Reproductive technologies to prevent transmission of mitochondrial DNA disease

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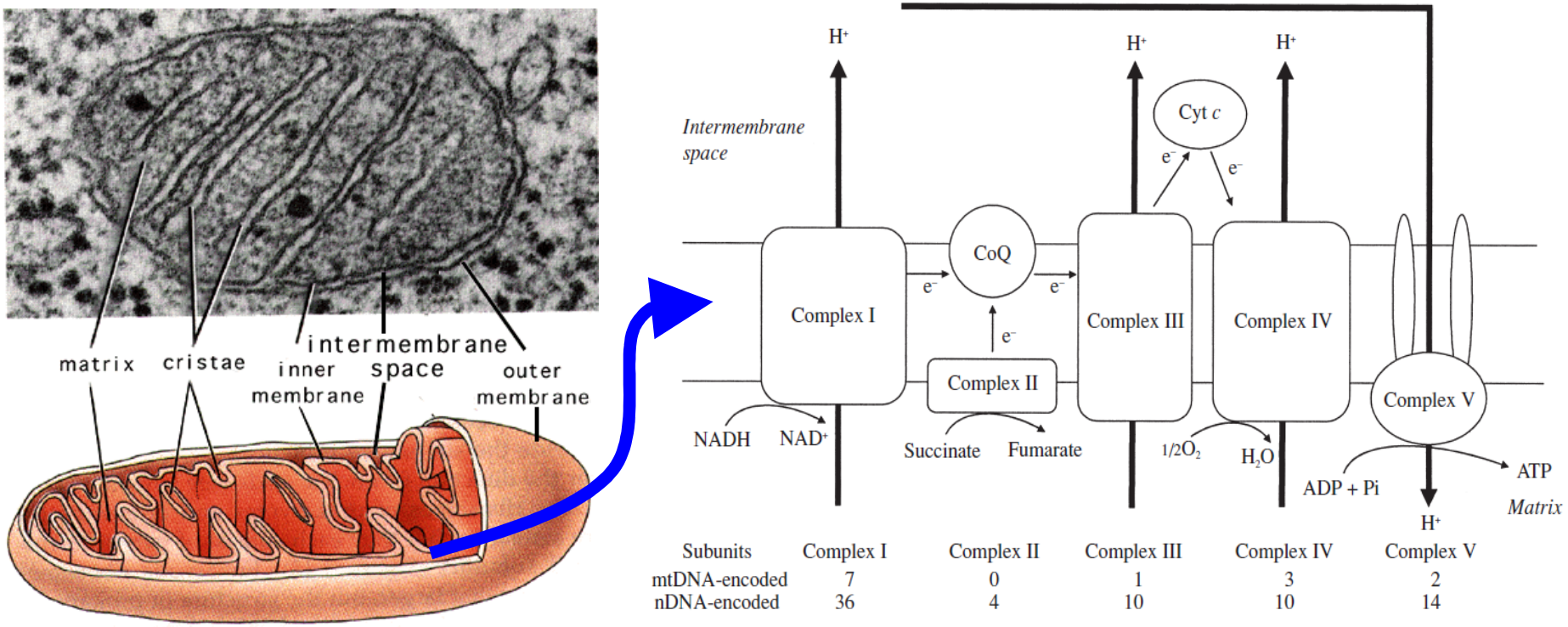
Mitochondria

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



Subunits	Complex I	Complex II	Complex III	Complex IV	Complex V
mtDNA-encoded	7	0	1	3	2
nDNA-encoded	36	4	10	10	14

Wei et al 2009

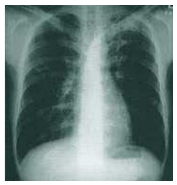
mtDNA mutations

- 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

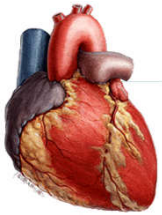
Diseases caused by mutations in mtDNA

Non-Neurological

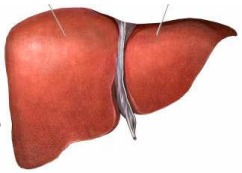
Respiratory Failure



Cardiomyopathy

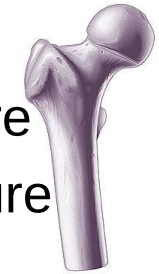


Liver Failure

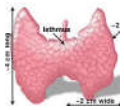


Short Stature

Marrow Failure

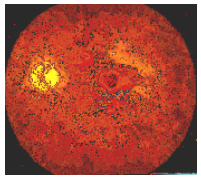


Diabetes

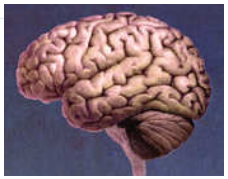


Thyroid Disease

Neurological

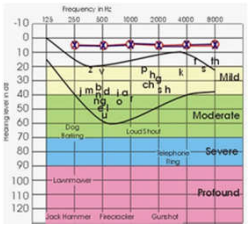


Optic Atrophy / Retinitis Pigmentosa

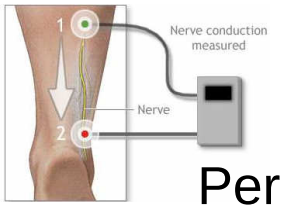


CVA / Seizures /

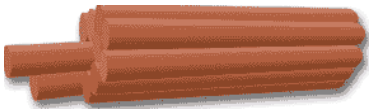
Developmental delay



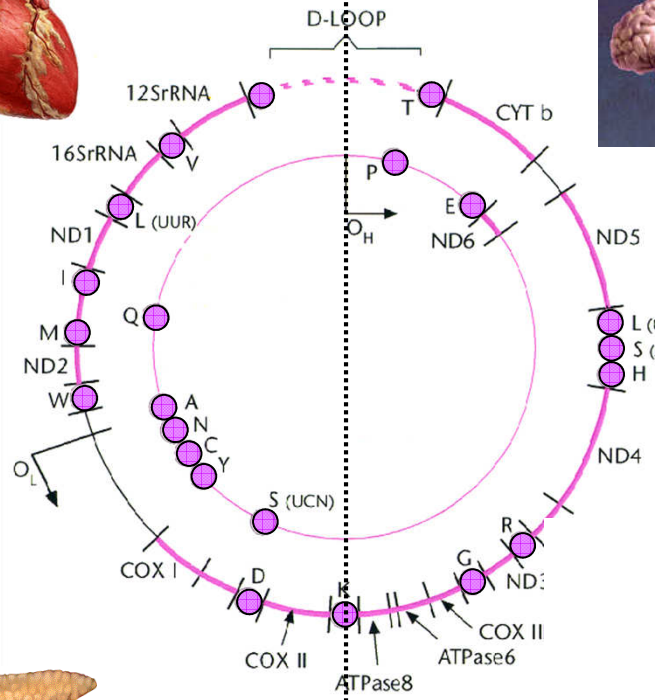
Deafness



Peripheral Neuropathy



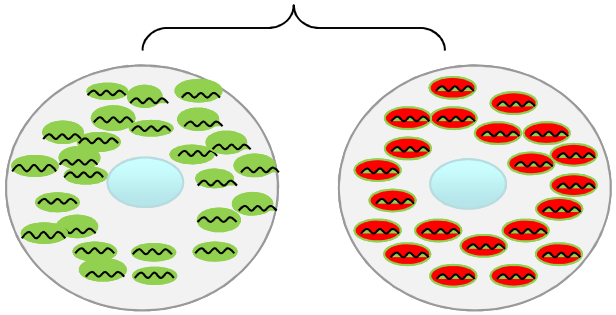
Myopathy



mtDNA mutations

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

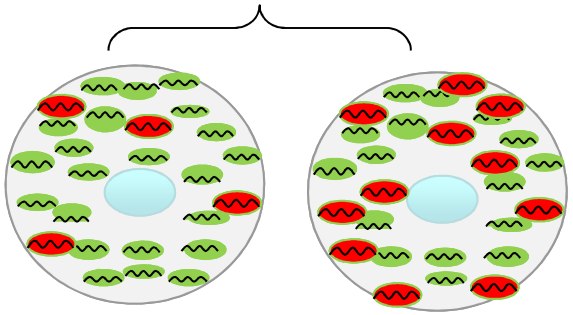
Homoplasmy



0% mutation

100% mutation

Heteroplasmy

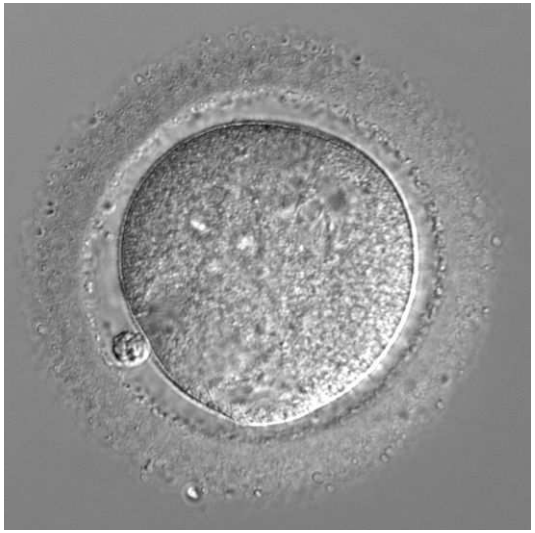


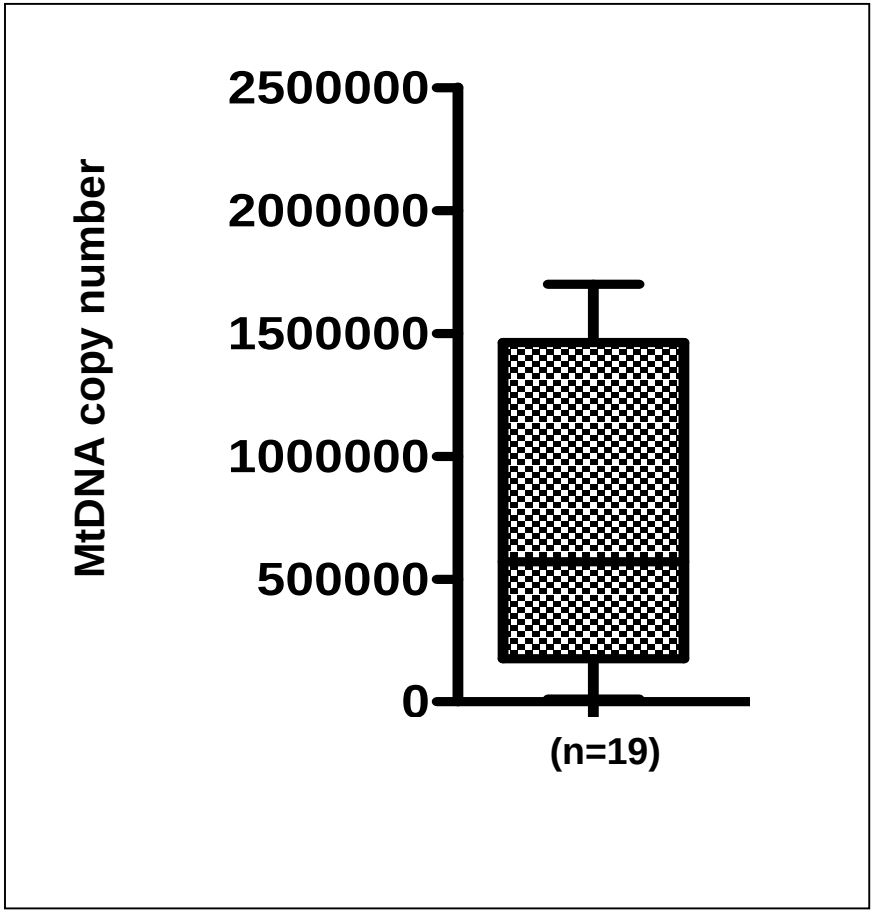
mix of mutated and normal mtDNA

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

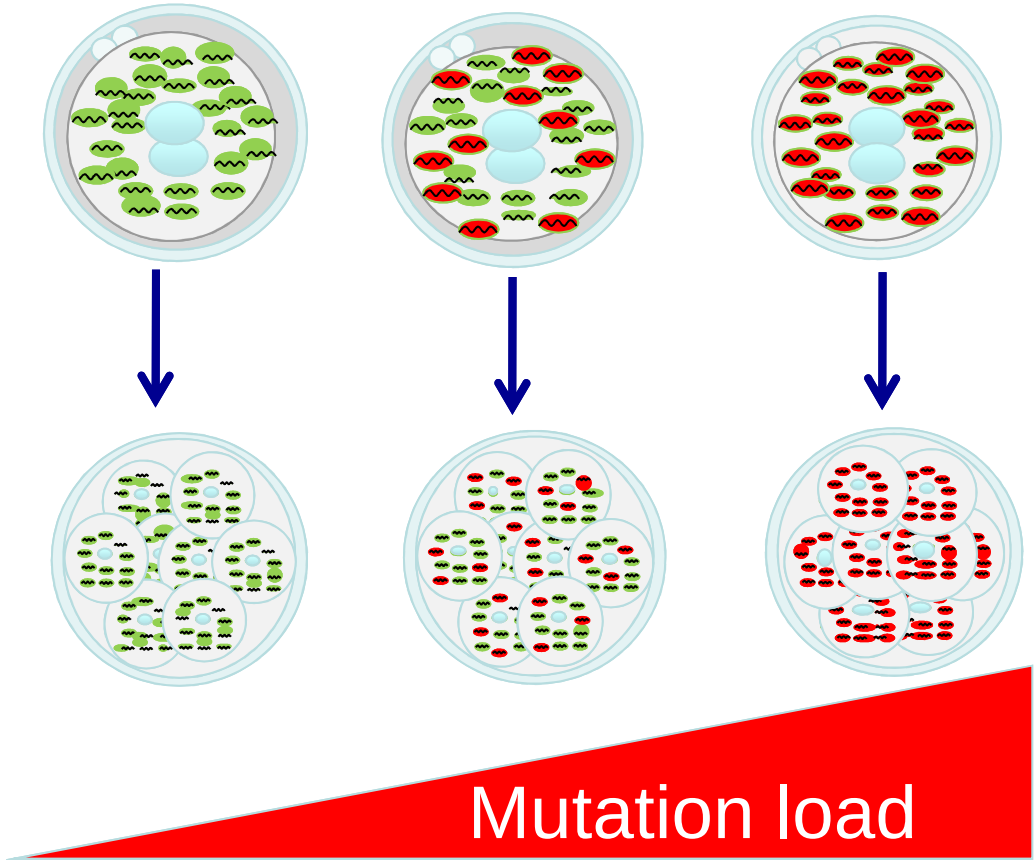
mtDNA: inherited only from our mothers

Human eggs contain abundant stock of mtDNA

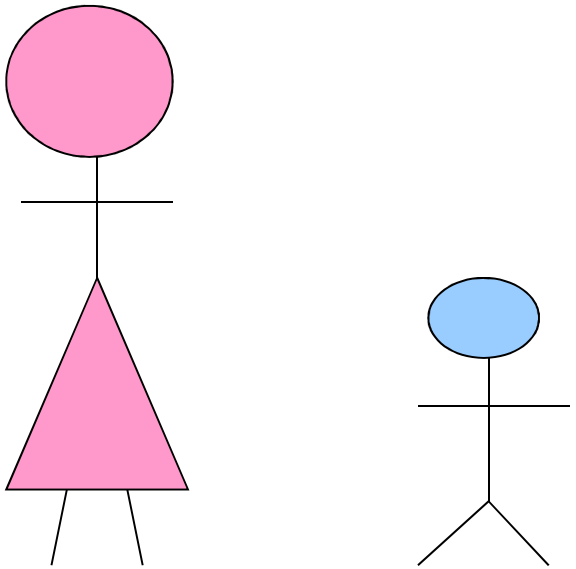




Wide variation in mtDNA mutation loads between eggs and embryos

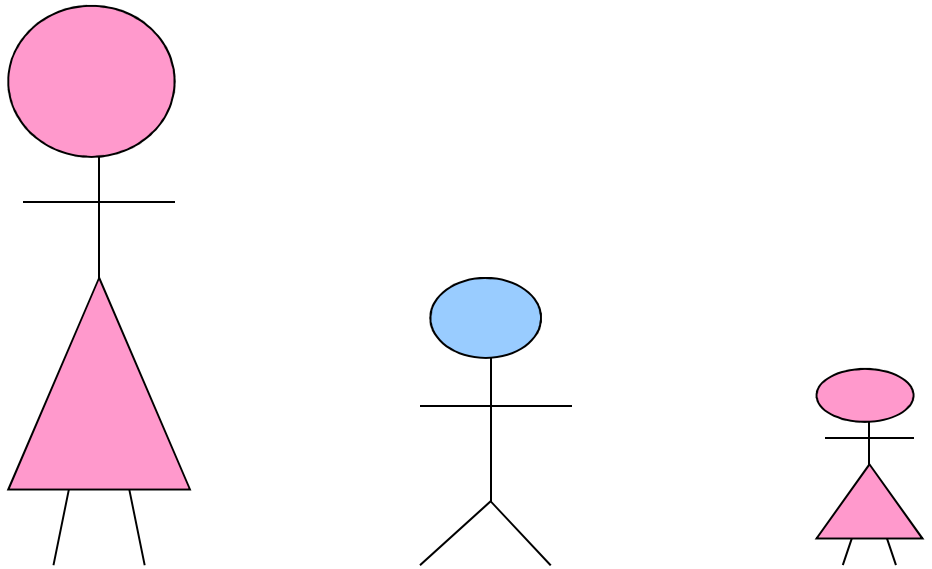


Reproductive consequences



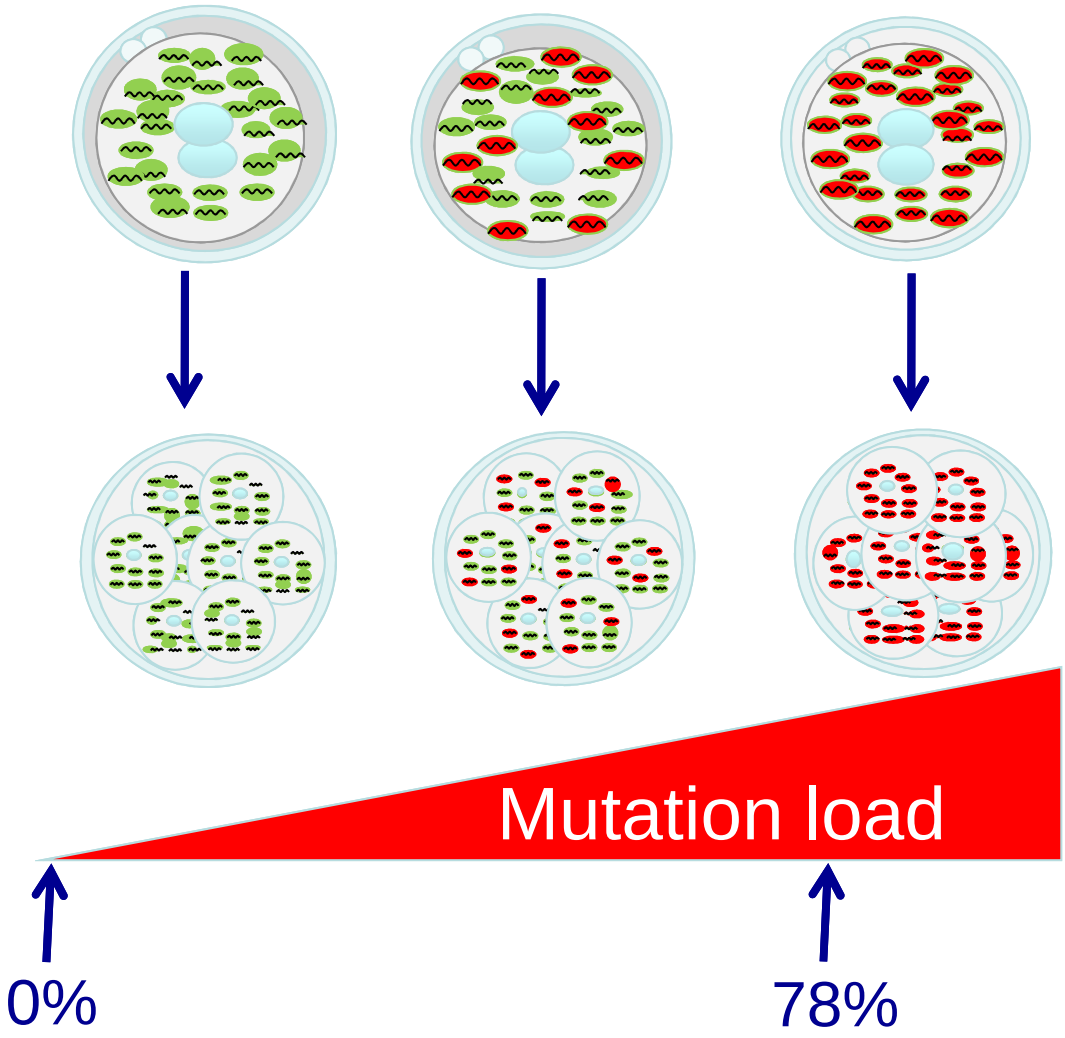
Mutation Load	Mother	Son 78%
	Mild clinical symptoms	Died aged 7 years

Reproductive consequences



Mutation Load	Mother 38%	Son 78%	Daughter 0%
	Mild clinical symptoms	Died aged 7 years	

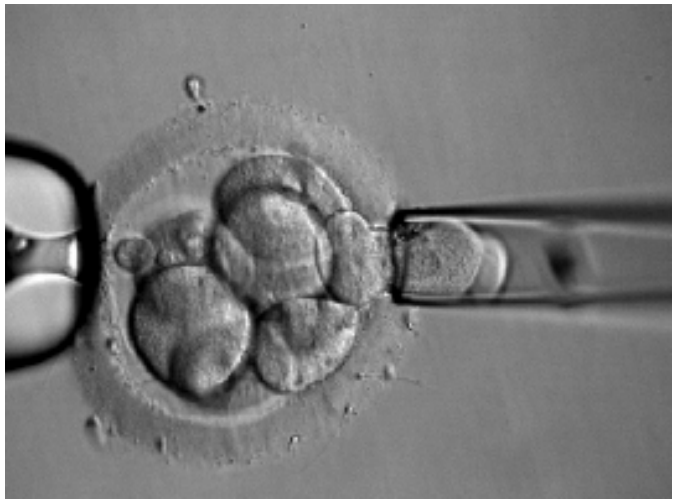
Wide variation in mtDNA mutation loads between eggs and embryos



What are the options for reducing risk of transmitting mtDNA mutations?

- Egg donation
- Prenatal diagnosis
- Pre-implantation genetic diagnosis (PGD)

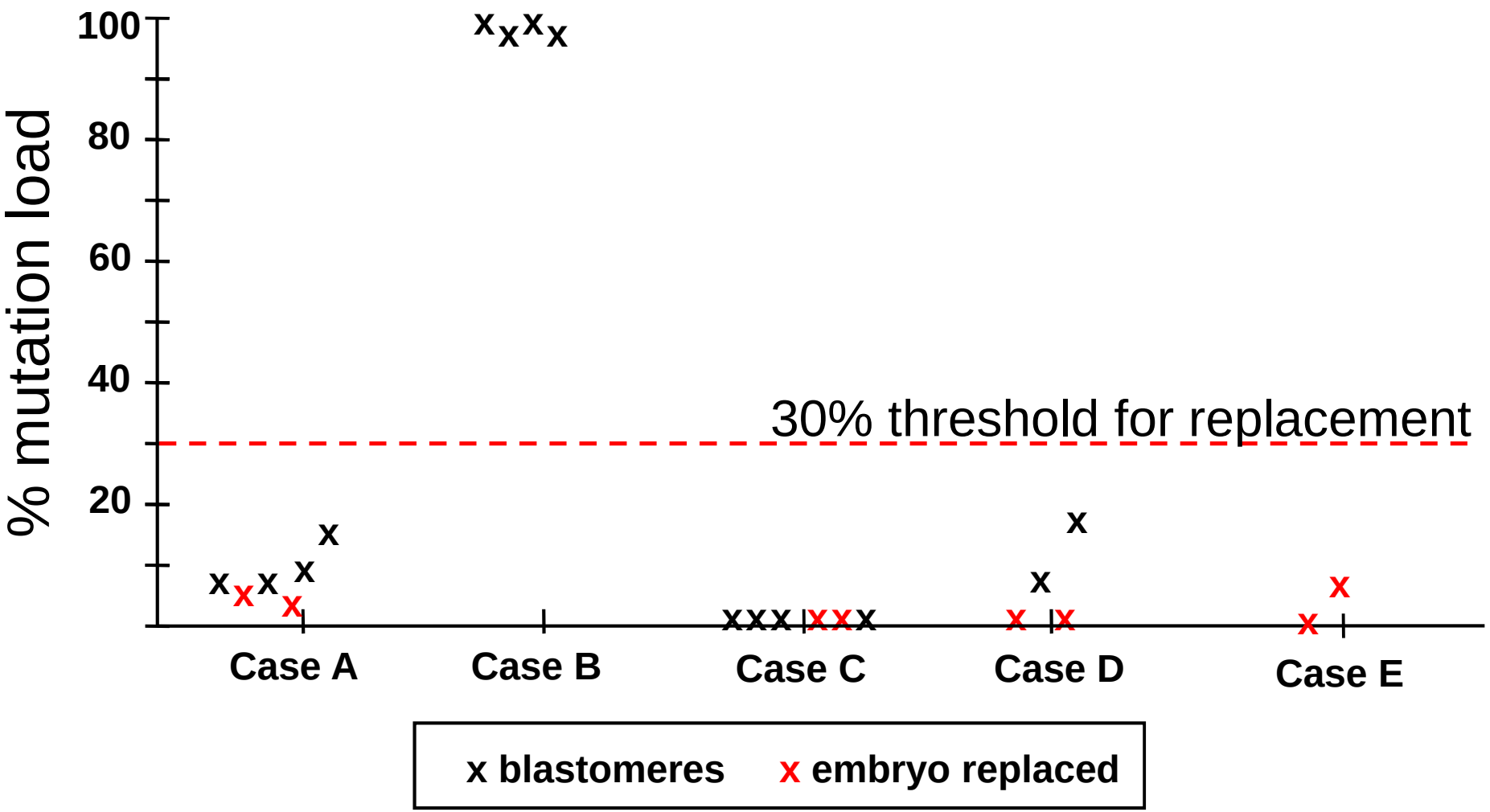
PGD can be used to detect mtDNA mutations



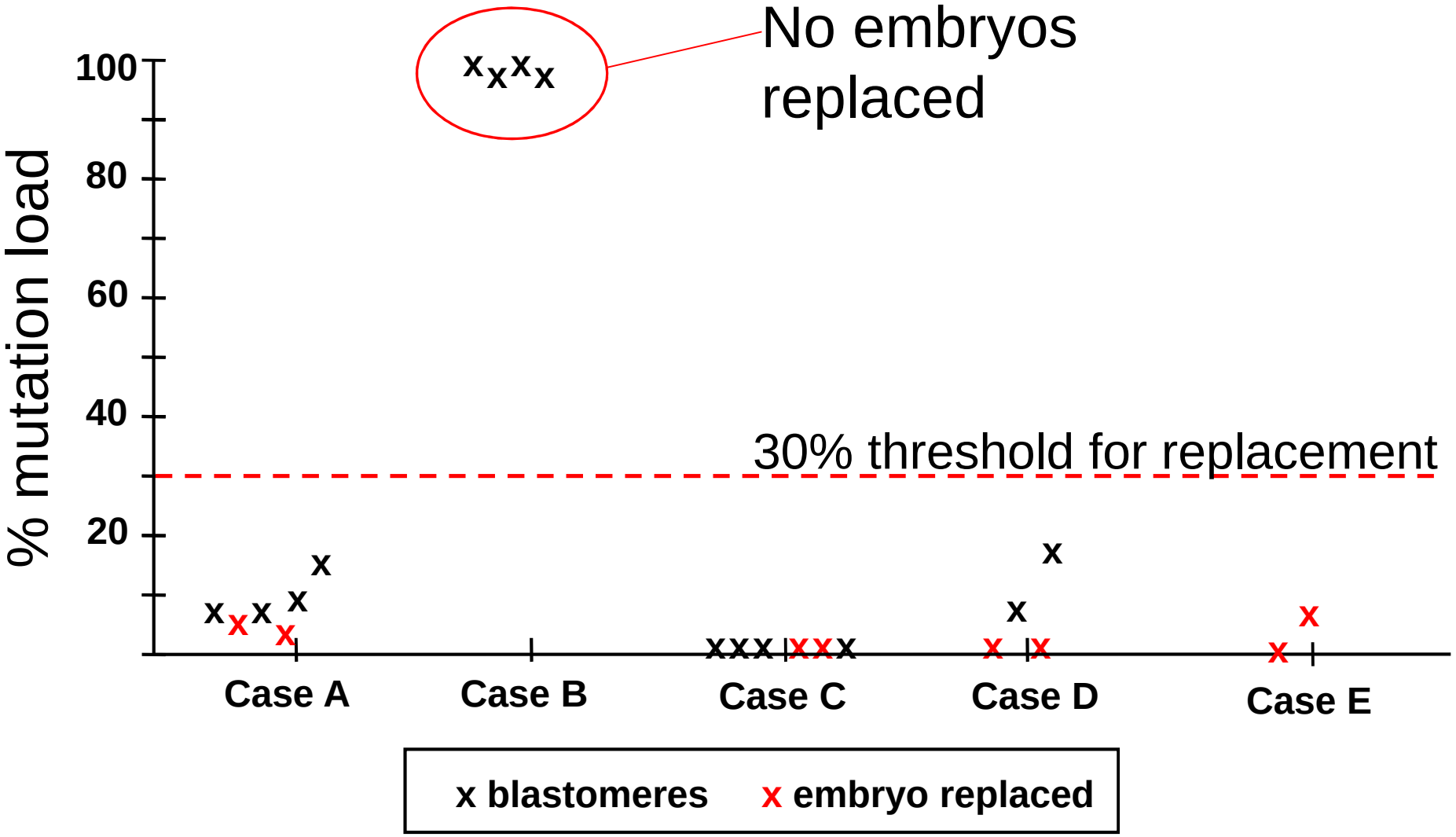
Single cell removed and sent to diagnostic lab for analysis

	Mutation level	**Risk of developing mitochondrial disease**
No mutation	0%	No risk
Low-level mutation	<30%	Lifetime risk extremely low
Intermediate-level mutation	31-70%	Risk rises with increasing mutation level
High-level mutation	>70%	High risk of severe disease

Summary of cases at Newcastle Fertility Centre

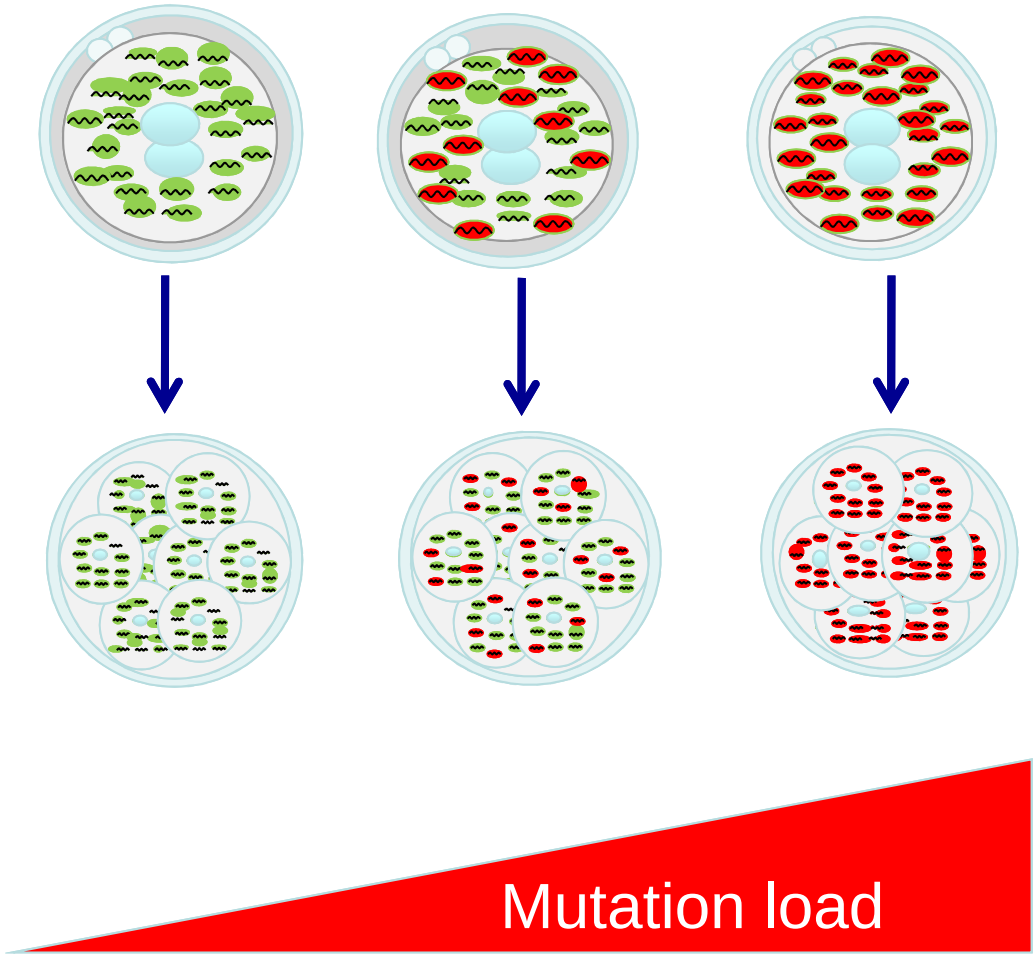


Summary of cases at Newcastle Fertility Centre



What can we offer in cases where all embryos have a high mutation load?

Are there alternative strategies?



Can we uncouple the inheritance of nuclear and mtDNA?

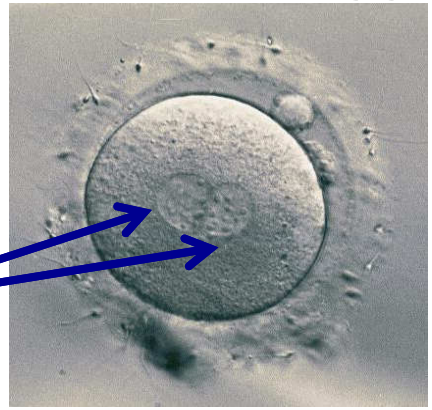
Not feasible to replace the mitochondria

Transplantation of the nuclear DNA

wild-type mitochondria mutant mitochondria

Pronuclear transfer

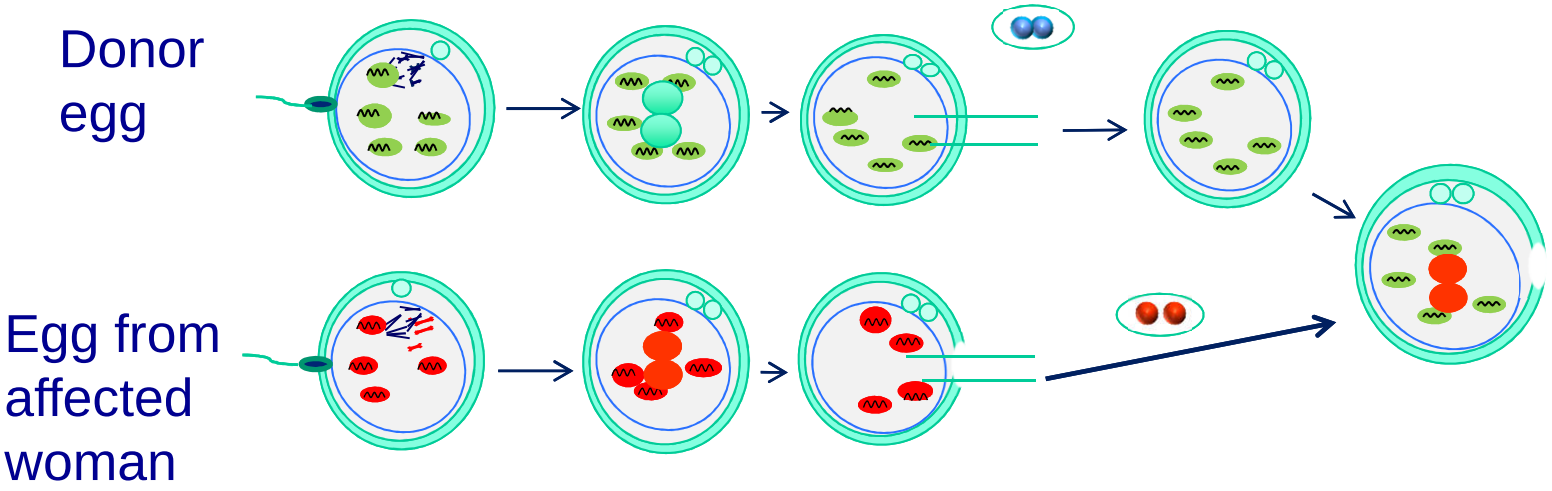
Fertilised egg



Pronuclei

- 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)

Pronuclear transfer strategy

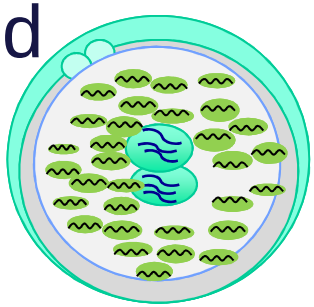


wild-type mitochondria mutant mitochondria

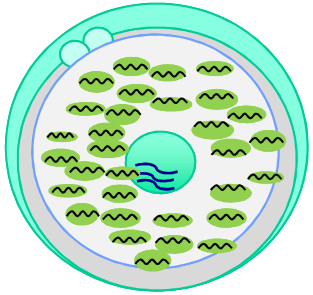
Challenges

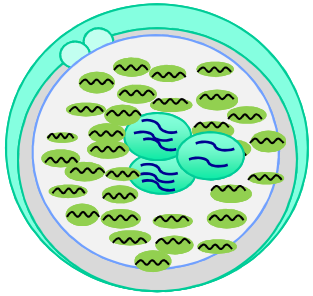
Research Material

– No ready supply of normally fertilised eggs available for research



– Initial experiments performed on abnormally fertilised human eggs (mono-pronucleate and tri-pronucleate)





Getting started: Legal challenges

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****"?*

Getting started: Legal challenges

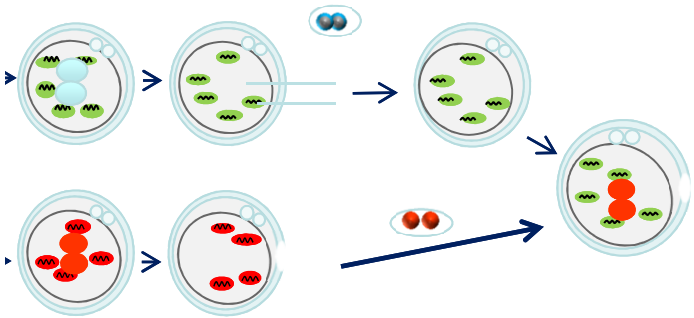
Pronuclear transfer involves:

- *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

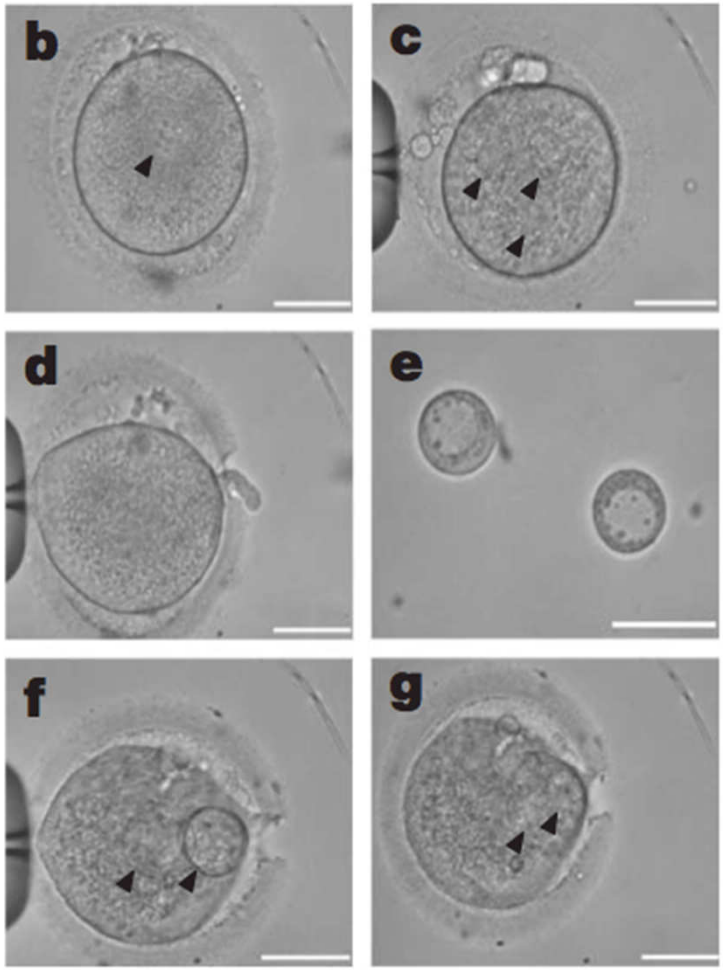
And then came the three parents

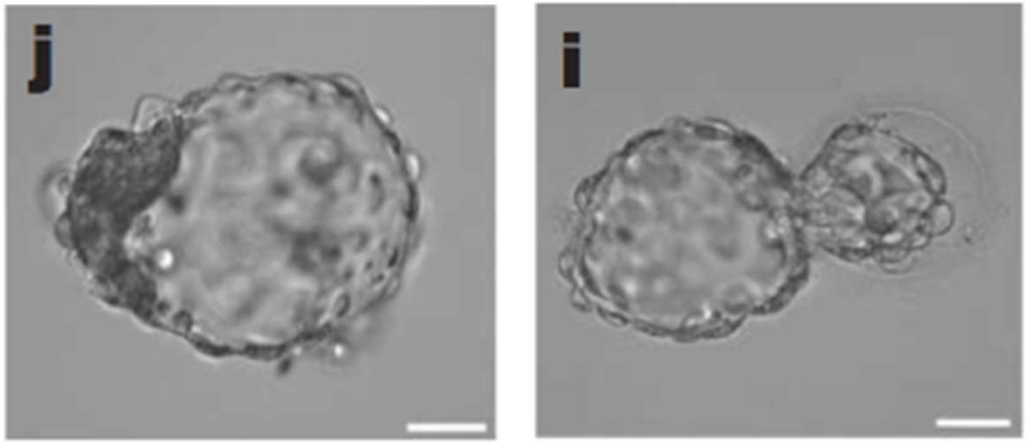
Back in the lab: Pronuclear transfer



- Is it technically feasible in human fertilised eggs?
- Can reconstituted fertilised eggs develop?
- Can we minimise the level of mtDNA carryover?

Monitor embryo development



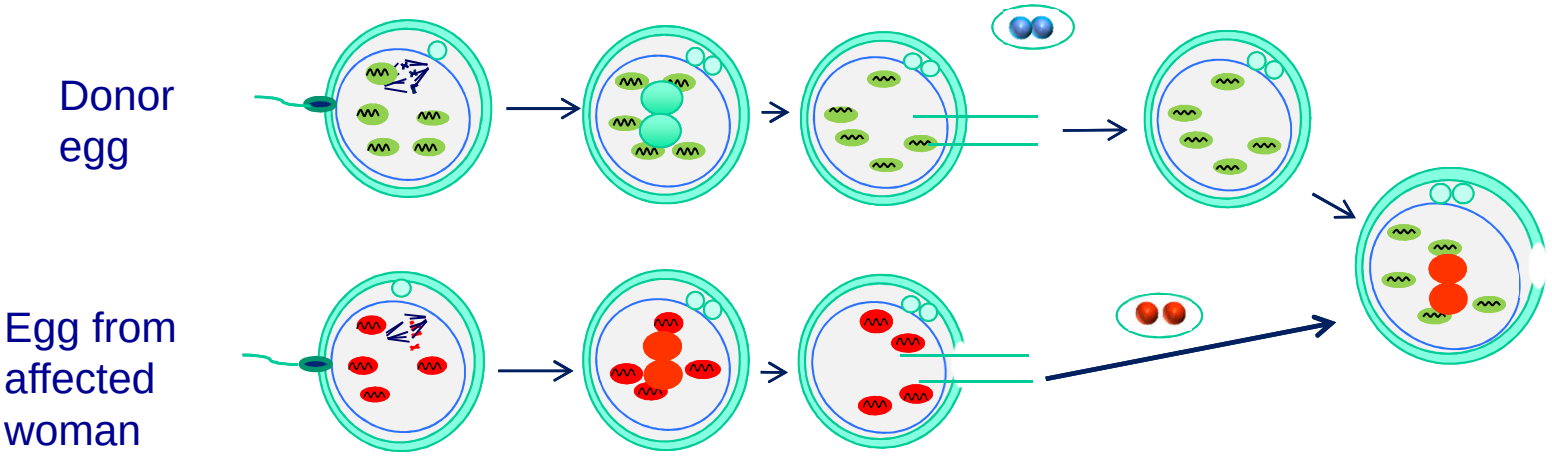


Embryo development:
(abnormally fertilised eggs)

- Unmanipulated controls: 17%
- Pronuclear transfer: 8%

Craven et al, 2010, Nature, vol. 465

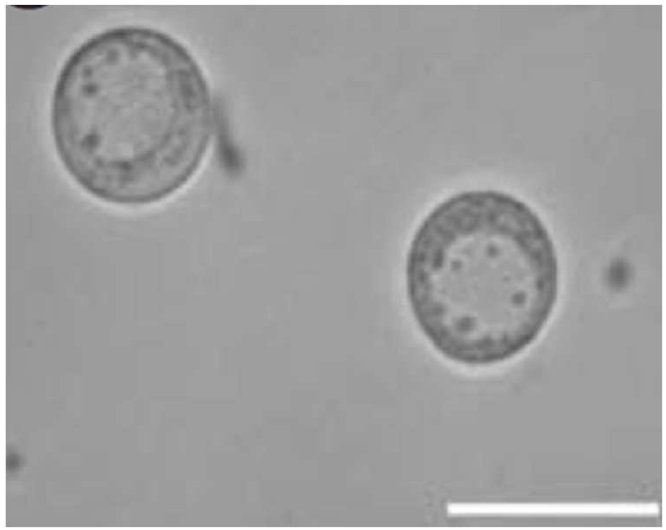
How much mtDNA are we transferring with the pronuclei



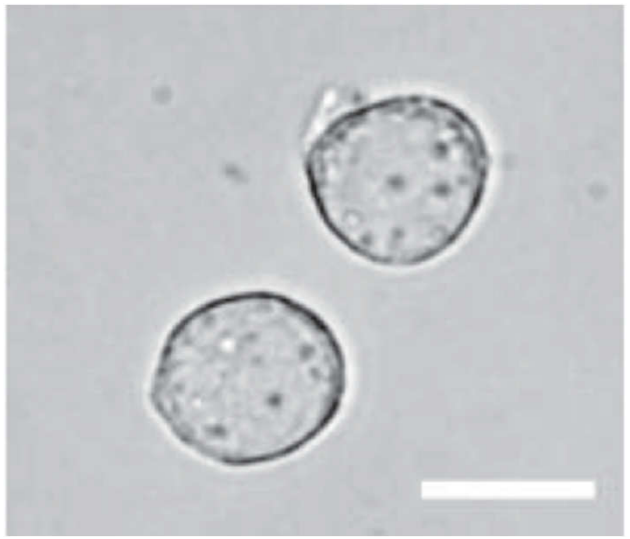
wild-type mitochondria mutant mitochondria

Optimisation of the procedure to minimise carry over of mtDNA

Before



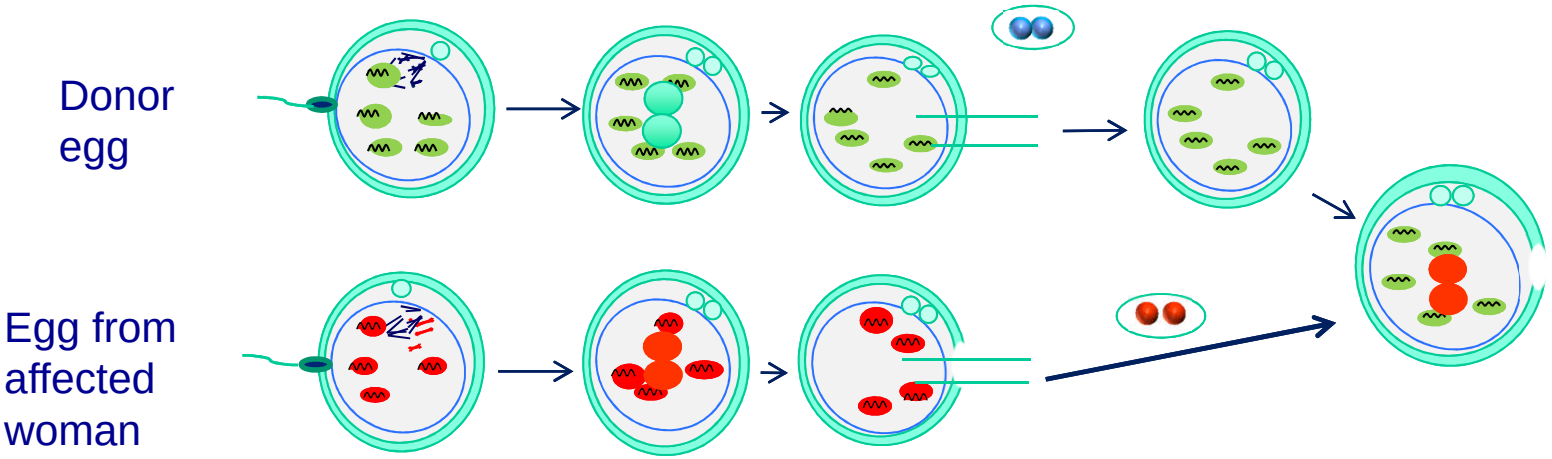
After



mtDNA carry-over	8.1± 7.6%	<2%

Craven et al, 2010, Nature, 465

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



wild-type mitochondria mutant mitochondria

Ongoing work: Testing Safety and Efficacy

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)

Legal and regulatory landscape

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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