Replacing the mitochondrial genome: When might it be appropriate and when is it not

Peter Braude OBE FRCOG FMedSci
Emeritus Professor of Obstetrics and Gynaecology
Division of Women’s Health
King’s College London
CONFLICT OF INTERESTS DECLARATION

I have served as external advisor to HFEA, Nuffield Council on Bioethics, UK Parliament, and Singapore National Bioethics Committee

I no longer work in any clinic

I don’t sell any products

I do not have shares in or work for any company related to IVF, PGD or PGS or mitochondrial donation

I will try and present facts and data openly available

www.oc2017.cme-congresses.com
IVF rule change could allow 'three parent' babies

28 February, 2014 | By The Press Association

Draft rules allowing the introduction of new treatments that could see the creation of babies with three genetic parents have been announced by the government.

Are three-parent babies the first step towards a Blade Runner future?
Mitochondrial transfer isn't necessarily part of the pro-choice package. It has ethical implications worth thinking about

Zoe Williams
theguardian.com, Friday 28 June 2013 12.09 BST
Jump to comments (322)

'Three-Parent Babies' Should Evoke Hope, Not Fear

Posted: 03/10/2014 9:10 pm EDT | Updated: 03/10/2014 9:59 pm EDT

Revolutionary new IVF treatment means babies could be born with DNA of three parents

Babies with three genetic parents could be born in the UK as early as next year after the Government paved the way for controversial new fertility treatments.

By Jo Wiley
Published Fri, February 28, 2014
Meet the world's first 'three-parent baby': Boy - delivered by US medical team in Mexico - carries a tiny piece of genetic code from a third donor 'parent' to avoid inheriting a disease from his mother

- Three-person baby technique lets parents with genetic mutations edit the mother's egg so they can have a healthy baby

First baby born using 3-parent technique to treat infertility

Exclusive: Mexico clinic plans 20 'three-parent' babies in 2017
Mitochondrial Donation

- Genetic disease - risks and patterns
- What’s unusual about mitochondrial genetic disease?
- New technologies for avoiding MGD?
- How ethical & how safe?
- Why is mitochondrial transfer being used to overcome some IVF failure?
Two Types of Genetic Risk

**Sporadic:**
occurs intermittently, often age related aneuploidy
loss / gain of whole chromosome (Down t21, Turner XO)
sometimes new mutation

**Recurrent:**
associated with particular inherited disorder
cystic fibrosis; Huntington; haemophilia, mitochondrial disease
The function of individual genes and the way that genes are inherited determines how our characteristics manifest.
Autosomal **Recessive** Genetic Disease

1:4 free of disease; 1:2 carriers – but asymptomatic; **1:4 will be affected**

- Cystic fibrosis
- Spinal muscular atrophy
- Sickle cell disease
- Tay-Sachs disease
- Fanconi Anaemia
Autosomal Recessive Genetic Inheritance

Symptomless Carriers
Autosomal **Dominant** Genetic Disease

Huntington’s Disease
Achondroplasia
Marfan Syndrome
Neurofibromatosis
Retinoblastoma
Autosomal dominant inheritance – e.g. Huntington’s, hypercholesteolaemia, achondroplasia

Appears in every generation - 50:50 chance of passing on disease
Reproductive options for those with serious genetic risk

- **Reproductive roulette**
- Gamete donation
- Adoption
- Remain childless
- Prenatal diagnosis and termination of pregnancy
Preimplantation Genetic Diagnosis can be used to avoid disease transmission.
Maternal inheritance with variable expression

Mitochondrial DNA Disease

Diagram showing the inheritance pattern of mitochondrial DNA diseases in a family. The pattern demonstrates how maternal inheritance can result in variable expression in offspring.
Unlike nDNA double helix with two copies each of ~24,000 genes, mtDNA is a small loop with only 37 genes.
Genetic control of the Electron Transport Chain
Tissues that are highly energy dependent
Mitochondrial DNA diseases

Strange acronyms for strange disorders

Genetic defects of the human mitochondrial genome were first described in 1988

- **MELAS** (Mitochondrial Encephalomyopathy; Lactic Acidosis; Stroke)
  - $TRNL1$ (a tRNA gene) mutation
- **MERRF** (Myoclonic Epilepsy; Ragged Red Fibers)
  - $8344A>G$ $TRNK$ (a tRNA gene) mutation
- **NARP** (Neuropathy; Ataxia; Retinitis Pigmentosa)
  - $8993T>G$ $MTATP6$ (subunit 6 of mitochondrial ATP synthase)
- **LHON** (Leber’s Hereditary Optic Neuropathy)
  - $11778G>A$ 50% males; 10% females affected: Homoplasmic
Mitochondrial disease mutations

- m.1555A>G – deafness
- m.1624C>T – LS
- m.3243A>G - MELAS/MIDD/CPEO
  m.3271T>C – MELAS
- m.3460G>A - LHON
  several mutations - MELAS
- m.4300A>G – cardiomypathy
- m.5545C>T – multisystemic disorder, RC deficiency
- m.7445A>G, m.7472Cins – deafness, myopathy
- m.8344A>G, m.8356T>C – MERRF
- m.8993T>G/C, m.9176T>G/C – NARP/MILS
- m.14709T>C – myopathy, weakness, diabetes
- m.14484T>C – LHON
- m.14459G>A, m.14487T>C – LS
- m.13513G>A and other mutations – MELAS, LS and overlap syndromes
- m.11777C>A – LS
- m.11778G>A – LHON
- m.10158T>C, m.10191T>C, m.10197G>A – LS/Leigh-like syndrome
What makes mitochondrial disease unusual

Thousands of mitochondria in each cell and thousands of molecules of mtDNA in each mitochondrion

Homoplasmy: a single mtDNA type

Heteroplasmy: two or more mtDNA types

30% mutation load: no disease

70% mutation load: disease

Severity of the disease depends on the proportion of mutated mitochondria
What makes mitochondrial disease unusual

Mitochondrial inheritance
Leber’s hereditary optic neuropathy (LHON)

- **I**: 
  - **II**: 
    - **III**: 
      - **IV**: 
        - **Affected**: 44%  2%  85%  2%  15%  52%  0%  2%
Options for women carrying mitochondrial disease

• *Play the lottery* as expression is variable
  - not all children will be equally affected

• *Donor oocytes* – eggs have unaffected mtDNA

• *PGD*
  – Relevant where nuclear genes causative
  – Can be a problem with mtDNA heteroplasmy
Testing Nuclear DNA is Different from testing Mitochondrial DNA.

**Nuclear DNA**
- Bad gene **present** or **not present**
  - **unaffected**
  - **affected**

**Mitochondrial DNA (mtDNA)**
- Mixture of **mutated** and **normal** mtDNA (heteroplasmy) in each cell
  - Less likely to be affected
  - More likely to be affected
Determining the mutation level below which disease will not manifest and embryos are eligible for transfer

- If set at <10% mutated mtDNA, unlikely any embryos for transfer
- If set too high >40%, disease still may manifest later in life
- How strictly to adhere to set limit?

Hubert J.M. Smeets RBMOnline 2013 Preventing the transmission of mitochondrial DNA disorders: selecting the good guys or kicking out the bad guys
Disease may not be eliminated but probability reduced

Smeets et al 2011
Disease may not be eliminated but probability reduced

Estimated probability of symptoms with 95% CI (ratio=0.477)

Risk Reduction

Smeets et al 2011
Characteristics of families and retrieved oocytes in women carrying pathogenic mtDNA mutations (carriers) or wild-type mtDNA (healthy)

Could we do something else?

Get healthy mitochondria from somewhere else?
- Inject them – ooplasms transfer – too few
- Move the chromosomes to a disease free egg: spindle probably too unstable

- Move the chromosomes into a healthy donated egg after fertilisation when they are more stable packaged in a pronucleus
Dealing with Inherited Mitochondrial Diseases

Egg Abnormal mitochondria

Donated egg
Normal mitochondria
Dealing with Inherited Mitochondrial Diseases

- **Egg Abnormal mitochondria**
- **Zygote Abnormal mitochondria**
- **Donated Egg Normal mitochondria**
- **Zygote Normal mitochondria**
Dealing with Inherited Mitochondrial Diseases

- Egg Abnormal mitochondria
- Zygote Abnormal mitochondria
- Donated Egg Normal mitochondria
- Enucleated zygote Normal mitochondria
Dealing with Inherited Mitochondrial Diseases

Egg Abnormal mitochondria

Zygote Abnormal mitochondria

Donated Egg Normal mitochondria

Reconstructed zygote Normal mitochondria
Dealing with Inherited Mitochondrial Diseases

- Egg Abnormal mitochondria
- Zygote Abnormal mitochondria
- Donated Egg: Normal mitochondria
- Reconstructed zygote: Normal mitochondria
- Cleaving embryo: Normal mitochondria
Dealing with Inherited Mitochondrial Diseases

- Egg Abnormal mitochondria
- Zygote Abnormal mitochondria

- Donated Egg Normal mitochondria
- Reconstructed zygote Normal mitochondria
- Cleaving embryo Normal mitochondria

ProNuclear Transfer into oocyte
Mitochondrial Gene Replacement in Primate Offspring and Embryonic Stem Cells

Masahito Tachibana, Michelle Sparman, Hathaitip Sritanaudomchai, Hong Ma, Lisa Clepper, Joy Woodward, Ying Li, Cathy Ramsey, Olena Kolotushkina, and Shoukhrat Mitalipov

Maternal Spindle Transfer

Female with mutant mtDNA → Abnormal mitochondria

Maternal nuclear genome → Discard

Father sperm → IVF (three genetic sources)

Healthy female egg donor → Normal mitochondria

Oocyte (cytoplasm only) → Combine

IVF (three genetic sources) → Embryo with normal mitochondria

No mitochondrial disease
She's got my eyes, her father's nose and the donor's chance of not contracting my disease...
What are the **BIG ISSUES**

- Are there now three parents?
- Is this the same as cloning?
- Is this germ-line therapy?
- Isn't this a step too far when other options exist?
- Is this technology safe to use?
- Has this technique a place in the IVF clinic?
THREE-PARENTS
In **reproductive cloning**, all embryos are created to be identical.
Germ-line gene therapy

Where the genes of an egg or a sperm or early embryo are altered and so that all the cells of the resulting baby will be altered including those of the ovary and the testis.

The change passes down the generations.

Exception is mitochondrial disease which does not pass down through males – through female only.
Isn't this a step too far when other options exist?

- There is no cure for mitochondrial disease
- Prenatal Diagnosis & TOP not an option for some patients
- Many couples reluctant to use a donor egg as they want to have their own genetic child
- PGD will not work for patients with mutant homoplasmy or high mutant heteroplasmy as the proportion of mtDNA too high to select unaffected embryos
Is mitochondrial gene therapy safe?

Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2016 update

Report to the Human Fertilisation and Embryology Authority (HFEA)
November 2016

Review panel Chair: Dr Andy Greenfield, Medical Research Council (MRC) Harwell Institute and HFEA member
Can it work?

Blastocyst development After PNT

Hyslop et al., Nature. 2016 Jul 27 538, .542
Are PNT blastocysts normal?

Gene expression analysis
single cell RNA-Seq

Screen for chromosomal abnormalities - Array CGH

Evidence so far indicates that PNT blastocystcs are indistinguishable from controls.
What are the risks?

Figure 2. Pronuclear transfer (PNT)

1. Patient’s egg with abnormal mitochondria fertilised with partner's sperm
2. Patient’s zygote with abnormal mitochondria
3. Patients’ pronuclei removed from zygote and transferred to enucleated zygote, which has normal mitochondria.
4. Cleaving embryo with normal mitochondria and maternal paternal genome can be transferred to the uterus

Possibility of Carryover and Reversion
Carryover in PNT and MST embryos

• PNT using normally fertilised oocytes
  carryover usually <2%; none>5% (Hyslop et al 2016)

• MST using activated oocytes
  carryover <2% (avg 0.2%) (Yamada et al 2016)

• MST using fertilised eggs from mutation carriers
  carryover 25/26 <1%; 1=<2% (Kang et al 2016)
<table>
<thead>
<tr>
<th>Mutation</th>
<th>At biopsy</th>
<th>At birth</th>
<th>Comments</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1 m.8993T&gt;G</td>
<td>0% &amp; 0%</td>
<td>0%</td>
<td>First report. Two embryos transferred</td>
<td>Steffann et al., 2006</td>
</tr>
<tr>
<td>2 m.8993T&gt;G</td>
<td>2.5%</td>
<td>4%</td>
<td>3-5% cord blood &amp; placenta; buccals 5% at age 4½y</td>
<td>Thorburn et al 2010*</td>
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<tr>
<td>3 m.3243A&gt;G</td>
<td>5% &amp; 13%</td>
<td>5%</td>
<td>Two embryos transferred; 15±5% placenta, 5±1% cord blood</td>
<td>Monnot et al., 2011</td>
</tr>
<tr>
<td>4 m.3243A&gt;G</td>
<td>12%</td>
<td>15%</td>
<td>47% blood, 52% urine @ 1½m; 46/42%@18m</td>
<td>Treff et al., 2012/Mitalipov et al., 2014</td>
</tr>
<tr>
<td>5 m.8993T&gt;G</td>
<td>0%</td>
<td>0%</td>
<td>'Healthy son', no further details</td>
<td>Sallevelt et al., 2013</td>
</tr>
<tr>
<td>6 m.8344A&gt;G</td>
<td>53% &amp; 59%</td>
<td>63%</td>
<td>Two embryos transferred; no further details</td>
<td>Steffann et al., 2014</td>
</tr>
<tr>
<td>7 m.3243A&gt;G</td>
<td>0%</td>
<td>0%</td>
<td>Male; measured in cord blood, urine, saliva</td>
<td>Heindryckx et al., 2014</td>
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<tr>
<td>8 m.36**G&gt;A</td>
<td>2%</td>
<td>7%</td>
<td>Female, measured buccal and urine cells</td>
<td>Newcastle group 2016*</td>
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<tr>
<td>9 m.83**A&gt;G</td>
<td>48%</td>
<td>Not available</td>
<td>Male; &lt;60% generally asymptomatic</td>
<td>Newcastle group 2016*</td>
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<tr>
<td>10 m.130**T&gt;C</td>
<td>1%</td>
<td>0%</td>
<td>Male; undetectable in cord and peripheral blood</td>
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What are the risks?

Figure 2. Pronuclear transfer (PNT)

1. Patient’s egg with abnormal mitochondria fertilised with partners sperm
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4. Cleaving embryo with normal mitochondria and maternal paternal genome can be transferred to the uterus.

Donated egg fertilised Normal mitochondria
Zygote Normal mitochondria
Zygote enucleated Normal mitochondria
Derivation of embryonic stem cell lines to explore the fate of carried over mtDNA

• **MST using activated oocytes** (Yamada et al 2016)
  8 ES cell lines: 4=0%; 4=0.2%-1.7% and 0% by passage 6
  **but** one went from 1.3% to 53.2% at passage 36

• **MST using fertilised eggs from mutation carriers** (Kang 2016)
  3/18 reverted 100% mutated mtDNA by passage 10

• **PNT using fertilised oocytes** (Hyslop et al 2016)
  4= low levels <7%;
  1= upward drift to 52% by passage 12
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Panel’s Recommendation

Cautious and specific implementation with long-term follow-up

Since PGD is licensed for use in mtDNA disorders and often results in embryos being transferred with significant levels of mutant mtDNA (but is still useful as a risk reduction strategy), PNT and MST is safe enough to be used in a similar way as a risk reduction strategy where PGD is in appropriate or unlikely to work.

BUT:

- Patients must be fully informed of the theoretical risk and PND offered
- Information about haplogroups of donor and recipient should be collected
- Patients should be encouraged to take part in long-term follow-up
- The panel could not recommend its use be extended to otherwise healthy individuals with fertility problems but not genetic disease
Mitochondrial Transfer Technology Applied to Infertility

World-First in Ukraine as 'Three-Parent' Baby Born to an Infertile Couple

Three parents, one baby, and a lot of controversy.

PETER DOCKRILL   19 JAN 2017
Molecular Human Reproduction vol.4 no.3 pp. 269–280, 1998

Ooplasmic transfer in mature human oocytes

Jacques Cohen¹,², Richard Scott¹, Mina Alikani¹, Tim Schimmel¹, Santiago Munné¹, Jacob Levron², Lizi Wu³, Carol Brenner¹, Carol Warner³ and Steen Willadsen¹

¹The Institute for Reproductive Medicine and Science of Saint Barnabas, Livingston New Jersey, USA, ²Department of Obstetrics and Gynecology, Tel Hashomer, Tel-Aviv, Israel, and ³Department of Biology, Northeastern University, Boston, Massachusetts, USA
Outcome of 37 attempts of OI at St Barnabas (1996-2001)

13 pregnancies

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</tr>
<tr>
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<td>4/2</td>
</tr>
</tbody>
</table>

+ 1 preclinical loss (XO)

(1) XO TOP: (2) XX

(1) OI; (2) OD

Chen et al RBMOnline 33, 737 (2016)
Control of events during early cleavage of the mouse embryo: an analysis of the ‘2-cell block’

By MARTIN J. GODDARD and HESTER P. M. PRATT

From the Department of Anatomy, University of Cambridge
Cytoplasmic factors influence mitochondrial reorganization and resumption of cleavage during culture of early mouse embryos

A.L. Muggleton-Harris and J.J.G. Brown

MRC Experimental Embryology and Teratology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK

Outcome for Blocking and Non-Blocking mouse strains
Alleviation of the ‘2-cell block’ and development to the blastocyst of CF1 mouse embryos: role of amino acids, EDTA and physical parameters

David K. Gardner¹ and Michelle Lane

Alleviation of the two-cell block of ICR mouse embryos by polyaminocarboxylate metal chelators

T. Matsukawa, S. Ikeda, H. Imai and M. Yamada*

Laboratory of Reproductive Physiology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Involvement of Superoxide Radicals in the Mouse Two-Cell Block

YOICHI NODA, HISASHI MATSUMOTO, YOH UMAOKA, KENICHI TATSUMI, JUNJI KISHI, AND TAKAHIDE MORI

Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Kyoto, Japan

Superoxide dismutase and thioredoxin restore defective p34cdc2 kinase activation in mouse two-cell block

Satoshi Natsuyama², Yoichi Noda², Masakane Yamashita³, Yoshitaka Nagahama³, Takahide Mori³

¹ Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Kyoto, Japan

Release of two-cell block by reduction of protein disulfide with thioredoxin from Escherichia coli in mice

S. Natsuyama, Y. Noda, K. Narimoto, Y. Umaoka and T. Mori

Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

Hypoxanthine Causes a 2-Cell Block in Random-Bred Mouse Embryos

D. LOUTRADIS, D. JOHN, and A. A. KEISSLING³

Departments of Obstetrics, Gynecology, and Reproductive Biology
Harvard Medical School
Boston, Massachusetts 02115

Two-Cell Block to Development of Cultured Hamster Embryos Is Caused by Phosphate and Glucose

SCOTT A. SCHINI and BARRY D. BAVISTER²

An improved culture medium supports development of random-bred 1-cell mouse embryos in vitro

C. L. Chatot, C. A. Ziomek, B. D. Bavister*, J. L. Lewis and I. Torres

Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545, USA; and *Department of Veterinary Science, University of Wisconsin, Madison, WI 53706, USA
What factors might be added in OT

Cohen et al. MHR 1988

1. Mitochondria from the donor supplement pool of mitochondria
2. Internal pool of inherited (maternal) mRNAs may be boosted
3. Other organelles (ribosomes, proteins, spindle organizing units)
4. Specific consequences by altering a single mechanism
   e.g. change in the polarization of mitochondria
In our opinion, the presented technology is highly experimental and it would be wise to delay its widespread medical application until further studies in animal models and donated human material indicate the best approaches.

“The transfer of small amounts of donor ooplasm (15%–5) probably includes mRNAs, proteins, mitochondria, as well as other factors and organelles.”
Cytoplasmic Transfer Abroad - Medical Tourism Guide
Details of leading clinics and hospitals performing Cytoplasmic Transfer to foreign patients.

Successful Parents Ukraine
Surrogacy & Egg Donations Agency, Ukraine
An agency that coordinates egg donors, surrogacy and fertility treatments in the Ukraine. Provides European egg donors, Ukraine surrogacy services, PGD, and many other services relevant for couples looking to have a child.

Instituto Bernabeu
Fertility Clinic, Spain
Instituto Bernabeu is staffed by recognised fertility and assisted reproduction specialists. Thousands of patients from over 60 different countries have entrusted Instituto Bernabeu with their dream of becoming parents.

Bahceci IVF Centers, Istanbul, Turkey

Successful Parents India
Surrogacy and Egg Donation Centre, India
Successful Parents India is a branch of a European based head office of Successful Parents Agency. The Agency operates in India and accepts clients for fertility treatments in New Delhi: surrogacy, egg donation (Indian and Caucasian egg donors), PGD (not sex selection), as well as many other services.

Published on November 23, 2015

British Cyprus IVF
First baby born using 3-parent technique to treat infertility
Outcome of 37 attempts of OI at St Barnabas (1996-2001)

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(1) XO TOP: (2) XX

6/8 showed no evidence of donor mtDNA

Chen et al RBMOnline 33, 737 (2016)
MST for infertility

Infertile female with unexplained infertility

Mitochondria presumed to be defective

Embryo with all mtDNA from donor
