kept to below two per cent of the mutated mtDNA:

> Additional safety experiments were performed to determine whether there was any potential for mitochondrial DNA carry-over to result in the original mitochondrial DNA from the mother's egg increasing back up to substantial

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39 Professor St John, *Committee Hansard*, 17 May 2018, p. 41; Professor St John, *Submission 31*, [p. 2]; Dr Ian Trounce, *Submission 47*, [p. 1].

40 Murdoch Children's Research Institute, *Submission 23*, p. 5; Professor Mary Herbert, *Submission 49*, p. 3.

41 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 6.

42 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 6.

43 Wellcome Centre for Mitochondrial Research, *Submission 45*, [p. 2].

> levels. Their data showed that this did not happen so long as the procedure ensured no more than 2% of the mother's mitochondrial DNA was present in the embryo after mitochondrial transfer.[44]

3.56 Professor Carroll advised the committee that he considered that, if good techniques are used, the risks of the baby acquiring a genetic disease were low:

> My view is that, once you're doing the mitochondrial procedure, the risks of carryover that are sufficient to contribute to the baby having any genetic disease is so low that I don't think it's a necessary part of the procedure... I think that the mitochondrial donation procedure leads to so few mitochondria, using good techniques, being donated to the new embryo that it's unlikely to be propagated.[45]

3.57 The Fertility Society of Australia (Fertility Society) also acknowledged that, whilst a small risk exists, it is worth taking if it means that a child will not be born with a fatal disease:

> Based on the scientific advice of our membership, we believe that the balance of safety versus risk has been addressed. There is no question that new technology does occasionally bring negative results, but given the fatal nature of Mitochondrial disease we believe that miniscule risk is worth taking.[46]

The potential of sex-selection

3.58 As noted above, some researchers have raised a risk of mitochondrial disease re-emerging in the children or the children of daughters born to mitochondrial donation techniques because of mtDNA carryover.[47]

3.59 Dr Peter McCullagh, a British medical practitioner who followed the developments in the UK, noted that there had been a proposal to restrict clinics to selecting males embryos for clinical implantation to mitigate this risk:

> To ensure that the mutated mtDNA is not transmitted to any children leading to a risk of transgenerational impacts, it has been proposed that licences to undertake mitochondrial transplantation should be restricted to British clinics which commit to gender selection for males. There have been warnings that, even if the first generation of females is not clinically affected, mitochondrial coded disease may nevertheless emerge in later

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44 Murdoch Children's Research Institute, *Submission 23*, p. 4. See also Louise A Hyslop, Paul Blakeley, Lyndsey Craven, Jessica Richardson, Norah M E Fogarty, Elpida Fragouli, Mahdi Lamb, Sissy E Wamaitha, Nilendran Prathalingam, Qi Zhang, Hannah O'Keefe, Yuko Takeda, Lucia Arizzi, Samer Alfarawati, Helen A Tuppen, Laura Irving, Dimitrios Kalleas, Meenakshi Choudhary, Dagan Wells, Alison P Murdoch, Douglass M Turnbull, Kathy K Niakan, Mary Herbert, *Nature*, vol. 534, pp. 383–386.

45 Professor Carroll, *Committee Hansard*, 17 May 2018, p. 25.

46 Fertility Society, *Submission 27*, p. 2.

47 Associate Professor Dowling, *Committee Hansard*, 17 May 2018, p. 52; Dr McCullagh, *Submission 46*, [p. 6].

> ones. Birth of a clinically normal infant may not necessarily guarantee similar normality in the following generation.[48]

3.60 That argument found favour with the National Academies of Sciences, Engineering and Medicine (NASEM) in the United States of America. A report by the NASEM for the USA Food and Drug Administration recommended that initially only male embryos should be transferred because there was a 'need to proceed slowly and to prevent potential adverse and uncertain consequences of MRT [mitochondrial replacement techniques] from being passed on to future generations'.[49]

3.61 Under the American proposal, female children would only be able to be born after adequate follow-up and satisfactory findings in male children.[50] This is because mtDNA cannot be inherited through the male line.

3.62 Mr Sean Murray from the Australian Mitochondrial Disease Foundation (AMDF) told the committee that only selecting male embryos could be considered in Australia as an interim safeguard measure:

> I think the recommendation was made as a risk mitigation there, because, as I explained before, in my situation I can't pass on my mitochondrial DNA to my children, and I think that that's the rationale behind that. So that could definitely be viewed as a safeguard measure while we figure this out in more detail.[51]

3.63 Some submitters noted that a prohibition on implanting female embryos would halve the efficiency of the techniques and would potentially require women to undergo additional ovarian hyperstimulation to produce additional eggs.[52]

3.64 Many of the scientists the committee spoke to considered that it was not a necessary prohibition. Professor Thorburn noted that, even though it was considered, the UK ultimately decided not to impose such a prohibition because it was not considered to be necessary.[53]

3.65 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan and Filipovska advised the committee that, in their opinion, the same risks existed for male and female embryos, meaning that there was no clear reason to prohibit the implantation of female embryos.[54]

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48 Dr McCullagh, *Submission 46*, [p. 6].

49 National Academies of Sciences, Engineering and Medicine (USA), *Mitochondrial Replacement Techniques: Ethical, Social and Policy Consideration*, February 2016, quoted in NHMRC, *Submission 4*, p. 5.

50 NHMRC, *Submission 4*, p. 5.

51 Mr Murray, *Committee Hansard*, 17 May 2018, p. 6.

52 Professor Thorburn, *Committee Hansard*, 17 May 2018, p. 15; Dr Balasubramaniam, *Submission 52*, [p. 4].

53 Professor Thorburn, *Committee Hansard*, 17 May 2018, p. 15.

54 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, pp. 6–7.

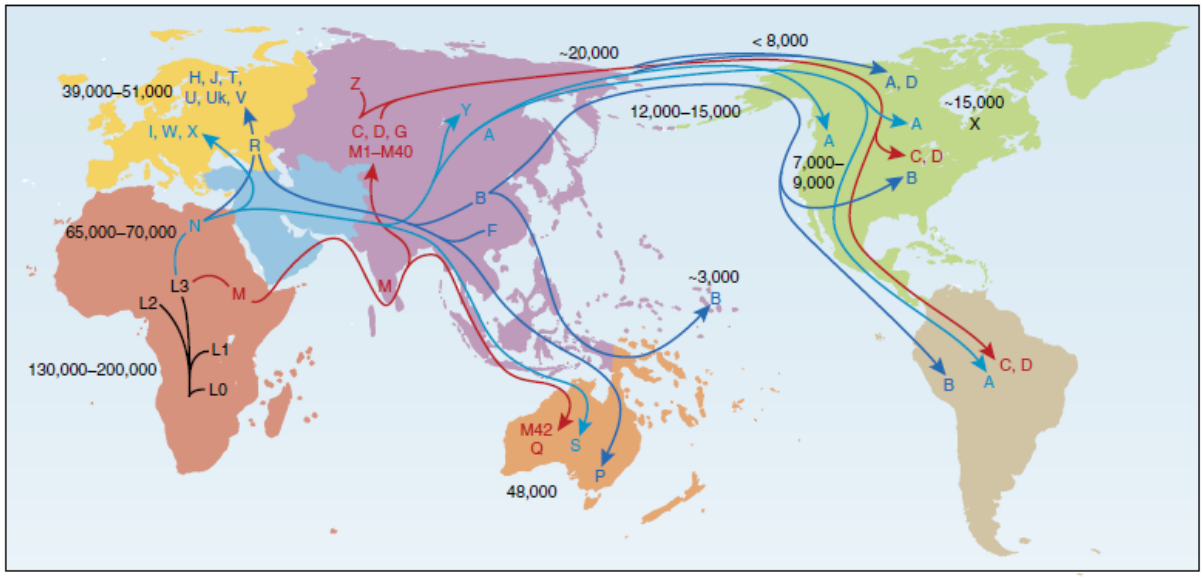
3.66 Some submitters considered that while a degree of risk exists, the question whether to implant female embryos should be considered by the prospective parents after counselling.[55]

mtDNA matching

3.67 As noted in chapter one, mtDNA is maternally inherited. Different people have different mtDNA if they come from a different haplogroup (also sometimes called a haplotype). A haplogroup corresponds to the common maternal origins of the species. In humans, there are about 25 different major variations of the mtDNA sequence and they largely correspond to continental population groups.[56]

3.68 A visual representation of the distribution of those haplogroups is included below.

Figure 3.6—Haplogroup distribution



Source: Professor Robin Lovell-Badge, *Submission 58—Attachment 1*, p. 1061.

3.69 Submitters to the inquiry noted that a person's haplogroup can influence a number of factors related to a person's health, including 'sperm motility, infection resistance, susceptibility to neurodegenerative disease and ageing'.[57]

3.70 Some submitters have expressed concern that a failure to match the haplogroup of the egg donor to the haplogroup of the mother's nuclear chromosomes may lead to potential negative health effects on the child born of the technique. Professor St John explained to the committee the nature of his concern:

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55 Professor Carolyn Sue, Director, Kolling Institute of Medical Research, Mitochondrial Disease Research Centre, *Committee Hansard*, 17 May 2018, p. 26; Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 7.

56 Professor St John, *Submission 31*, [p. 3]; Associate Professor Dowling, *Submission 25*, p. 3.

57 Murdoch Children's Research Institute, *Submission 23*, p. 6. See also Associate Professor Dowling, *Submission 25*, p. 4.

> We know from certain studies in both human and mouse models that, if you increase the genetic distance between the source of the eggs from which the chromosomes are coming and the donor egg itself, that can influence the outcome and the phenotype of the offspring and the cells you're trying to make.[58]

Incompatibility between different haplogroups

3.71 There were two areas of concern where it was suggested there could be negative consequences of unmatched haplogroups. The first of these was that mtDNA from a different haplogroup may be inconsistent or incompatible with the mother's chromosomes and this may in turn affect potential gene expression.

3.72 The first mechanism was raised by Professor St John, Associate Professor Damian Dowling and Dr Ian Trounce who drew the committee's attention to studies that primarily used mice and fruit flies and indicated that a failure to match the mother's mtDNA haplogroup with the egg donor's mtDNA haplotype had the potential to lead to changes in gene expression.[59]

3.73 Professor St John advised the committee that studies on mouse stem cells had demonstrated using mtDNA from distantly related haplogroups could have an effect on the health of the mouse.[60]

3.74 Associate Professor Dowling similarly observed that different variations may alter the efficiency of the gene expression and 'in theory could therefore affect individual performance'.[61] He noted though that some mtDNA mutations could be beneficial in some environments, for example the same mutation that causes the debilitating Leber's Hereditary Optic Neuropathy (LHON) is the same mutation that can help humans to survive at high altitudes in oxygen deficient environments.[62]

3.75 Other submitters were less certain that mtDNA matching was necessary. Professor David Thorburn, Head of Mitochondrial Research and Diagnostic Laboratories at the Murdoch Children's Research Institute and Victorian Clinical Genetic Services questioned that the studies on mice and fruit fly would necessarily translate to humans:

> There are experiments in animals, particularly in inbred mice, flies and worms, which suggest that there could be some degree of incompatibility between distantly related haplogroups...They tend to be more distantly related than humans are, and they tend to be inbred rather than outbred, so

58 Professor St John, *Committee Hansard*, 17 May 2018, p. 41.

59 Professor St John, *Committee Hansard*, 17 May 2018, pp. 41–42; Professor St John, *Submission 31*, [p. 3]; Associate Professor Dowling, *Submission 25*, p. 3; Dr Trounce, *Submission 47*, [p. 1].

60 Professor St John, *Committee Hansard*, 17 May 2018, pp. 41–42; Professor St John, *Submission 31*, [p. 3].

61 Associate Professor Dowling, *Submission 25*, p. 3.

62 Associate Professor Dowling, *Submission 25*, p. 3.

> you can measure outcomes very accurately. My personal view is that this is unlikely to be an issue…[63]

3.76 Professor Thorburn also pointed to studies conducted with Macque monkeys and the limited human evidence that is available to indicate that unmatched mtDNA haplogroups have not led to health problems:

> It's very reassuring that when this has been done in the macaque monkeys, there hasn't been any evidence seen for this drift of maternal versus donor haplogroups happening. Those monkeys have been healthy when studied—at least the males have been shown to be fertile; the female has just reached reproductive age I think—and the limited information of the one child born from this technique in Mexico, which was a terrible regulatory process, has had about the same amount of the mutation in cord blood and cheek wash and all those sorts of non-invasive tests of tissues and placenta. So the available evidence suggests that it hasn't been seen in primate models—monkeys or humans.[64]

3.77 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan and Filipovska note that while there is still some uncertainty, they consider that the 'likely risks are relatively low'.[65]

Nuclear-mitochondrial interaction

3.78 The second area of concern where there may be negative consequences of not matching haplogroups, is that there may be an evolutionary link or interaction between the mtDNA and the nuclear DNA and breaking the link between the two may lead to potential negative consequences.

3.79 Associate Professor Dowling explained in his submission that evolutionary theory indicates that the mtDNA and the nuclear DNA have evolved together. He suggested that in this way the nuclear DNA and the mtDNA were like pieces of a jigsaw that would not necessarily be compatible with other mtDNA and may cause negative health consequences in the offspring.[66]

3.80 Associate Professor Dowling expressed concern about this and pointed to a number of studies that indicated there may be effects on humans as a result of mitochondrial donation. However, he noted that it was uncertain whether creating novel mitochondrial and gene combinations would lead to health benefits or detriments:

> The evidence to date suggests that it's more likely there will be effects than no effects by creating novel combinations of mitochondrial and nuclear genotype as mitochondrial donation will do. However, it's not clear. We have, at this stage, no way to predict whether or not the effects will actually be advantageous to the child and improve the performance of what the child

63 Professor Thorburn, *Committee Hansard*, 17 May 2018, p. 15.

64 Professor Thorburn, *Committee Hansard*, 17 May 2018, p. 18.

65 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 5.

66 Associate Professor Dowling, *Submission 25*, p. 4.

> would have been in the event that it hadn't originally carried the pathogenic mtDNA mutation, or whether it will result in a decrease in performance in the child. The majority of evidence suggests the negative effects are more common than the positive effects, but it can go either way.[67]

3.81 In particular, Associate Professor Dowling pointed to a 2018 meta-analysis study that suggested that 'humans showed stronger effects' than other animals to mitochondrial donation.[68] The meta-analysis referred to by Associate Professor Dowling estimated 'negative effects in at least one in every 130 resulting offspring born to the therapy'.[69] Professors Thorburn, Christodoulou, Sue, Carroll, Ryan and Filipovska submitted that if that estimate is correct, the resulting risks are 'lower that the approximately 3% risk for any couple of having a child with some kind of genetic anomaly'.[70]

3.82 Other submitters disagreed that this would be a problem. The Progress Educational Trust dismissed the suggestion that disrupting co-evolution could lead to adverse consequences:

> Some have argued that mitochondrial donation could disrupt relationships that have developed between mitochondrial and nuclear DNA via co-evolution, and that this could have adverse consequences. There is little evidence for this view.
>
> There have been experiments on animals where co-evolved relationships between mitochondrial and nuclear DNA were deliberately disrupted. However, this has only been shown to have a mildly adverse effect in two situations, and neither of these situations is applicable to mitochondrial donation in humans.[71]

3.83 The Progress Educational Trust was clear that while the nuclear genes have an effect on the mtDNA, the effect only operates in one direction:

> It is known that nuclear gene products can and do leave the nucleus and have an effect on mitochondrial DNA. However, the reverse is not true – there is no evidence of mitochondrial gene products leaving the mitochondria and having an effect on nuclear DNA.

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67 Associate Professor Dowling, *Committee Hansard*, 17 May 2018, p. 53.

68 Associate Professor Dowling, *Submission 25*, p. 4; Ralph Dobler, Damian K Dowling, Edward Morrow, Klaus Reinhardt, 'A systematic review and meta-analysis reveals pervasive effects of germline mitochondrial replacement on components of health', *Human Reproduction Update*, 2018, pp. 1–16.

69 Ralph Dobler, Damian K Dowling, Edward Morrow, Klaus Reinhardt, 'A systematic review and meta-analysis reveals pervasive effects of germline mitochondrial replacement on components of health', *Human Reproduction Update*, 2018, p. 2.

70 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 5.

71 Progress Educational Trust, *Submission 48—Attachment 1*, p. 3 (footnotes removed).

> The relationship between the nucleus and the mitochondria is therefore one-sided. This makes it highly unlikely that donated mitochondria could relate to the nucleus in a dysfunctional way.[72]

3.84 This view was supported by Professor Carolyn Sue, Director of the Mitochondrial Research Centre at the Kolling Institute of Medical Research, who pointed to other clinical situations where no haplogroup matching exists and continues without consequence:

> My feelings are that with any tissue donation—such as liver transplants, heart transplants and bone marrow transplants—there is no haplogroup matching, so these are models that existed in patients that I see in hospital every day. Patients get better from their transplants. They go around with different parts of DNA—both nuclear and, in this case, mitochondrial DNA. But everybody forgets about that. We know that there are patients who have mixed components of mitochondrial DNA accepting therapies, benefiting from therapies and having their lives improved by these techniques. I see mitochondrial donation as something like this.[73]

3.85 This view has been supported by the experimental data from the Wellcome Centre for Mitochondrial Research in the UK that found no difference in gene expression between the control group and the pronuclear transfer embryos.[74]

3.86 As noted above, the scientific reviews conducted in the UK recommended that haplogroup matching be used as a precautionary step. Submitters to the inquiry generally agreed that, in the interests of caution, mtDNA matching should be considered.[75]

3.87 However, Professor Thorburn explained that the regulator in the UK did not mandate that haplogroup matching must be undertaken and instead left the decision to the families involved:

> What they concluded—and I agree with it—was this should be mentioned in discussions with the families, that there may be advantages in matching the haplogroup but that it shouldn't be a barrier to families choosing an unmatched donor, because it greatly restricts the number of donors that would be potentially available.[76]

3.88 Leaving the decision to about whether to use haplogroup matching, after counselling, to the prospective parents was endorsed by the Australian Academy of Sciences, Murdoch Children's Research Institute and Victorian Genetic Clinical

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72 Progress Educational Trust, *Submission 48—Attachment 1*, p. 3.

73 Professor Sue, *Committee Hansard*, 17 May 2018, p. 23.

74 Wellcome Centre for Mitochondrial Research, *Submission 45*, [p. 3].

75 Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 6.

76 Professor Thorburn, *Committee Hansard*, 17 May 2018, p. 14. See also HFEA, *Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2016 update*, November 2016, additional information received 30 May 2018, p. 8.

Services and Professors Thorburn, Christodoulou, Sue, Carroll, Ryan and Filipovska.[77]

Is an additional scientific review required?

3.89 Four safety and efficacy reviews have been conducted in the UK, the last of which was published in 2016.[78] The safety and efficacy reviews were carried out by an expert panel of members who had 'no direct interests in the outcome of the review'.[79] The scientific reviews took evidence from a range of domestic and international experts including from the USA and the Netherlands. Based on those factors, questions were raised at the committee's public hearing about whether there was a need for an Australian scientific review to be conducted.

3.90 The AMDF did not consider that an Australian scientific review was necessary:

> …in terms of a suggestion of a review of the science, gauging the public reaction and public opinion on this and looking at the ethics, that is something that we are partly doing right now. I think the AMDF, certainly, would draw upon the exhaustive and lengthy experience and process that was undertaken in the UK, where three independent scientific reviews were undertaken in relation to the science of mitochondrial donation… From the foundation's point of view, from a science perspective, we can certainly rely on the science that has been undertaken around the world. I don't know that there is any Australian nuance to the science of mitochondrial disease; I don't think anything changes around the science because we're here in Australia [80]

3.91 The Murdoch Children's Research Institute and Victorian Clinical Genetic Services noted that Australia could adopt most of the outcomes from the process undertaken in the UK rather than attempting to recreate the process from scratch.[81]

Australian clinical capacity

3.92 Discussions of the safety of the science, must also take into consideration the safety of implementing the science in the Australian clinical context.

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77 Murdoch Children's Research Institute, *Submission 23*, p. 14; Australian Academy of Sciences, *Submission 35*, p. 5; Professors Thorburn, Christodoulou, Sue, Carroll, Ryan, Filipovska, *Submission 59*, p. 7.

78 HFEA, *Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2016 update*, November 2016, additional information received 30 May 2018. The reviews were carried out in 2011, 2012–13, 2014 and 2016.

79 HFEA, *Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2016 update*, November 2016, additional information received 30 May 2018, p. 55.

80 Mr Murray, *Committee Hansard*, 17 May 2018, p. 8.

81 Murdoch Children's Research Institute, *Submission 23*, p. 2.

3.93 A number of witnesses and submitters to the inquiry indicated that the Australian Assisted Reproductive Technology (ART) industry currently has relevant clinical skills necessary to deliver mitochondrial donation.[82]

3.94 The Fertility Society submitted that 'Australia has been on the leading edge of development in ART for the last 4 decades' and that in Australia 'we are fortunate in having embryologists who have the skills and expertise to undertake the necessary techniques to allow Mitochondrial transfer'.[83]

3.95 Professor Christodoulou submitted that the UK facility licenced to undertake mitochondrial donation has offered to work with Australian centres which may wish to offer these techniques, to provide any necessary instruction or guidance.[84]

3.96 In addition to the expertise of embryologists who would undertake the donation techniques, another ART function is the necessary counselling procedures to ensure that potential users of this technology understand the risks and ethics, and are therefore able to provide informed consent. The Fertility Society submitted that the Australian ART industry 'has demonstrated rigorous counselling and consenting processes for standard IVF treatment as well as the more complex issues around PGD [pre-implantation genetic diagnosis]'.[85]

Committee view

3.97 The committee understands that there are a number of possible mitochondrial donation techniques that may be used to ensure that women living with a mitochondrial disease do not pass it on to their children.

3.98 The committee acknowledges that these techniques – like any new reproductive technique – involves uncertainty and a degree of risk. However, the committee heard evidence that suggests the risks are manageable and proportionate relative to the serious risks posed to the wellbeing of a child if it inherits mitochondrial disease.

3.99 The committee considers that the scientific studies that have been conducted in the UK indicate that mitochondrial donation is a procedure that can be safely performed, and that these studies included contribution from international experts in this field.

3.100 However, it is not the role of a Senate committee to make definitive scientific findings. The committee therefore believes that formal endorsement of the UK scientific findings should be made by a panel of Australian experts with relevant

82 Monash Biomedicine Discovery Institute, *Submission 19*, [p. 3]; Monash Children's Research Institute, *Submission 23*, p. 14; Fertility Society, *Submission 27*, p. 1. See also Professor Sue, *Committee Hansard*, 17 May 2018, p. 24; Professor Carroll, *Committee Hansard*, 17 May 2018, p. 24.

83 Fertility Society, *Submission 27*, p. 1.

84 Professor Christodoulou, *Submission 12*, pp. 2–3.

85 Fertility Society, *Submission 27*, p. 2.

scientific knowledge. This panel would be appropriately constituted and overseen by the NHMRC.

3.101 Evidence provided to this committee indicates there are some areas for continued scientific consideration of emerging issues such as mtDNA carrying over during mitochondrial donation and haplogroup matching.

3.102 Whilst the committee recognises that there is still some dispute about the potential effects of mtDNA carryover, the committee considers that it is reasonable to consider the introduction of mitochondrial donation in Australia, subject to further consultation.

3.103 The committee agrees that haplogroup matching requires further scientific assessment, noting that such a step could reduce the pool of available donors.

3.104 The committee understands that the Australian ART industry performs at world-leading standards, and has the capacity to adapt existing skills to undertake these techniques for safe treatment in a clinical setting. The committee also understands that such clinics have indicated strong support for mitochondrial donation, and are ready to support the implementation should it be made lawful. It should be noted that these clinics could receive a financial benefit from any legislative change that would permit mitochondrial donation in Australia. The committee heard in evidence that local clinics could serve as a 'southern hemisphere' hub for mitochondrial donation if this technique was legalised:

> So if Australia were to follow, there would probably be two choices—a north and a south. It would probably attract people.[86]

3.105 The committee is aware that a preferred method for mitochondrial donation does not appear to have yet been identified. Because additional research is being performed in this field around the world, the committee considers that serious consideration needs to be given to the way any possible regulation is framed to permit the safest and most up-to-date scientific techniques to be used in Australia. The committee's views on regulatory issues are considered in greater detail in chapter five.

3.106 The committee acknowledges that, separate to the scientific safety of mitochondrial donation, there are significant ethical issues to consider prior to any decision on whether to allow mitochondrial donation in Australia. These will be discussed in the following chapter.

86 Dr Petra Wale, Board Member, Fertility Society, *Committee Hansard*, 17 May 2018, p. 46.