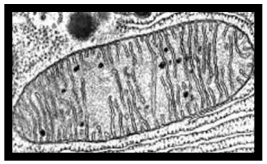
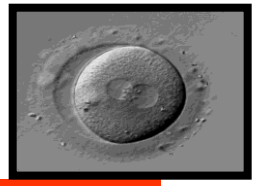
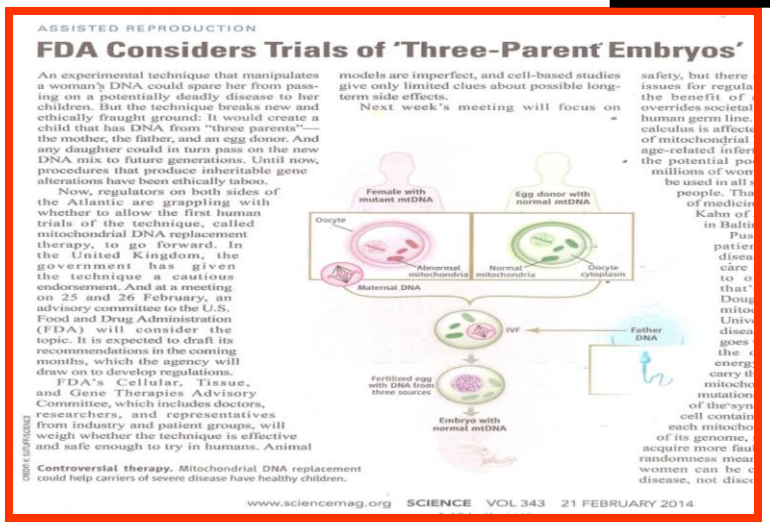
mtDNA disorder prevention: pros and cons of the different options







ASSISTED REPRODUCTION

FDA Considers Trials of 'Three-Parent' Embryos'

An experimental technique that manipulates a woman's DNA could spare her from passing on a potentially deadly disease to her children. But the technique breaks new and ethically fraught ground: It would create a child that has DNA from "three parents"—the mother, the father, and an egg donor. And any daughter could in turn pass on the new DNA mix to future generations. Until now, procedures that produce inheritable gene alterations have been ethically taboo.

Now, regulators on both sides of the Atlantic are grappling with whether to allow the first human trials of the technique, called mitochondrial DNA replacement therapy, to go forward. In the United Kingdom, the government has given the technique a cautious endorsement. And at a meeting on 25 and 26 February, an advisory committee to the U.S. Food and Drug Administration (FDA) will consider the topic. It is expected to draft its recommendations in the coming months, which the agency will draw on to develop regulations.

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Next week's meeting will focus on safety, but there issues for regula the benefit of overrides societal human germ line calculus is affect of mitochondrial age-related infer the potential po millions of wom be used in all people. Tha of medicin Kahn of in Balti Pus patien disea care to o that' Doug mito Univ disea goes the energ carry t mitoch mutation of the'syn cell contain each mitoch of its genome, acquire more fau randomness mea women can be disease, not disc

Female with mutant mtDNA
Egg donor with normal mtDNA
Oocyte
Abnormal mitochondria
Normal mitochondria
Oocyte cytoplasm
Maternal DNA
IVF
Father DNA
Fertilized egg with DNA from three sources
Embryo with normal mtDNA

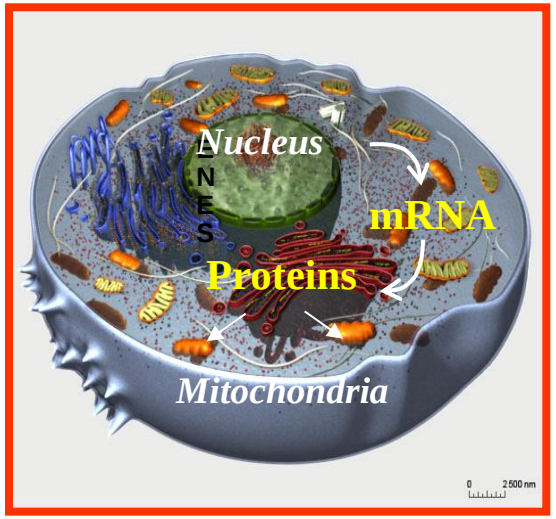
Controversial therapy. Mitochondrial DNA replacement could help carriers of severe disease have healthy children.

www.sciencemag.org SCIENCE VOL 343 21 FEBRUARY 2014

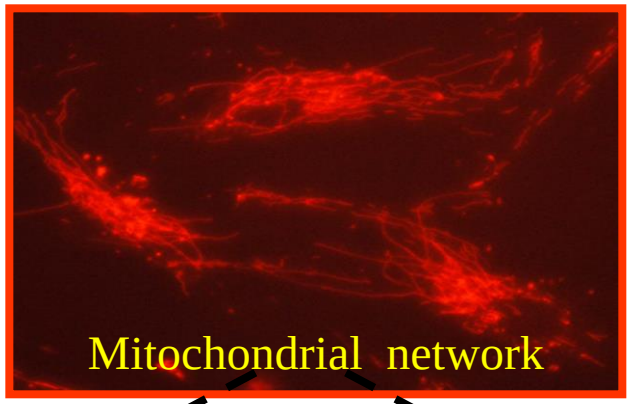
Jean-Paul Bonnefont

Université Paris Descartes / IHU IMAGINE
UMR1153 Equipe « maladies mitochondriales »
et
Assistance publique-Hôpitaux de Paris / GH Necker-Enfants malades
Laboratoire de génétique moléculaire

Nucleus-mitochondria crosstalk

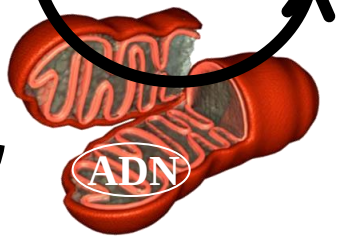


~ 1000 genes needed
for a mitochondria



Fuel
(O_2)

Energy
(ATP)

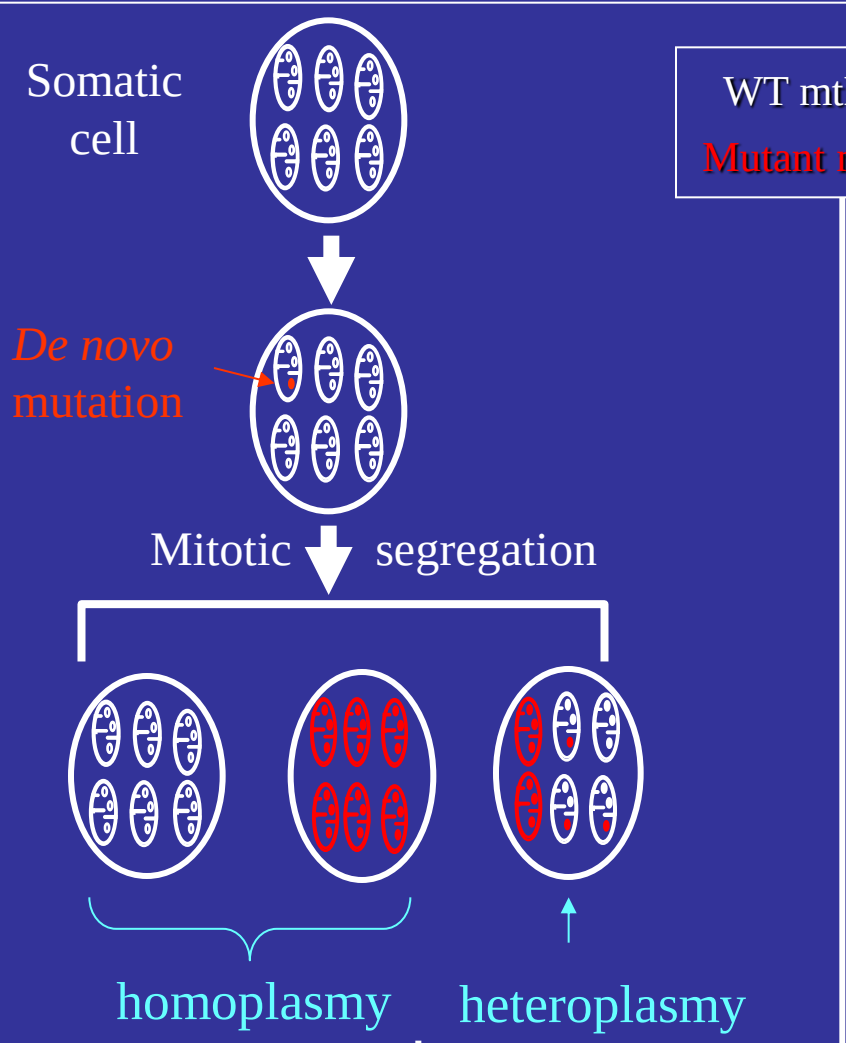


Nuclear genome
~ 20 000 protein-coding genes

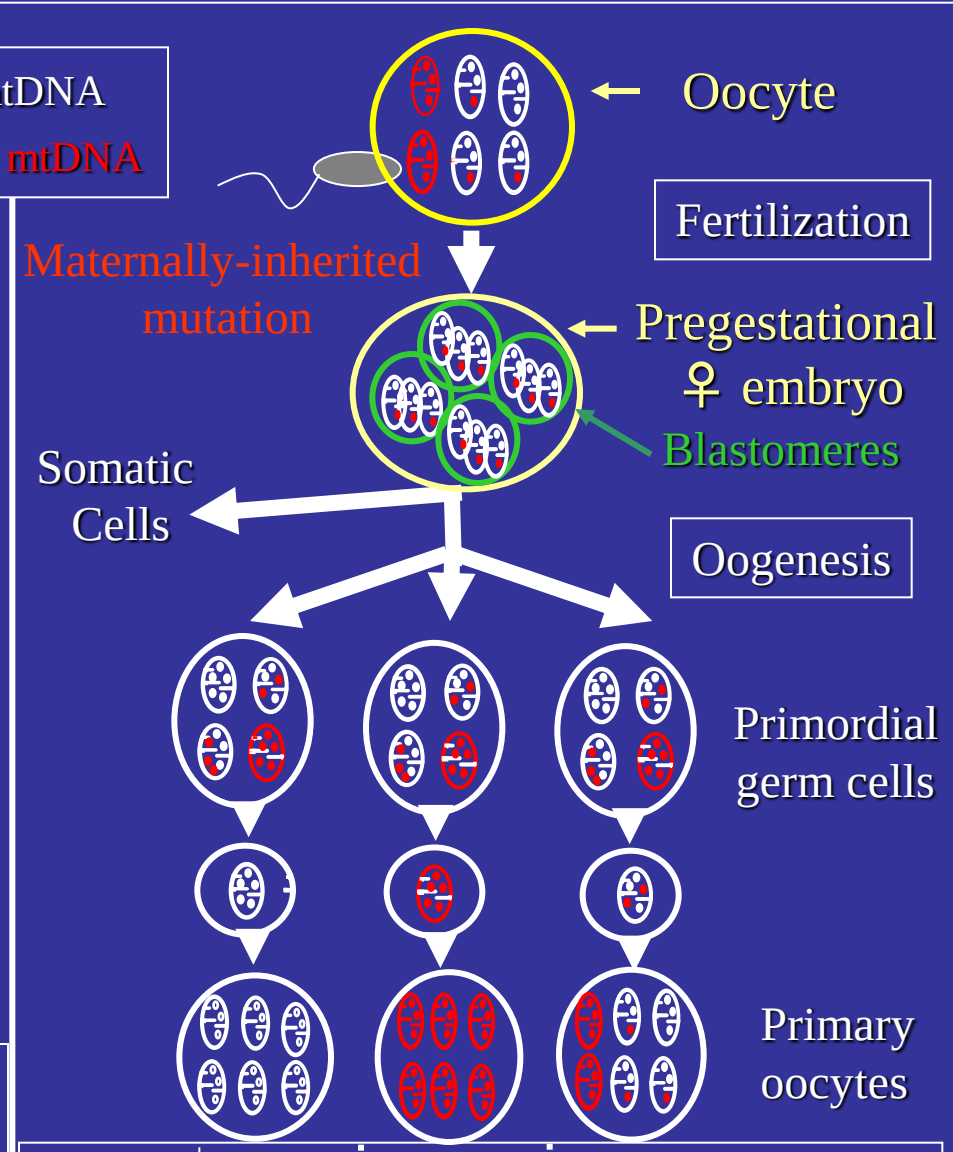
Mitochondrial signals
modulate the nuclear genome expression

Mitochondrial genome
13 protein-coding genes

Several copies of mtDNA in a mitochondria: A complex pattern of segregation of mtDNA mutations



Tissue function	OK	↓	↓ only over a tissue-dependant threshold of mutation



Embryo	healthy	affected	healthy or affected

m.3243A>G

gène **tRNA$^{Leu(UUR)}$**

↓

MELAS

Myopathy
Encephalopathy
Lactic
Acidosis
Stroke-like episodes

m.3460G>A
or m.11778G>A
or m.14484T>C

ND1, ND4, ND6 genes

↓

LHON

Leber Hereditary
Optic Neuropathy

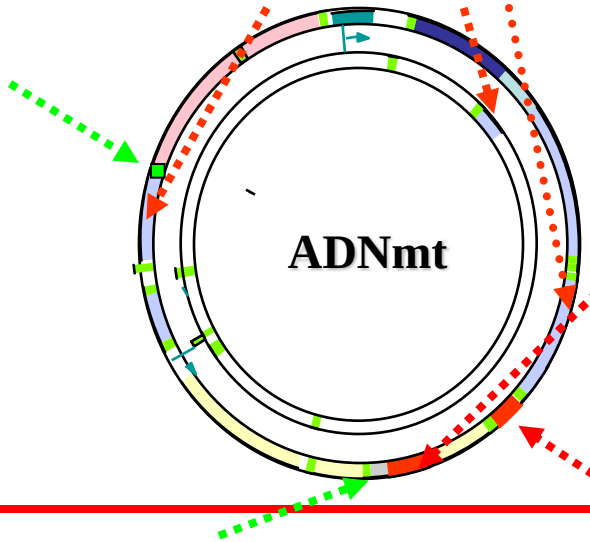
m.8993T>G/C
or m.9185T>C

ATPase 6 gene

↓

NARP

Neurogenic muscle weakness
Ataxia
Rétinitis
Pigmentosa



m.8344T>G
tRNA^{Lys} gene

→

MERRF

Myoclonic Epilepsy
Ragged Red Fibres

m.10197G>A

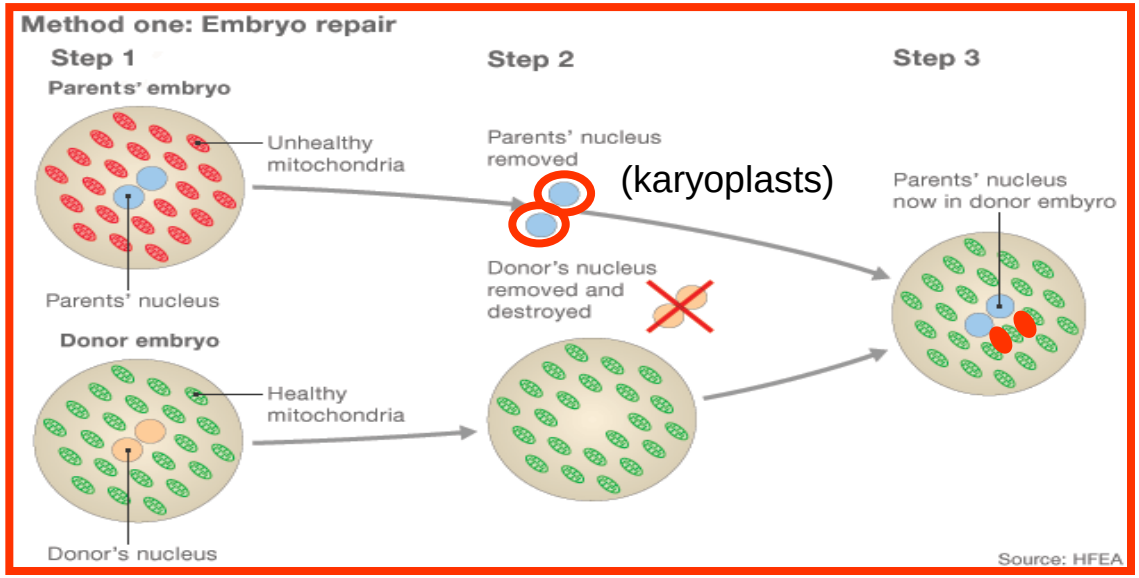
ND3 gene

↓

LEIGH Sd

Nuclear genome transfer from à mtDNA carrier into a mtDNA mutation-free recipient

- Aim: to achieve wild-type homoplasmic embryos in females carrying a mtDNA mutation
- Method: to transfer nuclear genetic material
  - from oocytes or preimplantation embryos, retrieved in a mtDNA carrier individual,
  - to enucleated oocytes or preimplantation embryos donated by a mtDNA mutation-free individual



Rationale behind nuclear genome transfer procedures:
1/Reliability of PND/PGD methods would be uncertain

1/ The mutant load assessed from one human blastomere (day 4)

- accurately reflects the mutant load of the whole embryo,

(Steffann et al. Cell Report 2014)

- remains stable throughout the embryo fetal development

(Monnot et al. Hum Mut 2010)

2/ The mutant load assessed in a given fetal tissue (10-32 gestation weeks)

- accurately reflects the mutant load in all fetal tissues

(Steffann et al. J Med Genet 2007; Monnot et al. Hum Mut 2010)

3/ The mutant load assessed in cord blood cells at birth

- accurately reflects the mutant load measured in amniocytes

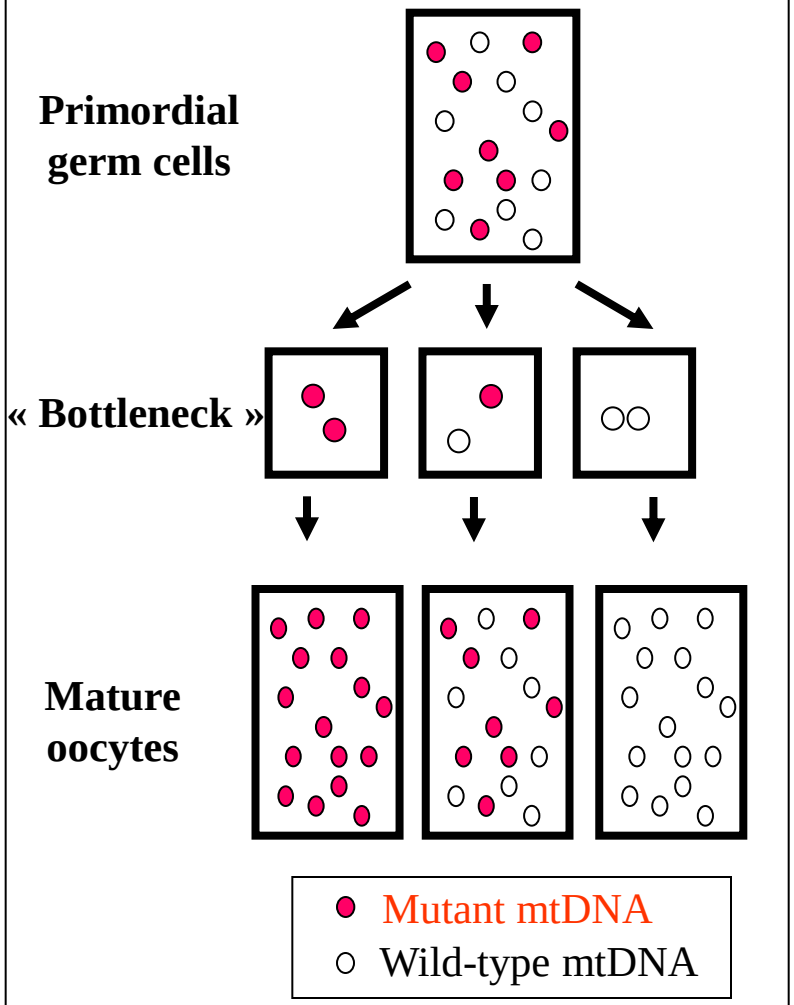
(personal data)

irrespective of the mtDNA mutation type

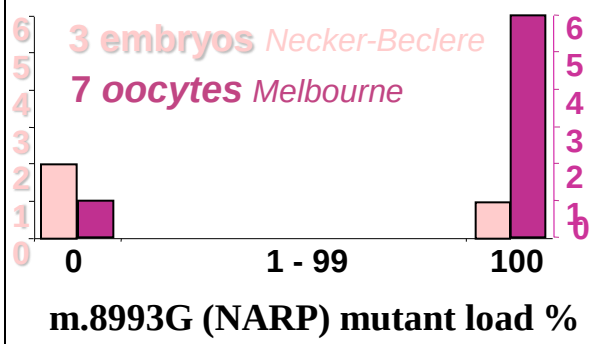
Rationale behind nuclear genome transfer procedures:

2/ the probability to have a healthy offspring through PND/PGD methods would be uncertain

mtDNA segregation throughout oogenesis



Mutant load in **oocytes** /embryos from carriers



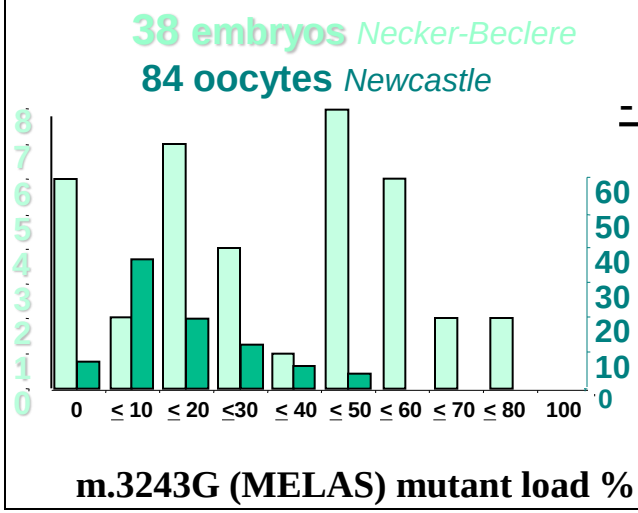
Probability of

- wt homoplasmic embryo

30 % (3/10)

- Heteroplasmic (≤30 %)

0 %



Probability of

- wt homoplasmic embryo

6 % (7/122)

- Heteroplasmic (≤30 %)

72 % (89/122)

Rationale behind nuclear genome transfer procedures:

3/ The predictive value of a embryofetal mutant load for the postnatal outcome would be poor

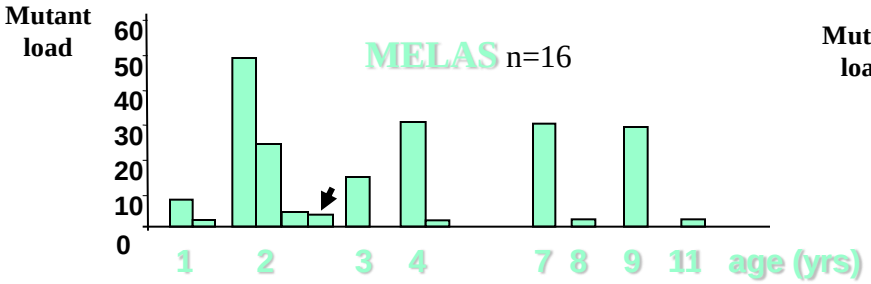
Postnatal data

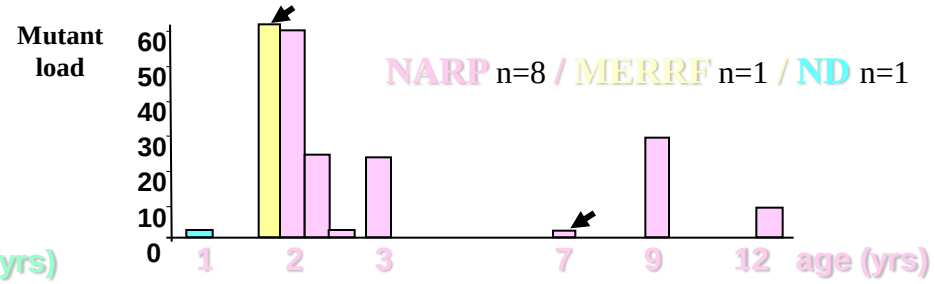
mutant load threshold for disease expression

- MELAS (m.3243G) ≥ 60 %
- NARP/Leigh (m.8993T>G/C) ≥ 60 %

PND-PGD Necker 2005-2015

- Attitude: mutation load - < 30 %: PND continuation of pregnancy PGD reimplantation
  - 30-60 % : PND/PGD discussion with the couple
  - > 60 %: PND termination of pregnancy PGD embryo discarded
- Number: 53 as PND 43 and PGD 10 (12 different mutations)
- Results: *Follow-up of children born after a PND or PGD procedure operated at Necker*





26 children (PND 23 , PGD 3) , all being symptom-free ⟶ « success » rate 50 % (26/53)

PND/PGD of mtDNA mutations :issues

1/ Frequency of high levels of embryofetal heteroplasmy

PND: termination of pregnancy (50%)

PGD: impossibility of embryo transfer

2/ Residual risk of disease in embryos/fetus with « low » heteroplasmy

3/ Low probability of healthy offspring in mutant homoplasmic women

Other approaches ?

Cytosolic transfer

→ Excess of chromosomal anomalies

→ Prohibited by FDA in USA

Nuclear genome transfer

A number of approaches devoted to nuclear genome transfer from à mtDNA carrier into a mtDNA- mutation free recipient

- **Pronuclei transfer between zygotes**

  - Mouse — Japon, 2005, *PNAS*

    Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation

    Akitsugu Sato*†‡, Tomohiro Kono§, Kazuto Nakada*†¶, Kaori Ishikawa*†, Shin-Ichi Inoue*, Hiromichi Yonekawa‡, and Jun-Ichi Hayashi*‖

  - Human — Newcastle, UK, 2010, *Nature*

    Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease

    Lyndsey Craven[1], Helen A Tuppen[1], Gareth D Greggains[2,3], Stephen J Harbottle[2], Julie L Murphy[1], Lynsey M Cree[1], Alison P Murdoch[2,4], Patrick F Chinnery[1], Robert W Taylor[1], Robert N Lightowlers[1], Mary Herbert[2,3,4], and Douglass M Turnbull[1,4,5]

- **Meiotic Spindle transfer between oocytes**

  - Monkey — Portland, USA, 2009, *Nature*

    Mitochondrial gene replacement in primate offspring and embryonic stem cells

    Masahito Tachibana[1], Michelle Sparman[1], Hathaitip Sritanaudomchai[1], Hong Ma[1], Lisa Clepper[1], Joy Woodward[1], Ying Li[1], Cathy Ramsey[1], Olena Kolotushkina[1] & Shoukhrat Mitalipov[1,2,3]

  - Human — New-York, USA, 2013, *Nature*

    Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants

    Daniel Paull[1], Valentina Emmanuele[2], Keren A. Weiss[1], Nathan Treff[3], Latoya Stewart[1], Haiqing Hua[1,4], Matthew Zimmer[1], David J. Kahler[1], Robin S. Goland[4], Scott A. Noggle[1], Robert Prosser[5], Michio Hirano[2], Mark V. Sauer[5,6]* & Dieter Egli[1]*

    Portland, USA, 2013, *Nature*

    Towards germline gene therapy of inherited mitochondrial diseases

    Masahito Tachibana[1], Paula Amato[2], Michelle Sparman[1], Joy Woodward[1], Dario Melguizo Sanchis[1], Hong Ma[1], Nuria Marti Gutierrez[1], Rebecca Tippner-Hedges[1], Eunju Kang[1], Hyo-Sang Lee[1], Cathy Ramsey[1], Keith Masterson[2], David Battaglia[2], David Lee[2], Diana Wu[2], Jeffrey Jensen[1,3], Phillip Patton[2], Sumita Gokhale[4], Richard Stouffer[1,2], and Shoukhrat Mitalipov[1,2]

- **Polar body genome transfer in oocytes or zygotes**

  - Mouse — Shanghaï, Chine + Harvard, USA, 2014, *Cell*

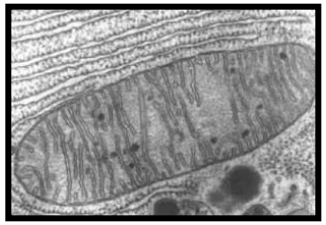
    Polar Body Genome Transfer for Preventing the Transmission of Inherited Mitochondrial Diseases

    Tian Wang,[1,4] Hongying Sha,[1,4,*] Dongmei Ji,[2,4] Helen L. Zhang,[3] Dawei Chen,[2] Yunxia Cao,[2,5] and Jianhong Zhu[1,5,*]

Nuclear genome transfer: issues

Selection of recipient oocytes/embryos

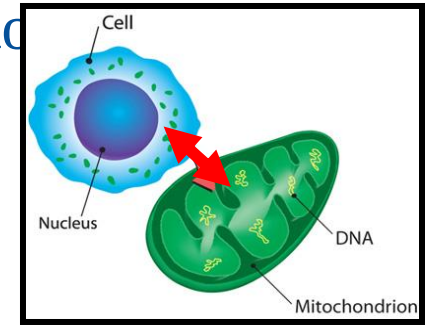
Relatives ? Non relatives ?



Rate of success

Impact on development: *long-term risks?*

- Dysruption of physical links between nucleus and cyto



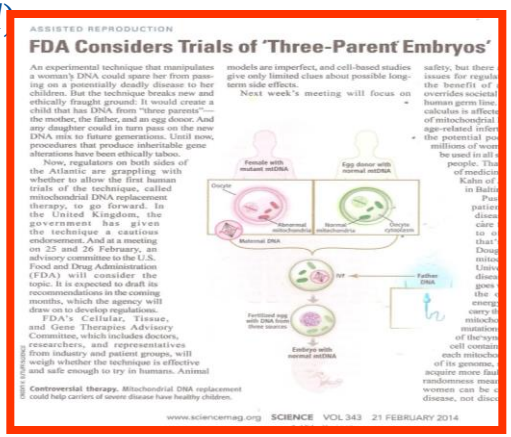
- Impairment of nucleus-mitochondria crosstalk ?

(Nagao et al., 1998, *Genes Genet. Syst.*, Johnson et al., 2001 *Nat Genet.*
Carelli et al., 2003, *Trends Genet.*, Roubertoux et al., 2003, *Nat Genet.)*

- Tolerance of the « contaminant » heteroplasmy ?

(active maintenance of homoplasmy in mammals…)

murine models: Acton et al., 2007, *Biol Reprod*, Sharpley et al., 2012, *Cell*


ASSISTED REPRODUCTION

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Female with mutant mtDNA
Egg donor with normal mtDNA
Oocyte
Nucleus
Abnormal mitochondria
Normal mitochondria
Oocyte cytoplasm
Maternal DNA
IVF
Father DNA
Fertilized egg with DNA from three sources
Embryo with normal mtDNA

Controversial therapy. Mitochondrial DNA replacement could help carriers of severe disease have healthy children.

DREW KUNTZINGER

www.sciencemag.org SCIENCE VOL 343 21 FEBRUARY 2014

Ethics / Psychology: 3-parent embryos

Jeffrey Kahn, Bioethics, Baltimore *« by mixing new DNA into the germ line, we're not treating humans. We're creating humans. There's not a model for that… »*

Legal *(most countries):* prohibition of genome modifications impacting the germ cell line in human

Laboratoire de génétique moléculaire

IHU IMAGINE

- S. Monnot
- N. Gigarel
- JP. Bonnefont
- R Frydman
- A. Munnich
- A Rotig
- J Steffann

Biologie de la Reproduction

N. Frydman

- L. Hesters
- G Tachdjian

Gynécologie-Obstétrique

- L. Grunfeld
- R. Fanchin
- A. Benachi

Gynécologie-Obstétrique

- L. Salomon
- P. Roth
- Y. Ville

GH Necker-Enfants Malades

Paris, France

Foetopathologie

- J Martinovic

Hôpital Antoine-Béclère

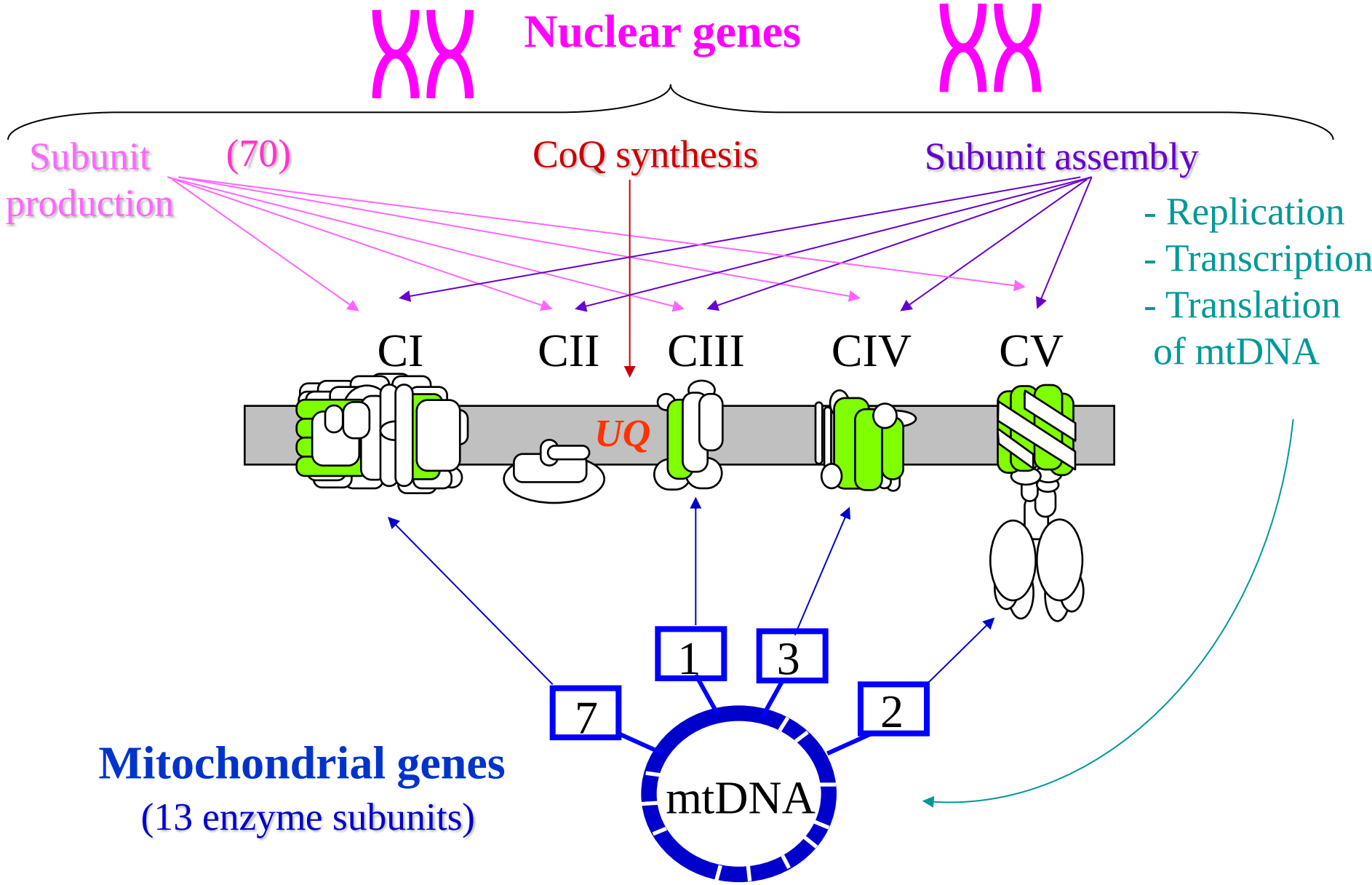
Clamart, France

Molecular Physiology and Biophysics

DC. Samuels

Vanderbilt University, Nashville, USA

The 5 respiratory chain enzymatic complexes: a double origine, nuclear and mitochondrial

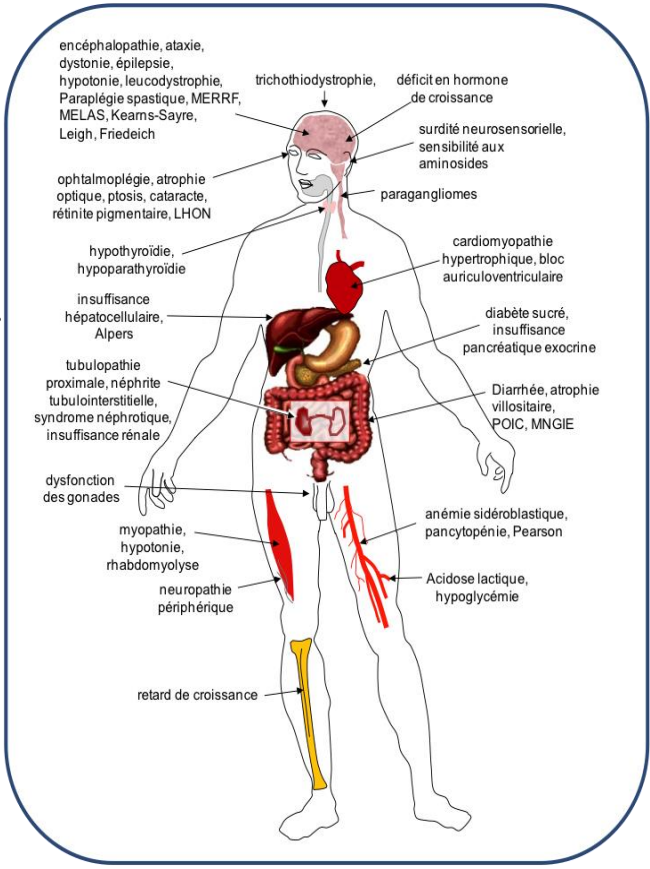


Les cytopathies mitochondriales

Incidence ~ élevée (1/5 000-10 000 enfants)

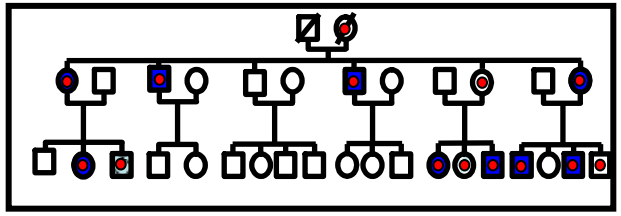
Atteinte pléiotropique

–neurologique, centrale et périphérique
–neurosensorielle (atrophie optique, rétinopathie, surdité…
–musculaire
–cardiaque
–endocrinienne (diabète sucré…)
–Hépatique
–Rénale



Pronostic: bénin à gravissime

Hérédité - mendélienne: 3/4
- maternelle par mutation de l'ADNmt: 1/4



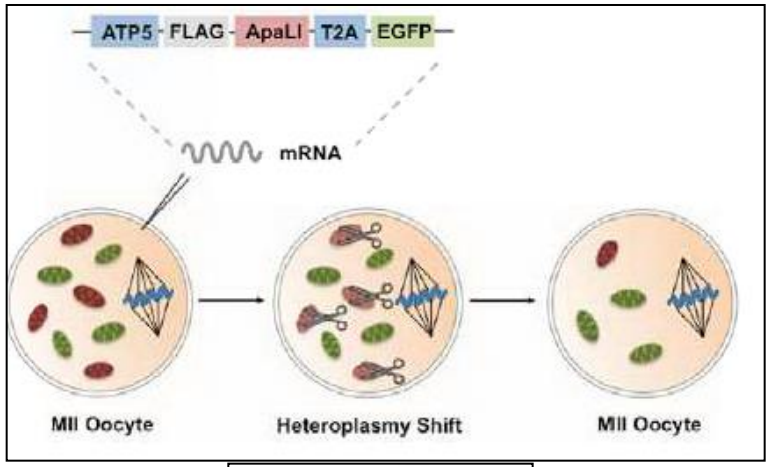
Traitement ~ 0

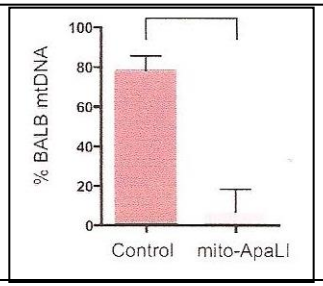
Comment prévenir la transmission intergénérationnelle ?

Transfert de pronuclei *vs* fuseau *vs* GP

- Transfert de PN
  - PN ~ faciles à visualiser
  - plus volumineux que fuseau ↑ risque de lésion cellulaire
  - nombre de centrosomes anormal ↑ risque d'aneuploidie
- Transfert de fuseau
  - difficulté visualisation + isolement chromosomes MII
  - réactivation ovocyte avant fécondation
- Transfert de GP
  - GP faciles à visualiser
  - peu de mitochondries: ↓ risque de contamination du receveur par de l'ADNmt muté

Les alternatives au transfert nucléaire





Genome editing mitochondrial

Selective Elimination of Mitochondrial Mutations in the Germline by Genome Editing

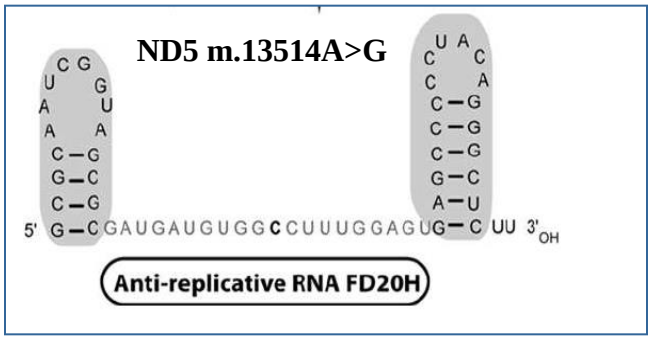
Pradeep Reddy,[1,14] Alejandro Ocampo,[1,14] Keiichiro Suzuki,[1] Jinping Luo,[1] Sandra R. Bacman,[2] Sion L. Williams,[2] Atsushi Sugawara,[1] Daiji Okamura,[1] Yuji Tsunekawa,[3] Jun Wu,[1] David Lam,[1] Xiong Xiong,[4] Nuria Montserrat,[5] Concepcion Rodriguez Esteban,[1] Guang-Hui Liu,[6,7,8] Ignacio Sancho-Martinez,[1] Dolors Manau,[9] Salva Civico,[9] Francesc Cardellach,[10] Maria del Mar O'Callaghan,[11] Jaime Campistol,[11] Huimin Zhao,[4] Josep M. Campistol,[12] Carlos T. Moraes,[2,13] and Juan Carlos Izpisua Belmonte[1,*]

Miami, USA 2015, Cell *(161, 459–469)*

2 approches
- Mitochondria-targeted restriction nucleases
- Mito-TALENs (transcription activator-like effector nucleases)

La thérapie anti-génomique

I Tarassov, UMR7156, Strasbourg



petit RNA spécifique de l'ADN muté

↓

ralentit la réplication des molécules mutées

↓

diminution du taux de mutation

Les mitochondries comme traitement de l'infertilité?

Prélèvement des cellules dites « progénitrices » d'ovocytes (située en périphérie de l'ovaire)

↓

Extraction des mitochondries

↓

Injection dans l'ovocyte maternel pour le « ré-énergiser »

↓

7 mai 2015 : naissance du premier bébé avec le traitement AUGMENT d'ovascience
Dr Casper, Toronto, Mount Sinai Hospital

METHODE 1 : transfert de pronuclei entre zygotes

MODELE : Souris

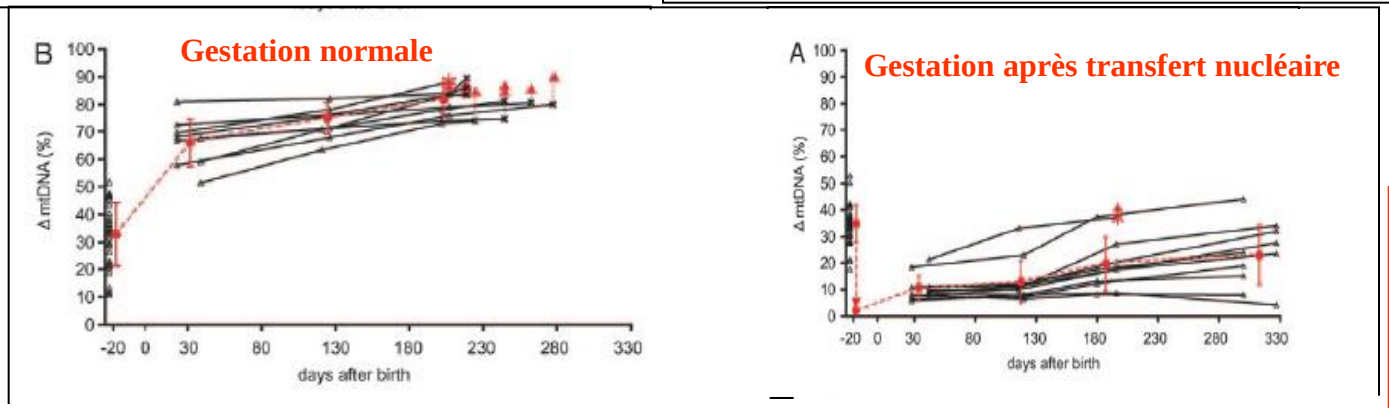
Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation

Akitsugu Sato*†‡, Tomohiro Kono§, Kazuto Nakada*†¶, Kaori Ishikawa*†, Shin-Ichi Inoue*, Hiromichi Yonekawa‡, and Jun-Ichi Hayashi*‖

Japon

2005, *PNAS*

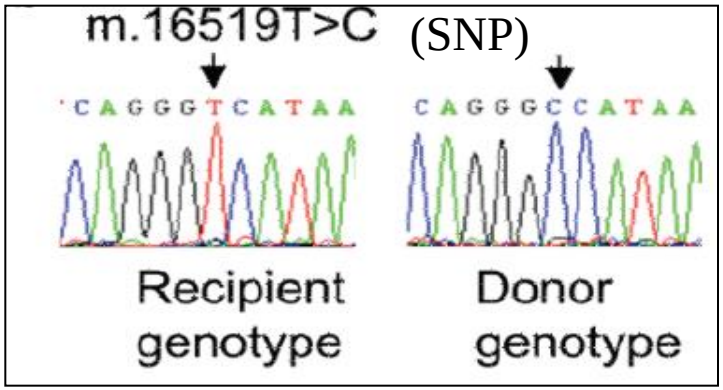
(102:16765-70)



Evolution du taux de mutation de l'ADNmt (pré et postnatal) sans ou après transfert nucléaire

- zygotes transférés 39
- naissances 11
- hétéroplasmie moyenne
  - naissance: 11 %
  - J300: 23 % (+ 12%)

MODELE : Humain



Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease

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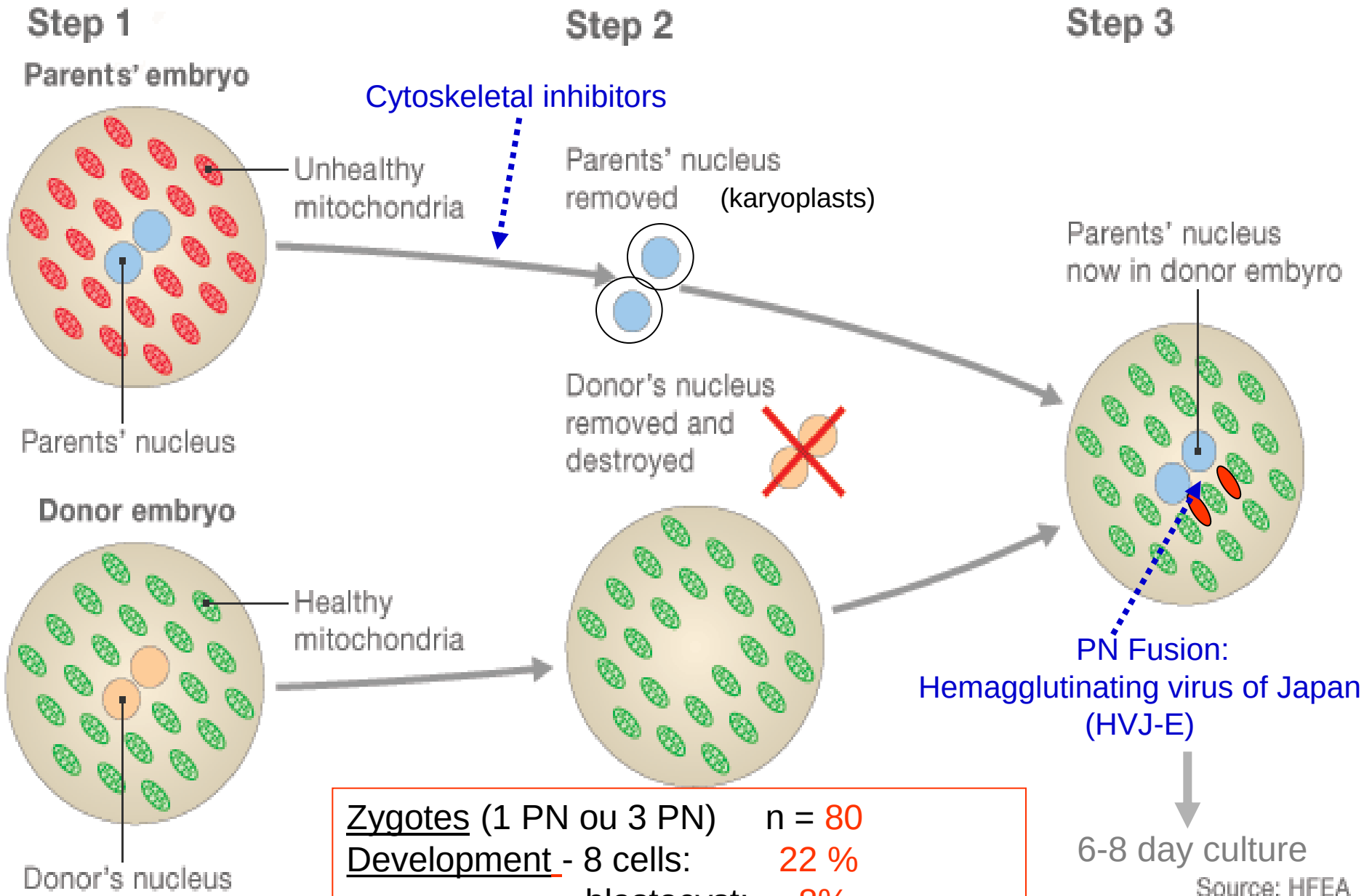
Newcastle, UK 2010, *Nature*

(465:82-85)



Zygotes (1 PN ou 3 PN) n = 80
Development - 8 cells: 22 %
- blastocyst: 8%
Heteroplasmy: < 2 % (0 – 10 %)

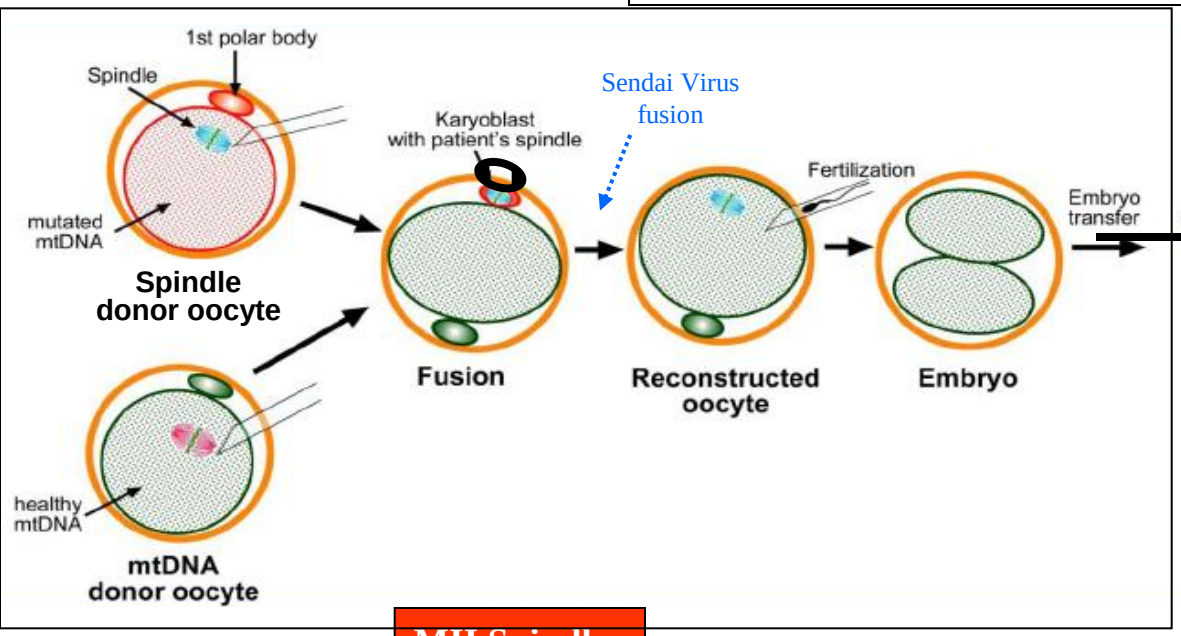
METHODE 2 : transfert du fuseau méiotique entre ovocytes

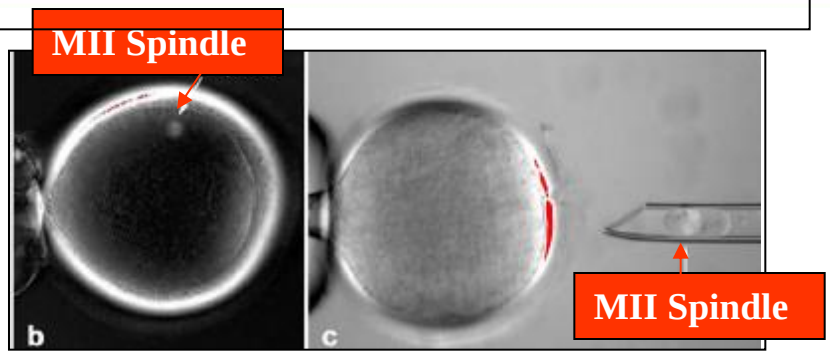
MODELE : Primate

Mitochondrial gene replacement in primate offspring and embryonic stem cells

Masahito Tachibana[1], Michelle Sparman[1], Hathaitip Sritanaudomchai[1], Hong Ma[1], Lisa Clepper[1], Joy Woodward[1], Ying Li[1], Cathy Ramsey[1], Olena Kolotushkina[1] & Shoukhrat Mitalipov[1,2,3]

Portland, USA — 2009, *Nature* (461:367-72)



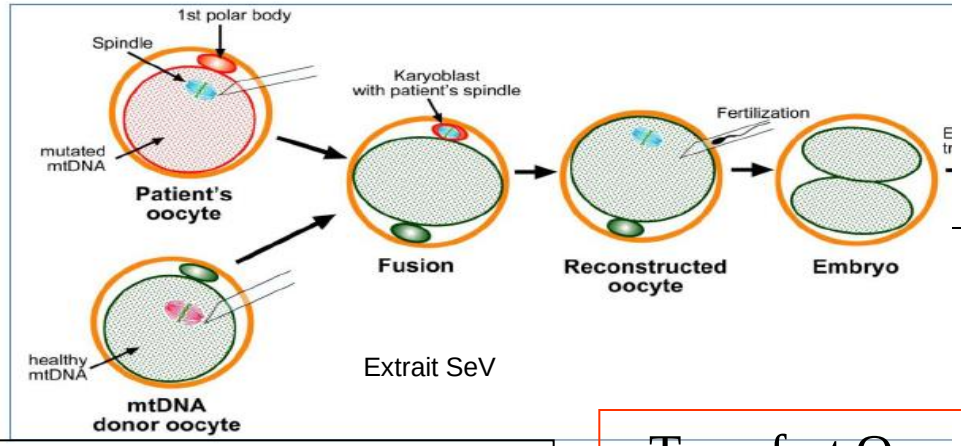


Transfert Fuseau ovo:	15
Implantation:	4
Naissance:	3
	(20 %)
Hétéroplasmie	< 3 %

OK avec un recul de 3 ans

METHODE 2 : transfert du fuseau meiotique entre ovocytes

MODELE : Humain



Extrait SeV

→ Cellules souches

Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants

Daniel Paull[1], Valentina Emmanuele[2], Keren A. Weiss[1], Nathan Treff[3], Latoya Stewart[1], Haiqing Hua[1,4], Matthew Zimmer[1], David J. Kahler[1], Robin S. Goland[4], Scott A. Noggle[1], Robert Prosser[5], Michio Hirano[2], Mark V. Sauer[5,6]* & Dieter Egli[1]*

2013, *Nature* (493: 632-7) New-York, USA

- Transfert Ovo MII 18
- parthénogenése
- Blastocyste: 7 (40 %)
- Hétéroplasmie < 1%

Problème:
Réactivation spontanée de la méiose (50 %)

Towards germline gene therapy of inherited mitochondrial diseases

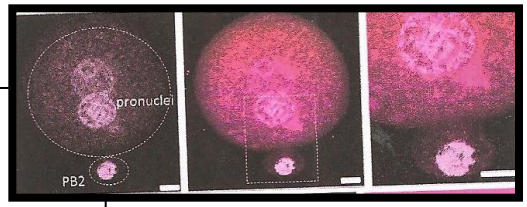
Masahito Tachibana[1], Paula Amato[2], Michelle Sparman[1], Joy Woodward[1], Dario Melguizo Sanchis[1], Hong Ma[1], Nuria Marti Gutierrez[1], Rebecca Tippner-Hedges[1], Eunju Kang[1], Hyo-Sang Lee[1], Cathy Ramsey[1], Keith Masterson[2], David Battaglia[2], David Lee[2], Diana Wu[2], Jeffrey Jensen[1,3], Phillip Patton[2], Sumita Gokhale[4], Richard Stouffer[1,2], and Shoukhrat Mitalipov[1,2]

2013, *Nature* (493: 627-631) Portland, USA

- Transfert Ovo MII 64
- Fécondation. 44
- Blastocyste: 19 (30 %)
- Hétéroplasmie < 1%

METHODE 3 : transfert de globule polaire

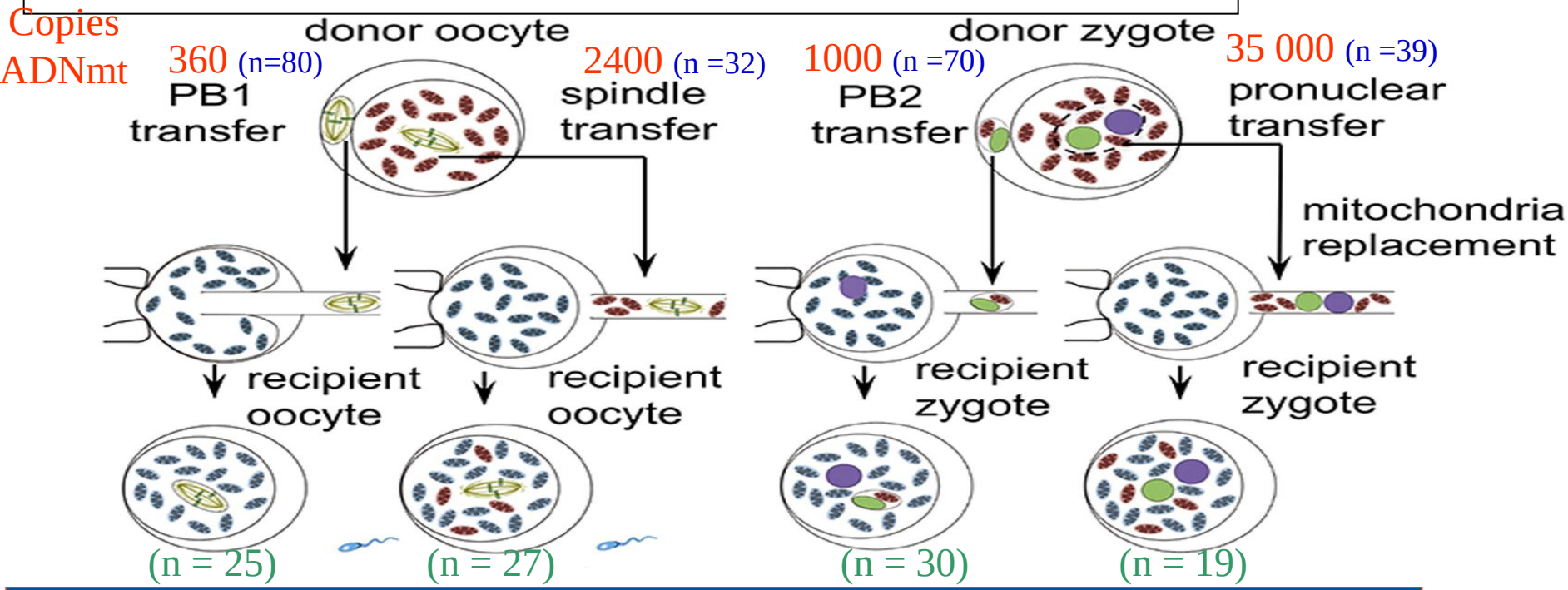
MODELE : Souris



Polar Body Genome Transfer for Preventing the Transmission of Inherited Mitochondrial Diseases

Tian Wang,[1,4] Hongying Sha,[1,4,*] Dongmei Ji,[2,4] Helen L. Zhang,[3] Dawei Chen,[2] Yunxia Cao,[2,5] and Jianhong Zhu[1,5,*]

Shanghaï, Chine + Harvard, USA 2014, *Cell* (157:1591-604)



Blastocyste	89 %	87 %	55 %	80 %
Hétéroplasmie	**0%**	**10%**	**<2%**	**20%**

BBC NEWS
HEALTH

27 June 2013 Last updated at 23:33 GMT

UK government backs three-person IVF

By James Gallagher
Health and science reporter, BBC News

The UK looks set to become the first country to allow the creation of babies using DNA from three people, after the government backed the IVF technique.

It will produce draft regulations later this year and the procedure could be offered within two years.

Experts say three-person IVF could eliminate debilitating and potentially fatal mitochondrial diseases that are passed on from mother to child.

Opponents say it is unethical and could set the UK on a "slippery slope".

They also argue that affected couples could adopt or use egg donors instead.