

Three-person babies, not three-parent babies

Louise Hyslop

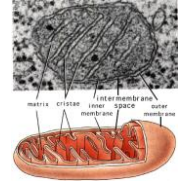


Newcastle Fertility
Centre @ Life



Mitochondria

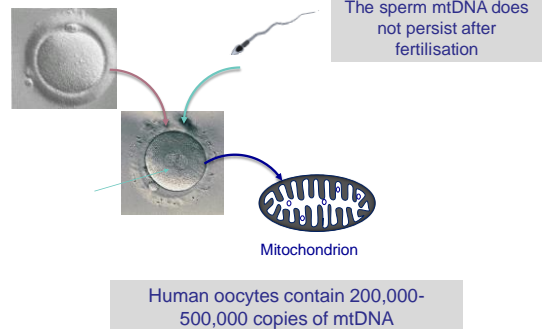
- Mitochondria produce energy in the form of ATP
 - Provides 90% of the cells energy requirements
- Mitochondria contain own DNA (mtDNA)
 - Multiple copies of mtDNA in each cell
 - mtDNA can have mutations



Pathogenic mtDNA mutations

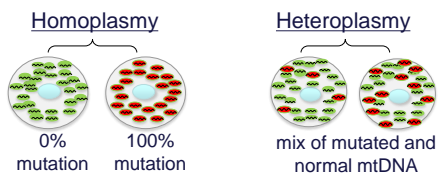
- Cause a broad spectrum of multi-system diseases
- Affect tissues with high energy requirements
- Disease onset can occur in childhood or later in life
- Prevalence of disease is estimated to be ~1 in 5000

Mitochondrial DNA (mtDNA) is maternally inherited



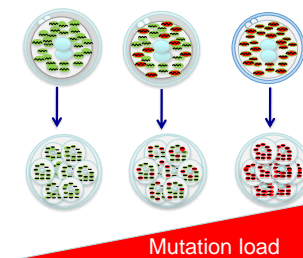
Inheritance of mtDNA mutations

Mutations can be present in all, or just some copies of mtDNA

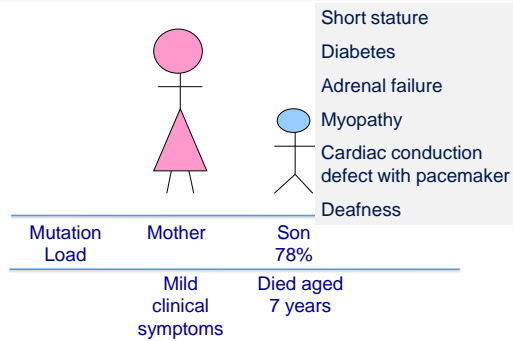


Severity of disease is determined by the ratio of mutated to non-mutated mitochondrial DNA

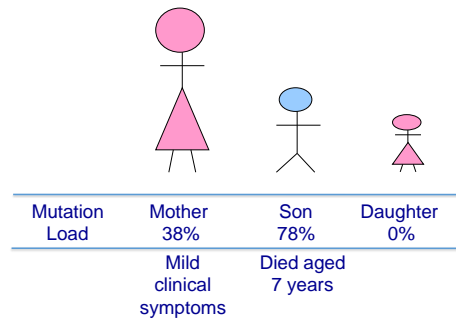
Wide variation in mtDNA mutation loads between eggs and embryos



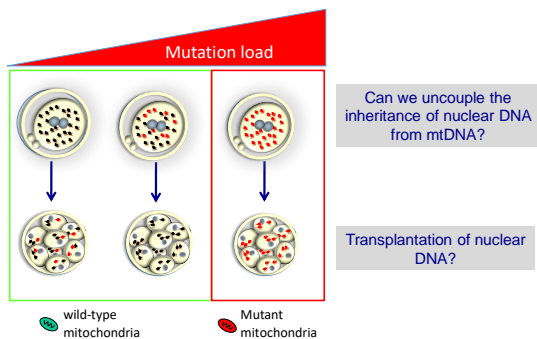
Reproductive consequences



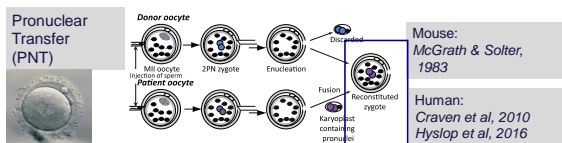
Reproductive consequences



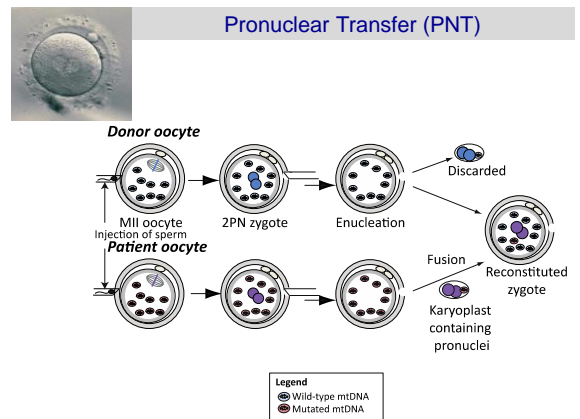
Are there alternative strategies for cases with high mutations loads?



Transplantation of nuclear DNA: current options



Pronuclear Transfer (PNT)

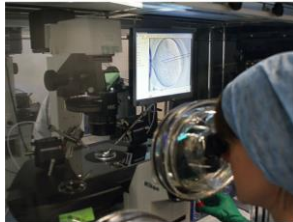




Control of
environmental
conditions during
manipulations

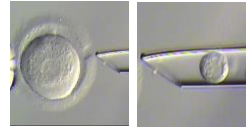
Isolator-based workstation
with controlled

- Temperature
- CO₂
- O₂



Human PNT: The technical challenges

Step 1
Removal of
pronuclei



Chemical inhibitors
used to
depolymerize
the actin and
microtubule
networks

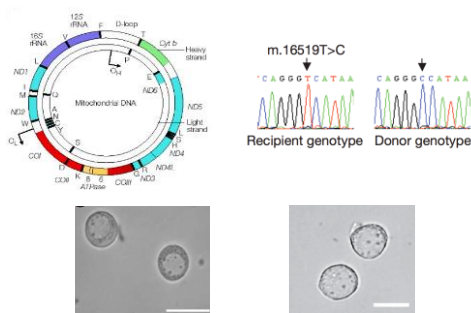
Timing

- Survival improved if performed shortly after appearance rather than just prior to syngamy

Pipette size

- big enough to remove the PN without damaging them
- small enough to minimise the amount of surrounding cytoplasm

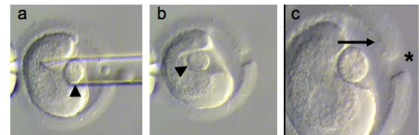
Mitochondrial DNA carryover



Craven et al, 2010 & Hyslop et al, 2016, Nature

Human PNT: The technical challenges

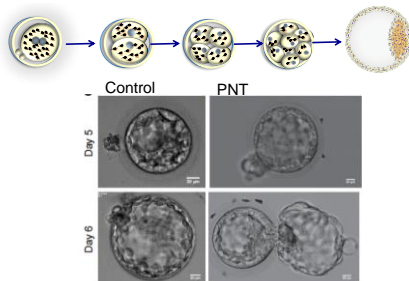
Step 2
Fusion



Fusogen: Inactivated viral envelope protein
(HVJ-E)

>90% survival

Can pronuclear transfer embryos develop to the blastocyst stage?

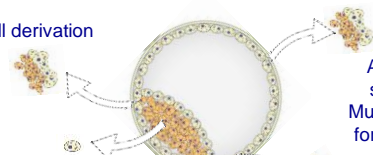


- Blastocyst development reduced compared to controls
- Blastocyst quality was similar to controls

Further studies on PNT blastocysts

Maximising the amount of information we can obtain
from individual blastocysts

ES cell derivation

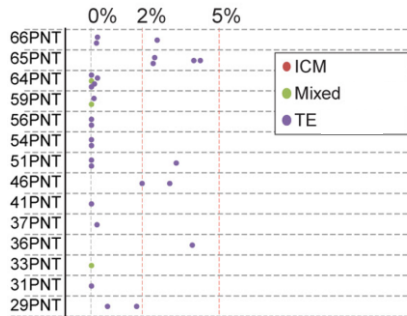


Aneuploidy
screening:
Multiple chunks
for array-CGH

Gene expression in
single cells by
RNA-Seq

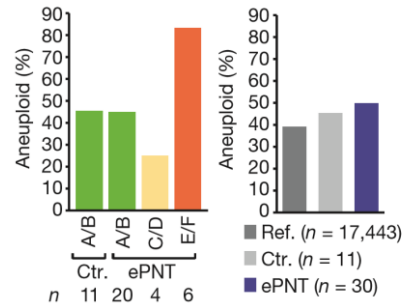
Multiple chunks for
Analysis of mtDNA
carryover

MtDNA carryover in PNT blastocysts



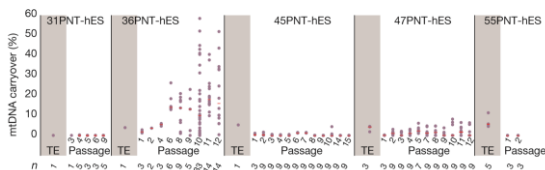
<2% in majority of PNT blastocysts

Aneuploidy screening of PNT blastocysts



Not significantly different from controls and reference population

ES cell derivation



Next Steps towards clinical treatment

HFEA have defined requirements for licensing pronuclear transfer and spindle transfer for use in clinical treatment

- Evidence of competence of the embryologist
- Process and equipment validation documentation
- Schedule of quality indicators
- Procedures for the follow-up of children born

Permitted only in cases where there is risk of serious mtDNA disease

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