Reproductive technologies to prevent transmission of mitochondrial DNA disease

Louise Hyslop
Mitochondria

- Produce > 90% of the energy our cells need
- Contain own DNA (mitochondrial DNA / mtDNA)
- Multiple copies of mtDNA in each cell

Wei et al. 2009
mtDNA mutations

- DNA mutations are like spelling mistakes in the genetic code
- Bad mistakes can affect energy production
- Very serious consequences for organs that require a lot of energy such as the brain and heart
Diseases caused by mutations in mtDNA

Non-Neurological
- Respiratory Failure
- Cardiomyopathy
- Liver Failure
- Short Stature
- Marrow Failure
- Diabetes
- Thyroid Disease

Neurological
- Optic Atrophy / Retinitis Pigmentosa
- CVA / Seizures /
- Developmental delay
- Deafness
- Peripheral Neuropathy
- Myopathy
Mutations can be present in all, or just some copies of mtDNA.

**Homoplasmy**
- 0% mutation
- 100% mutation

**Heteroplasmy**
- Mix of mutated and normal mtDNA

Severity of disease is determined by the ratio of mutated to non-mutated mitochondrial DNA.
mtDNA: inherited only from our mothers

Human eggs contain abundant stock of mtDNA
Wide variation in mtDNA mutation loads between eggs and embryos
Reproductive consequences

<table>
<thead>
<tr>
<th>Mutation Load</th>
<th>Mother</th>
<th>Son</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild clinical symptoms</td>
<td>Died aged 7 years</td>
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</table>

78%
Reproductive consequences

<table>
<thead>
<tr>
<th>Mutation Load</th>
<th>Mother 38%</th>
<th>Son 78%</th>
<th>Daughter 0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild clinical symptoms</td>
<td>Died aged 7 years</td>
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</table>
Wide variation in mtDNA mutation loads between eggs and embryos.
What are the options for reducing risk of transmitting mtDNA mutations?

- Egg donation
- Prenatal diagnosis
- Pre-implantation genetic diagnosis (PGD)
PGD can be used to detect mtDNA mutations

Single cell removed and sent to diagnostic lab for analysis

<table>
<thead>
<tr>
<th>Mutation level</th>
<th>Risk of developing mitochondrial disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mutation</td>
<td>No risk</td>
</tr>
<tr>
<td>Low-level mutation</td>
<td>&lt;30% Lifetime risk extremely low</td>
</tr>
<tr>
<td>Intermediate-level mutation</td>
<td>31-70% Risk rises with increasing mutation level</td>
</tr>
<tr>
<td>High-level mutation</td>
<td>&gt;70% High risk of severe disease</td>
</tr>
</tbody>
</table>
Summary of cases at Newcastle Fertility Centre

- Case A: % mutation load
- Case B: % mutation load
- Case C: % mutation load
- Case D: % mutation load
- Case E: % mutation load

30% threshold for replacement

x blastomeres  x embryo replaced
Summary of cases at Newcastle Fertility Centre

No embryos replaced

30% threshold for replacement

x blastomeres  x embryo replaced
What can we offer in cases where all embryos have a high mutation load?
Can we uncouple the inheritance of nuclear and mtDNA?

Not feasible to replace the mitochondria

Transplantation of the nuclear DNA

Are there alternative strategies?

Mutation load

wild-type mitochondria  mutant mitochondria
Pronuclear transfer

- Proven to be compatible with development in mice (McGrath and Solter, 1983; Meirelles & Smith, 1997)

- Proven to prevent transmission of a mitochondrial DNA deletion in mice (Sato et al, PNAS, 2005)
Donor egg

Egg from affected woman

wild-type mitochondria

mutant mitochondria

Pronuclear transfer strategy
Research Material

- No ready supply of normally fertilised eggs available for research

- Initial experiments performed on abnormally fertilised human eggs (mono-pronucleate and tri-pronucleate)
In the UK, all procedures involving the creation of human embryos are regulated by the Human Fertilisation and Embryology Authority (HFEA).

Paragraph 3(4) of Schedule 2 to the HFE Act 1990:
“a Licence under this paragraph cannot authorise altering the genetic structure of any cell while it forms part of an embryo”

Would pronuclear transfer alter the genetic structure of the embryo?
- Problem: what is meant by the term “genetic structure”?
Pronuclear transfer involves:

- *Substitution of mtDNA without altering the sequence of the DNA*

- *Change in the genetic composition but not the structure*

Research licence granted 18 months after application
And then came the three parents
• Is it technically feasible in human fertilised eggs?
• Can reconstituted fertilised eggs develop?
• Can we minimise the level of mtDNA carryover?
Embryo development: (abnormally fertilised eggs)
- Unmanipulated controls: 17%
- Pronuclear transfer: 8%

How much mtDNA are we transferring with the pronuclei

Donor egg

Egg from affected woman

wild-type mitochondria  mutant mitochondria
Optimisation of the procedure to minimise carry over of mtDNA

Before

mtDNA carry-over 8.1± 7.6%

After

<2%

Conclusion: Pronuclear transfer is a feasible option for reducing the risk of transmission of mutated mtDNA

![Diagram showing pronuclear transfer process with donor egg and egg from affected woman, illustrating wild-type and mutant mitochondria.]
Effects of pronuclear transfer on embryo development of *normally* fertilised eggs

– Can reconstituted embryos develop with **high efficiency**?

– Are these reconstituted embryos *normal*?
  • Are the manipulations harmful to embryos?

**Requires a source of normally fertilised eggs donated specifically for research**
Sources of eggs for research

Altruistic donation
• women 21-35 years
• reimbursed for expenses

“Egg share for research”
• women undergoing IVF treatment
  * Financial contribution towards treatment costs (Fee paying patients)
  * An extra cycle of treatment if required (NHS-funded patients)
What needs to happen before this can be offered in clinical treatment?

– Law would need to be amended
  • Explicitly for preventing transmission of mtDNA disease
  • Treatments would require a licence from the HFEA

– HFEA need to examine the safety and efficacy data from the normally fertilised eggs
Legal and regulatory landscape

- Public consultation
- Debate and votes in Houses of Parliament on regulations
- Detailed regulations agreed and adopted by the HFEA
- Examination of the safety and efficacy data by the HFEA
- Application to the HFEA for licence to use new technique in clinical treatment
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- Helen Tuppen

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Egg donors and patients