Ethical and Social Considerations of Mitochondrial Replacement Therapy (MRT)

Committee on Ethical and Social Policy Considerations of Novel Techniques for Prevention of Maternal Transmission of Mitochondrial DNA Diseases

Public Workshop
March 31-April 1, 2015
Ethical and Social Considerations of MRT

Kinship

• Value of / preference for genetically-related children
  • Existence of alternative approaches to parenting
  • Influence of reproductive autonomy in the US
• DNA from three individuals
• Social considerations
  • Identity
  • Parenthood
  • Ancestry
First-in-human research for the purpose of creating children

- Informed consent for an unborn child; consent for future generations
- Children and women are bearers of risk
- ‘Disease prevention’ vs ‘reproductive opportunity’ (non-identity)
  - Curing a child otherwise born with mito disease or creating a new person?
- Allowable risk vs perceived benefit?
Ethical and Social Considerations of MRT

**Risk to women (potential mothers and egg donors)**
- Ovarian hyperstimulation syndrome (OHSS)

**Risk to embryo**
- Epigenetic modification
- Reagents used (e.g.: dissolving agent in MST)

**Risk to offspring**
- Creation of disease through alleviation of another
- Birth, developmental, long-term defects
  - Haplotype incompatibility (sterility?)
  - mtDNA carryover $\rightarrow$ heteroplasmy

**Risk to future generations**
- mtDNA carryover $\rightarrow$ mtDNA bottleneck $\rightarrow$ ↑ heteroplasmy in oocytes
Moral status of oocytes and embryos

• Manipulation and/or destruction of oocytes vs embryos
  • (MST or PB1T) vs (PNT or PB2T)
• Considerations of relative safety of each technique
Ethical and Social Considerations of MRT

Fairness, equity, and access

• Fee-for-service assisted reproductive technologies
• Increased demand for egg donors; payment
• Availability of alternative options (adoption or egg donation)
  • Desire for genetically related children?
Ethical and Social Considerations of MRT

**Downstream applications**

- Disease threshold for inclusion criteria?
- Creation of an obligation for at-risk families?
- Acceptable range of potential genetic modifications introduced?
  - Treatment vs enhancement
- Impact on acceptance of nDNA germline modification?
Germline modification

- Working definition: “human inheritable genetic modification” (FDA)
- nDNA vs mtDNA: a distinction for germline modification?
  - Distinction between replacement and editing of DNA?
- Controls: therapeutic vs enhancement
Maternal Spindle Transfer (MST)

1. The spindle of chromosomes is removed from the donor egg and discarded.

2. The spindle of chromosomes is removed from the intending mother’s egg and transferred to the ‘enucleated’ donor egg; the intending mother’s egg is discarded.

3. The reconstructed oocyte contains the intending mother’s nuclear DNA and donor’s mitochondrial DNA.

4. The egg is then fertilized with the intending father’s sperm.

5. The embryo develops in vitro and is transferred to the womb of the woman who will carry the child.
Maternal Spindle Transfer (MST)

**Potential risks**

mtDNA carryover: PBT < **MST** < PNT (estimated <1%)

**Technicality of procedure:**
- Spindle-chromosome complex sensitive to manipulation; higher risk of chromosomal abnormalities than in PNT
- Visualization of spindle
- Operator dependent

**Reagents:** treatment of oocytes with cytoskeletal inhibitors for karyoplast removal; Sendai virus for fusion

**Ethical considerations**

Manipulation and destruction of oocytes

*nb: Embryos deemed not suitable for transfer may be discarded.*
# Maternal Spindle Transfer (MST)

## State of the science

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
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<tr>
<td><strong>Tachibana et al. (2009)</strong></td>
<td>Rhesus macaques <em>(Macaca mulatta)</em> 'genetically distant sub-populations'</td>
<td>• Developmental potential&lt;br&gt;• F1 health, mtDNA carryover</td>
<td>15 ST embryos transferred into 9 ♀: 6 with 1-2 blastocysts, 3 with 2 cleavage stage (4-8 cell) embryos</td>
<td>• Four healthy offspring born following blastocyst transfer (one set of twins, two singletons)&lt;br&gt;• 3% carryover of mtDNA</td>
<td>• The ST strategy will probably result in least amount of mtDNA carryover, as compared to other techniques&lt;br&gt;• ST could present a reliable approach to prevention of transmission of mtDNA mutations</td>
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<td><strong>Tachibana et al. (2013)</strong> (2009 follow-up)</td>
<td>Rhesus macaques</td>
<td>• Overall health&lt;br&gt;• Post-natal development</td>
<td>Routine blood and bodyweight measurements (birth – 3 years)</td>
<td>• Normal development&lt;br&gt;• No change in mtDNA carryover and heteroplasmy in blood and skin samples</td>
<td>• Oocyte manipulation and mtDNA replacement procedures are compatible with normal development&lt;br&gt;• Nuclear mtDNA interactions conserved within species</td>
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## Maternal Spindle Transfer (MST)

### State of the science, cont.

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<tr>
<td><strong>Tachibana et al. (2013)</strong></td>
<td>Human oocytes</td>
<td>• Developmental potential</td>
<td>106 donated oocytes: 65 ST, 33 control; Reciprocal ST followed by ICSI</td>
<td>• Significant portion of ST oocytes (52%) showed abnormal fertilization; remaining normally fertilized ST zygotes had comparable level of blastocyst development (62%) • &lt;1%/ND carryover of mtDNA in ST embryos</td>
<td>• Human oocytes are more sensitive to spindle manipulations than macaques • Compared to ST in macaque oocytes, ST in human oocytes resulted in a significant level of abnormal fertilization</td>
</tr>
<tr>
<td><strong>Paull et al. (2013)</strong></td>
<td>Human oocytes</td>
<td>• Preimplantation development</td>
<td>62 donated oocytes; parthenogenetically activated</td>
<td>• Efficient development to blastocyst stage (37% vs 32% control) • mtDNA carryover 0.5%, decreased to ND in blastocysts and eSC • Depolimerization prevents premature oocyte activation</td>
<td>• MST did not reduce developmental efficiency to blastocyst stage and resulted in carryover of &lt;1%, which decreased to ND • Spontaneous activation of oocytes can be avoided by cooling the spindle complex</td>
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## Maternal Spindle Transfer (MST)

### State of the science, cont.

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| Lee et al. (2012) | Rhesus macaque oocytes | • Developmental potential of ST embryos  
• Level of heteroplasmy in somatic tissues of preterm fetus (F₁) and oocytes (F₂), 135d post-embryo transfer | • 102 ST oocytes generated  
• Two singleton pregnancies generated using preselected ♀ embryos | • 63% of ST developed to blastocysts after fertilization  
• mtDNA carryover <0.5%/ ND in somatic tissues of F₁  
• 11/12 oocytes in each fetus (F2 generation) displayed ND levels of mtDNA heteroplasmy; one oocyte from each fetus contained substantial mtDNA carryover (16.2% and 14.1%) | • Confirms that MST results in low level of mtDNA carryover  
• Supports the observation that different mtDNA transmission mechanisms may exist for somatic and germline lineages |
Pronuclear Transfer (PNT)

1. The intending mother’s egg is fertilized by the intending father’s sperm.

2. The donor egg is also fertilized by the intending father’s sperm.

3. The pronuclei are removed from the single-celled zygote of the donor egg and discarded.

4. The pronuclei are removed from the intending mother’s fertilized egg and transferred to the enucleated fertilized donor egg. The enucleated fertilized egg of the intending mother is discarded.

5. The reconstructed embryo contains pronuclear DNA from the intending parents and healthy mitochondria from the donor.

6. The embryo develops in vitro and is transferred to the womb of the woman who will carry the child.

(Nuffield Council on Bioethics, 2012)


Pronuclear Transfer (PNT)

**Potential risks**

mtDNA carryover: PBT < MST < PNT (estimated <2%)

**Technicallity of procedure:**
- Easier visualization than MST (enclosed in karyoplast)
- Need to ensure inclusion of centrioles and other spindle assembly components
- Operator dependent

**Reagents:** treatment of zygotes with cytoskeletal inhibitors for karyoplast removal; Sendai virus for fusion

**Ethical considerations**

Manipulation and destruction of fertilized eggs
# Pronuclear Transfer (PNT)

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<td>Sato et al. (2005)</td>
<td>Mito-mice (ΔmtDNA: <em>Mus musculus domesticus</em>) • Wild-type mice: <em>Mus spretus</em></td>
<td>• Rescue from disease phenotype • mtDNA carryover</td>
<td>• 39 mito-mouse zygotes transferred into pseudo-pregnant females • 34 control (mito-mouse, no PNT)</td>
<td>• 11 mice born following PNT (9 control) • F₀ progeny rescued from disease phenotypes • Average carryover 11% at weaning, increased to 33% &gt;300d; estimated to be 43% at day 800</td>
<td>PNT is restricted to patients with mitochondrial diseases wherein pathogenic mtDNAs inherited maternally and do not possess significant replication advantages over wild-type mtDNA</td>
</tr>
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<td>Craven et al. (2009)</td>
<td>Human zygotes (abnormally fertilized – unipronuclear/tripronuclear)</td>
<td>• Developmental potential • mtDNA carryover</td>
<td>Pronuclei (2) transferred to enucleated recipient zygote: monitored 6-8 days <em>in vitro</em></td>
<td>• 22% developed past 8-cell stage, 8.3% to blastocyst stage (50% of unmanipulated control) • mtDNA carryover &lt;2%/ND</td>
<td>PNT has the potential to prevent mtDNA disease transmission and results in very low mtDNA carryover</td>
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# Pronuclear Transfer (PNT)

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<td><em>Turnbull group, unpublished</em></td>
<td>Human zygotes</td>
<td>• Developmental potential</td>
<td><em>Unavailable</em></td>
<td>• High rates of development to blastocyst stage&lt;br&gt;• mtDNA carryover &lt;2%/ND</td>
<td><em>Modifications to experimental protocol resulted in increased development to blastocyst stage</em></td>
</tr>
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Oogenesis & Formation of Polar Bodies

- The primordial germ cell (oogonium) undergoes mitosis in the fetus; at birth, the primary oocyte arrests in prophase of meiosis I (prophase I).

- Beginning at puberty, once per month, a primary oocyte completes meiosis I and begins meiosis II, before arresting at metaphase II. At this time the **first polar body** is produced. The resultant secondary oocyte and polar body are haploid.

- The secondary oocyte is ovulated. If fertilized by a sperm, the secondary oocyte completes meiosis II and the **second polar body** (haploid) is formed.
Polar Body 1 Transfer (PB1T)

1. The chromosome spindle is removed from the donor egg and discarded.
2. The 1st polar body is removed from the intending mother’s egg and transferred to the enucleated donor egg; the intending mother’s egg is discarded.
3. The reconstructed oocyte contains the intending mother’s nuclear DNA and donor’s mitochondrial DNA.
4. The reconstructed egg is fertilized with the intending father’s sperm.
5. The embryo develops in vitro (PB2 extruded) and is transferred to the womb of the woman who will carry the child.

(Wolf et al., 2014)
## Polar Body 1 Transfer (PB1T)

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<td>Wang et al. (2011)</td>
<td>Porcine</td>
<td>• Developmental potential</td>
<td>• Vitrified PB1 T</td>
<td>• 88.6% normal recombinant oocytes&lt;br&gt;• 9.3% cleaved ≥ 8-cell stage; those that cleaved had normal morphology</td>
<td>• Frozen-thawed PB1s support oocyte fertilization and embryonic development</td>
</tr>
<tr>
<td>Wang et al. (2014)</td>
<td>Mouse (Mus musculus)</td>
<td>• Developmental potential (in vitro &amp; in vivo)&lt;br&gt;• mtDNA carryover (F1 &amp; F2 generations)&lt;br&gt;• 25 PB1s &amp; 27 spindle-chromosome complexes transferred&lt;br&gt;• 14 PB1 and 18 ST embryos transferred to pseudopregnant ♀&lt;br&gt;• mtDNA carryover: tail tip/brain tissue and internal organs (F1) and toe tips (F2)</td>
<td>PB1/ST:&lt;br&gt;• 87.5%/85.7% developed to blastocyst&lt;br&gt;• 42.8%/44.4% live, healthy births&lt;br&gt;• ND/5.5% mtDNA carryover (tail tip/brain)&lt;br&gt;• ND/0-6.88% mtDNA carryover (internal organs)&lt;br&gt;• ND/7.1% mtDNA carryover (F2 generation)</td>
<td>• Proof for possibility of using MST in combination with PB1T to inc. chance of MRT success&lt;br&gt;• PB1T resulted in undetectable levels of heteroplasmic DNA in F1 and F2 generations</td>
<td></td>
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Polar Body 1 Transfer (PB1T)

Potential risks

mtDNA carryover: PB1T < PB2T < MST < PNT

Technicality of procedure: potentially easier to obtain polar bodies, as they are already enclosed in their own cell membrane; can be removed with only micropipette

Ethical considerations

Manipulation and destruction of oocytes

nb: embryos deemed not suitable for transplant may be discarded.
1. The intending mother’s egg is fertilized by the intending father’s sperm. \((not\ shown)\)

2. The donor egg is fertilized by the intending father’s sperm. \((not\ shown)\)

3. The **maternal** pronuclei from the donor zygote is removed and discarded, leaving a half-enucleated egg.

4. The 2\textsuperscript{nd} **polar body** from the intending mother’s zygote is transferred to the half-enucleated donor egg, which contains the paternal pronuclei and donor mtDNA.

5. The embryo develops in vitro and is transferred to the womb of the woman who will carry the child.
Polar Body 2 Transfer (PB2T)

**Potential risks**

*mtDNA carryover*: PB1T < PB2T < MST < PNT

**Technicality of procedure:**
- Identification of female pronuclei
- Potentially easier to obtain polar bodies, as they are already enclosed in their own cell membrane; can be removed with only micropipette

**Ethical considerations**

Manipulation and destruction of fertilized eggs

*nb: embryos deemed not suitable for transplant may be discarded.*
## Polar Body 2 Transfer (PB2T)

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</table>
| Wakayama et al. (1997) | Mouse (Mus musculus)   | • Integrity of PB genomes  
• Developmental potential  
• Effect of timing | PB2T with PB2 from same or different oocyte  
• Transfer of 30 compacted murulae or blastocysts to six pseudopregnant ♀ | • Reconstructed embryos had well-developed pronuclei  
• Developmental rate decreased as time of PB2 transfer after fertilization increased (70% when recently fertilized)  
• 18 live, healthy births | • The timing of transfer is important to success  
• PB2T supports full term embryo development and therefore could be used as an alternative source of female chromosomes |
| Wang et al. (2014)   | Mouse (Mus musculus)   | • Developmental potential  
• mtDNA carryover (F1 & F2 generations) | PB2/PNT:  
• 30 PB2s & 38 pronuclei transferred  
• 15 PB2 and 13 PNT embryos transferred to pseudopregnant ♀  
• mtDNA carryover: examined in tail tip/brain tissue and internal organs (F1) and toe tips (F2) | PB2/PNT:  
• 55.5%/81.3% developed to blastocyst  
• 40%/53.8% live, healthy births  
• 1.7%/23.7% mtDNA carryover (tail tip/brain)  
• ND-3.62%/5.5-39.8% mtDNA carryover (internal organs)  
• 2.9%/22.1% mtDNA carryover (F2 toe tip) | • PB2T results in very low level mtDNA carryover  
• PB1T, PB2T and ST could be readily used to exchange mtDNA |