Investigating the Level of mtDNA Carryover in Mitochondrial Replacement Embryos

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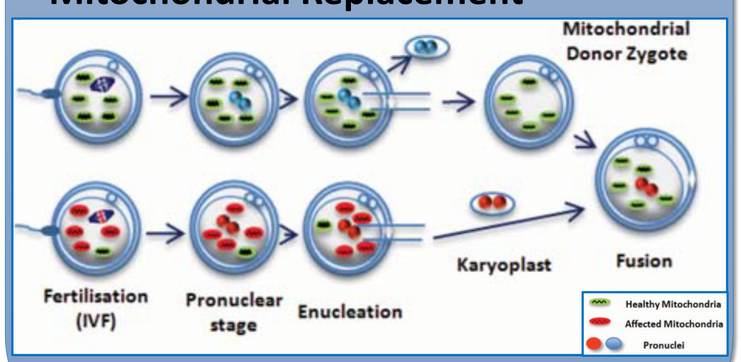
Introduction

Mitochondrial DNA (mtDNA) Disease

- mtDNA maternally inherited
- Can result in a range of isolated organ or multisystem disorders
- Currently no cure available
- Reproductive options for women:
  - Genetic counselling
  - Prenatal diagnosis
  - Preimplantation genetic diagnosis
- However, these techniques will not be suitable for all women with an mtDNA mutation

Mitochondrial Replacement



- Could be used to prevent transmission of mtDNA disease

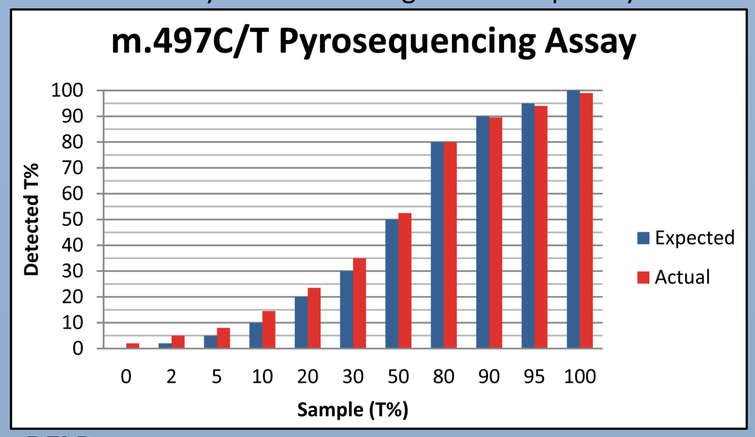
Aim

- Understand more about the safety and efficacy of mitochondrial replacement
- Need to consider the potential transfer of faulty mitochondria from the affected egg (carryover) during the manipulation
- Develop an assay that can detect minimal mtDNA carryover

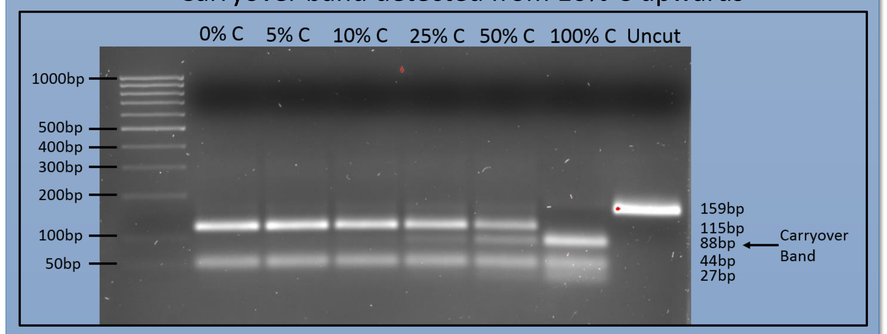
Results

Nucleotide m.497, Wild-type C, Variant T

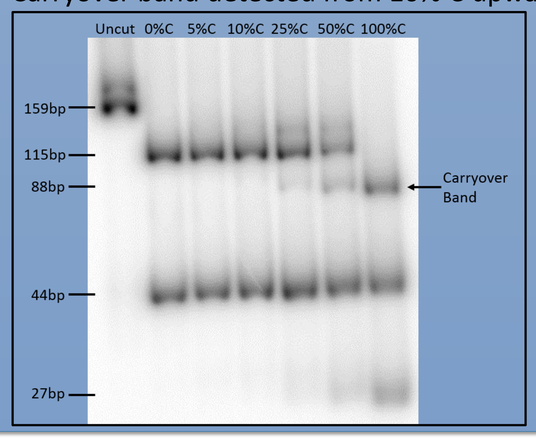
- **Pyrosequencing**
  - Accurately detected a range of heteroplasmy levels

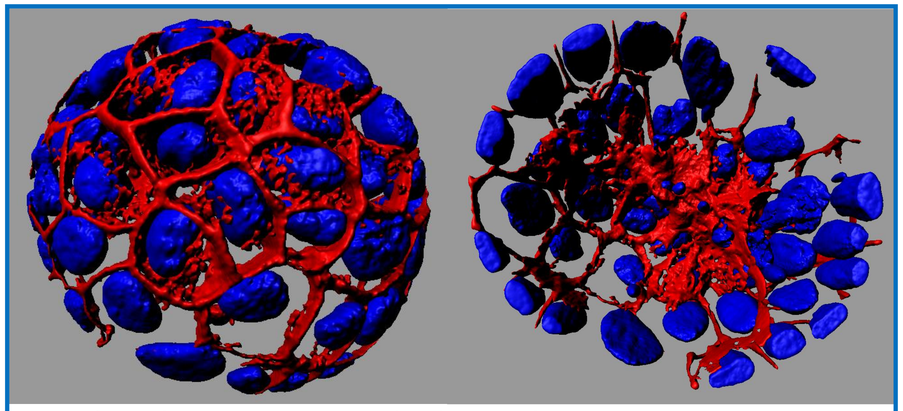


- **RFLP**
  - Carryover band detected from 10% C upwards



- **LHC-RFLP**
  - Carryover band detected from 10% C upwards





Confocal images on whole embryos

Phalloidin (red) – Staining cell membrane

Hoescht (blue) – Staining nuclear DNA

Methods

- Dilutions of wild-type: variant control DNA were set up to mimic the different heteroplasmy levels in mitochondrial replacement embryos
- 3 assays were tested to check their sensitivity against low heteroplasmy levels:
  - Pyrosequencing
  - Restriction Fragment Length Polymorphism (RFLP)
  - Last Hot Cycle-RFLP (LHC-RFLP)

Conclusions

- All 3 assays were able to detect mtDNA heteroplasmy
- For the m.497 variant, the pyrosequencing assay was able to accurately detect the lowest levels of heteroplasmy
- Mitochondrial replacement embryos will now be analysed using the m.497 pyrosequencing assay
- More data is needed to validate the alternative methods further

Reference

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

Craven, L., et al., *Mitochondrial DNA disease: new options for prevention.* Hum Mol Genet, 2011. 20(R2): R168-174