House of Commons

Science and Technology Committee

Mitochondrial donation

Correspondence received relating to the evidence hearing on 22 October 2014

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1 Introduction

Evidence hearing

1. On 22 October 2014, the Science and Technology Committee held a one-off evidence hearing on mitochondrial donation. The evidence hearing had been arranged following the Government announcement that it intended to place Regulations before Parliament to allow for the use of novel techniques to prevent serious mitochondrial disease. The evidence session aimed to examine the science and proposed regulation of mitochondrial donation.

2. The session intended to establish where the science stands at present and what further work may be needed before mitochondrial donation could be used in clinical practice. It also aimed to clarify how this practice may be regulated in the future.[1]

3. Written evidence submitted by the witnesses who gave evidence to the Committee has been published on the Committee's web pages.[2]

Other correspondence

4. The Committee received further submissions which it agreed to publish as correspondence online. These submissions are published below.

[1] http://www.parliament.uk/business/committees/committees-a-z/commons-select/science-and-technology-committee/news/141016-mit-ev/

[2] http://www.parliament.uk/business/committees/committees-a-z/commons-select/science-and-technology-committee/inquiries/parliament-2010/mitochondrial-donation/

Witnesses

The following witnesses gave evidence. Transcripts can be viewed on the Committee's inquiry page at www.parliament.uk/science.

Wednesday 22 October 2014

Question number

Professor Doug Turnbull, Director, Wellcome Trust Centre for Mitochondrial Research; **Professor Peter Braude**, King's College London; **Professor Robin Lovell Badge**, MRC National Institute for Medical Research; and **Dr Edward Morrow,** Senior Research Fellow, University of Sussex — Q1-25

Peter Thompson, Chief Executive, Human Fertilisation and Embryology Authority; **Robert Meadowcroft,** Chief Executive, Muscular Dystrophy Campaign; and **Professor Jonathan Montgomery,** Chair, Nuffield Council on Bioethics, and Professor of Health Care Law, University College London — Q26-42

Jane Ellison MP, Parliamentary Under-Secretary of State for Public Health, Department of Health; and **Professor Dame Sally Davies,** Chief Medical Officer, Department of Health — Q43-66

Published written evidence

The following written evidence was received from witnesses and can be viewed on the Committee's inquiry web page at http://www.parliament.uk/business/committees/committees-a-z/commons-select/science-and-technology-committee/inquiries/parliament-2010/mitochondrial-donation/.

MIT numbers are generated by the evidence processing system and so may not be complete.

1 Human Fertilisation and Embryology Authority (MIT 0004)
2 Medical Research Council (MIT 0005)
3 Muscular Dystrophy Campaign (MIT 0006)
4 Wellcome Trust (MIT 0008)
5 Professor Jonathan Montgomery (MIT 0028)

List of correspondence

6 Progress Educational Trust (MIT 0001)
7 Alison Murdoch (MIT 0003)
8 The Academy of Medical Sciences (MIT 0007)
9 The Free Church of Scotland (MIT 0009)
10 The Lily Foundation (MIT 0012)
11 James Lawford Davies (MIT 0013)
12 Christian Medical Fellowship (MIT 0014)
13 Professor Adam Eyre-Walker (MIT 0016)
14 Center for Genetics and Society (MIT 0017)
15 Professor Justin C St John (MIT 0018)
16 Human Genetics Alert (MIT 0019)
17 Council for Responsible Genetics (MIT 0020)
18 Comment on Reproductive Ethics (MIT 0021)
19 Mothers for a Human Future (MIT 0022)
20 David A Prentice, Ph.D. (MIT 0023)
21 The Christian Institute (MIT 0024)
22 Caroline Simons (MIT 0025)
23 Emily Huezo (MIT 0026)
24 Julian Malcolm (MIT 0027)
25 Professor Stuart A. Newman (MIT 0029)

Correspondence

Correspondence submitted by the Progress Educational Trust (MIT0001)

EXECUTIVE SUMMARY

- It would be unethical *not* to permit the use of mitochondrial donation to prevent the transmission of inherited mitochondrial DNA (mtDNA) disease.
- There is no reason to think that techniques involving mitochondrial donation raise any particular ethical problems in respect of translational treatments.
- There is no meaningful association between mtDNA and a person's identity, and this is the case even if we take the epigenetics of mitochondria into account.
- There is no good ethical, scientific or policy reason to encourage people to regard mitochondrial donors as kin to mitochondrial recipients.
- Mitochondrial donation can be characterised accurately as a form of human germline genetic modification, but this does not make it ethically problematic.
- There should be a centrally funded, long-term register of any mitochondrial donation procedures performed in the UK, and an (immortalised) cell line should be created from the mitochondrial donor at the time of donation.

ABOUT THE PROGRESS EDUCATIONAL TRUST

1. The Progress Educational Trust (PET) – www.progress.org.uk – is an independent registered charity (number 1139856) founded in 1992.
2. PET's fundamental objective is to create an environment in which ethically sound research and practice in genetics, assisted conception, embryo/stem cell research and related areas will thrive. The ultimate beneficiaries of all PET's work are families and individuals threatened by infertility and genetic disease, including people wanting an opportunity to give birth to healthy children.
3. PET's Patron is Baroness Mary Warnock, who chaired the UK Government's Committee of Inquiry into Human Fertilisation and Embryology whose 1984 recommendations continue to shape policy to this day.

INTRODUCTION

4. In this document, we refer collectively to pronucelar transfer (PNT) and maternal spindle transfer (MST) as 'mitochondrial donation'.
5. The most urgent ethical issue arising from new techniques to prevent the transmission of inherited mitochondrial DNA (mtDNA) disease is the harm that will result from *not* permitting the use of these techniques, if they can be demonstrated to work. A 2008 study of the working-age population of the North East of England found that 9.2 in 100,000 people have clinically manifest mtDNA disease, making it one of the commonest inherited neuromuscular disorders

and a common cause of chronic morbidity ('Prevalence of mtDNA disease in adults', Schaefer *et al*, 2008, *Annals of Neurology*).

6. mtDNA disease can affect the bone marrow, brain, ears, endocrine and exocrine pancreas, eyes, gut, heart, kidney, liver, muscles and peripheral nerves. Generic symptoms include deafness, diabetes, fatigue, neuropathy, movement disorders, myalgia, seizures, stroke-like episodes and visual impairment ('The neurology of mitochondrial DNA disease', McFarland *et al*, 2002, *Lancet Neurology*).

7. The lay public and experts alike have been consulted exhaustively on the ethics, efficacy and safety of mitochondrial donation in recent years – by the Nuffield Council on Bioethics, by the Human Fertilisation and Embryology Authority (HFEA), and by the Department of Health. The response to these comprehensive pieces of work has been prevailingly supportive of the Government passing regulations to permit use of mitochondrial donation.

ETHICS

8. If we do not permit the use of new techniques to avoid the transmission of mtDNA disease, then we will be forced to continue relying on old techniques.

9. These old techniques are severely flawed, consisting as they do of preimplantation genetic diagnosis (PGD, only reliable and suitable in a minority of instances), prenatal diagnosis (also unreliable and involves contemplating a termination of pregnancy), requiring the patient(s) to use an egg donor (when the latest HFEA figures confirm that there remains a national shortage of donors), or requiring that the patient(s) abandon all hope of having children to whom they are biologically related. If it is within our means to offer aspiring parents better options than these, then it would be unethical *not* to do so.

10. It is ethically legitimate for prospective parents to aim to avoid the transmission of mtDNA disease, given the severity of the health and social consequences such disease might have for their (genetically related) future children. Some people at risk of transmitting mtDNA disease will prefer to try to conceive a child who is both genetically related to them and healthy, and this preference is entirely legitimate and deserving of our support.

11. Prospective parents who are currently at risk of transmitting mtDNA disease may feel unable to try for children, and/or may terminate any unplanned pregnancy because of concern about transmitting such disease. Other prospective parents will seek to conceive naturally, but may suffer great anxiety about whether and how their pregnancy and their future child will be affected – not least because of the many uncertainties inherent in antenatal testing for mtDNA disease, and the attendant issues involved in antenatal testing *per se*. These are compelling reasons to permit mitochondrial donation.

12. All medical treatment is experimental, in the broad informal sense that it stands to be improved or supplanted pending the emergence of further evidence, but treatment is only 'experimental' in a formal sense if patients receiving it are knowingly participating in an experiment. The transition from laboratory to clinical use is not instantaneous, and the category of 'translation' is useful in describing this transition and ensuring that patients are duly appraised of what it entails. There is no reason to think that mitochondrial donation raises any particular ethical problems in respect of translational treatments.

IDENTITY

13. In our view, there is no meaningful association between mtDNA and a person's identity, except in the narrowest technical sense of the word 'identity'. To wit, mtDNA sequence variation can be of practical utility in tracing the matrilineal 'ancestry' of cytoplasm, and from this an element of

a person's familial ancestry might be inferred. But this fact, while interesting and useful to those with an interest in genealogy, does not confer any profound significance upon mtDNA.

14. What is generally meant by a person's 'identity' is their essential and distinguishing characteristics, their ipseity. Properly functioning mtDNA is irrelevant to a person's ipseity. The only circumstance in which the genetic makeup of a person's mitochondria can have a distinct bearing on their life, is if pathogenic mtDNA mutations compromise the generation of intracellular energy, resulting in mtDNA disease.

15. This situation does not constitute an identity with cultural value deserving preservation (as some would argue in relation to the culture and customs that have developed around, say, deafness or dwarfism) but rather is precisely the debilitating situation that mitochondrial donation seeks to avert. Indeed, in most contexts it is nowadays thought inappropriate to define people in terms of a disorder they happen to have, and doing so is often discouraged.

16. This argument still stands, even if the epigenetics of mitochondria – epigenetics being the science of enduring changes in the pattern of gene activity that do *not* involve alteration of the DNA sequence – is taken into account.

17. Recent evidence suggests that mtDNA can be methylated, meaning that the expression of mtDNA can be epigentically modified. But this is not thought to have any impact upon the expression of nuclear DNA, except inasmuch as late onset complications in mtDNA disease can be mediated by an epigenetic response ('Epigenetics, epidemiology and mitochondrial DNA diseases', Chinnery et al, 2012, *International Journal of Epidemiology*). mtDNA can therefore be said to be both genetically *and* epigenetically tangential to a person's ipseity.

18. One reason why it is sometimes assumed that mtDNA is bound up with a person's ipseity, is because of a more general misapprehension that DNA *per se* is inseparable from identity. This notion has been promulgated widely in recent years, with the forensic use of DNA variation to identify individuals among populations and with public dissemination of the results of the Human Genome Project. But despite the popularity of this notion, it is far from being a generally applicable truth, and mtDNA is one of the starkest examples of an instance where it does not apply.

19. The provenance of mtDNA is irrelevant, beyond the importance of the mitochondria functioning properly. Mitochondrial donation creates neither observable nor other shared characteristics between the donor and the person conceived following donation, except for the fact that – hopefully – neither the donor nor the resulting child will have mitochondrial disease.

PARENTHOOD AND KINSHIP

20. Contrary to what has been suggested in much of the media coverage of mitochondrial donation, the word 'parent' is in no way a helpful or accurate description of someone's relationship to another person to whom they have donated solely mtDNA.

21. It is worth recalling that 'parent' is a generic and informal rather than a scientific term – if it were used in a scientific study, it would require elaboration to specify what it meant. Inasmuch as the generic term 'parent' relates to our biology, it is of no use in describing a person from whom we inherit solely mtDNA.

22. As ever, a child's genetic connection to those adults from whom it has inherited nuclear DNA is coterminous with the connection between a child and a parent. And as ever, a child's genetic connection with the adult from whom it has inherited mtDNA is tangential to the connection between a child and a parent.

23. Nor does mitochondrial donation, for most people at the present time, create any psychosocial expectations around a connection of kinship or any connection analogous to kinship. If there is a risk in this area, then the risk is that policymakers and regulators will inadvertently bring about expectations of kinship in a misguided attempt to anticipate such expectations.

24. There is no good ethical, scientific or policy reason to encourage people to regard mitochondrial donors as kin to mitochondrial recipients. Mitochondrial donors should not be made to feel that they are under any obligation to have contact with any children born as a result of their donation.

25. Since no physical resemblance or other personal or observable characteristics (with the sole exception of effectively functioning mitochondria) will be inherited from mitochondrial donors, a dispassionate view of the resulting genetic link is the most appropriate and reasonable view.

GERMLINE GENETIC MODIFICATION

26. A mitochondrial donor's mtDNA is not only replicated throughout the tissues of the recipient's body, but will (if the recipient is female) be inherited by any children the recipient conceives.

27. Mitochondrial donation can therefore be characterised accurately as a form of human germline genetic modification, albeit a particular form of germline genetic modification in which DNA molecules are left completely intact. This precludes any risk that might be posed by intervening in the gene sequence within the molecule.

28. Because the provenance of properly functioning mtDNA is irrelevant to an individual's identity, altering mtDNA's provenance in a way that endures across generations is not ethically problematic. Neither a person conceived using mitochondrial donation nor anyone who knew them would notice any difference, if one mtDNA donor was chosen rather than any one of a thousand others.

29. There are in fact positive ethical reasons for seeking to change the mitochondrial germline. A change from mutated, potentially disease-causing maternal mitochondria to predominantly non-mutated, non-disease-causing mitochondria is of benefit to the future child, and has positive implications for the child's family and for subsequent generations.

SAFETY AND LONG-TERM FOLLOW UP

30. We recommend that there should be a centrally funded, long-term register of any mitochondrial donation procedures performed in the UK, and this register should be made accessible to researchers.

31. The provenance of mtDNA is medically useful knowledge in relation to the long-term follow-up of those receiving donated mtDNA, and may also have some as yet unanticipated medical utility. (By contrast, the provenance of the sperm used to create the enucleated zygote into which the karyoplast is transferred in PNT is unlikely to be of any medical utility, but this information is also worth recording in the interests of comprehensiveness.)

32. We also recommend that an (immortalised) cell line be created from the mitochondrial donor at the time of donation, so that the relationship between the cell nucleus and the donated mitochondria can be studied in the original cellular environment. Such a resource would be as important as long-term follow-up of the health of the recipient and their progeny.

33. After the removal of nuclear DNA from the donor egg or embryo during mitochondrial donation, it is highly unlikely that any of the donor's nuclear DNA would remain and be transferred. Genetic screening tests are available to confirm that all nuclear DNA has been

removed from the donor egg or embryo, and whether and in what circumstances such tests are warranted should be a decision for the prospective regulator of mitochondrial donation (the HFEA).

October 2014

Written evidence submitted by Professor Alison Murdoch MD FRCOG (MIT0003)

Professor Murdoch is the Person Responsible for the HFEA Research licence under which the scientific studies are currently being carried out in Newcastle. She is a consultant gynaecologist who established the clinical fertility service in the North East of England 25 years ago. In addition to this clinical experience, she has published widely on the subject and was a past Chair of the British Fertility Society. She is a past member of the Nuffield Council on Bioethics.

If the Regulations are passed and the ongoing scientific work provides the information that will be required by the HFEA, this clinic will apply to the HFEA for a Treatment Licence for mitochondrial replacement. The purpose of the evidence presented here is to provide facts about the clinical procedure of IVF and egg donation so that the risks and expectations of the outcome of mitochondrial transfer procedures can be placed in context.

FACTS ABOUT EGG DONATION AND IVF.

Egg donation

Mitochondrial donation requires the donation of eggs. Egg donation has been a standard procedure within fertility practice in the UK and worldwide for nearly 30 years. In the UK in 2012, 2733 (4.5% of all IVF) egg donation procedures for fertility treatment were carried out. [3]

In relation to the risk to an egg donor, there is therefore good evidence available. Potential donors are informed of the risk before giving consent to proceed. This is considered to be an acceptable clinical practice.

Availability of egg donors.

In Newcastle we have an established practice that recruits egg donors for both fertility treatment and for research. We are confident that, as long as the anonymity of donors is assured, we will have an adequate recruitment rate to implement a mitochondrial replacement treatment program.

Risks of egg donation:

Superovulation.

Donors are required give themselves daily injections to stimulate several eggs to grow. There are no significant side effects that are directly due to these drugs. Any symptoms that develop relate to the number of eggs that grow.

Ovarian Hyperstimulation Syndrome (OHSS).

Significant OHSS occurs in about 1 in 50 women who are superovulated for fertility treatment. Those who are at risk of OHSS can be predicted i.e. woman with polycystic ovaries who are

[3] http://www.hfea.gov.uk/104.html

likely to grow more than 20 eggs. [4] They would not be accepted as donors. Therefore the OHSS risk for egg donors is extremely small.

Egg collection.

Eggs are collected by a minor procedure that carries a very small risk of bleeding and infection (<1:1000 for fertility patients). Since a woman who had a higher risk (e.g. a woman with an underlying medical problem such as endometriosis) would not be recruited as a donor, the risk in the donor population would be less.

Future fertility.

Donors have been followed up after donation and the subsequent risk of infertility in donors appears to be the same as that of the population (about 1 in 10 couples are infertile).

In conclusion, the process of egg donation is known to carry a small risk. Accepting this, egg donation is an established clinical and research practice. The donation of eggs for mitochondrial transfer will be similar.

Reproductive 'success'.

From a patient perspective, the only reproductive success is a healthy baby. Human fertility is less kind. Therefore in the context of assessing the outcome of the proposed techniques to prevent transmission of mitochondrial disease, the following information is required.

- The fecundity (chance of pregnancy with one episode of unprotected intercourse) is highly variable but on average is no more than the chance of conceiving with one IVF treatment: about 1 in 4[5].
- The chance of miscarriage after the pregnancy test is positive is 1 in 7.
- The chance of a baby having a congenital abnormality after normal conception is about 2-3 in100[6]. This risk is very slightly increased if conception follows IVF[7].

In conclusion, human reproduction is inefficient and results in many abnormalities. Against this background, the proposed new techniques will never be 100% successful in establishing a pregnancy nor risk-free for the baby. In clinical practice such risks are balanced against the benefits.

October 2014

Correspondence submitted by The Academy of Medical Sciences (MIT0007)

In April 2011, the Academy and partner organisations wrote to the Secretary of State for Health calling for the introduction of regulations to enable new techniques that aim to prevent the hereditary transmission of mitochondrial disease caused by mutations in mitochondrial DNA to be used in clinical practice, if sufficient pre-clinical evidence for the safety and efficacy of these techniques is obtained.[8] We subsequently welcomed scientific progress made in this field in our

[4] K Jayaprakasan, M Herbert, E Moody, J A Stewart, A P Murdoch 2007 Estimating the risks of Ovarian Hyperstimulation Syndrome (OHSS) during egg donation for research. Human Fertility (10(3)183-187

[5] http://www.hfea.gov.uk/docs/FertilityTreatment2012TrendsFigures.PDF

[6] http://www.nepho.org.uk/rmso/surveys/congenital

[7] http://www.ncbi.nlm.nih.gov/pubmed/20063307

[8] The Academy of Medical Sciences (2011). *Treatments to avoid transmission of mitochondrial disease.* http://www.acmedsci.ac.uk/download.php?f=file&i=13427

response to the Human Fertilisation & Embryology Authority's (HFEA) public consultation on mitochondrial donation in December 2012.[9] **The Academy is in full support of Parliament introducing regulations as soon as possible to enable mitochondrial donation to be used to prevent the transmission of serious mitochondrial disease.** We welcome the House of Commons Science and Technology evidence session to consider the science of mitochondrial donation on 22 October 2014 and hope these comments are helpful.

In our response to the Department of Health's consultation on its draft regulations to permit mitochondrial donation in May 2014 we highlighted the requirement for long-term follow-up research on offspring born from mitochondrial donation to monitor the safety and efficacy of the techniques.[10] We made the recommendation that, if the regulations are enacted, the HFEA should produce standards for licensed clinics to conduct evaluations of treatments and that clinics should report the follow-up data to the HFEA. We also recognised the importance of safeguarding patients from the promotion of mitochondrial donation where the approach may not be necessary and were glad to see that the Department of Health's draft regulations to permit mitochondrial donation ensure the HFEA would regulate the administration of treatments to patients on a case-by-case basis.

The Academy was pleased to see that the HFEA has produced a third scientific review into the safety and efficacy of mitochondrial replacement techniques.[11] It is encouraging that the review found that the new evidence assessed does not suggest that these techniques are unsafe. We welcome the fact that the review clarified that the use of non-human primates is not a critical prerequisite for the induction of the techniques in humans, as we previously urged consideration of this requirement.[12]

The Academy would like to highlight that the HFEA's third scientific review into the safety and efficacy of mitochondrial replacement techniques restated that '*whilst further experiments need to be carried out prior to these techniques entering the clinic, complete reassurance will never come from experiments conducted in animal models and with human material in vitro. Therefore, it should be accepted that there will always be some risk and unknowns associated with the use of MST [maternal spindle transfer] or PNT [pronuclear transfer] in humans until it is tried in practice*'.

The Academy of Medical Sciences promotes advances in medical science and campaigns to ensure that these are translated into healthcare benefits for society. Our elected Fellowship includes the UK's foremost experts drawn from a broad and diverse range of research areas. A number of our Fellows have expertise in areas related to the study of mitochondria, mitochondrial disease and the development of potential treatments for these diseases. The Academy has been fully supportive of previous developments to bring potentially life-saving

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[9] The Academy of Medical Sciences (2012). *The Academy of Medical Sciences' response to the Human Fertilisation & Embryology Authority's public consultation on mitochondria replacement.* http://www.acmedsci.ac.uk/download.php?f=file&i=13428

[10] The Academy of Medical Sciences (2014). The Academy of Medical Sciences' response to the Department of Health's draft regulations to permit mitochondrial donation. http://www.acmedsci.ac.uk/download.php?f=file&i=29610

[11] The Human Fertilisation and Embryology Authority (2014). *Third scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2014 update.* http://www.hfea.gov.uk/docs/Third_Mitochondrial_replacement_scientific_review.pdf

[12] The Academy of Medical Sciences (2012). *The Academy of Medical Sciences' response to the Human Fertilisation & Embryology Authority's public consultation on mitochondria replacement.* http://www.acmedsci.ac.uk/download.php?f=file&i=13428

treatments for serious mitochondrial diseases into the clinic, including our statement of support for the opening of a new Centre for Mitochondrial Research at Newcastle, and our previously cited response to the HFEA's public consultation on novel mitochondrial donation techniques. The Academy warmly welcomes the work done by the HFEA to clarify the current safety and efficacy of mitochondrial donation and by the Department of Health in drafting regulations for permitting mitochondrial donation techniques to be introduced in clinical trials.

October 2014

Correspondence submitted by The Free Church of Scotland (MIT0009)

1. The Free Church of Scotland is a Presbyterian denomination with about 12,500 members and 100 congregations. We are responding to this call for written evidence because we disagreed with the HFEA recommendation that the Government should formulate regulations to enable mitochondria donation techniques to be used for treating patients. We believe that the techniques proposed are not in accordance with internationally agreed bans on creating human embryos for experimentation and destruction as well as altering the human germ line.

2. Summary of our main ethical objections

- Problems with nomenclature hiding the real nature of the procedures
  - Mitochondria donation is a misnomer.
  - Reclassifying these procedures as not constituting 'genetic manipulation' is dishonest.
  - Rather than being a form of 'treatment', this is a form of eugenics.

- Problems with the techniques themselves
  - The well-known problems attending harvesting large numbers of human eggs
  - We oppose the destruction of embryos required in pronuclear transfer.
  - The intrusion of a third or even a fourth party into the process of reproduction poses problems for the relationship of the chromosomal parents and the identity of any children produced.
  - Because the techniques are similar, to some extent, to that used in cloning, their use might stimulate interest in human cloning, which would be dangerous and unethical.

- Problem of altering the germ line
  Despite holding only a small part of the genetic material in a cell, the mitochondria contribute significantly to the genome.

- These techniques, ethically dubious and uncertain in their efficacy and effects, are unnecessary. Other lines of research should be followed.

- Introducing such legislation, far from putting Britain at the forefront of bioscience, will further isolate us in the world community.

3. Problems with the nomenclature

3.1 We believe the use of the term 'mitochondria donation' obscures the real nature of the procedures in a disingenuous and clumsy attempt to impress lay-people with the altruistic sound of 'donation'. What is involved is donation not of mitochondria and insertion of them into an egg or embryo to replace faulty mitochondria, but donation of eggs and sometimes of sperm and, in the case of pronuclear transfer, accompanied by destruction of embryos.

3.2 The HFEA and the Government have decided that these procedures do not come under the head of 'genetic manipulation'. This is playing with words. Just because the number of genes in the mitochondria is very small compared to the chromosomal genes, this does not make them any less important. Indeed the whole point of the procedure is that they are important enough to cause disease!

3.3 This cannot be represented as a form of treatment for genetically transmitted disease in order to alleviate human suffering. No individual will be 'treated' by these techniques. Instead it is a form of eugenics, attempting to improve the human gene pool.

4. Problems with the techniques used
While the motive of preventing suffering caused by diseases due to mitochondrial genetic abnormalities is laudable, the methods to be used give rise to grave ethical concerns.

4.1 Research into these procedures will require the donation of many human eggs, with all its attendant problems, such as risk to the health of the donor and risk of exploitation of vulnerable women as donors.

4.2 In Pronuclear Transfer (PNT) two embryos are created and destroyed to create a third embryo. Because we believe that the human embryo, even one with genetic abnormalities, is an early human being, we believe it should be treated with the utmost respect and not experimented upon or destroyed.

4.3 The intrusion of a third or even fourth party into the process of reproduction gives rise to various concerns, such as the relationship of the chromosomal parents and the identity of any children born and their relationship to the egg and mitochondrial donor.

4.4 Because the techniques are similar, to some extent, to that used in cloning (somatic cell nuclear transfer), their use might stimulate interest in human cloning. This technique has been shown to have grave dangers and is rightly banned as being unethical.

5. Alteration of the germ line

Although the mitochondria comprise a small part of the germ line, they do contribute significantly towards it and manipulating them will change the germ line. So far this has been illegal because of the uncertainty of the effects that might be produced in future generations and so would be a new departure in bioethics. Although the motive – to produce a child free from disease caused by faulty mitochondria – is at first sight laudable, it is nevertheless a form of eugenics and may therefore open the way for eugenic abuses in other fields. Also the technique itself may give rise to unintended harmful consequences in future generations.

6. These techniques are unnecessary

These techniques are unethical, are uncertain as to their effectiveness and outcomes and are unnecessary. The HFEA report itself estimates that only 10 cases per year would qualify in Britain. Because of the ethical difficulties and the uncertainty of effectiveness and long term effects, it would be far better to pursue research into therapeutic alteration of the faulty mitochondria.

7. Far from placing Britain at the forefront of the field, this new departure would isolate Britain as one of the few countries to legalise not only the production of embryos for research purposes and then their destruction but also to allow alteration of the germ line.

We respectfully urge the Committee to recommend that the Government do not bring forward regulations to allow these procedures to proceed and instead recommend research into other

methods of treating diseases caused by mitochondrial abnormalities, for instance by mitochondrial repair.

October 2014

Correspondence submitted by The Lily Foundation (MIT0012)

The Lily Foundation charity was set up in memory of baby Lily who died of a Mitochondrial Disorder aged 8 months old. The charity is committed to funding research to help develop effective treatments and we hope ultimately a cure for Mitochondrial Disease.

We also support affected families, many of which suffer from maternally inherited Mitochondrial Disease for which Mitochondrial Donation could offer them hope of having their own child, free from this devastating condition.

The Lily foundation is a small charity and having lost children ourselves to this disease, we both know first-hand how debilitating, painful and heart breaking it can be to watch your child suffer and ultimately lose their life to this condition.

Many families we support have lost children to Mitochondrial Disease and many continue to watch their child suffer a condition that can often not be managed effectively with available treatments. The pressure on the whole family can often be unbearable and many families just cannot face the risk of trying for another child in case they are also affected. This is where Mitochondrial Donation could change their lives.

Offering these families the chance to have their own healthy child and giving siblings (who may also carry the disease) the opportunity to have their own healthy children in the future, would be an incredible thing.

For many of our families, time is running out and further unnecessary delay only adds to their heartache.

Of course it is necessary to thoroughly consider the safely of this technique, however if there is no evidence to suggest this technique is unsafe, then please do not deny our families the opportunity to try this ground breaking technique.

Every novel medical procedure enters unchartered territory and may have unknown risks - nobody knows this more so than our families who continually seek out new treatments to try and relive their child's suffering and improve their quality of life whilst they are with us. We know this disease, and in our experience there is very little speculated risk that could be worse than mitochondrial disease itself.

Please do not deny our families the hope that they may be able to have their own healthy child unless there is clear evidence that the technique is unsafe.

October 2014

Correspondence submitted by James Lawford Davies (MIT0013)

1. I am a solicitor and partner in the firm of Lawford Davies Denoon. I am also an Honorary Lecturer in the Department of Biochemical Engineering at UCL, and was previously a

Lecturer in Law and Medicine at the Newcastle University, and a Visiting Research Fellow at Durham University Law School.

2. I specialise in the law relating to reproductive and genetic technologies, human tissue and cells, and related research. In 2004-5 I acted for Newcastle Fertility Centre in relation to their application for a research licence from the Human Fertilisation and Embryology Authority ("HFEA") to carry out pronuclear transfer to prevent the transmission of mitochondrial disease.

3. I note that the Committee intends to consider the science of mitochondrial donation, and that the Chair of the Committee has stated that "…it is right that the safety and efficacy of mitochondrial replacement techniques are subject to scrutiny in the House" and that this evidence session will "scrutinise further the scientific evidence on mitochondrial donation".

4. I would like to make brief submissions in relation to two matters which I believe provide important background and context.

SCRUTINY

5. There have been important recent reviews of mitochondrial replacement techniques, including the review by the Nuffield Council on Bioethics, the scientific reviews by the HFEA, and their recent public consultation. However, careful scrutiny of mitochondrial donation dates back many years.

6. For example, the Chief Medical Officer's Expert Group Report, *'Stem Cell Research: Medical Progress with Responsibility'* (2000, the 'Donaldson Report') considered and recognised the future potential use of the technique in treatment. It was then considered again by the Science and Technology Committee in their extensive 2005 Report *'Human Reproductive Technologies and the Law'*, and the Committee also supported further research in this area.

7. Perhaps the most notable and detailed review of the research came later in 2005 when an Appeal Committee of the HFEA considered an appeal by Newcastle University against a decision by the HFEA Licence Committee to refuse a licence for research involving pronuclear transfer on the basis that the proposed research was not permitted by the legislation. Detailed submissions were prepared by both parties to the appeal, and after a full day hearing, the Appeal Committee was satisfied both that the research could be lawfully licensed, and also that it was both necessary and desirable.

8. In support of the University's case, we obtained letters from leading scientists and researchers of the day who all expressed their endorsement for the proposed research. These included supportive submissions from, amongst others, the late Dame Anne McLaren, Lord Walton of Detchant, Professor Martin Bobrow, and Professor Peter Rigby. All of these submissions are noteworthy, and considered both the science and the merits of this research in detail. An in-depth review of their comments is beyond the scope of this document, but I should like to make one brief quotation from the Dame Anne McLaren's letter. Dame Anne was, amongst many other things, a member of the Warnock Committee which designed the framework for the regulation of embryo research in the UK. After

expressing her view that she saw no reason why the applicants should not be granted a research licence she stated as follows:

> *"Incidentally, my recollection of the Warnock committee, which met at a time when transgenic animals were being widely discussed and homologous recombination made gene therapy a plausible long-term goal, is that we all knew what was meant by the "creation of human beings with specific characteristics"* [which the Committee proposed be prohibited]*, and it would never have occurred to us that "health" would be considered a specific characteristic."*

9. Whilst I accept that the reviews of the past are in no way binding on the Science and Technology Committee, it would be unfortunate if the very thorough and lengthy review process which has preceded this one-off evidence session were to be over-looked or under-estimated. I also accept that considerations in relation to treatment may be different to research, but I would stress that this particular research has always been proposed and reviewed in the knowledge that treatment and human application of the technology was the ultimate goal – a goal intended to avoid the transmission of mitochondrial disease.

PERMITTING LICENCES FOR TREATMENT

10. These appears to be much confusion surrounding the purpose and effect of the draft Regulations appended to the Department of Health's '*Mitochondrial Donation'* consultation paper of February 2014. For example, in the recent Parliamentary debate on this issue, there are numerous references to the regulations "permitting PNT and MST", "permitting these procedures", "the Government's stated intention to allow the creation of three-parent embryos", and "legislating to allow techniques".

11. The draft Regulations do not permit PNT or MST, and do not allow these techniques to be used.

12. The draft Regulations permit the HFEA to *consider* applications for licences permitting the use of these techniques in treatment.

13. This is a very important distinction. If the draft Regulations become law, it does not follow that the HFEA will grant licences for the techniques to be used imminently, or indeed at all. By way of illustration, as described above, Newcastle University's initial application for a licence to carry out the research was originally refused by the HFEA and only permitted after further detailed review – a process which took well over a year.

14. As with all licence applications, the HFEA will consider whether the proposed activity is safe, and whether the applicant has the necessary staff, expertise, skill and equipment to perform the proposed activity. It follows that even if the draft Regulations are passed, there will be a further tier of in-depth scrutiny by the HFEA and their external advisors before any licence is granted permitting the use of these techniques. The draft Regulations simply allow this further review process to begin.

15. It is not in anyone's interests – least of all the HFEA or the researchers – to permit or proceed with an unsafe treatment.

October 2014

Correspondence submitted by the Christian Medical Fellowship (MIT0014)

The Christian Medical Fellowship (CMF) was founded in 1949 and is an interdenominational organisation with over 4,500 British doctor members in all branches of medicine, and around 800 medical student members. We are the UK's largest faith-based group of health professionals. A registered charity, we are linked to about 80 similar bodies in other countries throughout the world.

EXECUTIVE SUMMARY

1. The arguments most commonly put forward to justify the use of mitochondrial donation for humans are variants on the following two questions posed in a recent BioNews article by Prof Frances Flinter[13]:

Is it ethical to try and prevent the development of a treatment that might enable the birth of a healthy baby for a couple for whom there are no other options?

Is it ethical to avoid trying a treatment that could also avoid further tragedy in future generations?

Our submission challenges the presuppositions behind two questions, which are central to the justification of mitochondrial donation.

2. Are there 'no other options' for prospective parents?

Many women who want a healthy, *genetically related* child, can use pre-implantation genetic diagnosis. Adoption or IVF with the use of a donor's egg are other available alternatives. In these cases, a child would not be fully genetically related but also would not be put at grave risk by an experimental, irreversible procedure. Furthermore, promising alternatives to both maternal spindle transfer (MST) and pronuclear transfer (PNT) are already being pursued by scientists in the treatment of mitochondrial disorders that do not involve changing the germline (references below).

3. Will mitochondrial donation techniques 'enable the birth of a healthy baby'?

Evidence presented to the HFEA since 2011 suggests that the application of this technology will be more unpredictable, complex, risky and limited (to certain combinations of haplogroups) than is being claimed by the HFEA. Evidence to the FDA suggests the same. Even those who are involved closely in this research acknowledge that there may be significant incompatibilities, causing major abnormalities. Hence the HFEA recommendation to screen embryos of any females who might be born *following* MST or PNT.

4. Will the use of mitochondrial donation 'avoid further tragedy in future generations'?

The results from mice and invertebrates suggest that many deleterious effects would not be revealed until adulthood. Again, this is evidenced by the warning that if a woman has a daughter born using these new techniques, her own daughter will have to use embryo screening to avoid the risk of passing on mitochondrial disorders, because there is such a risk her daughter will have abnormal mitochondria!

5. We are concerned that those promoting mitochondrial donation are ignoring the risks to the health of women alive now. *Nuffield has noted that '**many more egg donors will need** to be found**…A shortage of egg donors is an acknowledged problem**'.* **Egg donation is ethically troubling and risky to women's health, and for this research, is of no benefit to them.**

[13] Professor Frances Flinter, BioNews 29 September, 2014. http://www.bionews.org.uk/page_455952.asp

6. Are these techniques a 'treatment' for mitochondrial disorders?
Jeffrey Kahn of Johns Hopkins University admits that this is not about saving or treating lives: '*We're not treating humans. We're creating humans.'* Mitochondrial disease will continue to appear randomly at birth within the population. Indeed, very few women (about ten per year in the UK) would be candidates for even considering these techniques prior to pregnancy.

7. Is it ethical to 'try a treatment' (ie. experiment) on humans?
It is argued – often justifiably - that no research is 100% guaranteed to be safe, hence the need for human clinical trials. However these techniques are different to any others permitted before because they change the germline and impact future generations in ways we do not know and cannot predict. Serious safety issues associated with mitochondrial donation have been identified, and it seems ironic that trying to create genetically related children free of mitochondrial disease for a few women will put their own daughters, and granddaughters, at risk.

Acting chairman of the FDA committee, Daniel Salomon: '*I think it is pretty ridiculous how little data there is to support any of this, and that worries me.*

8. Are there other ethical and practical concerns with using new mitochondrial donation techniques?

Mitochondrial transfer is genetic modification and this modification is handed down the generations. It cannot be compared with a blood transfusion or a transplant.

All three genetic parents would play some role in the child's biological and genetic heritage, therefore the question is how much should that be recognised? Have politicians, the media and the proponents of this research seriously underestimated the influence that mitochondria have? The severity of the disease itself reflects the importance of the mitochondria for humans. Children conceived in this way will inherit some vital traits from three parents and need to be informed of that.

RESPONSE FROM CHRISTIAN MEDICAL FELLOWSHIP

Are there 'no other options' for prospective parents?

9. Procedures already exist that enable couples to have a healthy child of their own. The desire by parents to have children *genetically related* to them, and of course free of mitochondrial diseases, is the justification for the interest in the novel techniques, maternal spindle transfer (MST) and pronuclear transfer (PNT).

10. However for women who want (note, 'want' not 'need') to have a healthy child, *genetically related* to both parents, pre-implantation genetic diagnosis (PGD) is an alternative for many (albeit with some ethical concerns). Indeed, PGD is recommended by the HFEA for any females who might be born *following* MST or PNT, because of the risk that they will have a child of their own with mutant mtDNA.[14]

11. Adoption or IVF with the use of a donor's egg are also available alternatives. In these cases, the child would not be genetically related to respectively both or one of its parents but neither would the child or his/her children be put at grave risk by an experimental, irreversible procedure.

[14] http://www.hfea.gov.uk/docs/Mito-Annex_VIII-science_review_update.pdf

12. Moreover, alternatives to both MST and PNT are already being pursued by scientists in the treatment of mitochondrial disorders, that do not involve changing the germline. These are already making useful progress, and as one article concludes: *'This opens up new avenues to understand and develop therapies for mitochondrial diseases'*.[15]

13. We question whether the **risks to the child** created using these experimental mitochondrial donation techniques can ever be justified by the desire for him/her to be genetically related to both parents, when alternative options can be considered for prospective parents.

Will these techniques 'enable the birth of a healthy baby'?

14. The HFEA review of the safety of these techniques, in June 2014, concluded that it is safe enough to create one baby from three parents. Assuming, that is, one considers the reviewers' double negative wording to say as much: '*The evidence [the panel] has seen does not suggest that these techniques are unsafe*.'[16]

15. However if the double negatives are removed then we are left with the words: 'The evidence [the panel] has seen does suggest that these techniques are safe.' So why do the HFEA not say this directly? The clear implication is that there may be evidence that panel has not seen, which further research might uncover, that would point to the opposite conclusion.

16. Presumably the HFEA panel is aware that there remain too many safety concerns and unknown risks to justify a green light to creating reconstituted human embryos in order to avoid passing on debilitating and life-threatening mitochondrial disorders. But at the same time they cannot bring themselves to say 'no' to it.

17. In fact, evidence presented to the HFEA since 2011 suggests that the application of this technology and modification of the mammalian egg may well be more unpredictable, complex, risky and limited (to certain combinations of haplogroups) than is now being claimed by the HFEA.

18. For example, nuclear-mitochondrial compatibility is essential for nuclear transfer. Several scientific journal articles have highlighted concern that disrupting the 'fine-tuned' relationship between the nuclear and mitochondrial gene complexes will adversely affect health of the offspring.[17]

19. The HFEA have been provided with evidence that nuclear-mitochondrial interactions are disrupted following nuclear transfer, leading to 'unhealthy' mitochondria and compromised cell function.[18]

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[15] Anonymous, Correcting human mitochondrial mutations, 13 March 2012, e! Science News, http://esciencenews.com/articles/2012/03/13/correcting.human.mitochondrial.mutations,

[16] Third scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2014 update http://www.hfea.gov.uk/docs/Third_Mitochondrial_replacement_scientific_review.pdf

[17] For example, Klaus Reinhardt, Damian K. Dowling, Edward H. Morrow. Mitochondrial Replacement, Evolution, and the Clinic. *Science*. 20 September 2013: Vol. 341 no. 6152 pp. 1345-1346. '..studies *in humans have only tracked health through to the blastocyst stage and in macaques to three years of age. The results from mice and invertebrates suggest that many deleterious effects of MR would not be revealed until adulthood.'* The same researchers note that studies on other organisms have found that mitochondrial replacement does indeed have a big (adverse) effect on genetic expression, but this has received little profile. See also http://www.hfea.gov.uk/docs/Mito-Annex_VIII-science_review_update.pdf

[18] Eg.. See St. John, J. C., R. E. Lloyd, et al. The consequences of nuclear transfer for mammalian foetal development and offspring survival. A mitochondrial DNA perspective. *Reproduction* 2004: 127(6): 631- 41. St. John, J. C., R. E. Lloyd, et al. The potential risks of abnormal transmission of mtDNA through assisted reproductive technologies. *Reprod Biomed Online* 2004 Jan; 8(1): 34-44).

20. The macaque study by Tachibana et al had provided critical justification for the HFEA to recommend to the UK Government that the PNT procedure should be safe for human trials to proceed and that regulations should be introduced to permit their use to create new humans.[19] Although the HFEA has since admitted that: 'Current *research using PNT in Macaques has yet to be shown to be successful'* they have instead concluded that safety tests are no longer required to be carried out on non-human primates.[20]

21. Even those who are involved closely in this research acknowledge that there may be significant incompatibilities, causing abnormalities: 'In *addition to the risk of aneuploidy and other effects of the technical procedures, concern has been raised about the implications of possible incompatibilities between the nuclear genotype of the parents and the donor mitochondrial genomes. The potential biological significance of this stems from the fact that the majority of proteins involved in mitochondrial metabolism are encoded by the nuclear genome.'* 'The *question of whether the manipulations associated with nuclear genome transplantation might induce epigenetic anomalies remains to be resolved'.*[21]

22. PNT has been so unsuccessful with monkeys (causing *increased* abnormalities) that the HFEA suggests that monkeys are not a good model for humans and instead mice, or humans themselves, should be used for trials.

23. Adverse experiences with germ line modification and somatic gene transfer should serve as a warning for the enormous risks that would await mitochondrial gene replacement in humans.[22]

Will the use of mitochondrial donation 'avoid further tragedy in future generations'?

24. The results from mice and invertebrates also suggest that many deleterious effects would not be revealed until adulthood. Thus the HFEA warns that if a woman has a daughter born using these new techniques, her daughter will have to use embryo screening to avoid the risk of passing on mitochondrial disorders, because there is such a risk her daughter will have abnormal mitochondria![23]

25. In other words, a mother can choose to use this technique instead of embryo screening but her daughter will have to use screening. 'Reproductive choice' only works for some. And why is this technique safe for the mother but not her daughter?

26. Those promoting mitochondrial donation appear to be ignoring the risks to women alive now. Very few have warned of the dangers to another – larger – group of women who will risk their health for this research, by providing their eggs.

27. Yet the Nuffield Council on Bioethics has warned that: ***'One of the major barriers mentioned by scientists*** *when assessing the potential for cell reconstruction techniques to become treatments is the fact that* ***many more egg donors will need*** *to be found to undertake the research required in order for the safety and efficacy of PNT and MST to be established, and if therapies are to be provided in future.* ***A shortage of egg donors is an acknowledged problem*** *in respect of donation for reproduction,* ***and it is not yet clear whether egg donors would be more likely to come forward in sufficient numbers to take part in mitochondrial donation for***

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[19] HFEA, Mitochondria public consultation 2012. Tachibana et al. (2013) *Nature* Vol. 493 issue 7434, p. 627-631.

[20] Para 3.6.2. http://www.hfea.gov.uk/docs/Mito-Annex_VIII- science_review_update.pdf_

[21] Craven, Murdoch, Herbert, Turnbull et al, Mitochondrial DNA disease: new options for prevention. Hum Mol Genet. Oct 15 2011; 20(R2): R168–R174. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3179382/

[22] Bredenoord, A., Braude, P. Ethics of mitochondrial gene replacement: from bench to bedside. *Br. Med. J.*. 2011;342:87-89. Also http://www.ncbi.nlm.nih.gov/pmc/articles/PMC543871/ A further example was the death of a healthy teenager in a clinical trial for gene therapy in 1999.

[23] http://www.hfea.gov.uk/docs/Mito-Annex_VIII- science_review_update.pdf

research or treatment use.' (my emphasis).[24]

28. Egg donation has significant health and ethical implications, including the health risk to the donor from powerful hormonal treatments, injections, invasive surgery, [25] and it is not for her own benefit.

29. In preliminary trials the Oregon team driving much of this new mitochondrial research used 106 eggs from seven women; one woman donated 28 eggs, indicating possible ovarian hyperstimulation syndrome (OHSS) which can be dangerous or even fatal.[26]

30. The two techniques used for mitochondrial diseases should be less wasteful of eggs than cloning techniques. However, 'fewer' still means many eggs. Every single embryo generated by the two techniques (spindle transfer and pronuclear transfer) ultimately needs at least two eggs, and probably more as the procedure is, first, unlikely to be 100% efficient and, second, at least half the embryos may be defective, if the published results are anything to go by.[27] So even more 'material' may be required in order to create an embryo considered suitable for transfer to a womb.

31. The team in the UK driving this new research, the Newcastle Fertility Centre, have not recently published information about the total number of eggs and embryos they have used in particular research projects. However a study at the Newcastle Fertility Centre, reported in *Human Fertility*, found that more than 20 eggs were collected from at least one in seven patients, 14.5% of these women were admitted to hospital and nearly all reported symptoms consistent with OHSS.[28]

In other words, between 1999 and 2003 a total of 49 women were admitted to hospital. Life-threatening complications occurred in two women.

32. Worryingly, there seem to be no definitive data on the number of women hospitalised for OHSS after egg donation, as a Parliamentary response reveals.[29] We know from an HFEA report[30] that just under half of 864 reported clinical incidents between 2010-2012 were due to OHSS. And: '*Each year approximately 60 instances of severe OHSS and 150 cases of moderate OHSS are reported to the HFEA.*'

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[24] http://www.nuffieldbioethics.org/sites/default/files/Novel_techniques_for_the_prevention_of_mitochondrial_DNA_disorders_compressed.pdf

[25] http://www.nlm.nih.gov/medlineplus/ency/article/007294.htm

[26] http://www.geneticsandsociety.org/downloads/Donna Dickenson Commercialization of Human Eggs in MT Replacement Research.pdf

[27] Tachibana et al. (2013) *Nature* Vol. 493 issue 7434, p. 627-631 found that 52% of embryos created through spindle transfer had chromosomal abnormalities – four times as many as control embryos. Craven et al. (2010) *Nature* Vol. 465, issue 7294: p. 82-85 reports that '*After transfer of two pronuclei, 8.3% of abnormally fertilized embryos developed to the blastocyst stage…This is approximately 50% of the blastocyst rate for unmanipulated abnormally fertilized embryos…*'

[28] http://www.publications.parliament.uk/pa/ld200708/ldhansrd/text/80110w0002.htm#08011053000119

[29] http://www.parliament.uk/business/publications/written-questions-answers-statements/written-question/Commons/2014-07-02/203642/

[30] http://www.hfea.gov.uk/docs/Adverse_incidents_in_fertility_clinics_2010-2012_-_lessons_to_learn.pdf

Are these techniques a 'treatment' for mitochondrial disorders?
33. None of the proposed techniques represents an actual cure for mitochondrial disease, which will continue to appear randomly at birth within the population. The techniques will not treat or save lives. These techniques can only be applied to families after they have been identified as being at risk of conceiving a baby with mitochondrial disease and will be used experimentally to create *new* lives - for women who want their child to be genetically related to them.

34. This is an important difference. Jeffrey Kahn of Johns Hopkins University correctly acknowledges that these techniques are not treatments for mitochondrial disorders but are experimental methods of creating new lives free of the disorders: '**We're *not treating humans. We're creating humans.*'**[31]

35. Very few women would be candidates for even considering these techniques. Only about 15% of mitochondrial diseases are even caused by mitochondrial DNA. So these techniques would not help 85% of the women with mitochondrial diseases. Moreover, mothers can pass on disorders without being clinically affected themselves. Most cases are not diagnosed until after birth as many are sporadic and/or miss a generation because of variable penetrance.

36. Government claims that about ten lives per year in the UK may be saved are based on estimates by the Wellcome Foundation that have not been verified. Earl Howe, Health Minister, said recently that: *'Numbers are based on advice from the Wellcome Centre for Mitochondrial Research at Newcastle University...based on the numbers of patients already undergoing some form of reproductive assistance each year in the form of either pre-implantation genetic diagnosis or prenatal testing...The* ***Department of Health has no written calculations that can be placed in the House of Lords library.***[32]

37. In other words, these techniques will not be of any benefit to most children or parents and children will still be born with mitochondrial disorders.

Is it ethical to 'try a treatment' (ie. experiment) on humans?

38. It is argued – often justifiably - that no research is 100% guaranteed to be safe, hence the need for human clinical trials. However these techniques are different to any others ever permitted before, and would be prohibited in most other countries in the world, because they would change the germline and impact future generations in ways we do not know and cannot predict. Germline genetic engineering is a rubicon that should not be crossed.

39. The HFEA understand this concern, so suggest putting in place follow-up studies of children born using these new techniques. However this would not be legally required and follow-up studies are notoriously difficult to carry out over the long-term, especially if descendants also need follow up. Families cannot be contained in a lab, or in one place, like animals. There has been no follow up of the few children in the US born from similar techniques in 1996-7 (which were subsequently banned), nor evidence of how many were aborted or suffered abnormalities.[33]

40. This led acting chairman of the FDA committee, Daniel Salomon to say: '*I think it is pretty ridiculous how little data there is to support any of this, and that worries me.*'[34]

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[31] http://www.sciencemag.org/content/343/6173/827.full

[32] House of Lords Answers to Written Parliamentary Questions, Hansard. 6th May 2014.

[33] http://www.nytimes.com/2014/06/29/magazine/the-brave-new-world-of-three-parent-ivf.html?_r=1

[34] http://www.nytimes.com/2014/06/29/magazine/the-brave-new-world-of-three-parent-ivf.html?_r=1

41. As we have noted in detail above (paragraphs 17-24), there are serious safety issues associated with mitochondrial donation and modification of the mammalian egg, which have been identified in animal studies, including decreased survival, inhibited growth, behavioural and fertility problems. This technique has also been tried in humans, resulting in an abortion and two stillbirths.[35]

42. It seems ironic that the primary rationale for permitting these techniques is to allow some women to have genetically related children free of mitochondrial disease. And yet there is a high likelihood that children created using these new experimental techniques will be put at **greater risk themselves,** and any abnormalities and problems will be **generationally transmissible,** and thus affect even more children.

Are there other ethical and practical concerns with using these new techniques?
Is it genetic engineering?

43. '*The Government has decided to adopt a working definition for the purpose of taking forward these regulations. The working definition that we have adopted is that genetic modification involves the germ-line modification of* ***nuclear*** *DNA (in the chromosomes) that can be passed on to future generations.*'[36]

44. However back in 2013 the Government took a different line: '*…as the aim is that children born as a result of mitochondrial donation, and their offspring, would be free of serious mitochondrial disease it would be a form of germline modification or germline gene therapy, as respectively recognised by the HFEA and the Nuffield Council on Bioethics.*'[37] There appears to be a deliberate lack of transparency here.

45. Lord Robert Winston says: '*Of course mitochondrial transfer is genetic modification and this modification is handed down the generations. It is totally wrong to compare it with a blood transfusion or a transplant and an honest statement might be more sensible and encourage public trust.*'[38]

46. The proposed techniques are unequivocally germline genetic modification as they would take place in the laboratory during IVF, and therefore be passed on to future generations with unknown consequences.

The role of the donated mitochondria or 'third parent'
47. Advocates of these techniques downplay the relevance of the mitochondria in the individual's genetic make-up, yet we can agree that there will be three adults with whom a baby shares a parental genetic connection, and there will be identifiable genetic material from a second female parent which will be passed down the female generations.

48. Organ donors do not enable a person to come into existence but instead enable an existing person to stay alive. By contrast, with mitochondrial donation three parents create a *new* child by MST or PNT. They would all be biological parents, albeit playing different roles.

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[35] Fertility and Sterility Vol. 80, Suppl. 3, September 2003 s56 abstract.

[36] https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/332881/Consultation_response.pdf

[37] Earl Howe, 18 December 2013, Hansard http://www.publications.parliament.uk/pa/ld201314/ldhansrd/text/131218w0001.htm

[38] http://www.independent.co.uk/news/science/exclusive-scientists-accuse-government-of-dishonesty-over-gm-babies-in-its-regulation-of-new-ivf-technique-9631807.html

49. So all three parents play some role in the child's biological and genetic heritage, therefore the question here is how much should that be recognised?

50. Others have argued that the contribution of the mtDNA is important in shaping a person's narrative and determining who a person will be.[39] A child has the right to know about the existence and identity of all of their genetic parents, as well as how they came into being. Therefore a person must be informed if they were born (created) using mitochondrial donation techniques

51. The New Scientist recently revised its position on the ethics of mitochondrial donation suggesting the role of mtDNA may have been underestimated. *'Recent research suggests that they play a key role in some of the most important features of human life. This raises the ethically troubling prospect … that children conceived in this way will inherit vital traits from three parents.'* [40]

CONCLUSION

52. While we do not agree in principle with the use of these techniques, for both ethical and practical reasons, as a minimum we strongly recommend that Government wait until these techniques have passed all necessary safety tests before they are permitted to be used on humans.

53. We suggest that funding would be more effectively invested into researching treatments for the many who are already living with mtDNA disorders, and for those who will continue to be born with such disorders.

October 2014

Correspondence submitted by Professor Adam Eyre-Walker (MIT0016)

Adam Eyre-Walker is a Professor of Biology at the University of Sussex. He is an evolutionary biologist who has written over 100 peer-reviewed scientific papers. In 2012 he was awarded the European Society for Evolution Biology's Presidents award for outstanding contributions to evolutionary biology. He became interested in this topic on reading the paper by Reinhardt et al. published in *Science* magazine.

Mitochondrial replacement and the interaction between the nuclear and mitochondrial genomes.

SUMMARY

It is has been suggested that we should be cautious about mitochondrial replacement (MR) because of the potential for deleterious interactions between the mitochondrial and nuclear genomes. I do not believe these interactions are something we should be particularly concerned about for two reasons.

1. Such deleterious interactions will occur during normal sex as well as during MR. They will be more common during MR, but probably not very much more common.

[39] Baylis F. The ethics of creating children with three genetic parents. *Reproductive BioMedicine Online* 2013;26:531-534.

[40] *Three-parent babies: It's more messy than we thought*, New Scientist, 18 September 2014 http://www.newscientist.com/article/mg22329871.600-threeparent-babies-its-more-messy-than-we-thought.html#.VDVfJWd0zcs

2. Evolutionary theory suggests that deleterious interactions between the mitochondrial and nuclear genomes are likely to be quite rare within a population.

MAIN TEXT

(this was originally posted on the author's blog http://www.lifesci.sussex.ac.uk/home/Adam_Eyre-Walker/Website/Blog/Blog.html - it has been edited to remove as much technical jargon as possible)

Recently Reinhardt et al. [1] have argued that using mitochondrial replacement (MR) therapy in humans should be approached with caution because of potential incompatibilities between the mitochondrial and nuclear genomes. They note that since the products of the mitochondrial and nuclear DNA interact, genes on these two molecules are expected to be co-adapted. They suggest MR might break this co-adaptation and lead to un-anticipated effects and possibly disease. In support of their argument they cite a number of studies in mice and insects that have demonstrated an interaction between the nuclear and mitochondrial genomes.

In thinking about MR it is important to differentiate between two possible sources of deleterious genetic effects associated with the process that are conflated, to some degree, at least in the evidence they present, by Reinhardt et al. [1]. First, there are effects that are independent of nuclear background – e.g. mtDNA X is always more fit than mtDNA Y in all nuclear backgrounds, and to the same degree. It seems unlikely that these effects will be a problem for MR since it is simple to avoid them – choose a donor who is healthy, and if you are concerned that the mtDNA might perform differently in a male, check that she has had a healthy male child.

Second, there is potential for the mtDNA to interact with the nuclear genome such that mtDNA X is fitter than Y in nuclear background A but (relatively) worse in nuclear background B. These interactions are more problematic because they are unpredictable. Such negative interactions can potentially occur during normal sex as well as through MR; in both cases the mitochondrial DNA is placed in a different nuclear background. The difference between normal sex and MR is that in normal sex we know that the mitochondrial DNA is (reasonably) compatible with the nuclear genome of the mother, because the mother is alive. Half of her genome will be inherited by her offspring. In effect, selection against deleterious mito-nuclear interactions establishes a disassociation between the interacting mitochondrial and nuclear mutations (they are less likely to be found in the same individual). However, because the nuclear and mitochondrial genomes segregate freely during meiosis this dissassociation will tend to break down. It is unknown exactly how much less likely negative mito-nuclear interactions are during normal sex relative to MR, but difference is not expected to very large simply because segregation breaks the association each generation. Furthermore, the frequency of negative mito-nuclear interactions may not differ between normal sex and MR in males, because in this case the mtDNA will find itself in a novel sex-genetic environment, to which it has not previously shown itself to be compatible. Ironically, MR could potentially lead to fewer incompatabilities than normal sex if donors were chosen that have had healthy children from several different fathers (a strategy which would probably be considered unethical).

If we assume that deleterious mito-nuclear interactions are more likely in MR compared to non-MR individuals we might ask how strong those are likely to be. Within a population strong deleterious mito-nuclear interactions are expected to be rare because natural selection will remove mutations that generate such interactions. We can therefore reduce the likelihood of negative mito-nuclear interactions by selecting a donor who is carrying a common mitochondrial DNA sequence. Selection is expected to be less effective on mito-nuclear interactions that are male specific, because the mitochondria are maternally inherited, and hence not subject to selection. However, selection will act against the nuclear variant involved in the interaction, so we still expect such interactions to be infrequent. This is in contrast to

mitochondrial effects on male fitness that are independent of nuclear background; selection is expected to be ineffective against such mutations. However, as we have argued above, we can avoid such effects simply by choosing MR donors that are healthy and/or have had healthy male children.

Although, strong incompatabilities are expected to be rare within a population, they can evolve to be strong between different populations or species, because mutations in different populations and species will not have experienced each other. In this context it is note-worthy that most of the evidence for mito-nuclear interactions comes from cases in which individuals were taken from distant populations (e.g. [2-6]), sub-species or species (e.g. [5,7,8]), although, there are exceptions (e.g. [9]). It is also noteworthy that the majority of mito-nuclear interactions that have been discovered are quite modest in magnitude, particularly within a population, and often the effects are positive. However, there are some exceptions, notably the case in *Drosophila melanogaster*, in which introgression of a mitochondrial DNA from Brownsville Texas onto a standard genetic background yielded sterile males.[3,10]

Finally it is worth noting that the degree of disassociation between the nuclear and mitochondrial variants that interact depends upon the strength of selection; the stronger the selection the greater the disassociation and the greater the difference in the frequency of deleterious interactions in individuals born to MR and normal sex. However, the stronger the strength of selection the lower the frequency of the mutation in the population. Hence mutations that interact strongly and would cause a difference in the frequency of deleterious interactions between the nuclear and mitochondrial genomes are expected to be rare in the population.

In summary, if there are mutations segregating within a population with deleterious mito-nuclear interactions then these will affect individuals born via normal sex and MR. It is likely that these effects will be more common amongst MR individuals, but the difference in the frequency of these effects between individuals born to MR and normal sex is expected to be modest. Furthermore, the effects are predicted to be small because nuclear and mitochondrial mutations that generate large deleterious interactions are expected to be removed by natural selection from the population, and the available evidence supports this conjecture. Finally, it seems likely that the risk of deleterious effects in MR could be minimised by choosing a donor with a common mitochondrial haplotype that has had healthy male and female offspring.

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October 2014

Correspondence submitted by the Center for Genetics and Society (MIT0017)

1. The Center for Genetics and Society (CGS) is a nonprofit public affairs organization based in the United States that works for responsible development, use and societal governance of human biotechnologies. CGS has been closely following the technological and regulatory developments surrounding so-called "mitochondrial donation" both in the U.K. and in the U.S. We welcome the opportunity to submit these comments to the Science and Technology Committee for consideration at its 22 October 2014 evidence hearing.

2. CGS was established in 2001 in recognition of the profound challenges arising from technological advances that can be used to modify the human germline. These range from safety risks that would be assumed by women and children; to ethical questions of consent, rights, and dignity; to critical social questions of equality and exploitation.

3. Numerous scientific, ethical, policy and public interest organizations have concluded that "no changes should be made to the DNA of the human germline." POSTnote 431 characterizes this conclusion as a "consensus."

4. If mitochondrial manipulation techniques are permitted in the U.K., this would be accomplished via a Parliamentary vote in favor of an exception to the current prohibition of human germline modification (a policy that has been codified as law in some 40 other countries as well). Although it is important for regulation to be nimble in response to technological innovation, we believe that in this case the current policy situation – that is, the legality of gene therapy and illegality of human germline modification – strikes the right balance of enabling benefits while minimizing harms.

5. Despite repeated assertions by the Human Fertilisation and Embryology Authority (HFEA) that "there is no evidence that the techniques are unsafe," significant and growing evidence suggests that this is incorrect. Especially given recent findings, it appears that the techniques are in fact likely to be unsafe.

6. Every ethical review of mitochondrial manipulation techniques that has been undertaken by the HFEA and the Nuffield Council on Bioethics (NCB) has asserted that they should be considered advisable only if additional research demonstrating acceptable levels of safety and efficacy were to be completed successfully. In the last year, there has been very limited advancement in this endeavor. Instead, other investigators have reported findings that suggest substantial challenges to any demonstration of safety. In other words, there is much needed research that remains to be done, and new research that has been done has exacerbated rather than assuaged concerns.

7. The NCB recommended that mitochondrial manipulation techniques initially be offered as part of research trials, and that "parental consent to follow up should be mandatory for participation in the trial and extend to future generations." The proposal on which Members of Parliament will be asked to vote, by contrast, would allow the HFEA to license fertility centers to offer the techniques, and does not provide for follow up of any children born after their use. This would make it impossible to adequately assess the success or failure of the techniques.

8. We assume that the Committee will be made aware of the current and emerging evidence about mitochondrial function that demonstrates the lack of evidence that mitochondrial manipulation techniques would be safe. Here are two recent reports, and an earlier letter raising still-relevant safety concerns:
    - mtDNA Segregation in Heteroplasmic Tissues Is Common In Vivo and Modulated by Haplotype Differences and Developmental Stage, *Cell Reports*, Joerg Patrick Burgstaller et al., 26 June 2014
    - Mitochondrial Replacement, Evolution, and the Clinic, *Science*, Klaus Reinhardt et al., 20 September 2013
    - Nuclear transfer to prevent mitochondrial DNA diseases, *The Lancet*, Joanna Poulton et al., 2 September 2006

9. In addition, we list several resources that review and reflect on scientific evidence underlying the growing concerns about safety of mitochondrial manipulation techniques:
    - Possessed! The powerful aliens that lurk within you, *New Scientist*, Garry Hamilton, 22 September 2014
    - Three-parent babies: It's more messy than we thought, *New Scientist*, editorial, 18 September 2014
    - Human Genetics Alert Submission to HFEA update on safety of MST/PNT, David King, January 2013

10. The recent article in *New Scientist* reviews the accumulating evidence suggesting that comparing mitochondria replacement to "replacing batteries," as has often been done in public and policy conversations about the proposed techniques, is not only far too simple but also highly misleading. In fact, mitochondria exert great influence on phenotypic traits. The *New Scientist* reporter writes,

    > "Most debate around the issue has worked on the assumption that mitochondria are simply cellular powerhouses. However, given their new-found influence over our bodies the implications of this technology may be far more radical than we have assumed."

11. Based on their review of these recent findings, the editors at *New Scientist* wrote that

    > "the emerging science and the issues it raises have not had a proper airing. They urgently need to be brought to parliament's attention, debated and settled before a decision is made."

    The editors in fact reversed their own former position in favor of the techniques, saying that the proposition has turned out to be "more messy than we thought."

12. POSTnote 431 notes that "allowing mtDNA replacement may have little effect on the consensus not to alter germ line nDNA." In support of this, it notes that in 2012, the NCB concluded that there is a "distinct material boundary" between mitochondrial DNA (mtDNA) and nuclear DNA (nDNA), and that this allows a "clear legal distinction" to be made. However, the new evidence about the complexity of mitochondrial function

highlights the difficulty of drawing a meaningful line between mtDNA and nDNA when it comes to germline manipulations.

13. Also in support of the assertion that "allowing mtDNA replacement may have little effect on the consensus not to alter germ line nDNA," POSTnote 431 notes that

> "prevention of serious mitochondrial disease is the *only* purpose for which the current law might allow an embryo with altered DNA to be implanted into a woman. Allowing an embryo with alteration to its DNA to be implanted for any other purpose would require the primary legislation to be re-written, a major undertaking."

However, the assumption that mitochondrial replacement would be undertaken only for the prevention of serious mitochondrial disease may be wrong. Shoukhrat Mitalipov, the principal developer of maternal spindle transfer, told *Science* magazine in February 2014 that he believes mitochondrial manipulation should be adopted as a treatment for general age-related infertility.

> "No studies clearly indicate the efficacy of treatment" with the technique, Mitalipov says. But clinical trials "would be the way to test it." He would like to go forward with trials in both disease carriers and infertile women. "Mitochondrial diseases are rare, but age-related infertility is a huge problem."

14. A Parliamentary vote to permit mitochondrial replacement in the U.K. would likely encourage its approval by the U.S. Food and Drug Administration. Although U.K. law would limit its use to preventing transmission of mitochondrial disease, this would not be the case in the U.S, where drugs, devices and procedures can be used for any purpose once they are approved. In other words, this germline-modifying technique would in fact be very likely to spread to additional uses, especially because, as Dr Mitalipov's observation that "age-related infertility is a huge problem" suggests, there could be a significant market for procedures that claim to treat it.

15. We believe that such prospects are necessary and appropriate considerations in the current debate, and that those in the U.K. who are being asked to approve mitochondrial DNA replacement should consider the consequences of such a decision for the international consensus against genetic modifications that would be inherited by future generations.

16. Given the significant safety concerns about the proposed techniques, along with the weighty social and ethical concerns they raise, we strongly recommend leaving the law as it stands.

October 2014

Correspondence submitted by Professor Justin C St. John (MIT0018)

BACKGROUND

I am the Head of the Centre for Genetic Diseases at MIMR-PHI Institute of Medical Research and a Professor in the Faculty of Medicine and Health Sciences at Monash University, Melbourne, Australia. The aims of my research program are to understand how the mitochondrial genome is transmitted and replicated from the point of fertilisation onwards. To do this, I use a number of assisted reproductive technologies including *in vitro* fertilisation,

intracytoplasmic sperm injection, mitochondrial supplementation and somatic cell nuclear transfer. I believe that I am one of a few scientists whose research program combines reproductive biology and mitochondrial genetics. Part of my research program also includes understanding how mitochondrial haplotypes influence chromosomal gene expression patterns during development. I use stem cell models to undertake this work.

INTRODUCTION

I welcome the introduction of innovative approaches to prevent the transmission of mitochondrial disease and do not oppose their use based on a religious belief. I also think that, if scientists can, they have a responsibility to prevent the inheritance of diseases, such as mitochondrial disease.

However, I am concerned that metaphase spindle and pronuclear transfer have not been fully tested. I would like to see further testing in large animal models, which have a physiology and size close to the human. My concerns are based on whether the transfer of a small amount of mitochondrial DNA accompanying the metaphase spindle or the pronuclei into the donor egg would be preferentially selected and affect the offspring. Currently, I think the evidence is inconclusive and I have written about this in the scientific literature (1-3).

I would also be very keen to see more work carried out on human embryos generated using metaphase spindle and pronuclear transfer. Comparisons between human embryos and embryos generated from large animals using these techniques could be made and appropriate predictions drawn in line with the outcomes from monitoring live born large animals.

In terms of selecting the appropriate mitochondrial donor, my understanding of the arguments that have been put forward is that a close match mitochondrial donor would be found, as would be the case for blood donation. We (my research group) have argued that more divergent sources of mitochondrial DNA can lead to changes in gene expression. This is based on using a mouse embryonic stem cell model system where all the cell lines possess the same chromosomal genome but each cell line is distinct as it has mitochondrial DNA from different genetic backgrounds (4). As long as the mitochondrial donor is from a very similar genetic background, no major affects would be anticipated but this requires confirmation. However, it is important to remember that every fertilisation event produces distinct characteristics, many of which are still not explained.

MAIN SECTION

As we inherit our mitochondrial DNA from the population present in the oocyte just prior to fertilisation, there is the potential for all offspring from women who are carriers of mitochondrial DNA defects to suffer from mitochondrial disease. However, there are a series of dilemmas for women who are carriers of mitochondrial DNA genetic defects. For example, the amount of the genetic defect present in individual oocytes cannot be determined prior to fertilisation without the use of invasive sampling procedures as mitochondrial DNA is randomly distributed (segregated) to individual oocytes when in their immature form. As result, there can be considerable variability in the presence of the genetic defect between cohorts of mature oocytes from the same individual (5). Preimplantation genetic diagnosis, which samples a single cell from a very early embryo for genetic defects, can determine the mitochondrial DNA content present in the cell being analysed. However, it remains to be determined whether this would be predictive of the other cells from the same embryo. Whilst the offspring of the present generation may not be affected by mitochondrial DNA disease, it is not clear whether subsequent females would be carriers, as their oocytes could possess high levels due to random segregation. Furthermore, the oocytes from some women would fall into a grey area, whereby they would be borderline for possessing sufficient defective mitochondrial DNA to contribute to mitochondrial DNA disease (6).

My group and I have been sceptical about the use of certain assisted reproductive technologies that have been proposed as appropriate procedures to reduce the risk of transmission of mitochondrial genetic defects (2,6). These include: i) germinal vesicle transfer, which involves the transfer of the chromosomes from an immature oocyte into an enucleated oocyte from the same stage of development; and ii) metaphase spindle transfer where the chromosomes of a mature fertilisable (metaphase II) oocyte are transferred into an enucleated mature oocyte. In each of these cases, the oocyte is then fertilised with the father's sperm. A third approach is pronuclear transfer, where the chromosomes from a fertilised oocyte are transferred into a pronuclear stage oocyte. Fig 1 is a representation of metaphase spindle transfer but is also indicative of all three approaches.

Our scepticism is also based on the safety of the procedure and the continued potential for transmission of the genetic defect. An increasing body of literature suggests that the transfer of chromosomes to another egg can affect the regulation of the expression of certain genes (epigenetic regulation) during development, which can have adverse effects on the offspring's health and survival (7). It is also highly likely that, as the chromosomes are being transferred, a small amount of mitochondria would accompany them and would be selected for during development, as has been the case with cytoplasmic transfer (8). Two key studies, one in

monkeys generated through metaphase spindle transfer (9) and one in human using pronuclear transfer (10), which have not set out to deliberately eliminate the mitochondrial DNA accompanying the chromosomes to be transferred, have been promising but not conclusive.

From our findings, it is clear that there are a number of mitochondrial DNA replication events that take place as an egg matures to the metaphase II stage when it has the potential to fertilise and develop into an embryo and an offspring (11) (Fig 2). Post-fertilisation, there are a series of mitochondrial DNA reduction events, which ensure that mitochondrial DNA copy number is reduced to an absolute minimum in early developing cells and specifically embryonic cells (Fig 2). Most interestingly, at the very first stage of differentiation in the embryo, the blastocyst stage, there are distinct replication events that take place. The trophectodermal cells, that give rise to the placenta, upregulate mitochondrial DNA replication, whilst the inner cell mass cells, which give rise to the fetus, continue to dilute out mitochondrial DNA copy. This ensures that specialised cells acquire the appropriate numbers of mitochondrial DNA in order to meet their specific demands for energy (Fig 2). From both our published and unpublished work, it is quite clear that each assisted reproductive technology regulates mitochondrial DNA copy number differently during preimplantation development prior to the blastocyst stage (11,12). However, mitochondrial DNA copy number tends to be similar following each technique at the blastocyst stage. Whilst one might presume that an important milestone has been met, it should be pointed out that, because there are different mitochondrial DNA replication and reduction events taking place with each of the techniques, there is the potential for any mitochondrial DNA that is carried into the egg to be preferentially selected in a technique dependent manner. **Consequently, it is essential that experiments are carried out to determine whether there is an early phase of preferential selection of accompanying mitochondrial DNA when metaphase spindle transfer or pronuclear transfer are performed.**

Work from my group and others has clearly demonstrated that with somatic cell nuclear transfer, where chromosomes have been transferred into an enucleated recipient egg, there is the potential for the accompanying mitochondrial DNA to a preferentially selected (6). Evidence from studies carried out using a wide ranging number of species demonstrates that the offspring can inherent anything from 0–59% of its total mitochondrial DNA originating from the donor cell. This suggests that the introduction of mitochondrial DNA into an egg accompanying the chromosomes has the potential to be selected. Most interestingly, the amount of mitochondrial DNA that is transferred with the metaphase spindle is very similar to the amount of mitochondrial DNA that accompanies a donor cell when it is introduced into an egg following somatic cell nuclear transfer. The reason for donor cell mitochondrial DNA transmission following somatic cell nuclear transfer lies with the failure of the donor cell to shut down

mitochondrial DNA replication in the preimplantation embryo. Consequently, key mitochondrial DNA replication factors, such as DNA polymerase gamma A and mitochondrial transcription factor A, are still expressed enabling for premature preferential replication of the mitochondrial genome. This promotes the persistence of accompanying mitochondrial DNA to the blastocyst stage and, if present in the inner cell mass, then there is the strong likelihood that this mitochondrial DNA will be transmitted through to the offspring. **I do not believe that we currently have sufficient evidence to demonstrate that the mitochondrial DNA accompanying the metaphase spindle or the pronuclei will be selectively removed from the egg.**

The late Keith Campbell and myself argued that the oocyte itself and the mitochondrial DNA present in the oocyte can affect the phenotype of the offspring. My group has shown using an embryonic stem cell model, where each line has the same chromosomes but different mitochondrial DNA haplotypes, that there is a direct affect on chromosomal gene expression (4). This is mediated by changes in DNA methylation patterns. It is, therefore, essential that we conduct experiments to resolve issues about how the mitochondrial DNA haplotype affects development and the health of the offspring. The most essential approach would be to use large animal models so that better understanding of nuclear-mitochondrial DNA interactions during development can be resolved, especially as large animals have longer periods of gestation and will thus undergo more mitochondrial DNA replication events. **It is not appropriate to merely suggest that the mitochondrion and the mitochondrial genome influence energy within the cells, they have a far more sophisticated role to play during development.** It is well documented in the literature that mitochondrial DNA haplotypes predispose or protect individuals against severe diseases such as cancer (13), diabetes (14), Parkinson's disease (15) and many other neurological disorders.

CONCLUSIONS

My view is that the techniques associated with metaphase spindle transfer, pronuclear transfer or polar body transfer should be refined to ensure that no mitochondrial DNA accompanies the chromosomes as they are introduced into the enucleated egg. This is a key step that needs to be resolved before any assisted reproductive technology is used to give rise to offspring. Furthermore, I believe that if we are to produce offspring, which are not affected by mitochondrial DNA defects, then it is essential we ensure that a female offspring does not have the potential to transmit mitochondrial DNA defects to future generations. In other words, we need to ensure that the transfer is free of contaminating mitochondrial DNA defects the first time this technology is employed so that the offspring does not have to undergo the same complications or have the same quandaries as the mother had.

I wholeheartedly support the development of these technologies and to find mechanisms to prevent the transmission of mitochondrial DNA defects from one generation to the next. I do not believe that we are in a position where we can wholeheartedly endorse the current technologies because of the potential transmission of accompanying mitochondrial DNA. I feel that it is essential to provide our patients with the knowledge that, if they are to undertake this sort of treatment option, that they will have unaffected children. Therefore, I would like to see further refining of these technologies before they are endorsed for human clinical use.

My view is that each of the techniques should be explored using large animal model systems (not affected by human embryo legislation) and human oocytes. Culturing human oocytes once fertilised to blastocyst and the derivation of embryonic stem cells would allow questions related to the safety, mitochondrial DNA transmission, and appropriate levels of gene expression and epigenetic regulation during development to be addressed. This would provide unequivocal evidence for the safety of these techniques as potential reproductive strategies for those female patients who are carriers of mitochondrial DNA disease but wishing to have families. The use of large animal models with a physiology similar to the human would provide significant knowledge about the safety of the technologies and the transmission of mitochondrial DNA during the later stages of development and in the offspring. Furthermore, the derivation of embryonic stem cells from patients who are sufferers of mitochondrial disease, without any form of intervention, would also provide excellent models to understand how this disease is propagated.

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Figure 1

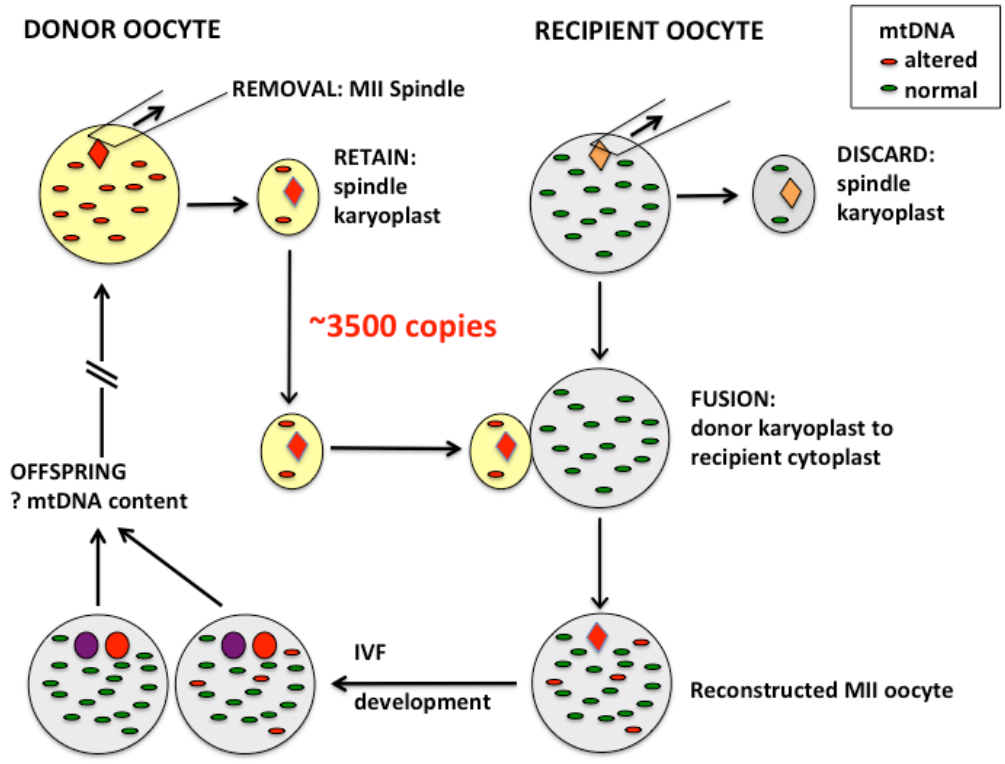

Fig 1: Representation of metaphase transfer. The processes of germinal vesicle, metaphase spindle and pronuclear transfer are similar. In each case, mitochondria carrying mitochondrial DNA, can be

transferred with the chromosomal DNA. With metaphase spindle transfer, the metaphase II spindle is removed from a mature oocyte that contains mutant mitochondrial DNA (donor). It is then transferred into an enucleated oocyte from an oocyte that has been donated by a female who is not a carrier of mutant mitochondrial DNA (recipient). The reconstructed oocyte is then fertilised through either *in vitro* fertilisation or intracytoplasmic sperm injection. After *in vitro* culture, it is transferred to the uterus of the 'chromosomal' mother. Adapted from (2).

Figure 2

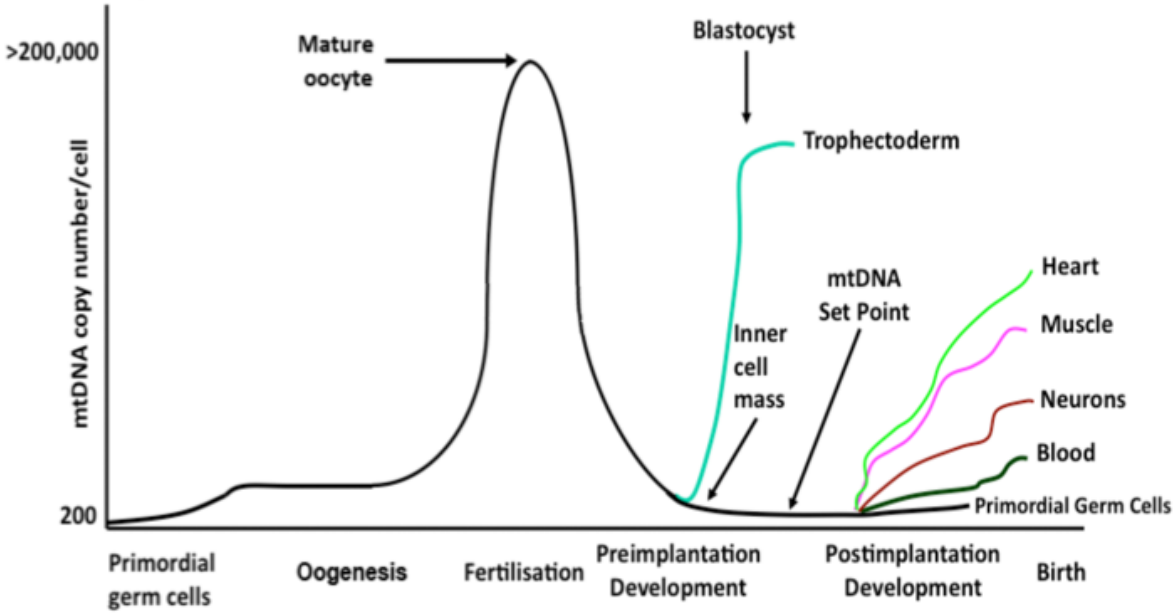

Fig 2: The regulation of mitochondrial DNA copy number during development. Mitochondrial DNA copy number increases during oogenesis but decreases during preimplantation development. At the blastocyst stage, replication is initiated in the trophectoderm. The inner cell mass cells continue to reduce copy number and establish the 'mitochondrial DNA set point' (mtDNA set point). When cells differentiate into specialised cell types, they increase copy number to match their needs for ATP. Adapted from (3).

October 2014

Correspondence from Human Genetics Alert (MIT0019)

Mitochondrial replacement: the need for a precautionary approach

SUMMARY

THE ETHICAL CONTEXT AND THE NEED FOR PRECAUTION

Our primary concern is that legalising the intentional manipulation of the human genome for the first time will open the door to subsequent genetic modification of humans, eventually for

enhancement purposes. Governments and international bodies have chosen to ban intentional inheritable changes to the human genome for a good reason.

However, this submission will concentrate on concerns about the safety of the procedures and the health of the children created through them. We argue that these scientific issues have not been dealt with properly by the HFEA, and that the techniques present major risks, which have not been properly considered. Given that the welfare of the child is the paramount consideration in the HFE Act, a precautionary approach to safety must be taken. The HFEA's approach, in contrast to that of the US Food and Drug Administration, has been the opposite of precaution. Precaution is especially necessary given that there already exist safe and reliable techniques for the avoidance of mitochondrial disease, notably conventional egg donation. Thus, the only benefit of the techniques is that the mother is genetically related to her child.

MITOCHONDRIA ARE NOT 'JUST BATTERIES'

The key piece of scientific misinformation that was crucial to the ethical misunderstanding of these techniques was the statement, endorsed by major scientific institutions, that mitochondria act as mere 'batteries' for cells. This argument is a classic example of the reductionist models of biology which dominate public debate. The assumption is that since nuclear DNA and mitchondrial DNA are separate comartments, so must their functions be. Even were it true that the functions of mitochondria are restricted to generating ATP, the idea that energy metabolism can somehow be isolated from the rest of the physiology of the organism, is biologically laughable. In reality, energy metabolism is entangled with many other aspects of cellular function. At the level of the the cell and whole organism, it is vital that the organism is able to sense the amount of calories available to it in the environment and adjust its physiology accordingly. Thus two extensive and extremely complex systems of molecular signalling exists between the mitochondria and the nucleus. Over the last few years it has become an established fact that because of their involvement in signalling with the nucleus, the effects of mitochondrial malfunction are central to the symptoms of aging-associated diseases, such as cancer and type 2 diabetes and variations in mitochondrial genes can affect susceptibility to those diseases. This scientific misframing of the central issue of the debate invalidates the results of the HFEA's consultation, the results of which were, in any case, far from the 'general public support' that the HFEA claimed.

THE RISKS OF EMBRYO MANIPULATION

IVF is known to increase the risk of certain epigenetic 'imprinting disorders' as well as low birth weight, which itself creates elevated risks of middle-age diseases. The late stages of egg maturation and the pre-implantation period of embryo development are the period at which the epigenome is most plastic and sensitive to environmental influence. In general, the more extreme and unnatural the manipulations, the greater the epigenetic damage; these manipulations are the most extensive yet proposed. We find it astonishing that, whereas the US Food and Drug Administration panel put these concerns at the centre of their deliberations, including calling witnesses to address them, the HFEA has resolutely refused to even acknowledge them.

EGG MITOCHONDRIA AND EPIGENETIC RISK

There is a flaw in the basic concept of nuclear transfer as a solution to mitochondrial disease, which derives from the simplistic assumption that nuclear states are unaffected my mitochondrial states. In fact, it is very possible that the nuclei of oocytes from mothers with mitochondrial mutations have epigenetic errors in their nuclear DNA. After all, if the epigenetic signalling mechanisms do not exist in eggs, they would be unique amongst cells of higher organisms; the question is how serious these errors are. Since these nuclei will be transferred to

donor eggs in MST/PNT, there is a real possibility that this will result in health problems in children.

CONCLUSION
It would be premature and wrong for Parliament to legalise these techniques before the safety issues raised here have been properly dealt with. The consequences of the lack of a precautionary approach may be felt not only by the families but by society at large for a long time to come.

Human Genetics Alert would like to thank the Science and Technology Committee for the opportunity to submit written comments to its hearing on mitochondrial replacement. We are an independent watchdog group which focuses on issues related to human genetics and reproductive technologies. The organisation was founded in 2001; we are a secular group that supports abortion rights.

1. Introduction: the need for sound science

The main emphasis of this submission is that a critical scientific flaw has undermined the public debate upon the ethics of mitochondrial replacement and the scientific assessment of its risks. Indeed, this same flaw underlies the very concept of mitochondrial replacement – that the molecular pathology associated with mitochondrial genetic diseases are restricted to mitochondria and that there are no abnormalities in the nuclei of eggs from women at risk of passing on those diseases. The flaw – nuclear DNA-centred genetic reductionism – has led the advocates of the technology and the media to minimise the sgnificance of mitochondria as 'just batteries', and the HFEA's science panel to ignore the crucial issue of epigenetic damage when discussing safety concerns.

Unfortunately, there is precedent for the precipitate legalisation of scientifically unsound techniques, on the basis of a public campaign of scientific misinformation. In 2007 and 2008, while the HFEA Act was being updated, the U.K. witnessed a massive campaign by scientists (including several U.K. Nobel Prize winners) to legalise the creation of human-animal hybrid embryos which, we were told, were vital to medical research. Despite warnings to the Committee by this organisation that such embryos were worthless for scientific research, the U.K. Parliament duly legalised their use. But in the following year, the Medical Research Council was forced to reject funding applications (from the same research centre that is spearheading the push for mitochondrial replacement), because they lacked scientific merit. Since then, that research agenda has been more or less abandoned. This fiasco was the most recent example of authoritative British scientific regulatory bodies and the medical establishment getting the science wrong, with disastrous results for public trust in them.

Parliament should learn from this experience and postpone legalisation of these techniques until there is evidence that they are safe to a standard of 'beyond reasonable doubt'.

2. The ethical context and the need for precaution

Our primary concern is that legalising the intentional manipulation of the human genome for the first time will open the door to subsequent genetic modification of humans, first for therapeutic purposes and then for enhancement purposes. Once this crucial ethical decision has been taken, it will become irrestible to proceed to these later steps: this is the reason why governments and international bodies have chosen to draw the line in this place. Thus the current decision is of historic importance, but has not received a proportionate amount of public attention. As we will argue below, the public debate has in fact been inadequate and distorted by misleading scientific information and ethical presentation.

However, the bulk of this submission will concentrate on concerns about the safety of the procedures and the health of the children created through them. We will argue that these scientific issues have not been dealt with properly by the HFEA, and that the techniques present major risks, which have not been properly considered. Given that the welfare of the child is the paramount consideration in the HFE Act, a precautionary approach to safety must be taken – in our view, the HFEA's approach, in contrast to that of the US Food and Drug Administration, has been the opposite of precaution. The need for precaution is especially clear given that there already exist safe and reliable techniques for the avoidance of mitochondrial disease, notably conventional egg donation. Thus, the only benefit of the techniques is that the mother is genetically related to her child. Whilst the desire to be the genetic mother of one's child is understandable, this is not a medical benefit for either mother or child, and cannot outweigh the risk to child's health or the consequences to society at large of opening the door to genetic modification of human beings.

3. Mitochondria are not 'just batteries'

The key piece of scientific misinformation that was crucial to the ethical misunderstanding of these techniques was the statement, endorsed by major scientific institutions, that mitochondria act as mere 'batteries' for cells, and that mutations in mitochondrial genes have no effect on an individual's identity. The analogy that was made is with a laptop computer; its batteries do not affect the programmes or data on the laptop. The purpose of this endlessly-repeated statement was to minimise the ethical significance of the changes to the germ line involved. (In these discussions, 'identity' was never clearly defined, but the general impression given was that it referred to visible physical differences, and perhaps personality.)

This argument is a classic example of the reductionist models of biology which dominate public debate. The assumption is that since nuclear DNA and mitchondrial DNA are separate comartments, so must their functions be. By understating the complexity of living organisms, and the problems involved in manipulating them, such reductionist models generally support the case in favour of adopting new techniques. But living organisms are simply not like computers: they are complex, whereas computers are merely complicated. Even were it true that the functions of mitochondria are restricted to generating ATP, the idea that energy metabolism can somehow be isolated from the rest of the physiology of the organism, is biologically laughable. One might imagine that such ambitious scientific claims would be backed up by detailed scientific evidence, but insofar as it is possible to determine the origin of what has now become an urban myth, the claim is entirely unsupported. The main quoted 'scientific' reference for the claim is a submission by the Medical Research Council and the Wellcome Trust, to the Nuffield Council enquiry, which contains no scientific references whatever for this claim. The MRC and the Wellcome Trust should examine the processes by which such scientifically misleading statements are issued on their behalf. As recognised medical authorities, they have a duty to get the science right.

In reality, energy metabolism is entangled with many other aspects of cellular function. At the level of the whole organism, it is vital that the organism is able to sense the amount of calories available to it in the environment and adjust its physiology accordingly. In fact, the failure to do this correctly, is, in the shape of obesity, not only a major problem for our societies but undoubtedly a central aspect of many people's personal identity. As we discuss below, this basic physiological imperative is reflected in the regulation by mitochondria of the epigenetic state of nuclear genes.

It is impossible to understate the significance of this scientific misframing to the ethical debate. The public was repeatedly told that the mitochondrial genome was, in essence, biologically insignificant compared to the nuclear genome, and that the changes being made are therefore trivial. (In fact, in terms of degrees of genetic manipulation, replacing the mitochondrial genome goes far beyond anything that has been done to date with genetic modification: it is equivalent

to changing the whole chromosome.) In itself, this misframing of the central issue of the debate invalidates the results of the HFEA's consultation, the results of which were, in any case, far from the 'general public support' that the HFEA claimed.

3.1 Mitochondria and cell signalling/regulation

In reality, the cells of all higher organisms, including humans, are a deep symbiosis between two different cells which came together over a billion years ago. One of these, the ancestor of mitochondria, was very efficient at producing energy from hydrocarbons and oxygen. But the ability of the organisms to live together required coordination of their activities: the growth of the overall cell entity, controlled by the genes in what is now the nucleus, must be regulated by the activity of the mitochondria, which respond to the availability of calories in the environment; and the activity of the mitochondria must respond to the needs of different types of cells, at different times. Thus two extensive and extremely complex systems of molecular signalling exists between the mitochondria and the nucleus: mitochondria to nucleus signalling is referred to as 'retrograde', nucleus to mitochondria as 'anterograde' signalling.

The overall purpose of retrograde signalling is to shut down the production of proteins by transciption of nuclear genes when calories are scarce and encourage it when they are abundant41. One way in which this works is that when calories are abundant, the mitochondria produce much ATP (the basic high energy currency of the cell) and acetyl-CoA. High levels of these lead to modification of the positively charged histone proteins which surround the negatively charged phosphate backbone of DNA by addition of negatively charged phosphate and acetyl groups. The opens up the structure of the histone-DNA complex (chromatin), allowing more transcription of the DNA. The reverse occurs when mitochondrial acitivity is low – the chromatin closes up and the DNA itself becomes modified by rhe addition of methyl groups attached to cytosines. DNA methylation is the crucial 'epigenetic marking' which allows cells to retain a memory of whether particular genes should be active or not. This memory can be maintained throughout the life of the organism, and in some cases can also be passed on to offspring through marking of the DNA in sperm and eggs. Changes in the markimgs underlie the process of differentiation whereby a fertilised egg produces hundreds of cell types as it grows. A complex network of transcription factors that are sensitive to mitochondrial signals control which precise genes are active. Mitochondria signal short term changes through the many signalling pathways in the cell that depend on the addition of phosphate groups to proteins, which control nuclear transcription factors that determine which specific genes are transcribed. In the last few years it has become apparent that mitochondria also produce small RNAs which are involved in separate signalling and cell regulation systems. Mitochondrial activity also determines fundamental aspects of cell chemistry: the 'redox potental' and pH. Because of these many effects of mitochondria they are becoming seen as 'master regulators of the life of the cell' 42.

Over the last few years it has become an established fact that because of their intricate involvement in signalling with the nucleus, and because of their activity in producing oxygen free radical molecules which can damage proteins and DNA, mitochondria are centrally involved in the changes that are part of normal aging. The effects of mitochondrial malfunction are central to the symptoms of aging-associated diseases, such as cancer and type 2 diabetes. Moreover, variations in mitochondrial genes can affect susceptibility to those diseases. And, as scientists are now increasingly coming to agree, the symptoms of mitochondrial genetic disease

41 Wallace DC and Fan W 2010 Energetics, epigenetics, mitochondrial genetics Mitochondrion 10 12-31.

42Horan, MP et al 2013 From evolutionary bystander to master manipulator: the emerging roles for the mitochondrial genome as a modulator of nuclear gene expression European Journal of Human Genetics 21:1335–1337

are also not just 'faulty battery problems' but arise partly because of epigenetic problems caused by retrograde signalling errors43.

4. The risks of embryo manipulation

Although it is difficult to disentangle the effects of assisted reproductive technologies (ART) from problems that derive from infertility, in humans, IVF is known to significantly increase the risk of two epigenetic diseases ('imprinting disorders') Beckwith Wiedmann Syndrome (BWS) and Angelman Sydrome (AS). More importantly, ART is associated with low birth weight even in singletons, which itself creates elevated risks of diseases which may only be manifested in middle age44. Many research studies have also demonstrated significant differences in epigenetic marks between ART- and naturally-conceived foetuses, which are not confined to imprinted genes. The significance of these differences is still unknown, but there is much concern that these differences will be manifested in a major legacy of health problems when children produced by IVF reach middle age. One recent scientific review of these issues was titled, 'Assisted reproductive technologies, epigenetics and long-term health: a developmental time bomb still ticking' 45.

Research has shown that epigenetic effects of ART manipulations can be traced to the effects of in-vitro culture and superovulation. Further problems can be seen in ICSI and, of course, most strikingly in the Large Offspring Syndrome and associate deformities in cloned cattle. Even transferring a naturally-produced embryo from one animal to another with no hormonal stimulation or in vitro culture has been shown to perturb epigenetic markings46. In general, the more extreme and unnatural the manipulations, the greater the epigenetic damage; the manipulations in MST/PNT are the most extensive yet proposed. All the literature on the subject notes that the late stages of egg maturation and the pre-implantation period of embryo development are the period in the life cycle of organisms at which their epigenome is most plastic and sensitive to environmental influence. In fact, it has been discovered that eggs are still acquiring epigenetic markings on imprinted genes after fertilisation, ie precisely in the period at which the physical manipulations are taking place in MST/PNT. It is, to say the least, unfortunate that this is the period in which ART massively manipulates the physical and hormonal environment of the embryo.

In this period, embryos are extremely sensitive to their surroundings and attempt to compensate for sub-optimal conditions by reprogramming their gene expression. In one recent piece of research the mitochondria in cultured mouse embryos were perturbed by the very minor and transient change of the main source of carbohydrate in the growth medium, thereby changing the redox potential of the cell. Although this had no effect on birth weight, significant differences in growth of the animals after birth were found, depending on the nutrient47. This is one illustration of the now well-accepted theory of the Developmental Origins of Health and Disease (DOHaD): that exposures of embryos and foetuses to certain environmental conditions, via their mothers, during the embryonic and foetal period can have permanent effects on the individual. It is thought that changes to metabolic and signalling patterns, made by embryos and foetuses in an effort to compensate for stress, can become 'set in stone', most likely through epigenetic memories, and later lead to disease, even though the stress that caused the pattern to be put in place no longer exists (see below).

43Chinnery et al 2012 Int J. Epidemiol **41** 177-187

44El Hajj N and Haaf T 2013 Epigenetic disturbances in in vitro cultured gametes and embryos : implications for human assisted reproduction Fertility and Sterility 99 632-640.

45Grace KS Sinclair KD 2009 Assisted, reproductive technology, epigenetics and long-term health: a developmental time bomb still ticking Seminars in Reproductive Medicine 27 409-16.

46Rivera RM 2008 Human Molecular genetics 17 1-14.

47Banrezes B et al 2011 PlosOne 6 e29388.

In ICSI, there are a set of epigenetic differences, above and beyond those present in IVF embryos48. Yet the physical manipulations involved here are considerably less severe than those involved in MST and PNT. ICSI involves a single penetration of the egg to inject a sperm in the periphery, but in MST/PNT, the donor egg is penetrated at its very core to remove its own chromosomes. The same is done to the mother's egg, with possible effects on it's DNA. Although this all appears in diagrams as simple, such manipulations dramatically disturb the egg's internal structures; and if we visualise the myriad molecular signalling systems that link the nucleus to the mitochondria and the rest of the cell as threads, nuclear transfer appears as a process of ripping the nucleus from the fabric of its functional support. It is really little short of miraculous that such a reconstructed embryo can survive at all, and long term damage to the molecular balance of the organism is to be expected.

As McEvoy et al note49:

> Nuclear transfer is not a robust technology in either murine or domestic animal studies and most reconstituted eggs never generate viable offspring. Initial enucleation of the oocyte, via a
> skilled yet crude excision process, and subsequent introduction of a donor nucleus is the equivalent of major transplant surgery and undoubtedly traumatizes both the cytoplasm and nucleus.

We would argue that the effects of the procedures are potentially more severe than transplant surgery, since they will potentially be felt in every cell of the child produced by these techniques. Indeed, one aspect of the present proposal is that it constitutes the first use of nuclear transfer manipulations in a clinical setting. We are not aware of any body of data that validates their use outside of the research setting, and we would have thought that this was essential for the most limited clinical use.

1. We find it astonishing that, whereas the US Food and Drug Administration panel put their concerns at the centre of their deliberations, including calling witnesses to address them, the HFEA has resolutely refused to even acknowledge them. The HFEA did eventually briefly mention them in the 2013 report, when they were raised by us in conjunction with the issues in section 5. Their response was an extraordinary blanket denial of the evidence compiled by many researchers and now widely acknowledged: “There was no evidence that such alterations, if they exist, have any far reaching effect on development or health”50! Although in 2011 it recommended (without mentioning the reason for the recommendation) experiments to determine the effects of manipulations, it now says that because MST has been show (in one paper) to be capable of producing blastocysts (the embryo stage at which they can be implanted) at reasonable efficiency, the experiments are no longer necessary.

> This is a perfect example of the HFEA's lack of precaution: as the FDA notes, it is perfectly clear that blastocysts can be produced - even by somatic cell nuclear transfer – which may appear perfectly normal under the microscope, but which harbour massive epigenetic defects which will cause major malformations in foetuses and offspring. In both recent papers on MST the manipulations caused such gross damage that the blastocyst production rate was still quite low, so it can be expected that those surviving embryos harbour more subtle defects.

As McEvoy et al note:

48Takashi Kohda and Fumitoshi Ishino 2013 Philos Trans R Soc Lond B Biol Sci 368(1609): 20120353

49Mc Evoy, TG et al 2001 Reproduction 122, 507-518

50HFEA Third scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2014 update.

> When such disruption coincides with the commencement of embryonic genome activation (from the two-cell stage onwards, depending on species) errors may increase even though, in contrast to some physical manipulations, genetic codes are conserved. Ironically, the subacute nature of at least some of the aberrant changes induced by in vitro production of embryos allows the changes to remain undetected in the short term. Blastocyst production, a hallmark for the efficiency of in vitro embryo culture systems, can often be achieved despite detrimental environmental effects. Indeed, Walker et al. (1992) reported that more blastocysts were produced from ovine zygotes in vitro than from equivalent zygotes in vivo. This finding should cause us to question the normality of blastocysts produced in artificial environments where subnormal embryos are perhaps less stringently de-selected than in dynamic conditions in vivo.

The FDA makes the same point: "Of particular concern is damage that might not be manifest as a failure to fertilize or implant, but would lead to unsuccesful pregnancies or safety concerns for the children produced."51

One recent review on this subject concludes: "From an epigenetic point of view, manipulation of oocyte and embryo should be restricted to a minimum, or the advantage of a specific technique must outweigh negative epigenetic effects." (ref 3). We must remark here that the minor benefit of allowing the mother to be the genetic parent of her child does, not in our view, outweigh such risks to the child.

We can only interpret the HFEA's refusal to consider these issues as a symptom of the same genetic reductionism that has characterised the public debate: the HFEA is assuming that all you need to produce a healthy cell is to make sure it has the right DNA. Disruptions to cell structure at crucial periods of sensitivity simply do not make it onto their radar – as long as the embryo can make it to the first hurdle (blastocyst formation) everything is sure to be fine.

5. Egg mitochondria and epigenetic risk

As we noted in section 3.1 above, there is an intricate signalling system in all human cells between mitochondria and the nucleus that has evolved over a billion years to permit the symbiosis and to provide the cell with a finely tuned mechanism for sensing the environment (particularly availability of calories) and responding appropriately. When mitochondria are damaged, by free radicals in aging or by a mutation in their own DNA, a significant element of the symptoms are due to the malfunctioning of the cellular retrograde/anterograde signalling systems, including epigenetic changes in the cell nucleus.

There is therefore a flaw in the basic concept of nuclear transfer as a solution to mitochondrial disease, which derives from the simplistic assumption that nuclear states are unaffected by mitochondrial states. In fact, it is very possible that the nuclei of oocytes from mothers with mitochondrial mutations have epigenetic differences in their DNA, or contain signalling molecules that will, at a later stage, cause such problems. Since these nuclei will be transferred to donor eggs in MST/PNT, there is a real possibility that this will result in health problems in children. After all, if the epigenetic signalling mechanisms do not exist in eggs, they would be unique amongst cells of eukaryotic organisms. The starting assumption must be that eggs containing mitochondria with a genetic defect do have such epigenetic perturbations; the question is how serious these are.

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51 FDA briefing on Oocyte Modification in Assisted Reproduction for the Prevention of Transmission of Mitochondrial Disease or Treatment of Infertility
http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/ucm380047.htm

There is one problem with this hypothesis. Shortly after fertilisation embryos undergo a wave of erasure of epigenetic markings inherited from sperm and eggs, in order that the embryo can start to behave as a 'totipotent' cell (ie capable of differentiating in to all other cell types), not as highly specialised differentiated cells (sperm and eggs). It has been thought that the only genes whose epigenetic markings are protected from this wave of erasure are a group of 100 – 200 'imprinted' genes. These genes often tend to be involved in regulating the growth of organisms, and unlike most genes, only one copy is permitted to be expressed: if the copy inherited from the mother is active, that inherited from the father is silent, and vice versa. Maternally active genes tend to suppress growth, paternal genes to increase it. If both or neither are expressed the result is an imprinting disorder, such as Beckwith Wiedermann Syndrome (BWS) or Angelman Syndrome (AS) which involve either over- or under-growth of the child, and many other symptoms. As noted above, perturbation of those genes is one of the known problems caused by ART manipulations, the Large Offspring Syndrome caused by cloning in ruminants being the rough equivalent of BWS.

Thus, when we presented this hypothesis to HFEA science panel in 2013, their response was that "pathologies associated with typical imprinting defects, such as Angelman or Beckwith Wiedermann syndromes, have not been noted to occur in children with mitochondrial disease". We therefore decided to check whether there were similarities between the symptoms of mitochondrial diseases and imprinting disorders. This is an inherently difficult comparison to make for a number of reasons. Firstly the symptoms of mitochondrial diseases are extremely varied, and as has often been noted, poorly understood. In fact the Newcastle group in a recent paper (ref 3) did attribute some of these symptoms to epigenetic signalling errors, but these were thought to arise as a result of the effects of mutant mitochondrial DNA, post-conception.

A second problem with such a comparison is that even the number of imprinted genes is only very roughly known: 100 – 200. So imprinting problems in some of the less well known genes, caused by mitochondrial genetic defects in eggs, could be present in the many unexplained symptoms of mitochondrial diseases. Not all imprinting disorders must necessarily have the same symptoms as BWS and AS.

Having said this, it appears that there are a number of notable similarities between mitochondrial and imprinting disorders, as noted by Hiendleder et al52. "A survey of perinatal clinical data from human subjects with deficient mitochondrial respiratory chain activity has revealed a plethora of pheotypes that have striking similarities with abnormalities commonly encountered in SCNT fetuses and offspring." (It should be noted that the imprinting errors in SCNT animals are similar to those in BWS). They cite a survey of pathologies of mitochondrial genetic disease patients performed by Kleist-Retzow et al53. Amongst the similarities between the two groups are rare symptoms such as polyhydramnios (excess amniotic fluid during gestation), ventricular septal defects, skeletal abnormalities, and, most significantly, intrauterine growth restriction – which may result in low birth weight as seen in human and mouse ART. Whilst by no means conclusive, these similarities certainly suggest the need for further research – the HFEA's responses definitely do not lay the issue to rest.

We will briefly deal here with the HFEA's second comment on this issue, which is particularly cursory and badly informed: 'Moreover, the mitochondria in growing oocytes are in a form that suggests that they are mostly inactive; therefore, on theoretical grounds the presence of mutant mtDNA in a growing and maturing oocyte was thought likely to be of little or even no consequence to the nuclear DNA (ref 10)'. Although it is true that mitochondria in eggs have a

52Hiendleder S. et al 2005 Reprod. Fertil Dev. **17** 69-83
53Von Kleist-Retzow JC et al 2003 Journal of Paediatrics 143 208-212.

structure which might be associated with inactivity, they are in fact active54 and vital for oocyte function. They are clearly primed and ready to spring into activity as soon as embryo development begins. There is abundant evidence that mitochondria are important for the developmental capability (quality) of eggs: indeed, it was precisely this correlation that led to the early attempts of Cohen et al to overcome infertility by injection of mitochondria from donor eggs. Furthermore, it can hardly be imagined that the dramatic growth of eggs in their final phase of maturation can be accomplished without active mitochondrial energy production, and during this phase, it is to be expected that all the usual retrograde epigenetic signalling is taking place. Moreover, mitochondrial signalling does not depend purely on whether they are active in producing ATP.

A further scientific finding that is of relevance to the hypothesis we suggested is that, contrary to the previous belief that only imprinted genes survive the erasure of epigenetic marks shortly after fertilisation, in fact the epigenetic markings of a considerable number of non-imprinted genes do survive, at least until the blastocyst stage55. At least theoretically it is therefore possible that incorrect versions of these marks in patients with mitochondrial genetic mutations might cause health problems.

It is therefore perfectly possible that epigenetic perturbations caused by mitochondrial DNA mutations can affect the mother's egg. It should be noted that these could be transmitted to offspring in more than one way:
1. Incorrect epigenetic marks on imprinted or nonimprinted genes in the nucleus of the mother's egg could be transferred to the offspring after MST/PNT.
2. Molecules in the nucleus of the mother's egg which control the epigenetic changes due to happen after fertilisation could either be incorrect or present at too high or too low levels. This could result in inadequate erasure of markings at non-imprinted genes or loss of markings at imprinted genes.

There are indications that there are mechanisms to coordinate the process with the sensing of the calories available, and this makes biological sense. The decision to reproduce is one involving a great deal of future energy expenditure for female mammals, and the mother must be able to sense the availability of calories in the environment. Thus it is no surprise that a consensus is emerging in studies of obesity and related metabolic syndromes like hyperglycaemia/diabetes that the inheritance of these conditions is at least partly epigenetic in character, ie via imprinting decisions. Fat mothers have fat children, but studies of the genes involved in this have failed to identify genes responsible for more than a small fraction of this inter-individual variation.

There is now considerable evidence that ovarian sensing of stress and calorie availablity leading to infertility is mediated, as would be expected, by mitochondria. For example, the damage to eggs done tobacco smoke can prevented by blocking the action of the mitochondrial protein, Bax56, and the same mechanism seems to be responsible for the effects on eggs of aging57. Even more significantly the effects of aging on egg mitochondria are reversed by a restricted calorie diet and this seems to depend upon the anterograde signally transcription factor PGC-1α58. Diabetic mice have impaired mitochondria and as do those on a high fat diet59

The results of the study mentioned above (ref 7), in which brief changes in main nutrient in the culture medium of embryos, thereby changing mitochondrial metabolism and the cell's redox,

54Van Blerkom J Mitochonrion 2011 11 797-813.
55Smallwood SA 2011 Nature Genetics 43 811–814. See also Borgel J et al Nature Genetics 2010 42 1093-100.
56Maitikainen t etal 2001 Nature Genetics 28 355-360.
57 Perez GI et al 2007 PNAS 104 5229-5234.
58Selesniemi K et al 2011 PNAS 108 12319-12324.
59Grindler NM and Moley KH 2013 Molecular Human Reproduction 19 486-494.

potential led to significant growth differences after birth, show that it is quite possible that mitochondria may be able to influence changes in epigenetic states in eggs.

Little is known about the link between mitchondria and the acquisition of genomic imprints in eggs and studies of the molecular mechanisms are only beginning to be published60. Unlike the situation in sperm, this process begins at puberty and continues until after the egg has been fertilised. Imprinting methylation marks are acquired as oocytes grow, in prepartion for ovulation. In diabetic mice, which are known have impaired mitochondria, errors in the methylation of the imprinted Peg3 gene in eggs were noted whilst other imprinted genes were unaffected61.

In one study62 using the Agouti mouse which transmits obesity cumulatively down the generations of the maternal line, it was possible to block the generation of increasing obesity by supplementing the mice's diet with the methyl donor folate: this is in accordance with the fact that in other cells, as part of the retrograde signaling response, mitochondrial metabolism produces the methyl donor S-adenosyl methionine (SAM), which affects the overall methylation level in DNA and thereby the epigenetic marking of genes involved in growth and energy metabolism. Although researchers have assumed that the critical energy sensing leading to epigenetic patterning occurs during gestation, there is in fact no reason to think that it could not also be occurring during egg growth and maturation.

In this context it is also important to note that the classic studies of transgenerational epigenetic inheritance in humans, the Dutch and Swedish famine studies, in which grandchildren of women who were pregnant during famine conditions exhibited clear differences in growth and metabolism, are all about growth and sensing of calorie availability. These show that epigenetic markings in one generation can be retained as they pass through the eggs of subsequent generation.

Finally, it seems likely that the effects of superovulation in creating epigenetic/imprinting differences happen through changing of the epigenome of eggs at a late stage in their growth (although there is some evidence that it operates via a mechanisms similar to the second possibility mentioned above. Superovulation in essence is a forcing of egg growth despite the mechanisms in the ovary which restrict the numbers of eggs that can mature at one time. Again, superovulation damages mitochondrial function in eggs63.

All these lines of evidence, whilst not conclusive, suggest that in eggs, like other cells, sensing of calorie availability and other growth-related signals involves the mitochondria. And if the mitochondria have genetic errors, the signalling may go wrong, producing incorrect epigenetic marks in the relevant growth and metabolism-related genes, as it does in all other cells. We believe that this hypothesis should be investigated and shown to be wrong, before MST/PNT are permitted.

6. Conclusion

This submission has pointed out that the entire public debate and consultation process surrounding mitochondrial replacement has been based on disastrously flawed scientific assumptions. The entire consultation process has been biased and should be repeated, especially since the results were far from the 'general public support' that the HFEA claimed.

60Tomizawa S 2012 Iternational Journal of Developmental Biology 56 867-75.
61Ge Z et al 2013 Biology of Reproduction 88 1-9
62 Waterland RA et al 2008 International Journal of Obesity 32 1373-1379.
63 Ge H et al 2012 Reproduction Fertility and Development 24 945-52.

We have also summarised evidence from the scientific literature indicating that: (i)The manipulations of eggs and embryos involved in MST/PNT have considerable potential to cause damage to the embryos that they construct; (ii) like all other cells with mitochondrial problems, the nuclear DNA of eggs derived from mothers carrying mitochondrial DNA mutations is likely to contain significant epigenetic errors.

The epigenetic damage resulting from these two sources, although it may not be evident at the blastocyst stage, has the potential to cause major health problems for the children born as a result of these techniques. Unlike the US Food and Drug Administration, the HFEA, inexplicably, has failed to even address these issues in a serious way.

Given the paramount importance in the HFEA Act of the welfare of the child, it is critical that we adopt a precautionary approach towards the health risks of these techniques. Statements from the HFEA that, 'there is no evidence that the techniques are unsafe', will not do. There is certainly considerable reason to think that the techniques might not be safe, and a precautionary approach would insist that until we are certain, beyond reasonable doubt, that they are safe, they should not be legalised. Failure to adopt such an approach will not only make both the clinicians and the HFEA vulnerable to medical liability suits, but will have a very significant impact upon the already shaky public trust in scientific regulatory bodies.

In summary, it would be premature and wrong for Parliament to legalise these techniques before the safety issues raised here have been properly dealt with. The consequences of the lack of a precautionary approach may be felt not only by the families but by society at large for a long time to come.

October 2014

Correspondence submitted by the Council for Responsible Genetics (MIT0020)

INTRODUCTION

The Council for Responsible Genetics is a public policy organization that represents the public interest and fosters public debate about the social, ethical and environmental implications of genetic technologies. We appreciate the opportunity to comment on oocyte modification in assisted reproduction for the prevention
of transmission of mitochondrial disease or treatment of infertility, and our current opposition to any procedure that alters human gametes.

COMMENTS

The first reported human pregnancy following cytoplasm transfer from donor oocytes into a woman's egg took place in 1997 (Cohen et al., 1997). Like many advances in assisted reproduction ooplasm transfer is designed to help women who seek a healthy pregnancy and that is a noble endeavor.
I offer three questions that should be answered before the procedure moves forward to gain approval and possibly becomes institutionalized.

1. Is ooplasmic transfer safe and effective for the offspring?

2. If the procedure is found to be generally safe but with some risks, do prospective parents have the authority to undertake the procedure, balancing risks and benefits, without additional oversight.

3. Are the potential benefits of ooplasm transfer for improving fertility or preventing the transfer of mitochondrial disease unique and sufficient to open the door to germ line genetic modification.

Question 1 is largely scientific; questions 2 and 3 are largely ethical. My remarks address question 1.

Most scientists who specialize in the biology of reproduction and who have written about cytoplasmic transfer have a clear message:

- Cytoplasmic transfer appears to be consistently associated with mitochondrial heteroplasmy (Scott and Alkani, 1998).
- Heteroplasmy—or babies born with two distinct female mitochondrial genomes, is a risk which must be understood before cytoplasmic transfer aka ooplasm transfer is considered for clinical practice (Lane, 2012).
- While an estimated 30 babies have been born using the technique there have been no systematic follow-up studies that examine the rate and degree of heteroplasmy in the newborn and in cases where it exists on its effect on the developmental health of the child.

  A recent review in Pub Med for the terms heteroplasmy and mitochondrial disease had 501 citations while ooplasm transfer in human cells had 58 citations. There is remarkably sparse empirical knowledge in animal studies and almost no human clinical studies on the safety and efficacy of ooplasmic transfer.

  There are no follow-up studies on the 30 children born through ooplasmic transfer. As one researcher wrote" "Transfer of oooplasm was thus applied with astonishing speed in humans in the absence of extensive research to evaluate the efficacy and the possible risks of the method." That was written in 2004 and things haven't changed (Levy et al. 2004).

The few published animal studies report a clear and present danger:

- Heteroplasmy created by the mixture of cytoplasm from different strains of mice resulted in physiological impairment, including disproportionate weight gain and cardiovascular system changes (Sharpley, 2012).
- Cytoplasmic transfer used in cattle produces heteroplasmic offspring (Ferreira et al. 2010).
- Some children born through cytoplasmic transfer have been identified as heteroplasmic (Levy et al. 2004).

- There is cross talk between mitochondrial DNA and nuclear DNA; it is not known but suspected that nuclear DNA cross talk between two mitochondrial genomes will affect the development of the offspring (Levy et al., 2004).
- The paternal genome may be especially susceptible to epigenetic alternations by foreign ooplasm (Liang et al. 2009).
- Mixing of two different mouse mitochondrial DNA within the same female germline can lead to offspring with neuro-psyuchiatric defects (Shapely et al. 2012)..
- While offering the prospect of treatment to some infertile couples, cytoplasmic transfer is "capable of generating unexpected abnormalities" (St. John, 2002).

Authors of the most current and comprehensive review article of mitochondrial DNA and heteroplasmy in the *Cold Spring Harbor Perspectives in Biology*, referring to ooplasmic transfer and other ART procedures wrote "all appropriate preclinical tests must be performed in an effort to reduce the risk for adverse outcomes" (Wallace & Chalkia, 2013, 44).

Many questions need to be answered before ooplasmic transfer could be considered safe and effective to the offspring. Until these questions are answered first by systematic animal studies (Acton et al. 2007), I can find no consensus within the scientific community to proceed.

Other methods for addressing the transfer of mitochondrial disease to offspring such as Pronuclear Transfer or Maternal Spindle Transfer introduce similar problems of heteroplasmy, which have not been resolved. As noted by Spikings et al. (2006): "Other techniques, such as germinal vesicle transfer and pronuclear transfer, have been proposed as methods of preventing transmission of mitochondrial diseases to future generations. However, resulting embryos and offspring may contain mtDNA heteroplasmy, which itself could result in mitochondrial disease. It is therefore essential that uniparental transmission of mtDNA is ensured before these techniques are used therapeutically."

There are ethical questions concerning germ line gene modification for ooplasm transfer, Pronuclear Transfer and Maternal Spindle Transfer, which hold equal if not greater weight than the scientific questions. These issues must be addressed more comprehensively by a separate national ethics commission, which should assess whether ooplasmic the three-parent genome is a stepping stone to a new eugenics (Rubenstein et al. 1995).

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October 2014

Correspondence from Comment on Reproductive Ethics (CORE) (MIT0021)

CORE is a public interest group founded in the United Kingdom in 1994, focusing on the ever-increasing ethical issues associated with assisted reproduction. We have quickly achieved a significant international profile, particularly among groups concerned with the welfare of women.

We have built up significant collaborations with women's rights groups, and were successful in 2005 in getting a ban passed against commercial trade in human eggs, through a European Parliamentary Resolution. It must be acknowledged that the human egg requirements for the current 3-parent embryo experiments, is but one of the weighty ethical concerns associated with these latest proposals.

As a founder member of CORE I have twice been invited by the Human Fertilisation & Embryology Authority (HFEA) to sit on ad-hoc committees to represent concerned public interest, including the most recent on what is termed by the HFEA and others 'Mitochondrial Donation'. This title in our opinion is inaccurate and avoids stating the reality of what is actually being proposed, namely germline genetic modification of the human embryo.

You will hopefully have received many submissions in opposition to what is being currently proposed, and we do not wish to repeat unnecessarily.

We wish to underline to the Committee that what is being planned in this field by the United Kingdom has rightly the opprobrium of the majority of scientists and countries worldwide, is included in many international formal documents of prohibition, and is recognized unequivocally as being human germline modification (passing on to future generations whatever unknowns might derive from such experiments).

We ask your Committee to investigate seriously the ethical and safety issues, and to try and take informed advice not simply from the scientists in the United Kingdom who wish to go ahead with the experiments but also from the expert scientists worldwide who shake their heads aghast at the casual way with which a mere handful of utilitarians in the United Kingdom are prepared to re-juggle the very building blocks of human life.

Just days ago I received a comment from a top academic scientist in the United States, Prof Stuart Newman, who directs a research programme in developmental biology, is co-founder of the prestigious Council for Responsible Genetics in Cambridge, MA, as well as co-author of Cambridge University Press's '*Biological Physics of the Developing Embryo*'.

Prof Newman commented on a Press Release issued last week from the Science Media Office promoting a Parliamentary hearing on this same issue in which Dr Dusko Ilic from Imperial College stated:

'*Worries that the mitochondrial genome will somehow affect the phenotype of the baby are baseless. Mitochondrial DNA encodes only 37 genes, whereas the nuclear genome codes for 20,000-30,000 genes. All mitochondrial genes code for proteins which play a role in metabolism, and speculations about other roles of mitochondrial genes such as their influence on the nuclear genome are only speculations and nothing else*.'

Prof Newman is adamant that this statement by Dr Ilic is not correct, citing a newly appearing paper, "***Disrupting Mitochondrial-Nuclear Coevolution Affects OXPHOS Complex I Integrity and Impacts Human Health***".[64]

Newman states that this is '*one of several in this vein in the past few years, and provides strong evidence that interruption of co-evolved relationships between the nuclear and mitochondrial genomes in a woman's egg increase susceptibility to disease, including type II diabetes.*'

This is but one single sample of the colossal body of informed opposition to the current proposals, but sadly in the United Kingdom the approach to debate and information gathering has been sloppy in the extreme and most panels discussing the proposed germline modification have been heavily biased a priori in favour. One exception is to be cherished, namely the 'New Scientist' who recently woke up to the reality of the dangers involved

One hopes sincerely that members of your Committee have the expertise to debate and understand this comment by Prof Newman and the others who have forwarded their concerns. And one hopes that you will make every effort to deconstruct the dangerous hype which circulates in the UK on this subject.

What is being proposed is the deconstruction of the very building blocks of life, combining material from 2 paternal and 1 maternal source at the very beginning of human life.

These proposals are intrinsically unsafe. No woman with mitochondrial disease would be cured by this procedure or any embryo carrying the disease. The latter is simply not allowed to live and the former will continue to be a carrier.

Furthermore, mitochondrial disease will continue to appear randomly in the population and we will be no closer to finding an ethical post-conception cure.

We would, however, have crossed the colossal boundary into human germline genetic modification.

We urge you to reject any move in such a direction.

October 2014

Correspondence submitted by Mothers for a Human Future (MIT0022)

1. Mothers for a Human Future is a United States nonprofit initiative focused on promoting awareness, advocacy, and activism about human biotechnologies that could

[64] http://www.ncbi.nlm.nih.gov/pubmed/25245408

fundamentally alter the human species. We have been active in raising awareness of these technologies in the United States, Europe, and among global advocates for children. We urge the Committee not to end the existing prohibition on human germline modification.

2. There is a long-standing international consensus that biotechnologies are to be used--with appropriate safeguards--to treat medical diseases, but are not to be used to manipulate the traits of future children. Human germline modification is prohibited by the Charter of Fundamental Rights of the European Union, the Council of Europe's Oviedo Convention on Biomedicine and Human Rights, other instruments of international law, and the domestic laws of 40 countries.

3. Parliamentary authorization of the use of mitochondrial manipulation techniques would create an exception to the existing prohibition on human germline modification.

4. Because germline modification could fundamentally alter the human species, no one country should unilaterally violate the international consensus against its use.

5. As noted in a recent editorial in *New Scientist*, the proposed techniques raise serious and growing efficacy, safety, and ethical concerns.

6. There is an urgent need for international public education, consultation, and decision-making on the appropriate global regulatory framework to promote responsible uses of this and other human biotechnologies.

7. In light of the profound safety, scientific, ethical, public policy, and societal issues raised by the proposed techniques, we urge the Science and Technology Committee to maintain the existing moratorium on human germline modification O

October 2014

Correspondence submitted by David A Prentice (MIT0023)

I am pleased to submit this written evidence for the Committee's consideration regarding the issue of creation of 3-parent embryos, a.k.a. "mitochondrial donation" or "mitochondrial replacement". I am submitting this written evidence in my personal capacity as a scientist, and also on behalf of the Family Research Council.

I am a cell biologist, currently working for the Family Research Council, a policy think tank in Washington, D.C., and as an adjunct professor of molecular genetics at a local university. For the previous 20 years, I was Professor of Life Sciences at Indiana State University and Adjunct Professor of Medical & Molecular Genetics at Indiana University School of Medicine, and I have done federally-funded laboratory research, lectured, and advised on these subjects extensively, in the U.S. and internationally.

EXECUTIVE SUMMARY

The use of mitochondrial donation, a.k.a. mitochondrial replacement, or creation of 3-parent embryos, has been proposed. The concept, as well as the techniques proposed to implement the concept, are flawed on numerous levels.

- The proposed techniques are all NON-THERAPEUTIC

- The proposed techniques treat human life instrumentally
- The proposed techniques treat women instrumentally
- The proposed techniques promote germline genetic modification
- The proposed techniques foster human cloning
- The proposed techniques are not safe
- The proposed concept gives no consideration to therapeutic alternatives

The Parliament should NOT approve this concept for clinical creation of human embryos.

BACKGROUND AND SUMMARY OF ISSUE

1. Proposals have been made for mitochondrial replacement therapy, via various techniques that use donor oocytes, or donor embryos, to provide genetically-healthy mitochondria, to replace genetically-mutated mitochondria in an affected oocyte. Mitochondria are the organelles within every cell responsible for generation of cellular ATP energy. These small organelles contain their own small DNA genome, encoding 37 genes including 13 essential polypeptides, 22 tRNAs and 2 rRNAs. The mitochondrial proteins are complemented by nuclear-encoded proteins, to form functional mitochondria.[65] Mutations in mitochondrial DNA can lead to various, often severe, diseases, all without current cure.[66] Approximately 1 in 6500 individuals are estimated to have a serious mitochondrial disorder.[67] Mitochondria are inherited through the maternal line, through the oocyte (the egg.)

2. Previously, several fertility clinics experimented with injection of ooplasm from a donor egg into the mother's unfertilized egg prior to fertilization *in vitro*. Over two dozen births attributed to ooplasm transfer were reported by several clinics between 1998 and 2002. Mitochondria were transferred as part of the ooplasm, resulting in offspring who carry genetic material from three separate individuals—the genetic mother and father, and the mitochondria/ooplasm donor; the researchers noted that this was "germline genetic modification".[68] Current terminology refers to these individuals as "3-parent babies". The U.S. FDA itself in 2002 called this "*de facto* germ line gene transfer".[69] Both terms are accurate, as the new individual has DNA from three parents, and the new DNA content can be passed to future generations.

3. The new proposals to avoid mitochondrial disease (meaning oocyte genetic modification by inclusion of donor mitochondria), also termed "mitochondria replacement", all involve creation of a new human embryo with altered genetics, due to tri-parental genetic contributions. The goal is creation of a new embryo with healthy mitochondria, to prevent inheritance of mitochondrial disease. The two main techniques considered are Maternal Spindle Transfer[70,71,72] and Pro-Nuclear Transfer.[73] An additional technique, similar in methods and

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[65] Taylor RW and Turnbull DM, Mitochondrial DNA mutations in human disease, Nat. Rev. Genetics 6, 389, 2005

[66] Schapira AHV, Mitochondrial diseases, Lancet 379, 1825, 2012

[67] Schaefer AM et al., Prevalence of mitochondrial DNA disease in adults, Annals of Neurology 63, 35, 2008

[68] Barritt JA et al., Amato P et al., Mitochondria in human offspring derived from ooplasmic transplantation, Human Reproduction 16, 513; 2001

[69] U.S. FDA Biological Response Modifiers (BRMAC) Briefing Document for Day 1, Ooplasm transfer as method to treat female infertility, May 9, 2002; http://www.fda.gov/OHRMS/DOCKETS/ac/02/briefing/3855B1_01.pdf(last accessed 15 Oct 2013)

[70] Tachibana M et al., Mitochondrial gene replacement in primate offspring and embryonic stem cells, Nature 461, 367, 2009

[71] Tachibana M et al., Towards germline gene therapy of inherited mitochondrial diseases, Nature 493, 627, 2013

[72] Paull D et al., Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants, Nature 493, 632, 2013

outcome that may be considered, is Embryo Cell Nuclear Transfer (Blastomere Nuclear Transfer).[74,]

4. Maternal Spindle Transfer Eggs from the intended mother (with mutated mitochondria) and eggs from a donor (with healthy mitochondria) are harvested. The nucleus is removed from an egg of the intended mother and from a donor egg. The nucleus from the intended mother is placed into the ooplasm of the donor egg, and the egg is fertilized with the intended father's sperm.

5. Pro-Nuclear TransferTwo single-cell embryos are created using IVF. Embryo #1 uses the intended mother's egg and intended father's sperm, and contains mutated mitochondria. Embryo #2 uses a donor egg and donor sperm or sperm from the intended father; this embryo has healthy mitochondria. The pronuclei (egg and sperm nucleus, prior to their fusion into a zygote nucleus) are removed from both embryos. The pronuclei from Embryo #1 (from the intended parents) are placed into the cytoplasm of the donor embryo; the recombined embryo now has the intended mother's and father's nuclear genetics and healthy mitochondria from the donor.

6. Embryo Cell Nuclear Transfer This technique is equivalent to somatic cell nuclear transfer (cloning of an adult), but transfers the nucleus of an embryo cell into an enucleated egg (cloning of an embryo). The donor egg has healthy mitochondria. Human somatic cell nuclear transfer (cloning of an adult) was recently demonstrated by the same labs that have worked on development of "mitochondrial donation" techniques.[75]

Arguments Against Approval

7. The proposed techniques are all NON-THERAPEUTIC.
No existing individual is treated using the proposed techniques for mitochondrial replacement. These are all non-therapeutic interventions. Rather, these techniques all create new human embryos with altered genetic composition, genetically designed individuals who will not inherit mitochondrial disease. Put another way, these techniques all evince a distinct lack of concern, even a disdain, for those individuals who currently suffer from mitochondrial disease, instead focusing solely on the design of new, genetically-correct individuals to take their place.

8. The proposed techniques treat human life instrumentally, including destruction of young human embryos for experimental purposes.
All of the proposed techniques manipulate young human life, treating new individuals as experiments. The Pro-Nuclear Transfer technique relies on destruction of two embryos (one completely healthy) to manufacture a third, recombined embryo.

9. The proposed techniques treat women instrumentally.
All of the proposed techniques rely on a significant number of donor eggs, with healthy mitochondria. The typical procedures used for the donation of eggs exposes young women to significant health risks from ovarian hyperstimulation syndrome, including uncertain long-term health risks due to lack of follow-up study of healthy egg donors.

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[73] Craven L et al., Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease, Nature 465, 82, 2010

[74] Bredenoord AL et al., Nuclear transfer to prevent mitochondrial DNA disorders: revisiting the debate on reproductive cloning, Reproductive BioMedicine Online 22, 200, 2011

[75] Tachibana M et al., Human embryonic stem cells derived by somatic cell nuclear transfer, Cell 153, 1228, 2013; AND Yamada M et al., Human oocytes reprogram adult somatic nuclei of a type 1 diabetic to diploid pluripotent stem cells, Nature 510, 533; 2014

10. The proposed techniques promote germline genetic modification.
All of the proposed techniques by definition alter the germline of any human embryo created by the techniques. These genetic alterations will be passed to the offspring of the modified individuals, with unknown consequences. The reality of these techniques as true germline genetic modifications is admitted even by proponents of the concept,[4,76] and is affirmed by the necessity of the Government to redefine germline genetic modification in order to bypass current conventions and regulations; to whit: "the Government's view is that the proposed mitochondrial donation techniques do not constitute genetic modification."[77]

11. The proposed techniques foster human cloning.
All of the proposed techniques foster the same manipulative techniques and skills used in somatic cell nuclear transfer, a.k.a. "Cell Nuclear Transfer", *i.e.*, human cloning, by micromanipulation of nuclei and of oocytes. The Pro-Nuclear Transfer technique and the Embryo Cell Nuclear Transfer technique are actually cloning of a human embryo, for the purpose of reproductive cloning (cloning for live birth). It should be instructive herein to consider the history of U.K. regulations in this respect. The Human Reproductive Cloning Act of 2001 made it an offense for a person to place in a woman "a human embryo which has been created otherwise than by fertilisation." The regulations were bent in the Human Fertilisation and Embryology Act of 2008 to allow embryo creation via "mitochondrial donation", producing "permitted eggs" and "permitted embryos", despite the fact that such eggs and embryos are products of genetic manipulation similar or identical to that in somatic cell nuclear transfer/cell nuclear transfer. It should come as no surprise that some of the most vocal proponents for creation and gestation of 3-parent embryos ("mitochondrial donation") have been scientists who provided both laboratory proof for creation of 3-parent embryos, AND for successful somatic cell nuclear transfer cloning.[6-8,11]

12. The proposed techniques are not safe.
There are significant concerns about the safety of mitochondrial transfer and oocyte modification. Ooplasm transfer is itself associated in mice with decreased viability of offspring as well as other subtle effects on growth and development.[78] Reinhardt *et al.* recently published a review paper enumerating several potential health hazards due to mitochondrial transfer and mitochondrial heteroplasmy.[79] The health risks they noted include decreased survival, decreased growth, and behavioral and fertility problems. New evidence continues to highlight the paucity of information we have regarding mitochondrial-nuclear interactions,[80] *e.g.*, in response to stress,[81] or in relation to signal transduction pathways.[82] While no comprehensive

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[76] See, e.g., Amato P et al., Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases, Fertility and Sterility 101, 31; 2014

[77] Mitochondrial Donation. Government response to the consultation on draft regulations to permit the use of new treatment techniques to prevent the transmission of a serious mitochondrial disease from mother to child, July 2014; at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/332881/Consultation_response.pdf

[78] Cheng Y *et al.*, Effects of ooplasm manipulation on dna methylation and growth of progeny in mice, *Biology of Reproduction* 80, 464, 2009

[79] Reinhardt K et al., Mitochondrial replacement, evolution, and the clinic, Science 341, 1345, 2013

[80] Friedman JR and Nunnari J, Mitochondrial form and function, *Nature* 505, 335, 2014

[81] Adachi Y and Sesaki H, Cyclin C: An Inducer of Mitochondrial Division Hidden in the Nucleus, *Dev. Cell* 28, 112, 2014; Cooper KF *et al.*, Stress-Induced Nuclear-to-Cytoplasmic Translocation of Cyclin C Promotes Mitochondrial Fission in Yeast, *Dev. Cell* 28, 161, 2014

[82] Garipler G *et al.*, Deletion of conserved protein phosphatases reverses defects associated with mitochondrial DNA damage in Saccharomyces cerevisiae, *Proc. Natl. Acad. Sci. USA* 111, 1473, 2014

follow-up was done for the children created by ooplasm transfer, early reports from one group noted potential chromosomal and behavioral abnormalities.[83]

13. The proposed concept gives no consideration to therapeutic alternatives.
Rather than the proposed techniques, which have numerous concerns scientific, ethical, and legal, there should be consideration of other possible techniques that would be truly therapeutic for such conditions of mitochondrial disease. An increasing number of possibilities exist. For example, there is recent evidence for transfer of mitochondria from donor bone marrow adult stem cells to other tissues,[84] as well as evidence for successful removal of mutant mitochondria via TALENs.[85] These techniques have distinct potential clinical applicability for individuals with mitochondrial disease. Genome editing techniques using the CRISPR-Cas9 system also show great promise at correcting genetic mutations,[86] and studies using targeted RNA import have shown efficacy correcting human mitochondrial mutations.[87]
Rapamycin drug treatment has shown remarkable success in effectively alleviating the symptoms of mitochondrial disease in a mouse model of Leigh syndrome.[88]

14. There are a host of scientific and ethical concerns about the proposed concept of creating 3-parent embryos and the techniques for mitochondrial donation, not the least of which is the unproven safety. We urge the Parliament NOT to approve this concept for clinical creation of human embryos.

Thank you for your consideration of these comments.

October 2014

Correspondence submitted by The Christian Institute (MIT0024)

EXECUTIVE SUMMARY

- The Christian Institute strongly opposes changing the law to allow Maternal Spindle Transfer (MST) and Pronuclear Transfer (PNT) techniques in the UK.
- There is widespread concern about the proposed techniques from a public safety perspective. Germline modifications overstep a profoundly important ethical boundary and cannot be shown to be safe. Contrary to extraordinary denials by the Department of Health, senior scientists have insisted that the plans do constitute genetic modification of human beings.

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[83] Barritt et al., Epigenetic and experimental modifications in early mammalian development. Part II. Cytoplasmic transfer in assisted reproduction. Human Reproduction Update 7, 428, 2001; AND Barritt et al., Rebuttal: interooplasmic transfers in humans, Reproductive Biomedicine Online 3, 47, 2001

[84] Prockop DJ, Mitochondria to the rescue, Nature Medicine 18, 653, 2012; Islam MN et al., Mitochondrial transfer from bone-marrow–derived stromal cells to pulmonary alveoli protects against acute lung injury, Nature Medicine 18, 759, 2012

[85] Bacman SR et al., Specific elimination of mutant mitochondrial genomes in patient-derived cells by mitoTALENs, Nature Medicine 19, 1111, 2013

[86] See, *e.g.*, Sander JD and Joung JK, CRISPR-Cas systems for editing, regulating and targeting genomes, Nature Biotechnology published online 2 March 2014; doi:10.1038/nbt.2842

[87] Wang G et al., Correcting human mitochondrial mutations with targeted RNA import, *Proc. Natl. Acad. Sci. USA* 109, 4840, 2012; Comte C et al., Mitochondrial targeting of recombinant RNAs modulates the level of a heteroplasmic mutation in human mitochondrial DNA associated with Kearns Sayre Syndrome, *Nucleic Acids Res* 41, 418, 2013

[88] Johnson SC *et al.*, mTOR Inhibition Alleviates Mitochondrial Disease in a Mouse Model of Leigh Syndrome, *Science* 342, 1524, 2013; Vafai SB and Mootha SK, A Common Pathway for a Rare Disease?, *Science* 342, 1453, 2013

- No woman or child will be cured of mitochondrial disease by the procedures. Instead, it is proposed to create embryos which are free of the disease by using an egg from another woman. This will give the resultant child three genetic parents. It has been widely described as a eugenic practice.
- 62% of respondents to the Government's 2014 consultation opposed the plans.
- Scientific understanding of the function of mitochondrial DNA is far from comprehensive and recent evidence suggests it might be far more important than the Department of Health has acknowledged.
- The Government has rejected a key safety recommendation of the HFEA Expert Panel that any children born as a result of the procedures should be subject to long-term monitoring. The Department says that this will not be mandatory because of legal "difficulties".
- The plans will divert resources away from cures for mitochondrial disorders.

INTRODUCTION

1. The Christian Institute is a non-denominational charity established for the promotion of the Christian faith in the UK and elsewhere. We have 32,000 supporters throughout the UK, including around 3,800 churches and church ministers from almost all the Christian denominations. We work across a wide range of areas, including public policy and medical ethics. We hold traditional, mainstream Christian beliefs about bioethics, including the sanctity of human life from conception.

2. The Science and Technology Committee has called for interested parties to present their views on the regulations before they are laid before Parliament. As a charity, we have been actively involved in issues of embryology and life over nearly two decades, with a particular interest in the Human Fertilisation and Embryology Act in 2008. We are very concerned that the Government is rushing ahead with techniques that will alter the human germ line in unknown ways, without taking full account of the evidence.

3. The Christian Institute has published resources over a number of years about the genetic modification of humans and the issues surrounding it. We are concerned to ensure that technological advancement does not overstep moral and ethical boundaries.

4. The Christian Institute strongly opposes both procedures (MST and PNT) because they are unethical and dangerous. We believe that a complete ban should remain on germline modification techniques.

LEGAL OBSTACLE TO PUBLIC SAFETY

5. The HFEA's Expert Panel recommended long-term follow-up monitoring of any children born as a result of the mitochondrial donation techniques.[89] However, the Government has rejected this, citing legal "difficulties".[90] How can the techniques possibly proceed in defiance of this key public safety protection?

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[89] *Nuffield Council on Bioethics*, 27 February 2014, see http://nuffieldbioethics.org/news/2014/draft-regulations-on-mitochondrial-replacement-techniques-echo-council/ as at 20 October 2014

[90] *Mitochondrial Donation*, Department of Health consultation document, February 2014, page 6 and para. 2.36

THE ROLE OF MITOCHONDRIAL DNA

6. The science of genetics is extremely complex and understanding of the intricate details is far from comprehensive. As yet, scientists only have a limited grasp of the role of DNA in causing disease, and its precise role in shaping our physical characteristics is not fully understood.

7. Proponents of MST and PNT argue that mitochondrial DNA is purely functional and plays a far less significant role than nuclear DNA in the shaping of a person. It is claimed that mitochondria and their genomes simply produce energy and therefore mitochondrial DNA has been likened to batteries in cells. However, Dr Ted Morrow suggests that this narrow view of the role of mitochondrial DNA is a misconception and is "not supported by current scientific evidence".[91] Professor Stuart Newman has said: "The mitochondria are … participants in the development of the organism. This clearly makes any person [brought into being from the procedure] a product of wholesale genetic engineering".[92]

8. Studies have suggested that the contribution of mitochondrial DNA is important in shaping who a person will be.[93] We would urge the Committee to seriously consider the New Scientist's U-turn in September 2014: "it appears that we may have seriously underestimated the influence that mitochondria have. Recent research suggests that they play a key role in some of the most important features of human life ... the emerging science and the issues it raises have not had a proper airing".[94] In light of the emerging evidence on the importance of mitochondrial DNA, these regulations must be dropped.

9. Mitochondrial DNA has been shown to have a potential influence on a range of traits, including cognition and memory, fertility, and ageing.[95] Garry Hamilton noted in the New Scientist: "Far from being passive power plants, it seems mitochondria influence some of the most important aspects of human life – from memory and ageing to combating stress and disease. They even have influence over the DNA in your cell nuclei, and change and evolve during your lifetime, giving you an unexpected source of adaptability to cope with a world in flux."[96] Furthermore, the interplay between nuclear and mitochondrial DNA is not fully understood, meaning that it is uncertain how donor DNA will interact with the nuclear DNA of the prospective parents in the procedures.

CHANGING THE GERM LINE AND DEFINING GENETIC MODIFICATION

10. The importance of mitochondrial DNA has been deliberately downplayed in the debate surrounding these procedures. The genes in mitochondrial DNA pass from a woman's egg into every cell of her offspring. Given this fact, there is no doubt that these procedures constitute germline modification. This is fraught with dangers and unknown consequences. If Parliament gives the go-ahead to MST and PNT, it will be a highly significant move: our country would become the first in the world to authorise these procedures.

11. Germline modification raises serious concerns. If modifications to mitochondrial genes are permitted, the Rubicon will have been crossed. These procedures go too far in intervening in

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[91] *BioNews*, 8 September 2014, see http://www.bionews.org.uk/page_451856.asp as at 22 October 2014

[92] *The Sunday Times*, 5 October 2014

[93] See for example Baylis, F, 'The ethics of creating children with three genetic parents', *Reproductive BioMedicine Online*, 26(6), 2013, pages 531-534

[94] 'Three-parent baby U-turn?' *New Scientist*, 20 September 2014, page 5

[95] See for example 'The micromanagers', *New Scientist*, 20 September 2014, page 43; Reinhardt, K; Dowling, D; Morrow, E, 'Mitochondrial Replacement, Evolution, and the Clinic', *Science*, 341(6152), 20 September 2013, pages 1345-1346

[96] 'The micromanagers', *New Scientist*, 20 September 2014, page 43

natural biological processes. If MST and PNT are legalised, it would set a precedent and it seems clear that this would pave the way for further genetic alterations to future generations. Even if stringent regulations are put in place, in reality it is far harder to turn back once the interference in embryos has been permitted. Just as tight regulations on euthanasia and abortion have gradually been weakened in countries around the world, it is not difficult to conceive of scientists pushing for modifications to nuclear genes in the future.

12. It is alarming that the procedures will change the germ line permanently for the children born as a result. Any adverse consequences arising from the alteration, including any mismatching, may not be reversible and could have unforeseen, far-reaching consequences. Since the implications of interfering with mitochondrial DNA stretch far beyond one generation, the prohibition on these techniques should remain in force.

13. The Government has adopted a false definition of genetic modification so as to exclude mitochondrial donation and make the procedures sound less controversial. This is untenable. The working definition the Government has adopted is that "genetic modification involves the germ-line modification of nuclear DNA (in the chromosomes) that can be passed on to future generations".[97] This definition excludes modification of the mitochondrial genome, thereby rendering the techniques apparently different from genetic modification. However, mitochondrial DNA cannot simply be ignored. Changing the overall genetic structure of an egg or embryo is undoubtedly a form of genetic modification, as senior scientists have insisted.

EUGENICS

14. The plans seek to improve the genetic makeup of children not yet born. This is unquestionably a eugenic practice. As Stuart Newman, Professor of Cell Biology and Anatomy in New York, has said: "One factor in placing these procedures on the social agenda seems to be the conflation of biological modification of people who do not yet exist with medical treatment of actual sick people. The attempt to improve future people is not medicine, however, but a new form of eugenics."[98]

15. Eugenics undermines human dignity and was rightly discredited decades ago. As Professor Calum MacKellar has said, the concept behind MST and PNT "questions the equality in value and worth of every possible future person".[99]

THE DESTRUCTION OF EMBRYOS

16. The research for MST and PNT will require thousands of embryos to be produced and experimented on. We hold that embryos represent the beginning of human life and are worthy of respect. Therefore these techniques should not be permitted. We should be protecting embryos at such a vulnerable stage, not creating life merely to experiment upon it. PNT utilises the destruction of life since it involves dismembering an early embryo. Yet embryos are not a random combination of genes; each one is the beginning of a human life and a unique fusion of genes with a complex interrelation of nuclear and mitochondrial DNA. If the regulations are permitted, they will take Britain further down the road of undermining the sanctity of human life.

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[97] *Mitochondrial Donation*, Government response to the consultation on draft regulations, Department of Health, July 2014, page 15

[98] Scolding, N, 'Three-parent babies – miracle or eugenics?' *Standpoint*, December 2013, page 41

[99] *BioNews*, 6 January 2014, see http://www.bionews.org.uk/page_385343.asp as at 22 October 2014

PUBLIC SAFETY CONCERNS

17. Although further experiments have been carried out in response to the Government's consultation procedure and the HFEA Expert Panel has looked at recent experiments, the safety of the procedures is still in doubt. Nuclear and mitochondrial DNA cannot yet be clearly delineated. Cells contain a fine balance and the full interaction of mitochondrial and nuclear DNA is not understood. Scientists have not shown that combining the nuclei and cytoplasm from two different individuals is safe for the reconstructed embryo and such combinations may compromise cell function.

18. Dr Ted Morrow has raised concerns relating to mismatching between the donor's mitochondrial DNA and the mother's nuclear DNA,[100] which have not been adequately addressed. Evidence from animals suggests that mismatching may occur between the nuclear DNA and the mitochondrial DNA. The side effects of any mismatching could be more serious than the mitochondrial diseases themselves.

19. If these regulations are approved, the UK will become the only country in the world to permit such a practice. The fact other countries are not going ahead strongly suggests that it is far more significant than just "changing a faulty battery in a car" – a comment from the Chief Medical Officer.[101] The UK is in danger of becoming a bioethical rogue state.

20. We would encourage the Committee to take note of the fact that an advisory panel to the Food and Drug Administration in the US also considered this issue in February 2014, when many members questioned the ethics of the procedure. Dr Evan Snyder, head of the FDA's Cellular, Tissues and Gene Therapies Committee, said himself: "There is just not enough preclinical data to suggest how to [treat patients] and how to do it safely."[102] Similarly, a professor of public health said: "There's so much at stake here in terms of the mistakes we could be making that you would need to have an overwhelming reason to enter into this arena".[103]

LACK OF PUBLIC SUPPORT

21. There has been a great deal of misinformation about the public's view on this issue. A majority of the public opposes the plans. The 2013 HFEA report of public opinion was publicised in a biased manner and sought to minimise the fact that over half the respondents in the public consultation said that the law should not be changed (558 out of 1,055 in answer to question 6). Furthermore, of the 1,857 responses to the Government's consultation earlier this year, 1,152 were opposed to the introduction of the new techniques and 700 were in support. This means 62% of respondents opposed the plans.

22. Similarly, public opinion polls which explain the issue show that the public have rejected the idea. A poll conducted by ComRes at the end of August 2014 found that while 18 per cent of those surveyed were in favour of the procedure, 46 per cent were opposed to the plans.[104]

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[100] *Guardian Online*, 'Safety concerns remain over three-person IVF', 22 July 2014, see http://www.theguardian.com/science/2014/jul/22/three-person-ivf-mitochondria-dna as at 22 October 2014

[101] *The Telegraph Online*, 28 July 2014, see http://www.telegraph.co.uk/health/10994805/Government-is-misleading-public-over-genetically-modified-babies-scientists-claim.html as at 22 October 2014

[102] *USA Today*, 26 February 2014, see http://www.usatoday.com/story/news/nation/2014/02/26/three-parent-dna-embryo/5837783/ as at 20 October 2014

[103] *New York Times Online*, 27 June 2014, see http://www.nytimes.com/2014/06/29/magazine/the-brave-new-world-of-three-parent-ivf.html?emc=edit_tnt_20140627&nlid=47387068&tntemail0=y&_r=2 as at 22 October 2014

[104] 'Three Parent Embryo Survey', *ComRes*, 20-22 August 2014, page 3, see http://www.comres.co.uk/polls/CARE_3-PARENT_CHILDREN_POLL_SEPTEMBER_2014.pdf

23. Scientists are already researching less controversial methods of addressing mitochondrial diseases. We recommend that the focus of investment and funding turns to finding treatments for those who are currently affected by mitochondrial disorders. Further research should be conducted into mutant mitochondria and the role of mitochondrial DNA rather than pushing forward regulations for controversial techniques that will do nothing for those already suffering and that may cause immense harm to future generations.

October 2014

Correspondence submitted by Caroline Simons (MIT0025)

INTRODUCTION

I have undertaken considerable research on the scientific, bioethical and legal aspects of mitochondrial donation during the past twelve months. This is the subject matter of my postgraduate dissertation, completed in partial fulfilment of the requirement for the degree of Master of Laws at Trinity College Dublin. I will be conferred with this degree in November 2014. I make this submission hoping to assist the work of this committee and in my personal capacity.

EXECUTIVE SUMMARY

This submission focuses on questions which arose during my research regarding the scientific assumptions on which the proposed techniques described as Mitochondrial Donation rely. I present scientific research and data which does not appear to have been considered previously in the context of the proposed techniques. I consider –

1. The incidence of mitochondrial and, in particular, mitochondrial DNA (mtDNA) related disease;
2. The assumptions regarding the inheritance mechanism of mtDNA mutations;
3. mtDNA-related disease in children;
4. Cytoplasmic/ooplasmic transfer in the US and EU;
5. Research regarding gene therapies for mtDNA disorders, one of which is in clinical trial for the most common mtDNA disease, and which do not appear to be discussed in the context of the proposed techniques;
6. The utility or otherwise of Preimplantation Genetic Diagnosis (PGD) as a diagnostic tool for mtDNA disorders;
7. The possibility of mito-nuclear haplotype mismatch.

> *'Given the lack of treatments and limitations of prenatal and preimplantation diagnosis, attention has focused on prevention of transmission of mitochondrial disease through germline replacement therapy. Because mitochondrial DNA is strictly maternally inherited, two approaches have been proposed.'* [105]

With few exceptions, scientific commentary on Maternal Spindle Transfer (MST) and Pronuclear Transfer (PNT) (the techniques described as 'Mitochondrial Donation'), has welcomed their promise for affected individuals. The scientific panel appointed by the HFEA advises that mitochondrial DNA (mtDNA) 'is inherited exclusively from the mother through the mitochondria present in her eggs,'[106] and that the proposed techniques 'have the potential to

[105] Amato P, Tachibana M, Sparman M and Mitalipov S, Three-parent in vitro fertilization; gene replacement for the prevention of inherited mitochondrial diseases. *Fertil Steril* 2014; 101(1): 31-5.

[106] HFEA, *Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: update* March 2013, 3.

be used for all patients with mtDNA (mitochondrial) disorders.'[107] Commentators speak in terms of the techniques offering the wonderful possibility of eradicating mitochondrial disease. Women who wish to avail of MST and PNT will have to undergo IVF, at some physical, emotional and financial cost to themselves and their families and with no guarantee of a child at the end. Children born of these techniques would have a modified genome and these modifications would be inherited by future generations. Close examination is required therefore, of the assumptions on which these techniques are based.

The Swedish National Council on Medical Ethics has monitored the development of these techniques. In a recent report it advised that, while the majority of its Council did not object to MST or PNT in principle, the current limited scientific basis for assessing the risks they pose make it unethical to use these techniques in clinical trials on humans.[108] I would agree with this conclusion and respectfully submit that further research may be required as follows -

1. *Incidence of mitochondrial disease*

Mitochondrial disorders are considered rare, but cumulatively affect significant numbers of people. It is estimated that at least 1 in 10,000 adults in the UK is affected by mitochondrial disease[109] and 1 in 6,500 children is born with a serious mitochondrial disorder.[110] These statistics do not indicate what proportion of adults and children suffer from disease caused by mitochondrial rather than nuclear gene mutations. They give no indication therefore, of the numbers of individuals who might hope to benefit from the proposed techniques. Further research is necessary to provide this information.

In Sweden and Australia it is estimated that 5 in 100,000 children have mitochondrial disease, with mutations in the mitochondrial genome accounting for 15% of that figure.[111]

Professor Turnbull estimated in 2012 that the research team at Newcastle would see 10-20 women annually who have such a high mtDNA mutation load that 'replacing the mitochondrial DNA appears to be their only option for having a genetically related healthy child.'[112] In 2014, the Department of Health advised that the research team at Newcastle estimated that the proposed techniques would 'prevent around 10 children a year suffering from serious mitochondrial disease.'[113] Responses to parliamentary questions a month later suggested that this number might be much higher, between 10 and 20, and increasing to about 80.[114] The basis for these numbers is unclear. The NHS provides a free clinical and diagnostic service for mitochondrial disease at specially designated centres in the UK. It made 17 prenatal diagnoses of mtDNA-related mitochondrial disorders between April 2007 and January 2013, an average of

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[107] Ibid. 5.

[108] The Swedish National Council on Medical Ethics Mitochondria replacement in cases of serious diseases – ethical aspects 2013: 2: Summary of a Report 11 November 2013, 9 www.smer.se/wp-content/uploads/2013/11/Mitochondria-replacement-sammanfattning-eng2.pdf

[109] Craven L et al. Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature* 2010; 465(7294); 82-5.

[110] Department of Health, Government response to the consultation on draft regulations to permit the use of new treatment techniques to prevent the transmission of a serious mitochondrial disease from mother to child. July 2014.

[111] DiMauro S, Davidzon G, Mitochondrial DNA and Disease *Annals of Medicine* 2005; 37(3) 222-32.

[112] Nightingale K, Working at the edge: a Q & A with Doug Turnbull. Medical Research Council, Insight, 3 December 2012. www.insight.mrc.ac.uk/2012/12/03/working-at-the-edge-a-qa-with-doug-turnbull/#more-1403

[113] Department of Health, A consultation on draft regulations to permit the use of new treatment techniques to prevent the transmission of a serious mitochondrial disease from mother to child. February 2014. Para 1.11; HL Deb 15 July 2013 cWA87.

[114] HC Deb 10 March 2014 c97W.

3 diagnoses per annum. It has been suggested that the availability of a free NHS nationally commissioned clinical and diagnostic service for mitochondrial disorders in adults and children makes it unlikely that these tests are done elsewhere and that the NHS figures are indicative of actual incidence.[115] I have been unable to ascertain if these NHS statistics include homoplasmic mtDNA-related disorders.

2. *mtDNA inheritance*

Prevailing scientific theory and genetic advice given to patients suggests that men with mtDNA mutations will not transmit those mutations to their offspring.[116] The Nuffield Council on Bioethics advised in 2012 that there was only one study[117], reported in 2002, in which paternal mitochondria were found to persist in the offspring and stated that there was 'no published evidence of father-to-child transmission of inherited disorder.'[118] Another paper published in 1983 reports evidence of three cases of paternal transmission of mitochondrial disease.[119] One scientist commenting on this study remarked that although the authors recognised that paternal transmission was possible, the prevailing scientific view of inheritance through the maternal line may have prompted them to offer other explanations as well. He emphasised the need to interrogate data that appear to be 'outside of the box'.[120] The midpiece of the sperm, which contains the paternal mitochondria, can be identified in the embryos of the majority of mammals, including humans, but its ultimate fate is unclear.[121] The preponderance of scientific research proposes that sperm mitochondria are eliminated in the preimplanted embryo, and that this elimination shortly after fertilisation is necessary to prevent 'potentially dangerous mitochondrial-genomic dysfunction.'[122] Some research proposes that when assisted reproductive technologies (ART) are performed, the rate of paternal mtDNA transmitted to the embryo may be increased and that the impact of paternal mtDNA cannot be ignored.[123] Although paternal transmission of mitochondria in animals is 'both common and recurring,'[124] the balance of scientific opinion is firmly of the view that paternal transmission of mitochondria in humans appears to be a rare event. Nevertheless, the mechanism of inheritance is imperfectly understood and further research is necessary to ascertain if paternal transmission of mitochondrial mutations might be significant, particularly in the context of ART, in some conditions and in some individuals. Paternal transmission of mtDNA mutations would not be avoided by the proposed techniques.

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[115] Nesbitt V et al. A national perspective on prenatal testing for mitochondrial disease Eu*ropean Journal of Human Genetics* 2014; 22(11):1255-1259.

[116] Taylor RW and Turnbull DM, Mitochondrial DNA mutations in human disease *Nat Rev Genet* 2005; 6(5); 389-402.

[117] Schwartz, M and Vissing, J Paternal Inheritance of Mitochondrial DNA *N Engl J Med* 2002; 347: 576-80.

[118] Nuffield Council on Bioethics, Novel techniques for the prevention of mitochondrial DNA disorders: an ethical review (2012) 19, para 1.10.

[119] Egger, J and Wilson, J Mitochondrial inheritance in a mitochondrially mediated disease *N Engl J Med* 1983; 309(3): 142-6.

[120] Gustafson AW, Letter to the Editor Paternal Inheritance of Mitochondrial DNA *N Engl J Med* 2002; 347; 2081-2.

[121] Ankel-Simons F, Cummins JM, Misconceptions about mitochondria and mammalian fertilization: Implications for theories on human evolution *Proc Natl Acad Sci* 1996; 93: 13859-13863.

[122] Song WH et al. Regulation of Mitochondrial Genome Inheritance by Autophagy and Ubiquitin-Proteasome System: Implications for Health, Fitness, and Fertility *Biomed Research International* 2014; Article ID 981867, 16 pages.

[123] Ming-Luo S, Schatten H, Sun QY, Sperm Mitochondria in Reproduction: Good or Bad and Where Do They Go? *Journal of Genetics and Genomics* 2013; 40: 549-556.

[124] Chinnery PF, Hudson G, Mitochondrial Genetics *Br Med Bull* 2013; 106(1): 135-59.

3. *Mitochondrial disease in children*

Mitochondrial disease occurs in three ways – first, where there is mutation in the mitochondrial genes, secondly, where the nuclear genes implicated in mitochondrial function are mutated and thirdly, where there is a build-up of mitochondrial damage. This damage is caused by outside factors such as viruses, the unintended effect of antibiotics or environmental toxins. More than 1000 nuclear genes are implicated in mitochondrial function.[125] In 2012, it was reported that 228 nuclear genes and 13 mitochondrial genes have been linked to human disease.[126] The scientific literature suggests that in the majority of cases, mitochondrial disease is probably caused by mutations in nuclear genes, particularly in the case of children.[127] Most of the 228 nuclear gene mutations that have been reported serious childhood disease.[128] The proposed techniques would not prevent transmission of mutations in the nuclear genes. Therefore the proposed techniques do not have the potential to be used for all patients with mitochondrial disorders.

Research indicates that at least a third of adults with mitochondrial disease have a sporadic disorder which is unlikely to be transmitted to future children.[129] A study published in 2004 has also cast doubt on the maternal inheritance of mtDNA mutation theory and attributed it to investigative bias. This study of 250 families concluded that for children with mtDNA mutations, maternal inheritance is the exception rather than the rule.[130]

4. *Cytoplasmic Transfer and 'three-parent children'*

The proposed techniques would not the first instances of generating embryos from eggs which might contain the mitochondria of two women. In the 1990s, some fertility clinics in the US performed cytoplasmic/ooplasmic transfer (CT) in the hope of 'rejuvenating' the eggs of women with certain fertility problems. This involved the injection of small amounts of cytoplasm from a donor's egg into the egg to be fertilised. The cytoplasm injected might contain mitochondria. Three of these clinics reported over two dozen births using this technique.[131] Donor mtDNA was found in amniocytes, placenta, and fetal cord blood of some of the offspring.[132] Donor mitochondrial DNA was found in one clinic in the blood of two of the children. The FDA intervened after a high incidence of birth defects or developmental anomalies was observed in the offspring produced by CT.[133] A follow-up of children produced by this technique began earlier this year. It appears prudent that account would be taken of this follow-up study before

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[125] Pagliarini DJ et al. A mitochondrial protein compendium elucidates complex disease biology *Cell* 2012; 134(1): 112-23; Broad Institute Human MitoCarta: 1013 mitochondrial genes www.broadinstitute.org/pubs/MitoCarta/human.mitocarta.html

[126] Koopman WJH, Williams PHGM, Smeitink JAM, Monogenic mitochondrial disorders *N Engl J Med* 2012; 366(12) 1132-1141.

[127] Thorburn DR, Mitochondrial disorders: prevalence, myths and advances *J Inherit Metab Dis* 2004; 27(3): 349-62 ; Debray FG, Lambert M, Mitchell GA, Disorders of mitochondrial function *Curr Opin Pediatr* 2008; 20(4): 471- 82 ; Goldstein AC, Bhatia P, Vento JM, Mitochondrial Disease in Childhood: Nuclear Encoded *Neurotherapeutics* 2013; 10(2): 212-26.

[128] For a list of the diseases which are known to be caused by mtDNA disorders, see Koopman (n 18) Supplementary Appendix.

[129] Chinnery PF, Inheritance of mitochondrial disorders *Mitochondrion* 2002; 2(1-2) 149-55; Thorborn (n 19).

[130] Thorborn (n 19).

[131] US Federal Drug Administration, BRMAC Briefing Document for Day 1 (9 May 2001) www.fda.gov/ohrms/dockets/ac/02/briefing/3855b1_01.pdf

[132] Song (n 15); Brenner CA et al. Mitochondrial DNA heteroplasmy after human ooplasmic transplantation *Fertil Steril* 2000; 74(3): 573-8; Barritt JA et al. Spontaneous and artificial changes in human ooplasmic mitochondria *Human Reproduction* 2000; 15(Suppl 2); 207-17; Barritt JA et al. Mitochondria in human offspring derived from ooplasmic transplantation: brief communication *Human Reproduction* 2001; 16(3)513-6.

[133] Barritt JA et al. Rebuttal: interooplasmic transfers in humans," *Reprod Biomed Online* 2001; 3(1): 47–8.

proceeding with the proposed techniques. Indeed, a follow-up of a sample size that would provide stronger statistical data should be possible, given the availability of CT in clinics in other jurisdictions, including, in the EU, Austria, Cyprus, Germany and Spain.[134]

5. *Gene therapy for mtDNA mitochondrial disorders*

There has been much discussion of the feasibility or otherwise of screening procedures to identify embryos without mitochondrial mutations for implantation, and scientists are divided on their efficacy. But there has been virtually no discussion, in the context of the proposed techniques, of gene therapies to address mitochondrial disease. The most common mitochondrial disorder is LHON, which is believed to affect 1 in 25,000 people. The mutation for this disease is homoplasmic, ie, it is carried in all of a woman's mitochondria. All of her children will inherit the mutation, but not all will develop the disease. 10% of her daughters and 50% of her sons will have a lifetime risk of blindness. It has been suggested that women carrying this mutation would be suitable candidates for MST and PNT given that the current testing or selection techniques cannot avoid transmission of the mutation. There are other possibilities on the horizon. Research on a gene therapy for one form of LHON (the form involving the ND4 subunit) has been found to prevent LHON vision loss in rodents and appears to be promising.[135] A Phase 1 clinical study in humans began in February 2014 and collection of the data for assessment of the outcome of that trial will be completed in December 2015.[136] This therapy uses the same method that has been successful in restoring sight in an adult gene therapy clinical trial for Leber congenital amaurosis and which has been extended to children and appears to have equal success. Scientists involved in these trials believe that gene therapy applications have vast potential for a wide range of other mitochondrial conditions.[137] Research into gene therapy to address other mitochondrial diseases is ongoing. Recent scientific papers describe techniques to deliver healthy mitochondria to affected cells.[138]This approach would not create inheritable modifications of the human genome.

6. *Preimplantation Genetic Diagnosis (PGD)*

Whether or not PGD is useful for the prediction of mtDNA disease depends on the mother being heteroplasmic and the mutation threshold for clinical expression of a mitochondrial disorder being known. The literature suggests that PGD for human heteroplasmic mutations appears to be safe and the results reliable if carried out on two blastomeres,[139] and that PGD followed by selection and implantation of the 'cleared' embryo gives carriers of heteroplasmic mtDNA a fair chance of having healthy genetically related children. There is no consensus about this in the scientific community.[140]However, it is noteworthy that the HFEA has licensed PGD testing for mtDNA mutations, including LHON which is a homoplasmic mutation.[141]

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[134] Cytoplasmic Transfer Abroad – Medical Tourism Guide http://m.health-tourism.com/cytoplasmic-transfer/

[135] Farrar GJ et al. Mitochondrial disorders: aetiologies, models systems, and candidate therapies *Trends in Genetics* 2013; 29(8); 488-97.

[136] http://clinicaltrials.gov/ct2/show/NCT02064569?term=Leber&rank=5

[137] Professor John Guy, University of Miami Miller School of Medicine http://med.miami.edu/news/clinical-trial-uses-gene-therapy-to-target-mutations-in-mitochon

[138] Kitani et al. Direct Human Mitochondrial Transfer: A Novel Concept Based on the Endosymbiotic Theory *Transplantation Proceedings* 2014; 46(4): 1233-6; Liu CS et al. Delivering healthy mitochondria for the therapy of mitochondrial diseases and beyond *International Journal of Biochemistry and Cell Biology* 2014; 53: 141-6.

[139] Sallevelt et al. Preimplantation genetic diagnosis in mitochondrial DNA disorders: challenge and success *Journal of Medical Genetics* 2013; 50(2): 125-32 ; Steffan et al. Data from Artificial Models of Mitochondrial DNA Disorders Are Not Always Applicable to Humans *Cell Reports* 2014; 7(4) 933-4;

[140] Mitalipov points to the limitations of PGD, which selects for the lowest ntDNA mutation level and may reduce, but not eliminate, the risk of transmitting mutations. He observes that PGD cannot ensure the production of any embryos with sufficiently low mutation levels to render them suitable for transplantation. Mitalipov et al.

7. *Mito-nuclear haplotype mismatch*

Apart from generating the energy required for the activities of the cell, and notwithstanding their characterisation in some discussion as a cell's batteries, it is generally accepted that mitochondria influence the expression of nuclear genes, including those implicated in cognitive abilities, fertility, ageing and longevity.[142] [143] It has been suggested that fundamental biological processes in humans depend on coordinated interaction between the mitochondrial and nuclear genomes in our cells, and that an understanding of this mito-nuclear interaction is required to inform scientific understanding of mtDNA-related disease. If mtDNA and nDNA typically have a symbiotic, highly specific and coordinated working compatibility in each cell, and if as has been said 'our genes show all the cardinal signs of selection for compatibility with mitochondria',[144] then is it not likely that MST and PNT could precipitate a mismatch in mtDNA and nDNA haplotypes? Some scientists certainly think so,[145] and a recent study of mice appears to support this view.[146] Some scientists believe that an nDNA mutation might express more seriously when combined with an incompatible mtDNA and so cause mitochondrial disorders in offspring created by the proposed techniques.[147] They conclude that mito-nuclear interaction is poorly understood and further research is required.[148]

8. *Conclusion*

In summary, current scientific knowledge indicates that 'Mutations that arise in [mtDNA] may be sporadic, maternally inherited or Mendelian in character.'[149] Most cases of mitochondrial disease occur as a result of mutations in the nuclear genes, particularly in the case of children, and the majority of cases of mtDNA-related mitochondrial disease do not occur as a result of maternal inheritance of faulty mitochondria. Thus, the fact that a woman already has a child with a mitochondrial disease is not a sufficient indicator that she might be a suitable candidate for proposed techniques. The generation of accurate clinical and genetic characterization of patient cohorts for a particular mitochondrial disease will greatly aid in defining the risk:benefit of any reproductive intervention. The resolution of such data will be improved using sequence data from the whole mtDNA genome (and eventually encompassing whole nuclear genome

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Limitations of Preimplantation Genetic Diagnosis for Mitochondrial DNA Diseases *Cell Reports* 2014; 7(4): 935-7; Amato et al. (n 1).

[141] HFEA, PGD conditions licensed by the HFEA www.hfea.gov.uk/cps/hfea/gen/pgd-screening.htm ; HFEA, New PGD conditions licensed by the HFEA between 1 April 2012 and 31 March 2013 www.hfea.gov.uk/cps/hfea/gen/List%20of%20New%20PGD%20Conditions%20Licensed%20by%20the%20HFEA.pdf

[142] Roubertoux Pl et al. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice *Nat Genet* 2003; 35(1): 65-9;

[143] Reinhardt, K, Dowling, DK & Morrow, EH. Mitochondrial Replacement, Evolution, and the Clinic', *Science* 2013; 341: 1345-6.

[144] Lane N, One baby, two mums *New Scientist* (London, 7 June 2008): 38-41.

[145] Reinhardt K, Dowling D, Morrow EH, Mitochondrial Replacement, Evolution and the Clinic *Science* 2013; 341(6152)1345-6.

[146] Burgstaller et al. mtDNA segregation in Heteroplasmic Tissues is Common In Vivo and Modulated by Haplotype Differences and Developmental Stage *Cell Reports* 2014; 7(6) 2031-2041.

[147] Moreno-Loshuertos R et al. Differences in reactive oxygen species production explain the phenotypes associated with common mouse mitochondrial DNA variants *Nat Genet* 2006; 38(11): 1261-8.

[148] Wolff JN et al. Mitonuclear interactions: evolutionary consequences over multiple biological scales *Phil Trans R Soc B* 2014; 369(1646) 20130443.

[149] Craigen WJ, Mitochondrial DNA Mutations: An Overview of Clinical and Molecular Aspects *Methods in Molecular Biology* 2012; 837(3) 3-15.

sequence data) to evaluate possible modulating effects of different mitochondrial haplotypes and nuclear backgrounds.

Mitochondria have been described by one scientist as the answer to the question of life, the universe and everything, or, if not the answer, he suggests that they make some sense of the shape of life.[150] The FDA in the US appears to have taken the view that further research is required before the proposed techniques could be considered for clinical application. The Swedish National Council is similarly cautious and has said that the limited scientific basis for assessing the risks these techniques pose makes it unethical to use them in clinical trials on humans. This submission raises certain issues in relation to the proposed techniques which may not have been considered previously. It respectfully suggests that further research is required before consideration is given to allowing clinical trials to proceed.

October 2014

Correspondence submitted by Emily Huezo (MIT0026)

MITCHONDRIAL DONATIONATION TECHNIQUES SHOULD NOT BE APPROVED WHATSOEVER BECAUSE:

1. *These techniques exploit women*

   Women and their bodies are treated as mere stuff that can manipulated and used in any which way. The dignity of the body of women is degraded to machinery whose only value is its mechanistic function. Women's bodies have are more than a machine and should be treated as such.
2. *The techniques exploit human life*

   Like the exploitation of women these techniques treat human life as merely experiments. No regard is given to the uniqueness of each human life, rather life is deemed as worthy only because of the worth imposed on it by scientific advancement. Human life is seen as something to be controlled and manipulated rather than as an unrepeatable and unique gift.
3. *Three parents are involved*
   Disregard is given to the unique relationship of a mother and father toward a child. A three parent embryo removes this uniqueness and makes for an unnatural relationship between the child and its parents. This may also have serious legal repercussions.

4. *These techniques cause decreased viability*

   According to Cheng Y et al., ooplasm transfer has been associated with decreased viability of offspring in mice.[151]
5. *Therapeutic alternatives should be considered*

   Therapeutic alternatives that do not destroy life and do not exploit women should be seriously considered before moving forward with the previously mentioned experiments.

October 2014

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150 Lane N, Mitochondria and the Meaning of Life: Power, Sex Suicide (Oxford University Press 2005) 321.

151 Chen Y et al., Effects of ooplasm manipulation on dna methylation and growth of progeny in mice, Biology of Reproduction 80, 464, 2009

Correspondence Submitted by Julian Malcolm (MIT0027)

SUMMARY

The Results of Mitochondrial Replacement Techniques are clear:

- Three parents
- One child
- Unnecessary and dangerous introduction of a myriad of new health risks

Mitochondrial replacement would attempt to prevent *one* health risk by introducing an entirely *new* health risk. We want to see real health solutions provided for children who suffer from Mitochondrial Disease but Mitochondrial Replacement are not the answer for these or any future patients and *ultimately will cause more problems than they will solve.*

REALITY

- A child who has been conceived using Mitochondrial Replacement Techniques would *bear the genetic traits of not two, but three parents.*

- *Mitochondrial replacement techniques will involve human cloning*, (also referred to as Somatic Cell Replacement)

- Mitochondrial Replacement Techniques will *provide zero therapeutic benefit to any current patients*

- Mitochondrial Replacement Techniques will *ensure that additional children will be born with a host of new health risks*.

- *The vast majority of mitochondrial disease cannot be addressed using mitochondrial replacement techniques*.

- Mitochondrial Replacement Techniques *could only be attempted in a* ***minority of the cases*** *of mitochondrial disease*. **These techniques would not be applicable to mitochondrial disease caused by nuclear DNA, which makes up the majority of cases**, nor would they prevent mitochondrial disease that arises due to spontaneous mutations or deterioration with age.

QUESTIONS:

- Is this technique merely the replacement the unhealthy mitochondria in a woman who carries the disease, with the healthy mitochondria from a donor woman, during the process of IVF?
- What are the potential health risks for intended mothers, donors and future or existing children?
- The United States Federal Drug Agency Advisory Committee has ruled these techniques to be unsafe. Why should US citizens' health be treated with more care and caution than for that of citizens in the UK?

ASSOCIATED CONCERNS, HEALTH RISKS AND OBJECTIONS:

There are four procedures for Mitochondria Replacement, each having serious concerns. These are:

Embryonic Transfer Procedures

- All Embryonic Transfer Procedures require the **destruction of human life**. A donor is **donating her offspring, not merely her eggs**.
- Mitochondria are "donated" not by a donor mother but by an embryo who has been **conceived for the explicit purpose of "biological harvesting**."
- The somatic cell nuclear transfer process in embryonic cell transfer **is quite plainly "human cloning."** There is no difference in this procedure than what would occur in adult cloning.

Non-Embryonic Transfer Procedures

- Ooplasm transplant has been demonstrated to be associated with **decreased viability and negative impacts on growth and development in children**. If the objective is to help ensure healthy development in children then ooplasm transplant is demonstrated to function in opposition to this end. (See Cheng Y et al., Effects of ooplasm manipulation on dna methylation and growth of progeny in mice, Biology of Reproduction 80, 464, 2009. Also see Reinhardr K et al. Mitochondrial replacement, evolution and the clinic, Science 341, 1345, 2013)
- Egg extraction process is already shown to be a problematic civic concern as the procedure poses health risks directly to the woman who acts as donor and raises the wider concerns associated with trafficking. The number of eggs required to garner success quite frankly exceeds what can be provided from amongst the pool of regionally available fertile women. This of course raises all sorts of concerns about the **commodification of women's bodies and the promotion of biological colonialism**.

ALTERNATIVE THERAPIES FOR MITOCHONDRIAL DISEASE

There are a wide range of non-problematic and more effective possibilities for prevention and treatment of mtDNA disease. These treatments include:

- Transfer of mitochondria from bone marrow
- Mitochondrial transfer from adult stem cells
- Removal of mutant mitochondria via TALENs
- Genome editing techniques using CRISPR-Cas9
- Targeted RNA import
- Rapamycin drug treatment

RESPONSES TO ARGUMENTS FOR MITOCHONDRIAL REPLACEMENT:

The personal testimonies of the parents whose children have endured this devastating disease is heart wrenching. Doubtless, the committee is already familiar with these stories and will be presented with further accounts. One often hears a retort somewhere along the lines that those who have not suffered a particular trial have no business impending the efforts of the afflicted to find relief. I wanted to take the unenviable task of replying to those real individuals who have suffered and express that reality that the methods of Mitochondrial Replacement will not alleviate the pain that they and their children are experiencing. What is more, these techniques will cause more pain for others.

We owe it to those who suffer to provide honest answers to their questions that are both empathetic and also take into consideration the whole of this problem. The questions below are posed in the good faith effort to summarize the public arguments that have called for support of Mitochondrial Replacement. The answers are also posed in good faith, in an effort to speak to the real and pressing personal concerns that have sparked the current review.

Q: Adoption is not something that I want to have to consider. I want to be able to look at my baby say, "she is mine, she has the same traits as me." That is important to me. Everyone else has the opportunity to do that with their children. Shouldn't I be able to have the same opportunity?

A: All parents who conceive undergo some level of uncertainty. That is the fear and joy of parenthood. There is always some element of surprise with a new baby and no one can guarantee who this child will be and what strengths and weaknesses they will carry. Neither is there any way to ensure that we can somehow level the field of parenthood to provide a false guarantee of equal level of uncertainty, with regards to health or any other personal outcomes. Of course we should work to prevent and cure health problems but not at the risk of making an artifice of our children's genetics. The science that would seek to pre-determine the particular traits of our children has been referred to as the pursuit of "designer babies." We instinctively know that there is something dangerous about this sort of scientific endeavor.

Q: All of the child's characteristics are contained in nuclear DNA. This isn't a three-parent baby. Isn't mtDNA an overwhelmingly small and inconsequential part of the total make-up human DNA?

A: It is simply not true that a child only get its characteristics from nuclear DNA. It is true that mtDNA accounts for less than 1% of human DNA but genetics are not simply a matter of quantity. Neither is this an accurate way to portray the role of mtDNA. Just because the vast majority of DNA is nuclear does not mean that there is not a large abundance of non-nuclear DNA in the body. The particular substances of DNA are of importance and it is important to understand that the coding region of the mtDNA is the region of a person's DNA that contains the most ancestral markers. In dealing with mitochondrial related disease itself we cannot neglect that mtDNA is so pivotal that it is essential for the survival of the individual. mtDNA cannot be treated as a negligible ingredient in the medical quest to treat mtDNA related illness. Furthermore, if you are so determined to have a baby with your genetics, why then would you insert someone else's genes into your baby? No manipulation of language will change the fact that this child will be tasked with parental identity issues that are unprecedented in human history.

Q: Don't you think it is unfair to refer to or to associate Mitochondrial Replacement with "designer babies"?

A: While the debate over "designer babies" typically involves the manipulation of particular or isolated DNA and mitochondrial replacement involves a broader introduction of gene line transfer it is the explicit objective in both scenarios that genetic traits will be altered in children. It is simply bad science to suggest that mtDNA replacement has no bearing on the characteristics of offspring when the intended effect is to change a portion of DNA that carries maternal inheritance patterns.

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Correspondence submitted by Professor Stuart A. Newman (MIT0029)

I hereby submit the statement below for consideration by the Members of the House of Commons Science and Technology Committee in their deliberations on the safety and efficacy of so-called "mitochondrial replacement" techniques.

Deceptive Labeling of Radical Embryo Construction Methods
Techniques now exist for generating infants which, if implemented, would constitute the first cases of large-scale human genetic engineering. These techniques are widely referred to – by their scientist-creators and other proponents, by journalists, by bioethicists, by members of regulatory panels, by legislators, and even by some critics of the procedure – as "mitochondrial transfer" or "mitochondrial replacement." These descriptions are not only scientifically inaccurate; they are also easing the way to public acceptance of these manipulations. What exactly are these techniques? An isolated nucleus from the egg of one woman is inserted into an enucleated (nucleus-lacking) egg of another woman. Done before fertilization, it is called "maternal spindle transfer" (MST). Done after, it is called "pronuclear transfer" (PNT). In fact, no transfer of mitochondria (the organelles that extract energy from fuel molecules and make it available for the cell's functions) is involved in these "three-parent" procedures. So why are they referred to as mitochondrial "transfer" or "replacement"?

The techniques are being promoted as a way of circumventing mitochondrial mutations, which can lead to severe disease. It is understandable that an affected woman who intends to become pregnant would seek to avoid passing down this genetic predisposition to her offspring. Methods such as MST and PNT represent radical interventions in the reproductive process that, if accurately portrayed, would stir fears in prospective parents and rightly attract the attention of legislators and regulators. The laboratory scientists and doctors for whom these women are clients (not patients – their own conditions are not being treated), thus have an interest in minimizing the perceived scale of what they are proposing to do.

Since it is true that nuclear genes of an affected woman or couple will eventually find themselves in the presence of mitochondria from a second woman, from the viewpoint of the first woman the mitochondria of her egg are "replaced." But this is only mitochondrial replacement in the sense that someone who moves into a new home may experience "refrigerator replacement," i.e., only by employing a highly idiosyncratic (and misleading) use of the term.

Focusing only on mitochondria ignores the other significant features of the second woman's egg such as its cytoplasmic and membrane composition and structure. Shifting attention in this fashion must raise questions about disingenuousness of the methods' proponents. In fact, the manipulation of the second woman's egg (i.e., the egg that will actually be implanted) constitutes a "genome transfer" or "genome replacement." Choosing a conceptual frame based solely on who is soliciting or paying for the procedure (i.e., the woman seeking to avoid passing on a genetic predisposition for mitochondrial disease) is not motivated by scientific or medical concerns.

In biological terms, both MST and PNT are very much like cloning by nuclear transfer, the methodology that produced Dolly the sheep. Like cloning, the techniques involve replacement of an egg's nucleus by a nucleus from another cell. When cloning, the

transferred nucleus is from a differentiated cell of a fully developed animal (or potentially, a person), making the resulting organism a genetic "copy" of the nucleus donor. When undertaking MST and PNT, the transferred nucleus is from an egg or a fertilized egg, so that the resulting organism will have a novel genome. Otherwise, however, the hazards of cloning also pertain to MST and PNT, since the manipulations are the same. Clones tend to die prematurely, as happened with Dolly, or exhibit enlarged organs and metabolic abnormalities. Some human embryos constructed by MST unexpectedly had unbalanced chromosomal duplications (aneuploidy). This is the case because unlike the sorts of cellular aberrations repeatedly encountered over the course of evolution – breaks in DNA, the unfolding of protein molecules – the experimental combination of fragments of two broken cells generated by cloning or the two proposed techniques have no inbuilt mechanisms to correct the range of functional and developmental defects inevitably associated with their construction.

It is unfortunate that few science journalists have the training or inclination to assume a critical stance toward the assertions of the scientists they interview. It is therefore common to see these procedures described in the popular and scientific press as the mere replacement of the 37 mitochondrial genes (compared to the 20-25,000 of the nucleus). The scientists who promulgate the transfer/replacement imagery and those bioethicists who do the same know better. Indeed, bioethicists should be scrutinizing the scientists' practice and language as opposed to promoting their fantasies and business models. Their collusion in these deceptions is inexcusable.

Moreover, anyone familiar with the relevant science would have been aware, over the period during which the techniques were being evaluated by the British Human Fertilisation & Embryology Authority (HFEA) and the U.S. Food and Drug Administration (FDA), of evidence that mitochondria are not (as the impact-minimizing refrain has it) mere energy-providing organelles. The very existence of mitochondrial DNA mutations affecting hearing, vision, pancreatic function and neuromuscular activity (the justifications of the entire enterprise), would be enough to tell us this. Indeed, in the past two years the evidence for the non-passivity of the mitochondria has become inescapable. Since mitochondria are active participants in cell function and organismal development, integration among coevolved nuclear and mitochondrial systems would contraindicate arbitrary mixing and matching. (The engines of a Jaguar and a Rolls-Royce do essentially the same thing, but they are not interchangeable.) This adds an array of hazards to MST and PNT that go well beyond those they share with cloning.

A prospective child made by MST or PNT would be the result of an evolutionarily unprecedented experiment with known, or easily anticipated, hazards. Juxtapose this against the fact that the biological identity and long-term health of the three biological parents undertaking MST or PNT are not directly at risk in the procedures. It is, therefore, entirely unwarranted to make their perspective (or more specifically that of the nuclear gene donor) the one from which the procedure is judged thereby allowing the techniques to be characterized as being of minimal impact. Rather, the perspective of the individual brought into being by the procedures should be paramount. Combining fragments of two damaged eggs to produce a human embryo is, despite the rhetoric of mitochondrial transfer and replacement, large-scale manipulation of nuclear genes. Its backdoor admittance to the repertoire of assisted reproduction techniques in the guise of

being a trivial tweak bodes ill for future attempts to regulate gene transfer methods for any other purpose.

A kind of *omertà* among scientists and bioethicists has prevented a significant number of them from representing to the HFEA and FDA, and the press, the gravity of these alterations. But the health implications and the eugenic outcomes these procedures would enable are too great to ignore.

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