Numerical identity: the creation of tri-parental embryos to correct inherited mitochondrial disease

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Abstract

Inherited mitochondrial disorders affect between 1 in 5000 to 1 in 8000 people. These are a heterogeneous group of maternally-inherited disorders, with an array of outcomes such as heart and liver failure, defects in energy metabolism, blindness, deafness, loss of motor skills and premature death.

Recently the Human Fertilisation and Embryology Authority provided advice to the UK Government to permit the use of enucleated donated oocytes with normal (wild-type) mitochondria (a currently prohibited IVF technique) to be used as recipients of nuclear DNA from intending mothers to overcome transmission of mitochondrial disorders.

In this short communication we present the basis for this radical new IVF technology, and discuss the implications for its use both in the context of treating a group of inherited disorders and the current New Zealand IVF legislation.

The recent outcome of the national consultation by the Human Fertilisation and Embryology Authority on possible changes to the UK Human Fertilisation and Embryology Act (HFEA) to allow the use of novel technologies for mitochondrial replacement to correct inherited mitochondrial disease raises a number of issues.

The HFEA “Advice to the Government” is with the UK government to consider whether to permit the new IVF technology, which is currently banned by the HFEA (1990). In essence the advice requests that donor oocytes with normal mitochondria be used to host the nuclear DNA from women with a history of inherited mitochondrial disease.

Two techniques have been proposed. The first involves the removal of the oocyte meiotic spindle (nuclear DNA) from the donor oocyte and replacing it with the meiotic spindle from the intending mother’s oocyte (the person at risk for transmitting mitochondrial disease), thereby providing healthy mitochondria. The reconstituted oocyte is then fertilised with sperm from the intending father or donor.¹

The alternative procedure involves pro-nuclear transfer to a donor oocyte whereby the intending mother’s oocyte is fertilised using IVF and the two pro-nuclei (one male and one female) are removed and transferred into the enucleated donor zygote at the same stage of development.²

In both situations the embryos then proceed to develop unaffected by mitochondrial disease. A third procedure cytoplasmic transfer, is not considered as it uses an admixture of intending mother’s oocyte cytoplasm and donor oocyte cytoplasm, which retains a significant proportion of mutated mitochondria, and is banned in the UK.
The proposed technologies will give the resulting child DNA from both intending parents plus DNA from the donor oocyte mitochondria. In essence, creating a three-parent baby.

Currently, neither of the two proposed techniques have been approved for use in treating inherited mitochondrial disorders in humans therefore the outcomes of the new techniques are not known. However, previous research using primates indicates that the transfer of the meiotic spindle to a host enucleated oocytes produced normal offspring.

Although this indicates the relative success of the technique the primates did not have any mitochondrial disorders and therefore the influence of mutated mitochondrial DNA could not be established. However, in a subsequent publication using human oocytes from normal donors and recipients these authors demonstrated comparable normal fertilisation rates between meiotic spindle transfer oocytes and controls but 52% of the meiotic spindle transferred oocytes had abnormal pro-nuclei development. Of the remaining 48%, 62% developed to the blastocyst stage.

Later work using abnormally fertilised pro-nuclear stage human embryos whereby the abnormal pro-nuclei were replaced by normal pro-nuclei demonstrated that 22% of the manipulated embryos developed to the 8-cell stage and 8% of those with two pro-nuclei developed to the blastocyst stage and from these data it was reported that less than 2% of the ‘foreign’ mitochondria had been transferred. However, once again the oocytes used did not contain any abnormal mitochondrial DNA.

Mitochondria are cell organelles responsible for approximately 95% of the energy requirements of the cell via the biosynthesis of adenosine triphosphate (ATP), by oxidative phosphorylation. Although the majority of DNA is in the chromosomes located in the nucleus, up to 1% of DNA is located in the mitochondria.

Typically mammalian cells contain $10^3$ to $10^4$ copies of mitochondrial DNA (mtDNA), which encodes for 2rRNAs, 22 tRNAs and 13 polypeptides; the latter are all involved in the respiratory chain. All other mitochondrial proteins, including those involved with replication, transportation and translation of mtDNA are encoded by nuclear genes targeted to the mitochondria by specific transport systems thereby creating a functional interdependence between nuclear and mitochondrial genomes.

Unlike nuclear DNA, mtDNA contains no introns and no protective histones and is error prone during replication. The accumulation of mtDNA mutations is approximately 10-fold greater than those identified in nuclear DNA and are widely associated with a number of cancers.

Although 39 of the 46 sub-units of complex 1 are encoded by nuclear genes, the entry of the proteins in to mitochondria is still not fully understood for about 20 of the sub-units. Currently over 100 pathogenic point mutations and 200 deletions, insertions and rearrangements have been described with tRNA mutations accounting for approximately 60% and polypeptide subunits affected by approximately 35% mutations.

It is estimated that inherited mitochondrial disorders are the most common inherited metabolic disorders affecting between 1 in 5000 and 1 in 8000 of the general population, with some presenting as well defined clinical syndromes but others with
unique phenotypes with variable onset and often multiple organ involvement particularly in those organs with high oxidative phosphorylation requirements.\textsuperscript{8,10}

Affected individuals are often heteroplasmic (a mixture of wild-type and mutant mtDNA), which creates a ‘threshold’ level for disease manifestation.\textsuperscript{8,11} Currently there are limited options for the diagnosis and treatment of this group of inherited diseases and reports of pharmacological agents and vitamin supplements have not made significant inroads in effective therapies.\textsuperscript{10}

Additional issues relate to the genetic counselling of this group of inherited diseases. Depending on the presentation and family history genetic counselling may need to resort to empiric risk especially when the mutation is unknown.

Where the mutation is unknown it has been proposed that the recurrence risk for children from an affected female would be in the order of 10 to 20\% and for an affected male the risk might be approximately 2\%.\textsuperscript{8}

For some of these disorders pre-implantation genetic diagnosis is possible using two of the pre-implantation embryo cells, however many of the different types of mtDNA mutations are inherited in a complex and poorly understood way causing issues relating to mutation load and difficulty in predicting the prognosis.

Recent research indicates that if the mutation load is 18\% or less then there would be a 95\% chance that the individual would not be affected.\textsuperscript{12} However, a heteroplasmic woman with this mutation load may produce embryos with mutation loads at 20 to 25\%, which would create a dilemma in decision-making and may have to rely on reproductive and family history.\textsuperscript{12}

As all human mtDNA is inherited from the mother via the oocyte (sperm mtDNA is not detectable following fertilisation and early pre-implantation development), the development of mitochondrial replacement therapies at the oocyte/zygote stages appears to offer the greatest hope for those families affected by these groups of inherited diseases.\textsuperscript{13} What then would be the issues?

First there is the issue of a three-parent child, both the HFEA and the Nuffield Bioethics Centre (UK) have indicated that the oocyte donor should remain anonymous—a decision contrary to the spirit of New Zealand’s HART legislation.

Second, although the intending mother’s nuclear DNA is transferred to a normal oocyte, mitochondria from her ‘affected’ oocyte may also be transferred with the cytoplasm surrounding either the spindle or the pro-nuclei.

What role will the intending mother’s mtDNA have in the host oocyte? Will the stress of the IVF procedures create an environment for mitochondrial fusion thereby creating hybrid mitochondria?\textsuperscript{14–16} possibly changing metabolic function and signalling cascades?

Little is understood about retrograde mitochondrial signalling to the nucleus, how important will this be in regulating mitophagy mediation in the oocyte/embryo\textsuperscript{17,18}? As little is known about how mitochondria segregate during cell division and tissue development, will heteroplasmic distribution of normal and mutant mitochondria still occur and present with differing disease patterns?
The ‘new’ (donor) mitochondria would be incorporated into all cells and therefore would result in the modification of the germ-line as well as somatic cells raising an issue regarding any impact of the therapy on future generations.\textsuperscript{19}

In New Zealand (as in the UK and Australia) legislation prohibits the modification of nuclear and mtDNA in oocytes, sperm and embryos as well as prohibiting somatic cell nuclear transfer (cloning). In addition, while pre-implantation human embryo research may be licensed to approved clinics in Australia under the “Research Involving Human Embryos Act (2002), current New Zealand policy is to allow research only on non-viable embryos.

Therefore, any developments and use in human reproduction would require a change in the Human Assisted Reproductive Technology Act (HART) (2004)\textsuperscript{19} in New Zealand, the HFEA (2008) in the UK and Australian legislation, which is where the HFEA is at present. In Australia, recent consideration relating to a review of both the Research Involving Human Embryos Act, (2002) and the Prohibition of Human Cloning for Reproduction Act, (2002) upheld section 13 of the latter Act in recommendation 7 in that “There should be no change to the current legislation in relation to the use of DNA from more than two persons”.\textsuperscript{20}

Notwithstanding the legislation, issues relating to consent should be considered, as the future child has not been involved in the treatment decision to modify its genome. Will there be issues connected to the three parent IVF and the perceived identity of the future child/children? In addition to the anonymity of the oocyte donor the HFEA and the Nuffield Bioethics Centre have proposed that the oocyte donor should have a similar status to tissue donors. How would this develop in a New Zealand context?

First, the HART Act requires donors to be identified and a register is maintained to facilitate donor identification for children born from gamete and embryo donation, enucleated oocyte donation would have to be considered in this context.

Second, the New Zealand Human Tissue Act (2008)\textsuperscript{21} does not cover reproductive tissue donations. Would this need to be amended as the UK proposal has recommended that oocyte donation be considered as a tissue donation?

Finally if the technique were used off-shore would donor oocytes from the host country be used rather than those from New Zealand adding an additional layer of complexity relating to donor identification, payment (commercial transactions for gametes and embryos is illegal in New Zealand) and future child identity.

**Competing interests:** Both authors declare that they have no competing interest related to this manuscript.

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