Clinical application of spindle nuclear transfer on poor embryo development

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embryotools

Oocyte quality

Assisted reproductive technologies (ARTs) can now circumvent many sub-fertility disorders, however, **efficacy is still limited** by the **number** and **quality of oocytes**;

Women affected by **ovarian dysfunctions, severe endometriosis, advanced maternal age, etc,** are often **challenging patients in IVF treatments;**

Frequently, some of these patients present **impaired embryo development** in repeated IVF cycles, which is mainly attributed to **poor oocyte quality;**

Oocyte quality is often **defined as the competence** of the oocyte **to develop into a** good morphology and euploid embryo with **chances to implant;**

Oocyte quality

The developmental competence is mainly dictated by chromosomal status of the oocyte and the cytoplasm, which is constituted by **innumerous organelles, mRNAs, proteins and other** factors that are crucial for embryo development;

As the most **numerous organelle in the oocyte**, **mitochondria** are thought to play an **important role in oocyte developmental competence** (Wolf et al., 2013)**;**

Wolf DP, Hayama T, Mitalipov S. EMBO J. 2017

Mitochondrial dysfuntions

Higher ATP levels in oocytes correlate with **better embryo development and implantation rates** (Van Blerkom et al., 2004, *Reproduction*);

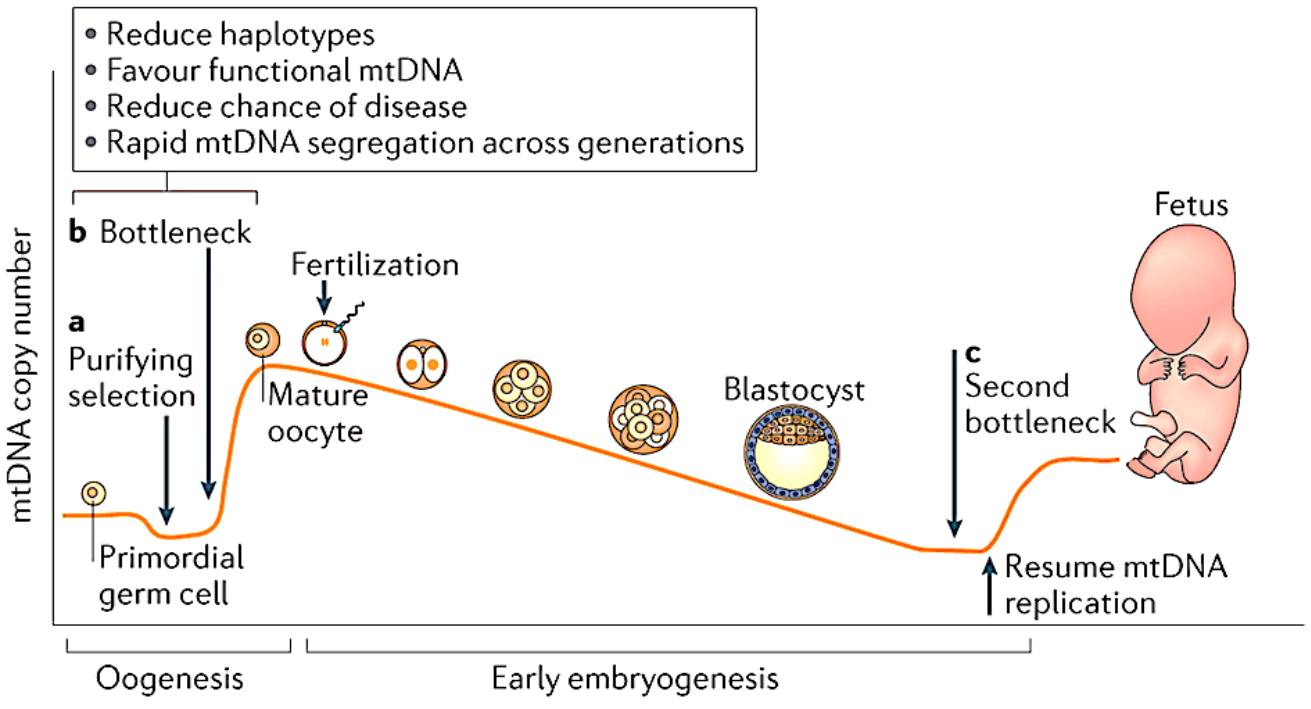
Decrease ATP synthesis in oocytes has **adverse effects on spindle formation, fertilization and chromosomal segregation, resulting in poor embryo development** (Chappel, 2013, *Obstet Gynecol Int*; Ramalho-Santos et al., 2009, *Hum Reprod Update*);

Mitochondria dysfunction is involved in poor developmental competence of oocytes **in older patients** (Chappel S. 2013*, Obstet Gynecol Int*);

Altered levels of **mtDNA are associated with female age** and **impact in aneuploidy and embryonic implantation potential** (Fragouli et al., 2015, *PLOS Genetics*)**;**

Mitochondria

Mutations and/or deletions in **mtDNA** have been **associated** with several **important diseases and dysfunctions** and pathophysiological processes, such as **aging**, **neurodegenerative** diseases, **diabetes** and obesity, and **infertility**;



Prashant Mishra & David C. Chan, *Nature Reviews Molecular Cell Biology* **15**, 634–646 (2014)

Strategies to enhance oocyte quality

Mitochondrial dysfunction is a major cause of decline in oocyte quality[1-4]

Donor oocyte cytoplasmic transfer resulted **in live births**, but was abandoned due to the **concerns with heteroplasmy**[4]

A limited survey-based uncontrolled follow-up study of children born after ooplasmic transplantation in a single centre

Serena H Chen [a], Claudia Pascale [a], Maria Jackson [a], Mary Ann Szvetecz [a], Jacques Cohen [b,*]

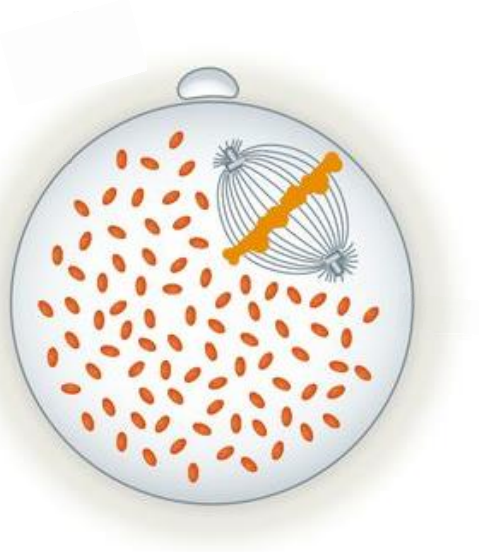
[a] *Institute for Reproductive Medicine and Science at Saint Barnabas Medical Center, Livingston, NJ 07039, USA;* [b] *ART Institute of Washington, Livingston, NJ 07039, USA*
* Corresponding author: *E-mail address:* jc@embryos.net (J. Cohen).

[1]Conti M, Franciosi F. ***Hum Reprod Update 2018***:*245-266.*
[2] Wolf DP, Hayama T, Mitalipov S. ***EMBO J. 2017.*** 36(17):2659.
[3]Van Blerkom, J. (2004). **Reproduction,** 128(3), 269–280.
[4]*Cohen J.* (1998) . ***Mol Hum Reprod*** (3):269-80.

Strategies to enhance oocyte quality

Mitochondrial dysfunction is a major cause of decline in oocyte quality[1-4]

Autologous mitochondrial transfer (AUGMENT) live births have been reported, but improvements on oocyte/embryo quality not proven;



Autologous mitochondrial transfer as a complementary technique to intracytoplasmic sperm injection to improve embryo quality in patients undergoing in vitro fertilization—a randomized pilot study

Elena Labarta, M.D., Ph.D.,[a,b] Maria José de los Santos, Ph.D.,[a,b] Sonia Herraiz, Ph.D.,[a,b] Maria José Escribá, Ph.D.,[a,b] Alicia Marzal, M.D.,[a] Anna Buigues, B.Sc.,[b] and Antonio Pellicer, M.D.[a,c]

[a]IVI-RMA Valencia and [b]IVI Foundation, Valencia, Spain; and [c]IVI Rome, Rome, Italy

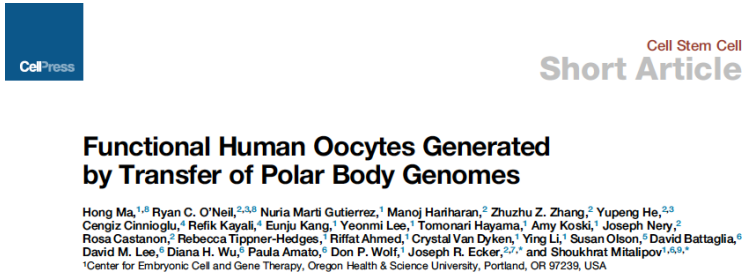
[1]Conti M, Franciosi F. ***Hum Reprod Update 2018***:*245-266.*
[2] Wolf DP, Hayama T, Mitalipov S. ***EMBO J. 2017.*** 36(17):2659.
[3]Van Blerkom, J. (2004). **Reproduction,** 128(3), 269–280.
[4]Cohen J. (1998) . ***Mol Hum Reprod*** (3):269-80.

Mitochondrial replacement techniques

Mitochondrial transfer is not sufficient to correct dysfunctional mitochondria or to **repair other cytoplasmic deficiencies** that may be **present in poor quality oocytes**;

An approach that may offer greater promise to **improve oocyte quality** is the **transfer of the nuclear genome** from an affected oocyte or zygote into a new healthy cytoplasm - **Mitochondrial replacement therapies (MRTs)**

GV transfer | Polar body transfer | Spindle transfer | Pronuclear transfer


Cell Stem Cell
Short Article

Functional Human Oocytes Generated by Transfer of Polar Body Genomes

Hong Ma,[1,8] Ryan C. O'Neil,[2,3,8] Nuria Marti Gutierrez,[1] Manoj Hariharan,[2] Zhuzhu Z. Zhang,[2] Yupeng He,[2,3] Cengiz Cinnioglu,[4] Refik Kayali,[4] Eunju Kang,[1] Yeonmi Lee,[1] Tomonari Hayama,[1] Amy Koski,[1] Joseph Nery,[2] Rosa Castanon,[2] Rebecca Tippner-Hedges,[1] Riffat Ahmed,[1] Crystal Van Dyken,[1] Ying Li,[1] Susan Olson,[5] David Battaglia,[6] David M. Lee,[6] Diana H. Wu,[6] Paula Amato,[6] Don P. Wolf,[1] Joseph R. Ecker,[2,7,*] and Shoukhrat Mitalipov[1,6,9,*]

[1]Center for Embryonic Cell and Gene Therapy, Oregon Health & Science University, Portland, OR 97239, USA


nature Vol 465|6 May 2010|doi:10.1038/nature08958

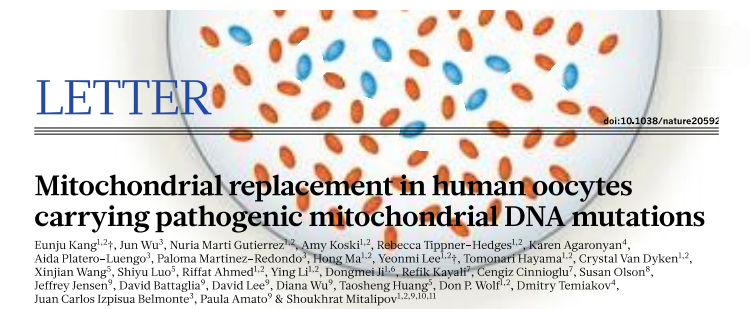
Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease

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


LETTER
doi:10.1038/nature18303

Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease

Louise A. Hyslop[1,2], Paul Blakeley[3], Lyndsey Craven[4], Jessica Richardson[1], Norah M. E. Fogarty[3], Elpida Fragouli[5], Mahdi Lamb[1], Sissy E. Wamaitha[3], Nilendran Prathalingam[1,2], Qi Zhang[1]†, Hannah O'Keefe[1], Yuko Takeda[1], Lucia Arizzi[1,2], Samer Alfarawati[5], Helen A. Tuppen[4], Laura Irving[1]†, Dimitrios Kalleas[1]†, Meenakshi Choudhary[2], Dagan Wells[6], Alison P Murdoch[2], Douglass M. Turnbull[4], Kathy K. Niakan[3] & Mary Herbert[1,2]


LETTER
doi:10.1038/nature20592

Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations

Eunju Kang[1,2]†, Jun Wu[3], Nuria Marti Gutierrez[1,2], Amy Koski[1,2], Rebecca Tippner-Hedges[1,2], Karen Agaronyan[4], Aida Platero-Luengo[3], Paloma Martinez-Redondo[3], Hong Ma[1,2], Yeonmi Lee[1,2]†, Tomonari Hayama[1,2], Crystal Van Dyken[1,2], Xinjian Wang[5], Shiyu Luo[5], Riffat Ahmed[1,2], Ying Li[1,2], Dongmei Ji[1,2], Refik Kayali[6], Cengiz Cinnioglu[7], Susan Olson[8], Jeffrey Jensen[9], David Battaglia[9], David Lee[9], Diana Wu[9], Taosheng Huang[5], Don P. Wolf[1,2], Dmitry Temiakov[4], Juan Carlos Izpisua Belmonte[3], Paula Amato[9] & Shoukhrat Mitalipov[1,2,9,10,11]


Vol 461|17 September 2009|doi:10.1038/nature08368 nature

Mitochondrial gene replacement in primate offspring and embryonic stem cells

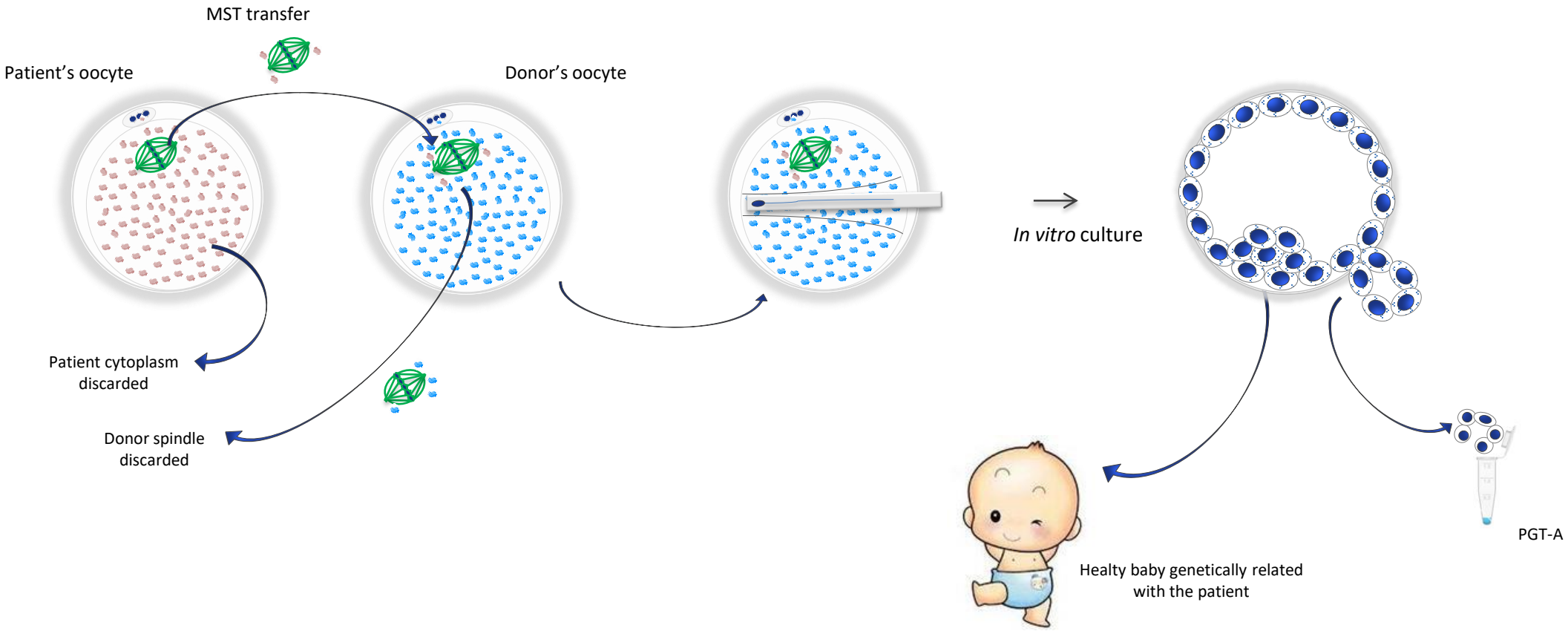
Masahito Tachibana[1], Michelle Sparman[1], Hathaitip Sritanaudomchai[1], Hong Ma[1], Lisa Clepper[1], Joy Woodward[1], Ying Li[1], Cathy Ramsey[1], Olena Kolotushkina[1] & Shoukhrat Mitalipov[1,2,3]


ARTICLE
doi:10.1038/nature11800

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]*

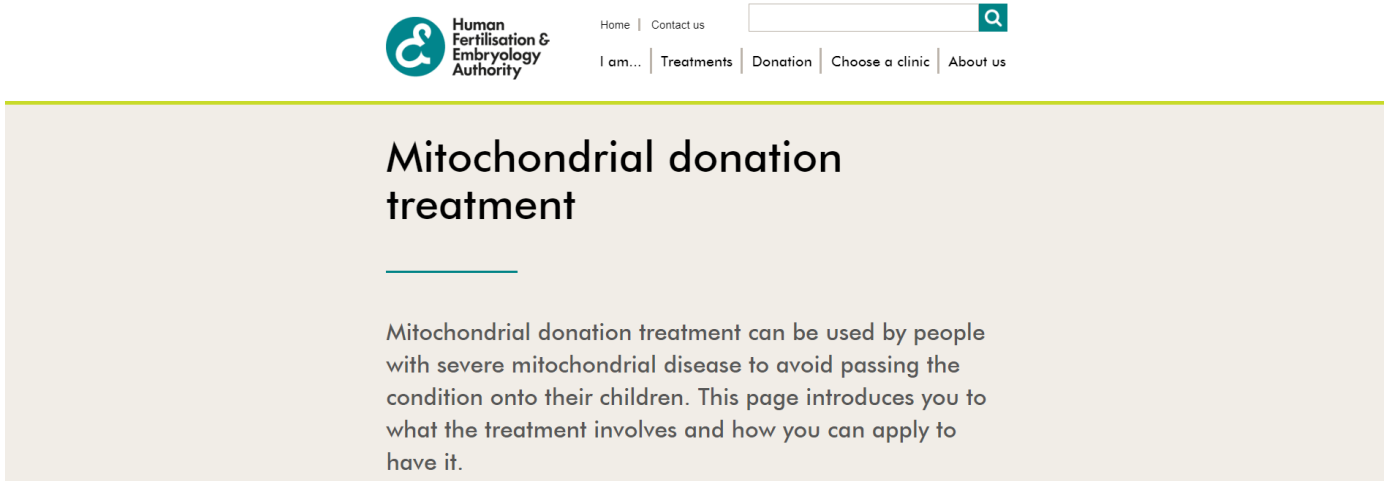
Maternal spindle transfer (MST)



Technically very demanding | **low mtDNA carryover** | Manipulation of oocytes **before fertilization** | Easier to **coordinate the spindle donor oocyte and the recipient cytoplast**

Prevention of mtDNA diseases

The **UK the first country** to regulate **mitochondrial donation to prevent** the **transmission of mtDNA diseases**;



- **"Assisted reproduction techniques that today are a routine in IVF** laboratories (IVF, ICSI, PGD/S) **all involved a degree of uncertainty when they were first performed in humans";**
- **"There is no compelling reason to think** that **cytoplasm/mitochondrial replacement techniques**, like spindle transfer, **are unsafe";**
- "**We reached the moment** when it is **important to move on and explore the feasibility of** MST in carefully controlled clinical trials".

Prevention of mtDNA diseases

embryotools

HARVARD LAW PETRIE-FLOM CENTER

Mitochondrial Replacement Therapy: Considering the Future of U.S. Policy

Join us for a panel discussion about the future of MRT policy in the U.S., during which speakers will review the latest technological developments, the regulatory barriers, and the ethical challenges affecting the clinical application of MRT. Learn more: http://petrieflom.law.harvard.edu/events/details/mitochondrial-replacement-therapy.

* Required information

Mitochondrial Replacement Therapy: The Road to the Clinic in Canada

Bartha Maria Knoppers, PhD;[1] Arthur Leader, MD;[2] Stacey Hume, PhD;[3] Eric A. Shoubridge, PhD;[4] Rosario Isasi, MPH;[5] Forough Noohi, MSc;[1] Ubaka Ogbogu, SJD;[6] Vardit Ravitsky, PhD;[7,8] Erika Kleiderman, LLB[1]

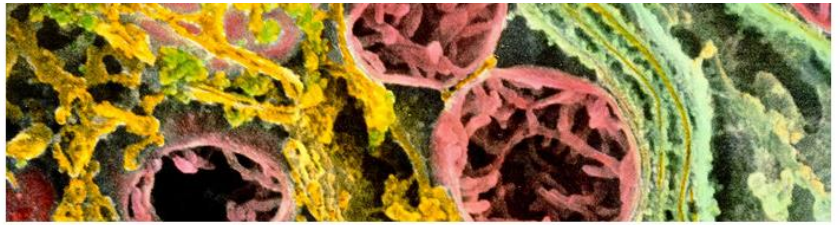
The Sydney Morning Herald

NATIONAL HEALTHCARE

'Three parent baby' IVF technique on track to become legal in Australia

AAAS Become a Member

Science Contents News Careers Journals



DNA mutations in mitochondria (seen here in pink), can cause devastating diseases that are passed on from mother to child. P. M. MOTTA, G. MACCHIARELLI, S.A. NOTTOLA/SCIENCE SOURCE

Singapore could become the second country to legalize mitochondrial replacement therapy

By Sandy Ong | Jun. 6, 2018 , 1:00 PM

Prevention of mtDNA diseases

First **live birth in 2016** of a boy using spindle nuclear transfer for preventing a mtDNA mutation causing **Leigh Syndrome**;

RBM ONLINE

ELSEVIER

Article

Live birth derived from oocyte spindle transfer to prevent mitochondrial disease

John Zhang [a,b,], Hui Liu [b], Shiyu Luo [c], Zhuo Lu [b], Alejandro Chávez-Badiola [a], Zitao Liu [b], Mingxue Yang [b], Zaher Merhi [d], Sherman J Silber [e], Santiago Munné [f], Michalis Konstantinidis [f], Dagan Wells [f], Jian J Tang [g], Taosheng Huang [c,*]*

[a] New Hope Fertility Center, Punto Sao Paulo, Lobby Corporativo, Américas 1545 Providencia, Guadalajara, Mexico
[b] New Hope Fertility Center, 4 Columbus Circle, New York, NY 10019, USA
[c] Division of Human Genetics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA
[d] Department of Obstetrics and Gynecology, Division of Reproductive Biology, NYU School of Medicine, 180 Varick Street, New York, NY 10014, USA
[e] Infertility Center of St Louis, St Luke's Hospital, St Louis, MO 63017, USA
[f] Reprogenetics, 3 Regent Street, Livingston, NJ 07078, USA
[g] Department of Obstetrics and Gynecology, The Mount Sinai Hospital, E 101st Street, New York, NY 10029, USA

Dr John Zhang completed his medical degree at Zhejiang University School of Medicine in China, and subsequently received his Master's Degree at Birmingham University in the UK. In 1991, Dr Zhang earned his PhD in IVF, and, after researching the biology of mammalian reproduction and human embryology for nearly 10 years he completed his fellowship training in Reproductive Endocrinology and Infertility at New York University's School of Medicine in 2001. Dr. Zhang continues his clinical research in minimal stimulation IVF, non-embryonic stem cell, long-term oocyte cryopreservation, and oocyte reconstruction by nuclear transfer.

KEY MESSAGE
We report a live birth after oocyte spindle transfer to prevent transmission of the mitochondrial disease, Leigh syndrome.

ABSTRACT

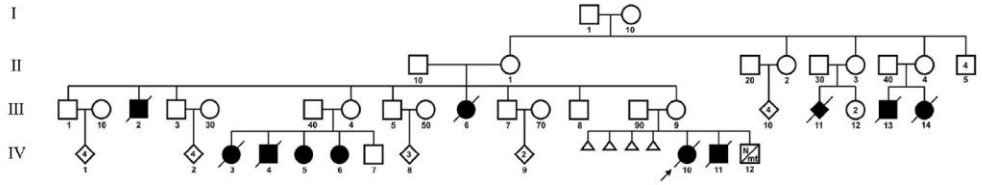
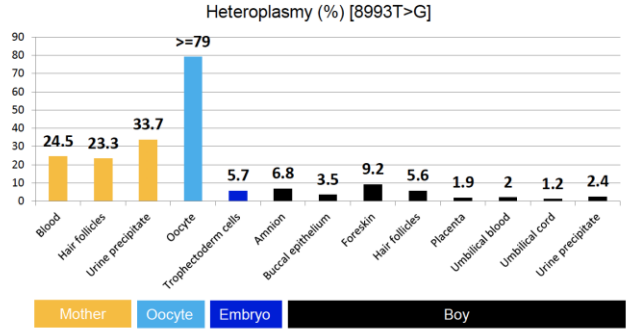


Figure 1 – Pedigree of the family with Leigh Syndrome (black fill indicates clinically affected individuals, blank triangle for miscarriage). The product of the nuclear transfer procedure was indicated by N/mt in order to indicate that the nuclear (N) genome and mitochondrial (mt) genome were from different individuals.



Patient's Haplogroup	I
Donor's Haplogroup	L2c
PGS	46 XY
Neonatal Pathogentic mutated mtDNA (%)	0-9.2

Research project

Hypothesis:

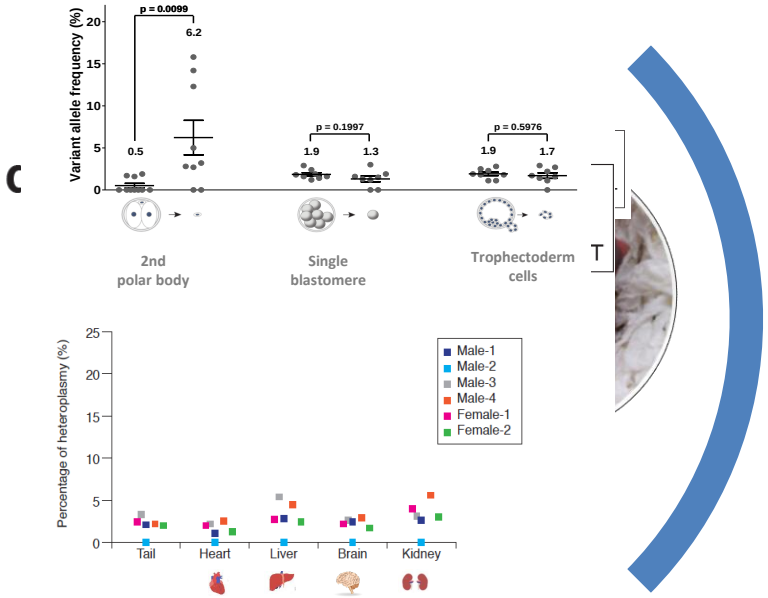
– ***Explore the efficiency and safety of the maternal spindle transfer (MST)*** *in the treatment of* ***infertility problems*** *related to* ***poor oocyte quality*** –

ACCIÓ
Generalitat de Catalunya

Unió Europea
Fons Europeu de Desenvolupament Regional

Research project

Proof of concept in the mouse model



- **MST feasible without impairing embryo development;**
- **Overcomes embryo development arrest in NZB oocytes;**
- **MST mice healthy and fertile over 5 generations (F5);**
- **Low (2-3%) heteroplasmy levels in embryos and organs;**
- **No heteroplasmy detected after F3;**

Costa-Borges et al., 2017; 2019 (in review)

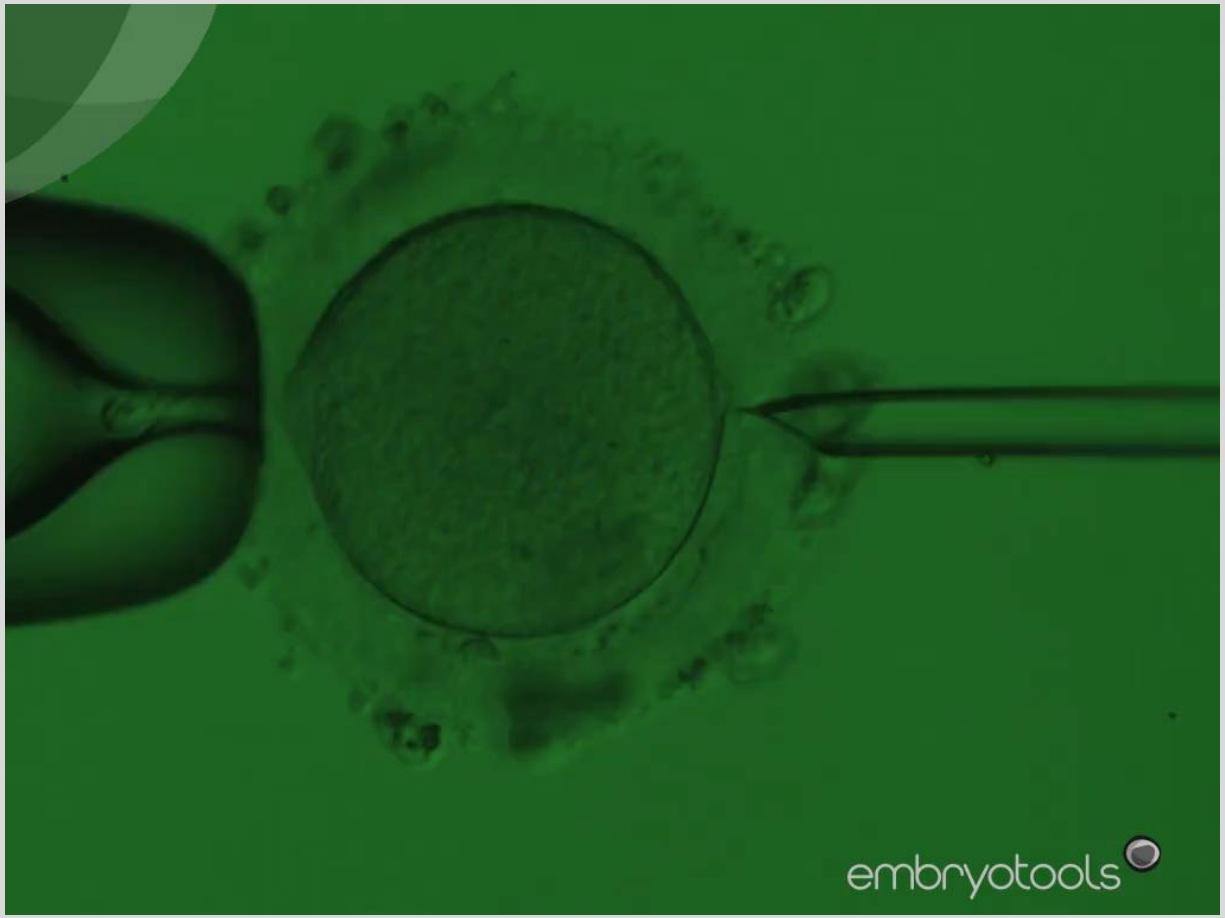
Translational Research into humans:

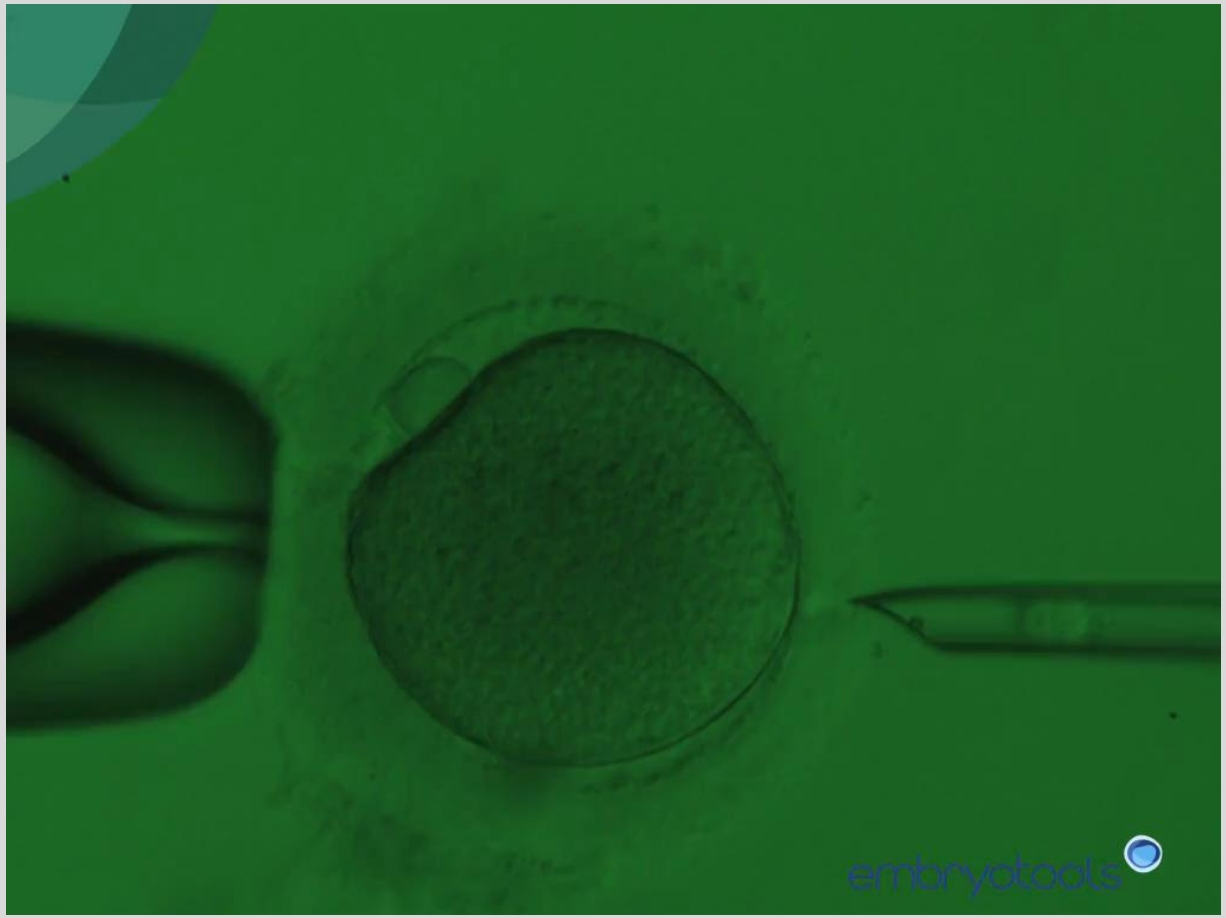
- **Pre-clinical validation | Pilot trial**

- ✓ **Authorized** by the **Greek Authority of Assisted Reproduction (ref. no.: 437/23.9.2016);**
- ✓ **Approved** by **the IRB of the IASO Maternity Hospital;**
- ✓ **Informed consent** was obtained from all **donors/patients participating** in the study;
- ✓ Experiments were **carried out** at the **Institute of Life (Athens);**
- ✓ **Progress updates delivered every three months;**
- ✓ **All treatments were free of charge;**

Dagan Wells, PhD
Katharina Späth, PhD

Enucleation and reconstruction techniques





Pilot trial | Inclusion criteria

ISRCTN registry

ISRCTN11455145 https://doi.org/10.1186/ISRCTN11455145

Spindle transfer for the treatment of infertility problems associated to poor egg quality: a pilot trial

Eligibility

Participant inclusion criteria:

1. Women under 40 y/o;
2. Diagnosed with infertility problems associated to impaired embryo development attributed to poor oocyte quality;
3. Several previous *in-vitro* fertilization (IVF) failed attempts;

Participant exclusion criteria:

1. Women over 40 y/o;
2. Couples diagnosed w/ severe male factor infertility

Target number of participants:

25 patients

Pilot trial | First pregnancy

ISRCTN registry

ISRCTN11455145 https://doi.org/10.1186/ISRCTN11455145

Spindle transfer for the treatment of infertility problems associated to poor egg quality: a pilot trial

Age: 32

Informed consents: ok

Indication: Low responder and endometriosis

Previous attempts: 4

Spindle transfer cycle: 3x MII (vitrified) **-> 2x** survived **-> 2x** MST -> **2x** fertilized **-> 1x** blastocyst

Ultrasounds: normal | Pre-Natal tests and amniocentesis (16 weeks): normal |

Samples	STR DNA Markers (Sizes in bp)																	
	D3S1358		VWA		D21S11		D8S1179		SE33		TH01		FGA		D18S51		Amelogenin	
Father	124	128	144		220	230	178	182	251	302	185	189	191	203	300		X	Y
Mother	132		136	156	224		174	182	255	310	185	196	191	203	300	320	X	
Donor	128	136	144		216	235	166	182	255		181		187	207	297	320	X	
Embryo	124	132	136	144	No amplification		178	182	251	255	189	196	191		300		X	Y
Amniotic Fluid 1st Aliquot	124	132	136	144	220	224	178	182	251	255	189	196	191		300		X	Y
Amniotic Fluid 2nd Aliquot	124	132	136	144	220	224	178	182	251	255	189	196	191		300		X	Y
Negative Control	No amplification		No amplification		No amplification		No amplification		No amplification		No amplification		No amplification		No amplification		No amplification	

Pilot trial | First pregnancy

ISRCTN registry

ISRCTN11455145 https://doi.org/10.1186/ISRCTN11455145

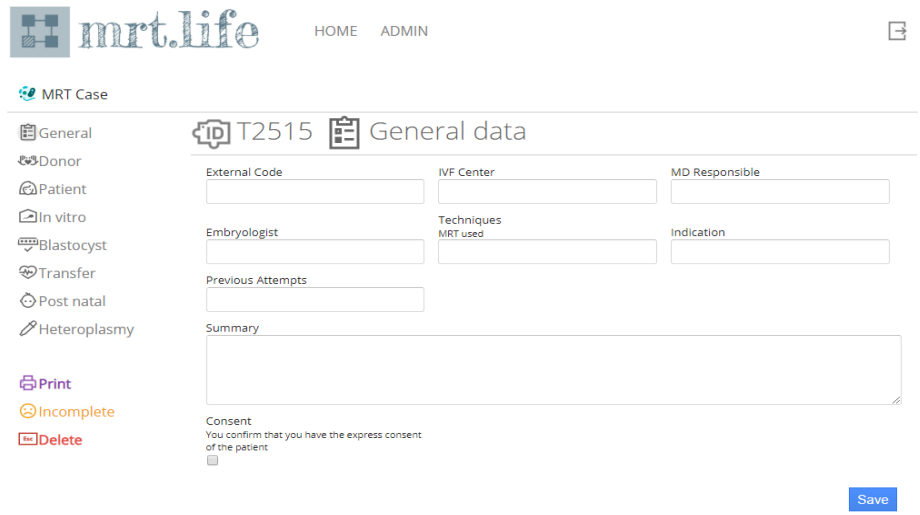
Spindle transfer for the treatment of infertility problems associated to poor egg quality: a pilot trial

April 9th 2019 – delivery at 39 weeks of gestation through C-section, healthy boy, 2.960 kg and 51 cm

Followed-up every 3 months

(neurological exams, heteroplasmy levels being evaluated over time)

Online **registry** open to follow up **all babies resultant from the application of MRT procedures**



Pilot trial | Second pregnancy

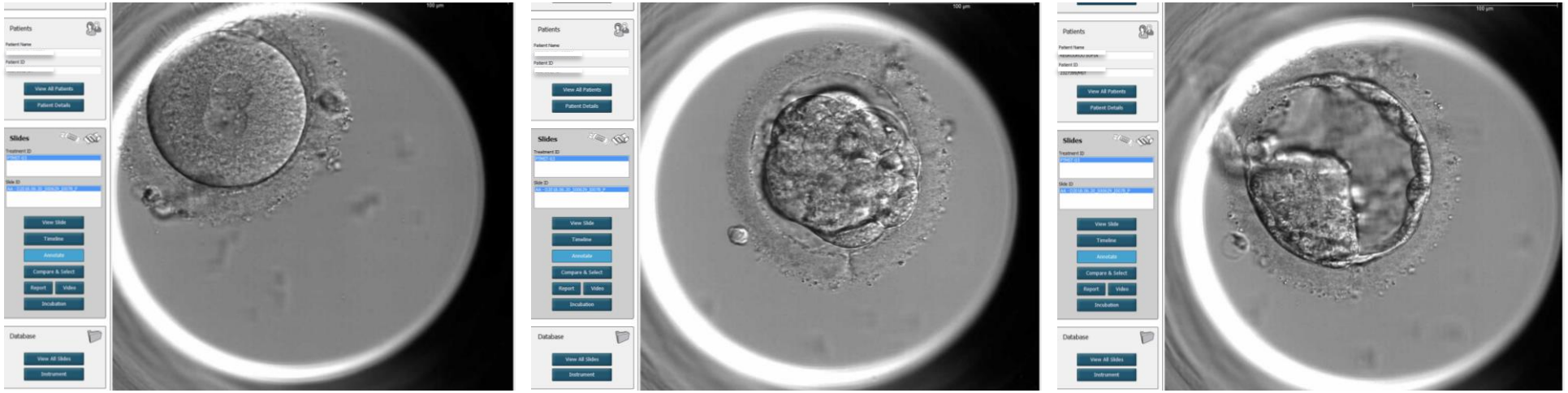
ISRCTN registry

ISRCTN11455145 https://doi.org/10.1186/ISRCTN11455145

Spindle transfer for the treatment of infertility problems associated to poor egg quality: a pilot trial

Age: 39
Informed consents: ok
Indication: un-explained infertility
Previous attempts: 11
Spindle transfer cycle: 10x MII (vitrified) **-> 8x** survived **-> 6x** MST -> **4x** fertilized **-> 2x** blastocysts
Molecular analysis: Chromosomally normal with mtDNA carryover **< 1%**

Ultrasounds: normal | Pre-Natal tests and amniocentesis (16 weeks): normal | Expected delivery: Oct2019



Pilot trial | Third pregnancy

ISRCTN registry

ISRCTN11455145 https://doi.org/10.1186/ISRCTN11455145

Spindle transfer for the treatment of infertility problems associated to poor egg quality: a pilot trial

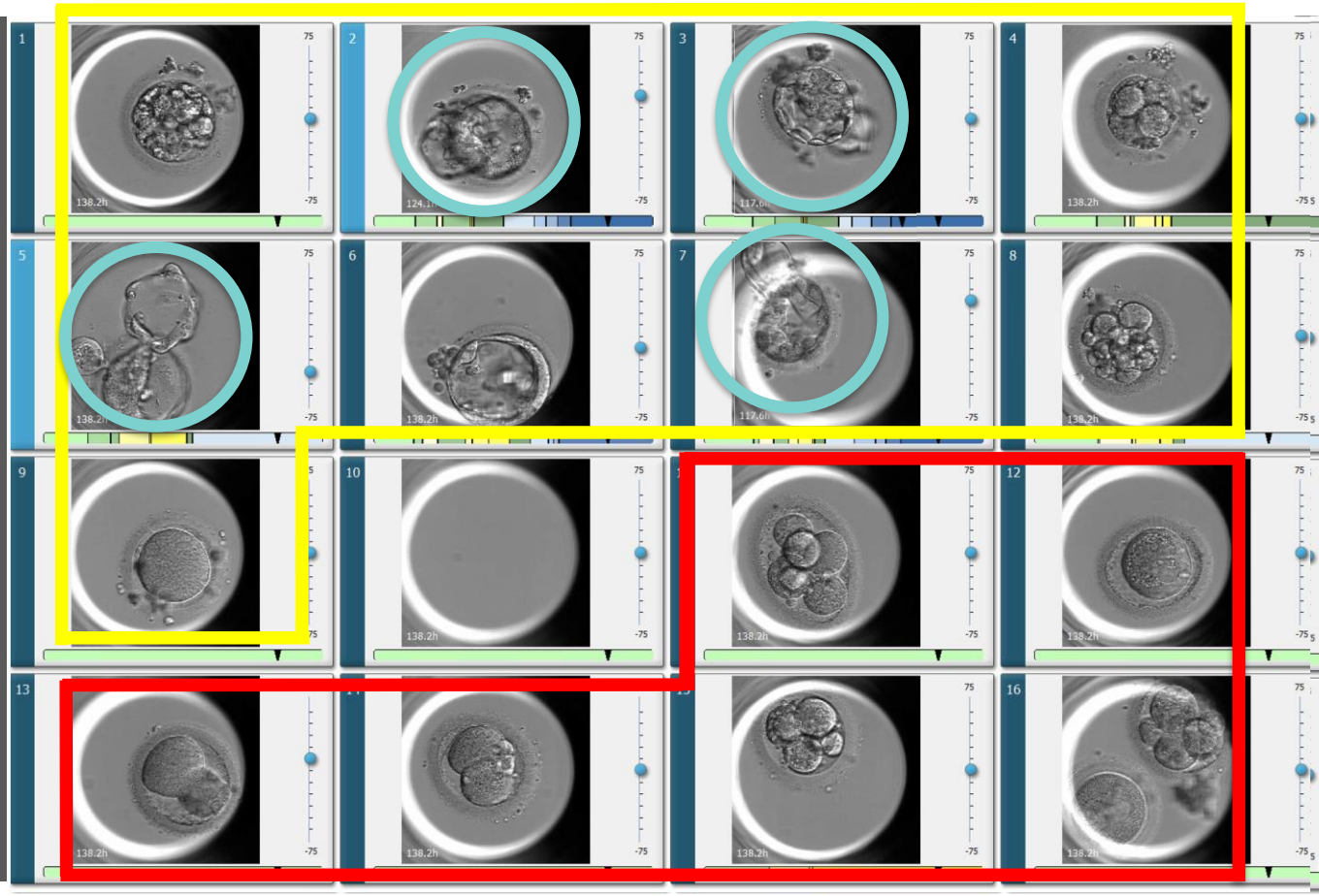
Age: 35
Informed consents: ok
Indication: PCOs | **Previous attempts**: 5
Collected 16x MII oocytes

Spindle transfer ( ): 9x MST

Control ICSI ( ) 7x MII

Molecular analysis: Chromosomally normal with mtDNA carryover < 1%;

Pregnant: 8 weeks



Pilot trial | Ongoing Results

No. of patients recruited: 20 | **Average age:** 37.3 (min 32 and max 40)
Average no. of previous IVF cycles: 4.9 (min 3 and max 11)

Mean no. of MII oocytes used MST/patient: 4.3 (min 1 and max 10, no. total = 100)

- **Successful MST rate:** 94.0% (94/100)
- **Fertilization rate:** 73.4% (69/94)
- **GQ Blastocyst developmental rate**: 57.9% (40/69)
- **% of euploid blastocysts/total biopsied**: 45.0% (18/40)
- **Pregnancy rates**: 75% (3/4)
- **Implantation rates**: 75% (3/4)

Abstract, **4613**, "**PRELIMINARY RESULTS FROM THE FIRST REGISTERED PILOT TRIAL WITH MATERNAL SPINDLE TRANSFER TO OVERCOME INFERTILITY**" accepted as oral number O-11 for oral presentation, **10/14/2019 11:45:00 AM, at the ASRM 2019 Scientific Congress**

Introduction of new technologies in reproductive medicine

Human Reproduction, Vol.29, No.3 pp. 413–417, 2014
Advanced Access publication on January 15, 2014 doi:10.1093/humrep/det463

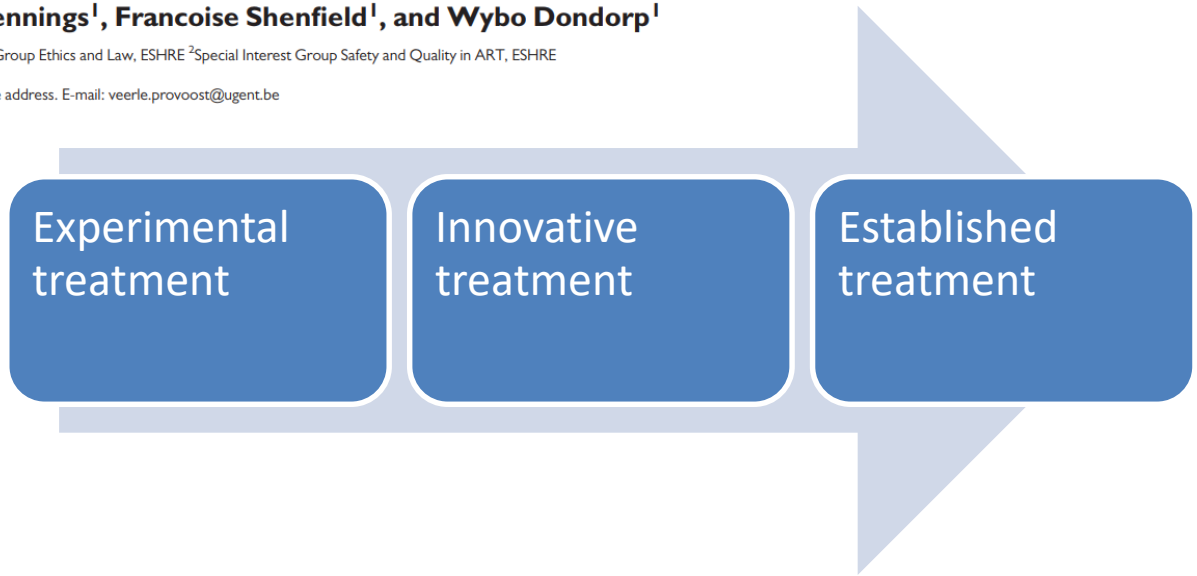
human reproduction ORIGINAL ARTICLE *ESHRE pages*

Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment†

Veerle Provoost[1,*], Kelly Tilleman[2], Arianna D'Angelo[2], Petra De Sutter[2], Guido de Wert[1], Willianne Nelen[2], Guido Pennings[1], Francoise Shenfield[1], and Wybo Dondorp[1]

[1]Special Interest Group Ethics and Law, ESHRE [2]Special Interest Group Safety and Quality in ART, ESHRE

*Correspondence address. E-mail: veerle.provoost@ugent.be



Experimental treatments:

	Current status of our project
There should be studies showing that the experimental treatment is safe in animals Experiments carried out in mice | 5 generations followed | ASRM prize | High impact journal	✓
Treatments involving laboratory procedures can be offered when there is at least clinical embryology data that indicate normal cleavage, embryo morphology and blastocyst formation. Pre-clinical validation in human donor oocytes | Normal fertilization, embryo development, blastocyst formation and aneuploidy rates | Very low heteroplasmy (<1%) | ASRM oral communication 2018 | Paper under-preparation (pending)	✓
Experimental treatments should always be embedded in a research setting, in which it is offered to a selected and limited patient cohort. Registered pilot trial being performed in a limited (25) and strictly selected patient cohort	✓
This requires the approval of a local ethics committee, and informed consent of the patient. License obtained from the Greek Authority in Assisted Reproduction | IRB approval | Treatments free of cost | Progress update reports delivered every 3 months | Informed consents obtained from both patients and donors	✓
Patients should be clearly informed of the experimental status and should receive information about (the lack of knowledge about) possible risks, alternative treatments etc. All patients informed both orally and with a written form (informative form) about the possible risks, alternatives, etc	✓

Conclusions

- **MST is feasible** and **does not adversely** affect the **spindle apparatus** or early **embryo development,** as long as, **all the steps of the protocol are optimized carefully**;

- **MST** can **enhance** the potential of developmentally **compromised oocytes** to develop up to the blastocyst stage, **without compromising euploidy rates and with very low (<1%) mtDNA carry-over levels**;

- **Preliminary** results indicate that **MST oocytes are able to implant and sustain a healthy pregnancy to term;**

- **Carefully controlled pilot trials** are expected to provide **more insights** into the feasibility of technique for **clinical applications to overcome infertility;**

Conclusions

- **MST may open** new hopes for **some** patients **with infertility problems refractory to current clinical strategies** – offering them higher chances of having a child genetically related to them;

- **MST can also be advantageous for donors** – **reduced phycological concerns – as resultant children would not be** genetically related to them | **reduce anonymity concerns;**

Multidisciplinary-team work

embryotools

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Irene Miguel-Escalada, PhD
Enric Mestres, MSc
Maria Garcia, MSc
Ivette Vanrell, MSc
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Unió Europea
Fons Europeu
de Desenvolupament Regional

ACCIÓ

Generalitat de Catalunya

This study was partially financed by the Acció-Generalitat de Catalunya and the European Regional Development funds (ERDF) Ref. RD 15-1-0011, Embryotools and the Institute of Life.

Thank you for your attention

Nuno Costa-Borges, PhD

Scientific Director | Co-Founder

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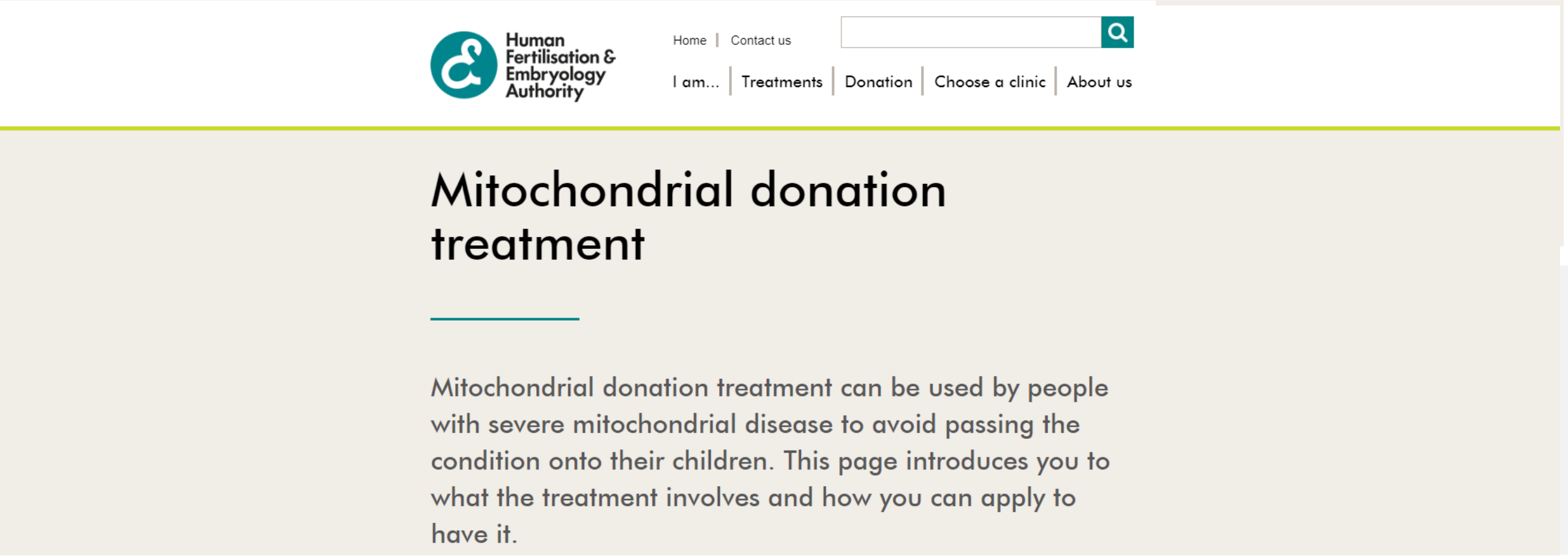
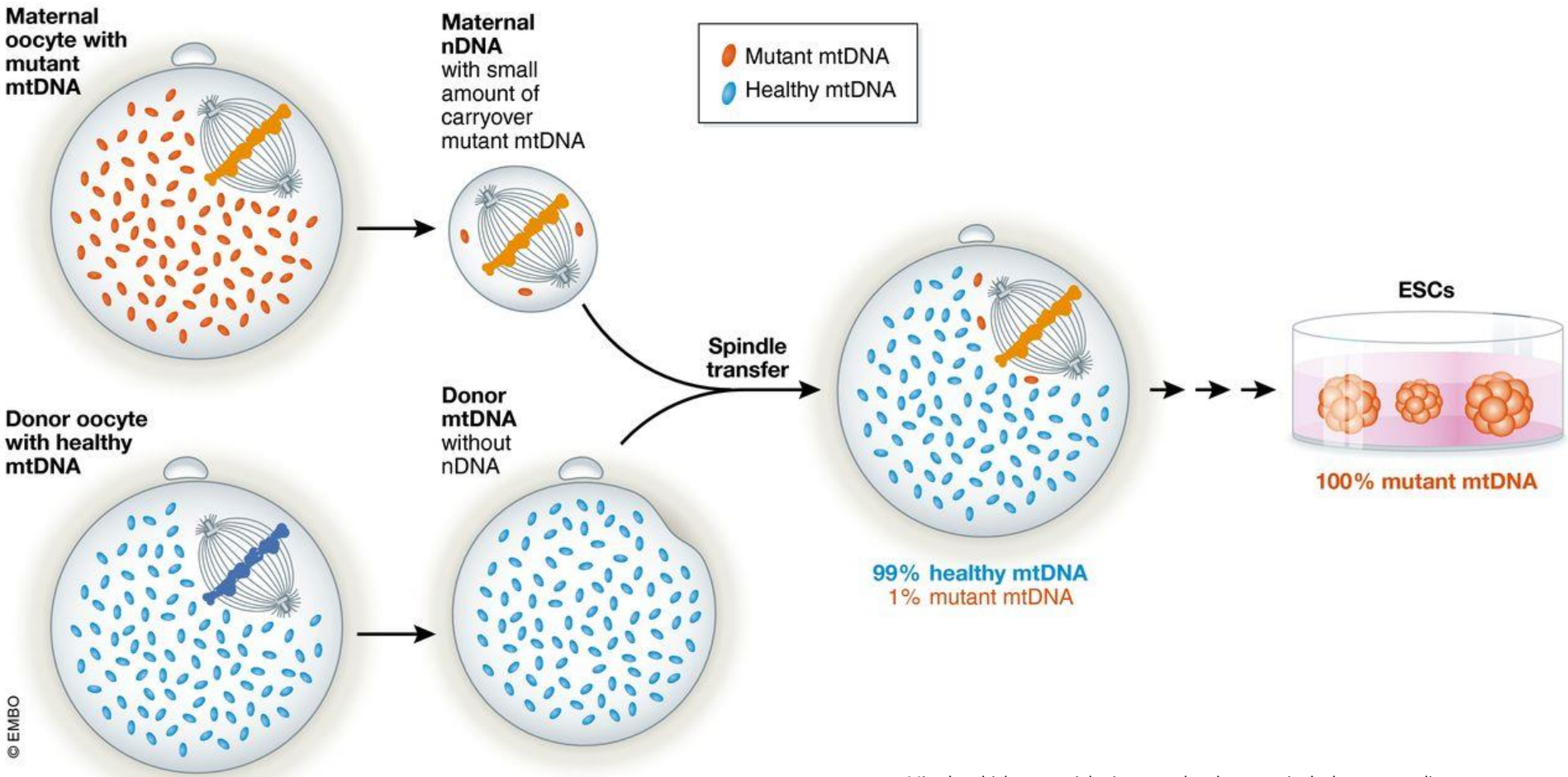
embryotools

Prevention of mtDNA diseases

The **UK the first country** to regulate **mitochondrial donation to prevent** the **transmission of mtDNA diseases**;



mtDNA reversion in hESCs



Mitochondrial genome inheritance and replacement in the human germline.
Wolf DP, Hayama T, Mitalipov S.
EMBO J. 2017 Sep 1;36(17):2659.

Previous statements

- 2018
- 2017
- 2016

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ESHRE News and Statements

9 July 2019

Moratorium on the use of spindle transfer as fertility treatment

ESHRE recommends extreme caution on the use of spindle transfer in human oocytes as a clinical application to address fertility problems. This treatment, also known as mitochondrial donation, involves the replacement of chromosomes of donor oocytes with the chromosomes of the patients, with the aim of correcting cytoplasmic disorders. This technique was originally developed for the treatment of women carrying life-threatening mitochondrial diseases to prevent the birth of affected children. After expert evaluation, it was made legal in 2015 in the UK, where each application, available only from licensed centres, is considered on a case by case basis and authorized only for those cases with a clear medical need and having no alternative.

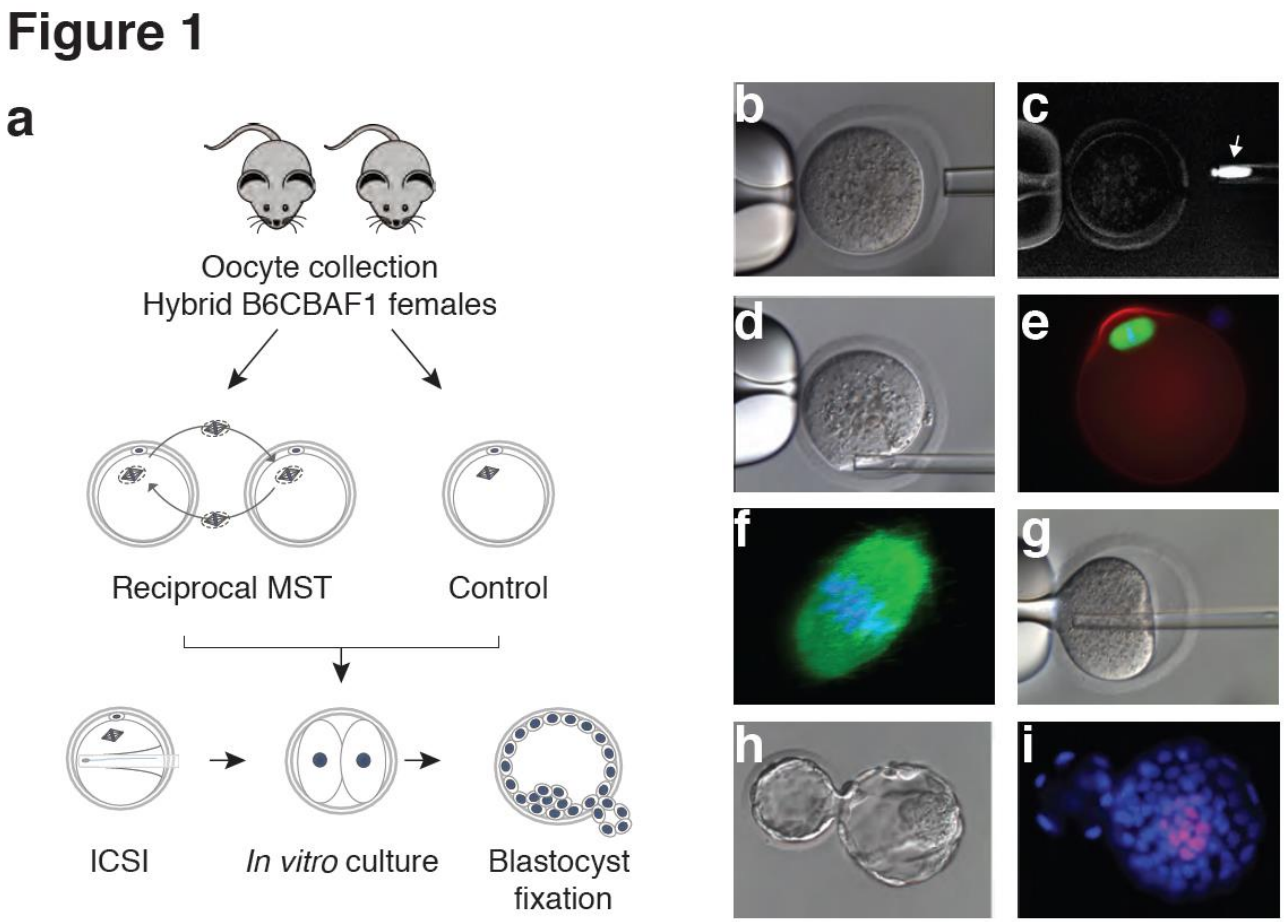
Recently, researchers from a Greek Institute reported the birth of a healthy boy following the use of spindle transfer in a 32-year-old woman without an inherited mitochondrial disease, who had failed four previous cycles of IVF. Whether there was an identifiable 'cytoplasmic disorder' is unclear.

In the absence of solid evidence proving that spindle transfer or other forms of cytoplasmic donation provides higher live birth rates than conventional assisted reproductive technology, the application of spindle transfer as a remedy for fertility treatment remains vague and unproven. The recent report in Science on the untested interplay between mitochondria and nucleus remains unclear in the possible generation of short and long term side effects (Science 364, eaau6520, 2019). The current lack of solid scientific evidence providing safety reassurance requires more study and continued vigilance.

At the present stage, and until this technology has been proven to be effective and safe, ESHRE strongly discourages the use of mitochondrial donation to alleviate an infertility condition. In line with the document issued by HFEA, the UK fertility regulator, and co-signed by ESHRE and other scientific societies, we support the need for a responsible use of evidence-based treatments in fertility practice (https://www.hfea.gov.uk/media/2792/treatment-add-ons-consensus-statement-final.pdf).

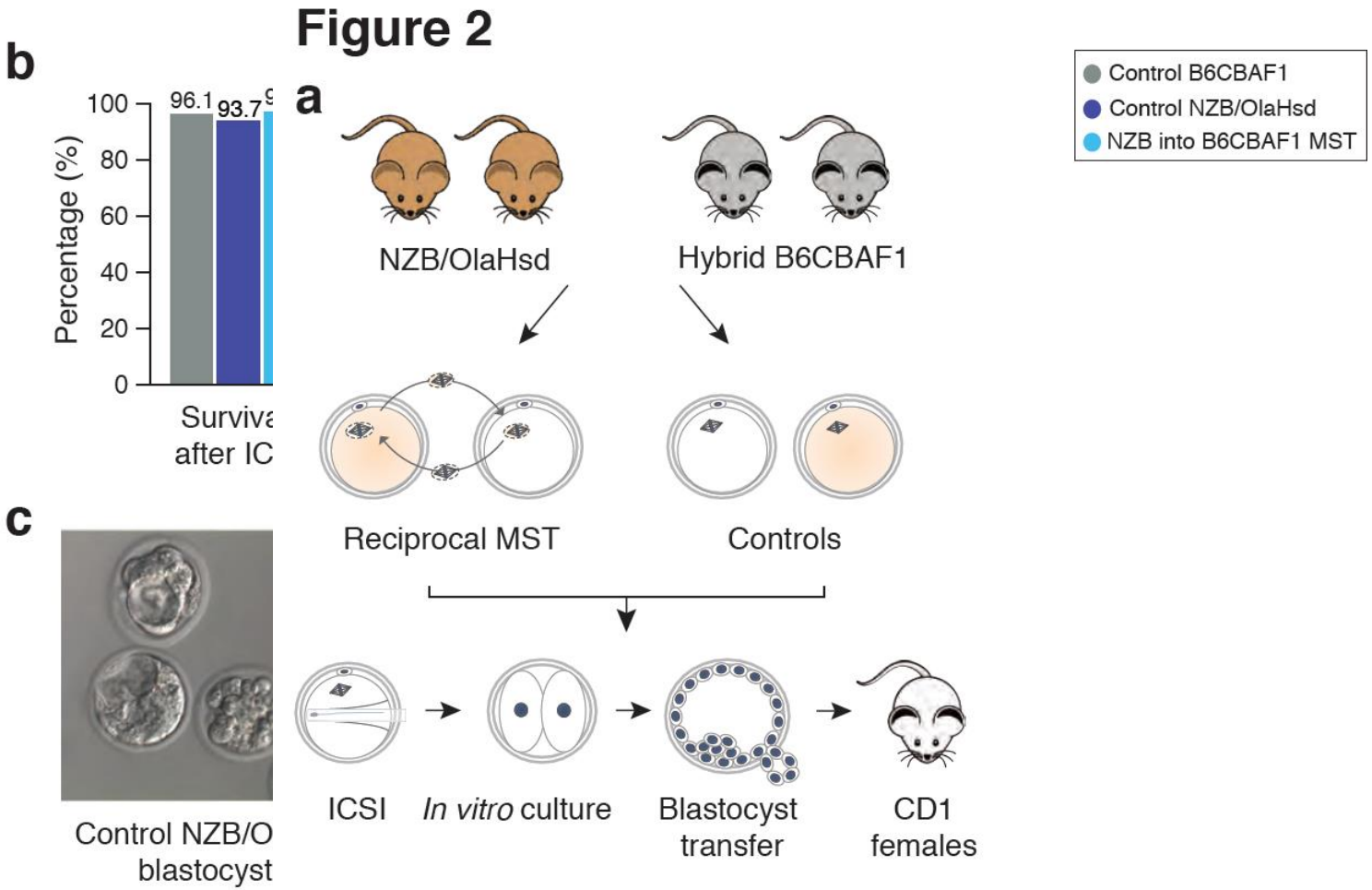
Proof of concept in the mouse

Maternal spindle transfer (MST) among sibling B6CBAF1 oocytes is feasible without impairing embryo development



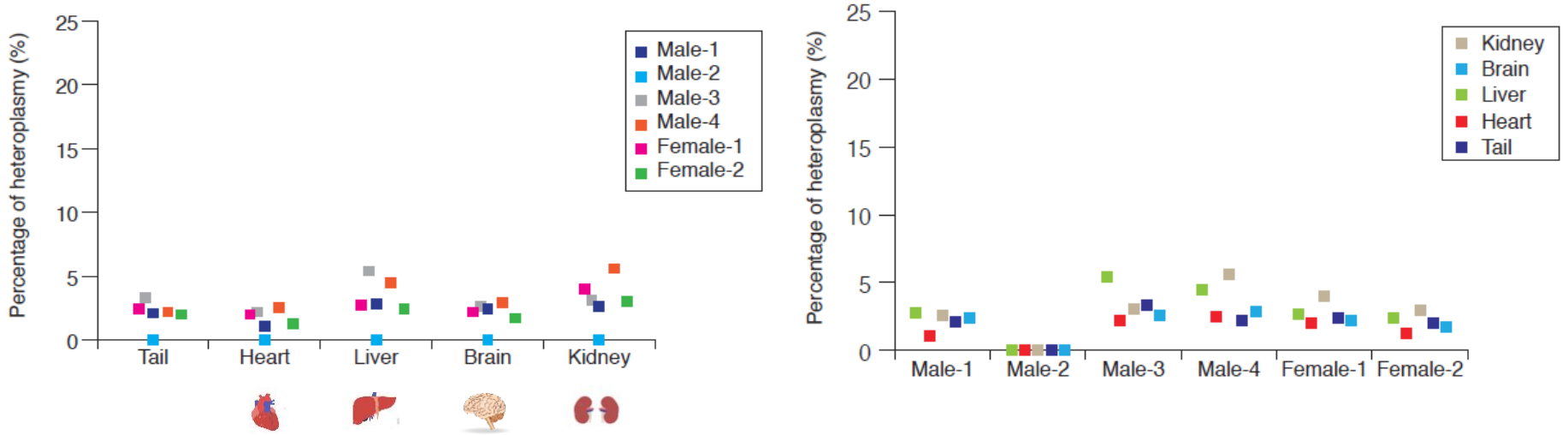
Proof of concept in the mouse

Spindle transfer overcomes embryo development arrest in NZB oocytes



Proof of concept in the mouse

mtDNA heteroplasmy is stable in adult organs of mice generated by MST



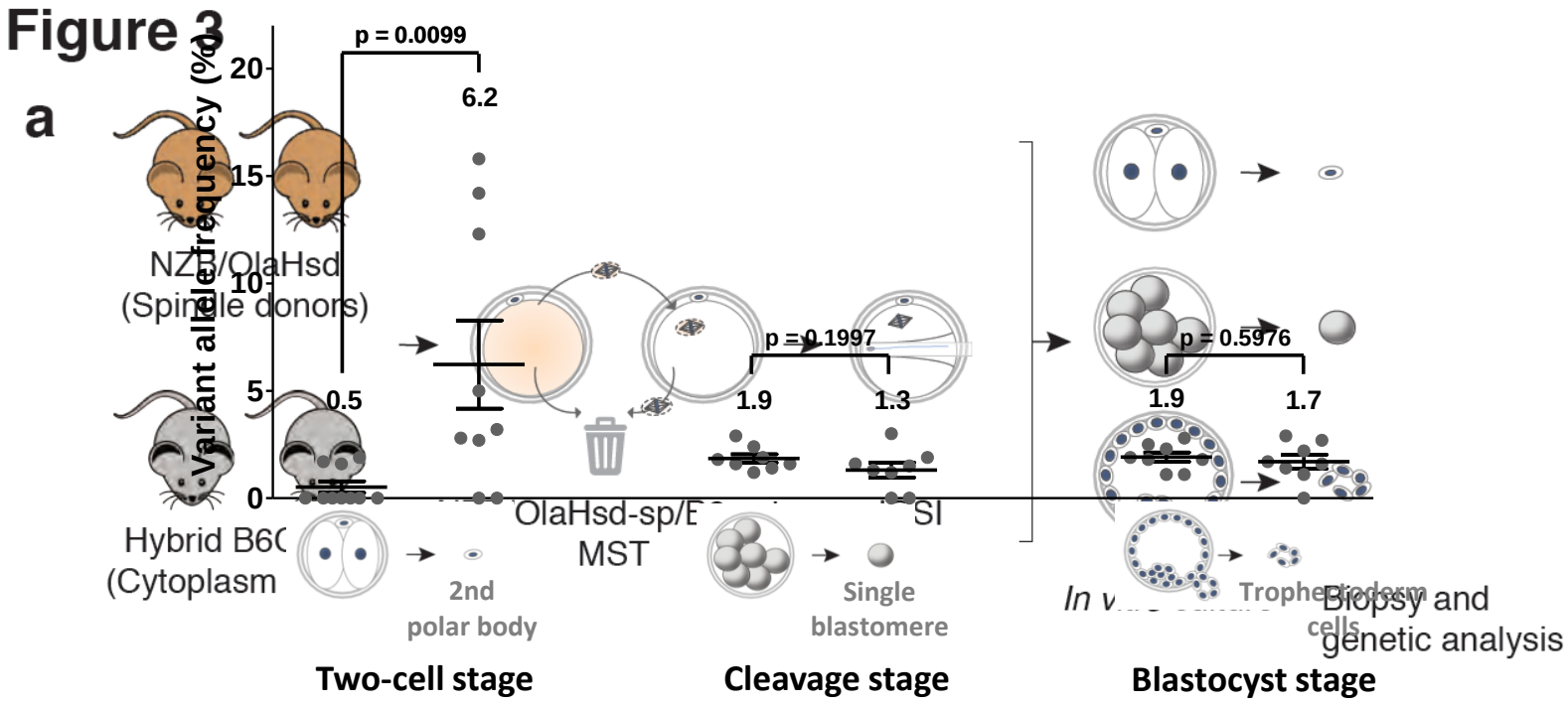
The **mean heteroplasmy level** for the MST-generated mice **was low at 2.1% ± 0.6%** (mean ± SD, n = 6, ranging from mean frequencies of **0.0% to 3.5% in individual mice.**

Analysis of heteroplasmy in MST male mice showed that the level of NZB mtDNA remained constant in all organs and equivalent to the levels found in the same organs of females.

Mice followed-up up to 5 generations (F5), no heteroplasmy detected after F3.

Proof of concept in the mouse

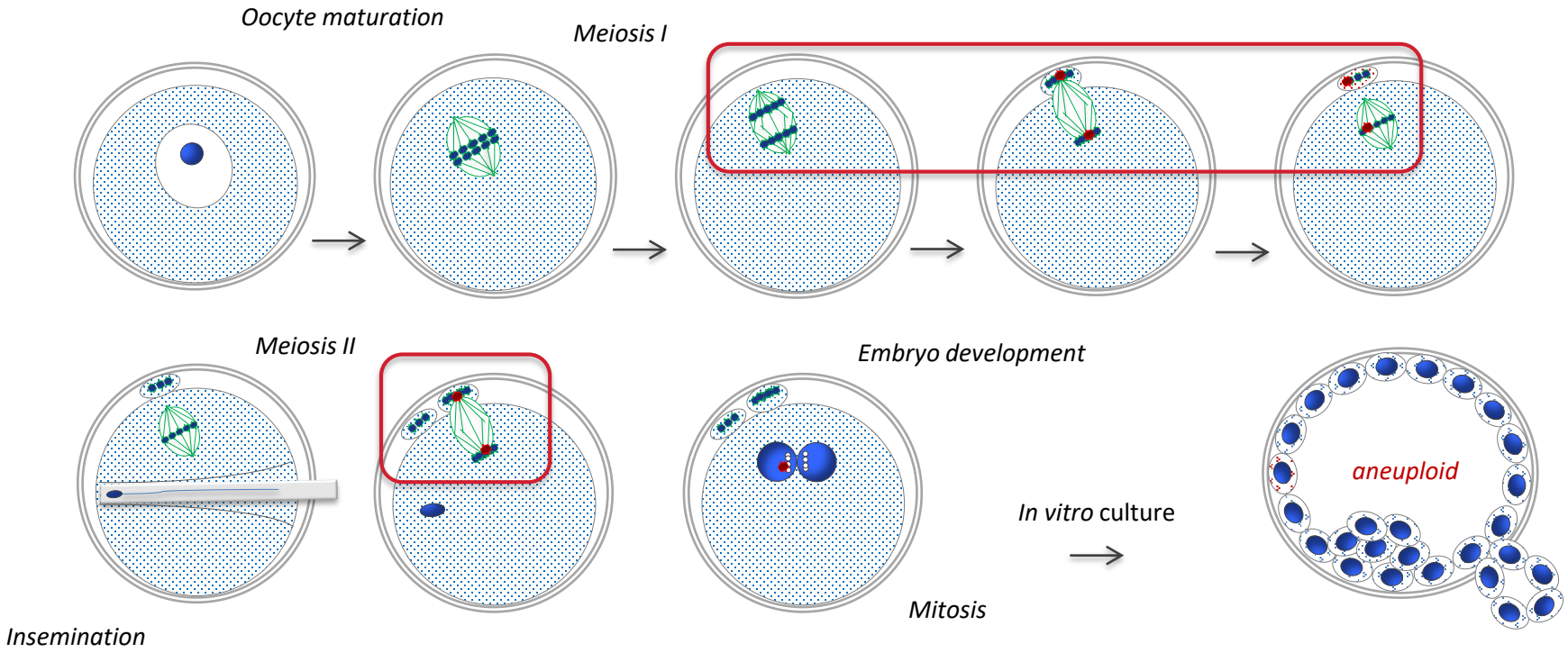
mtDNA carryover in biopsied cells and the complementary embryos



Heteroplasmy levels **were similar between all embryonic samples,** except for polar bodies; **Cleavage stage or blastocyst biopsy** are **preferable over biopsy of second polar bodies** as methods for determining the mtDNA carryover levels found in pre-implantation embryos;

Current research

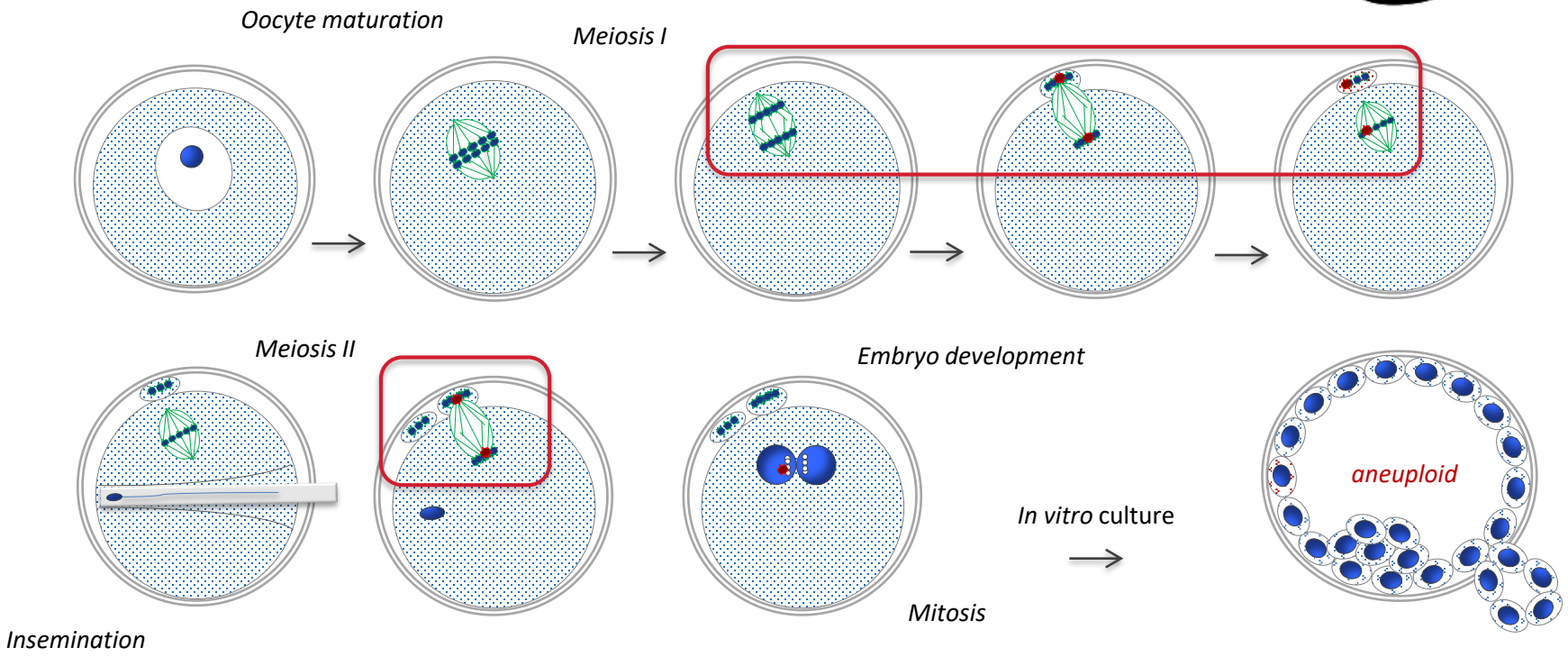
Can MST be valuable for advanced age patients?



The nature of the aneuploidy in aged oocytes can occur either due to **segregation errors in chromosomes** throughout **meiosis I** or in **chromatids segregation** during **meiosis II** at the time of second polar body extrusion;

Current research

Can MST be valuable for advanced age patients?



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Current research

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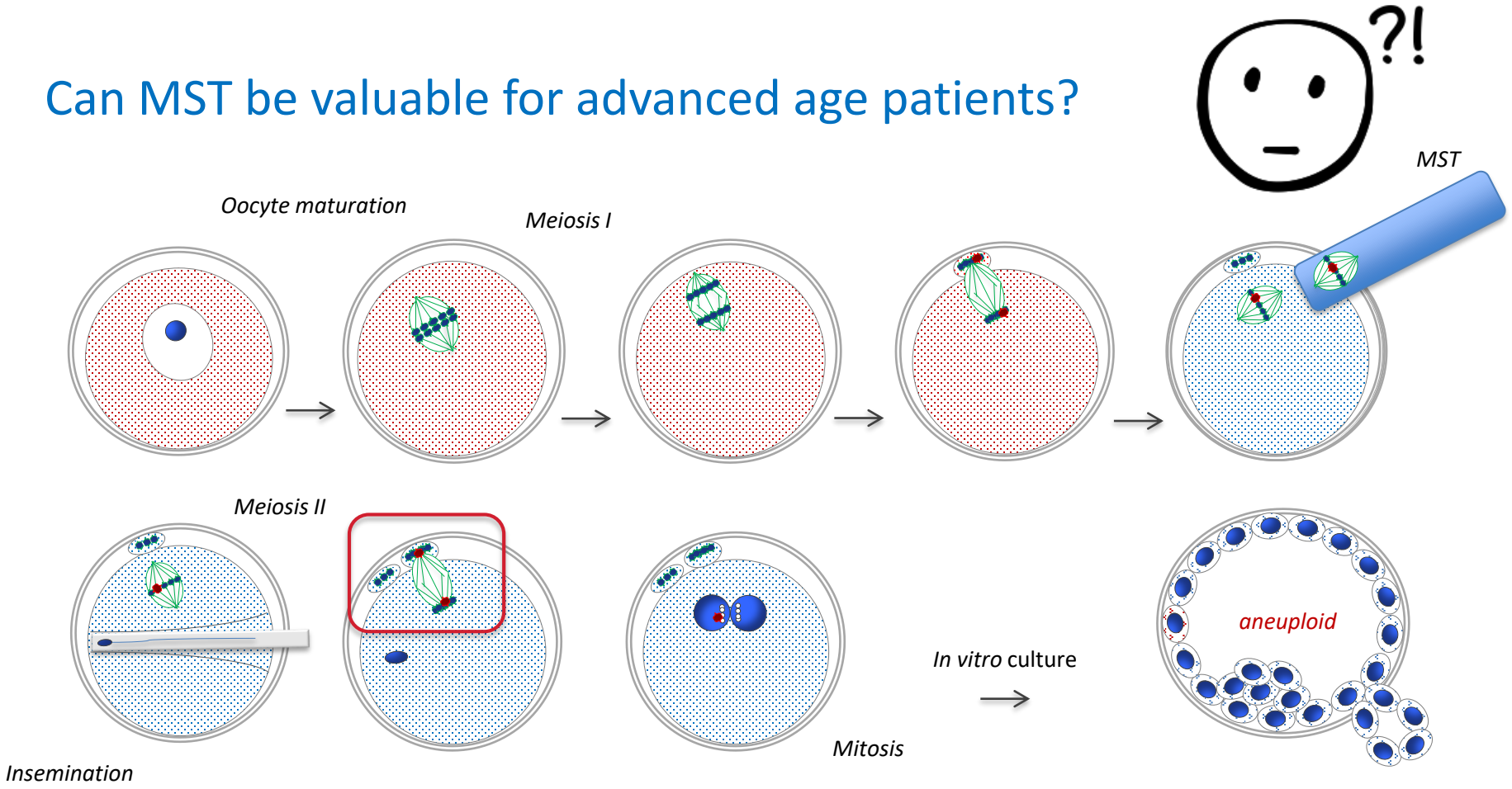
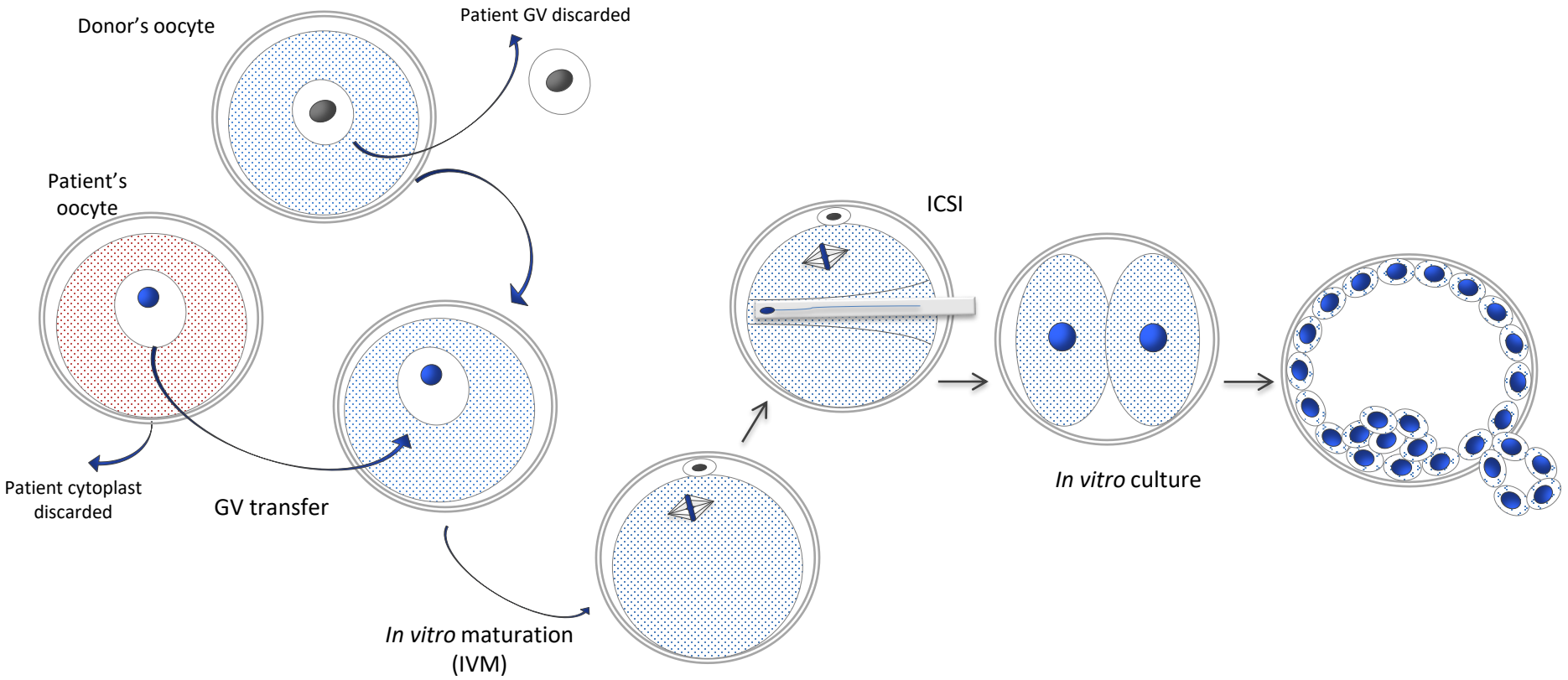


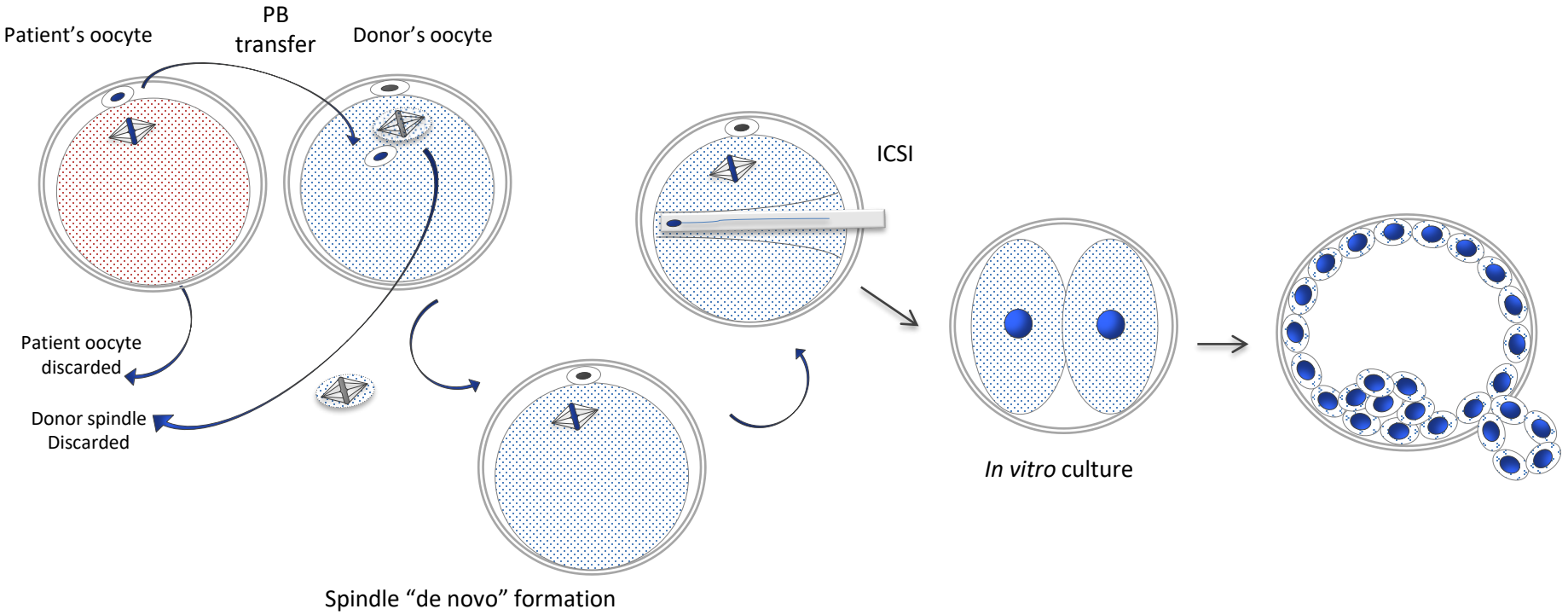
If errors occur during **meiosis I**, it is **likely** that the transfer of a MII-stage spindle into a more competent cytoplasm **may not be sufficient** to repair the **aneuploidies that are already present before MST is applied;**

Germinal vesicle transfer (GVT)



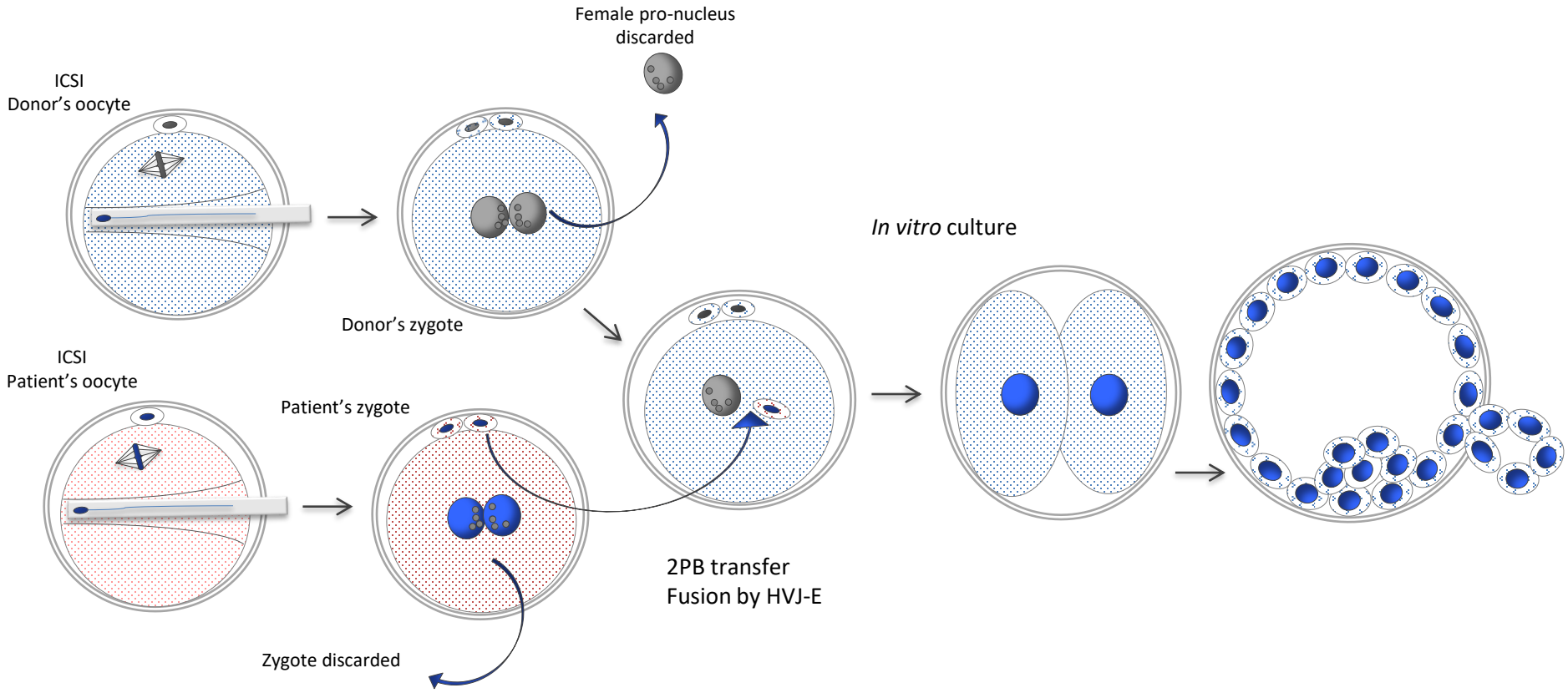
Technically GV is easy to manipulate | **Manipulation of oocytes before fertilization** | **high mtDNA carryover** due to uneven concentration of mitochondria in the perinuclear area | Limited by **low IVM efficiency** and **poor developmental rates after fertilization**

1st Polar body nuclear transfer (1PBT)



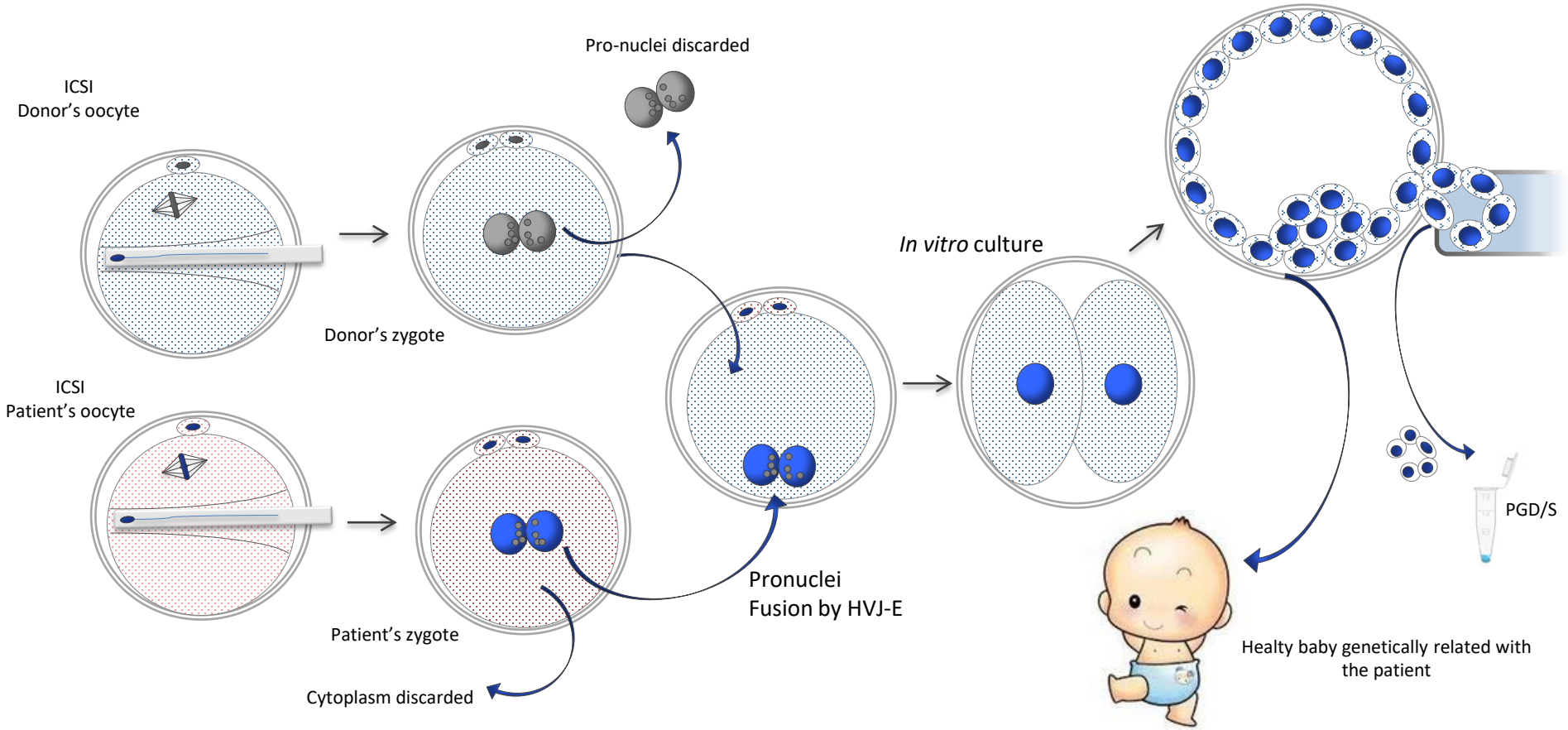
Technically demanding | Low mtDNA carryover within the 1st PB| Dependent on the chromosomal status of the 1PB - a brief lifetime | Manipulation of oocytes before fertilization

2nd Polar body transfer (2PBT)



Technically demanding | **Difficult synchronization between zygotes**| low mtDNA carryover | Dependent on the chromosomal status of the 2PB - a brief lifetime | **Difficult to distinguish male from female pronucleus** | **Ethical concerns – zygotes manipulation** |

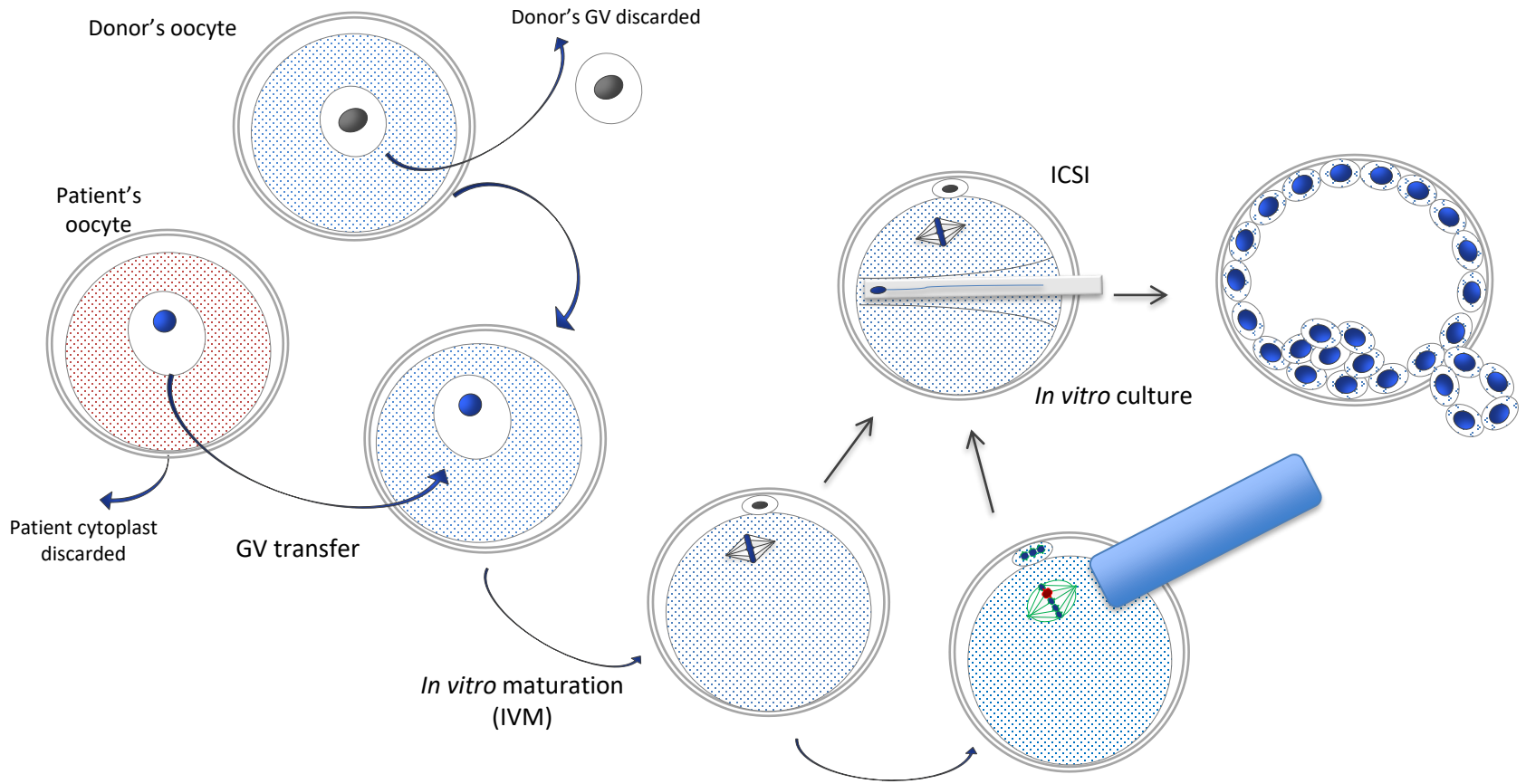
Pronuclear transfer



Technically easier | high mtDNA carryover due to uneven concentration of mitochondria in the peri-nuclear area | Difficult synchronization between donor and patient zygotes | Ethical concerns - zygotes manipulation | half of the embryos generated discarded

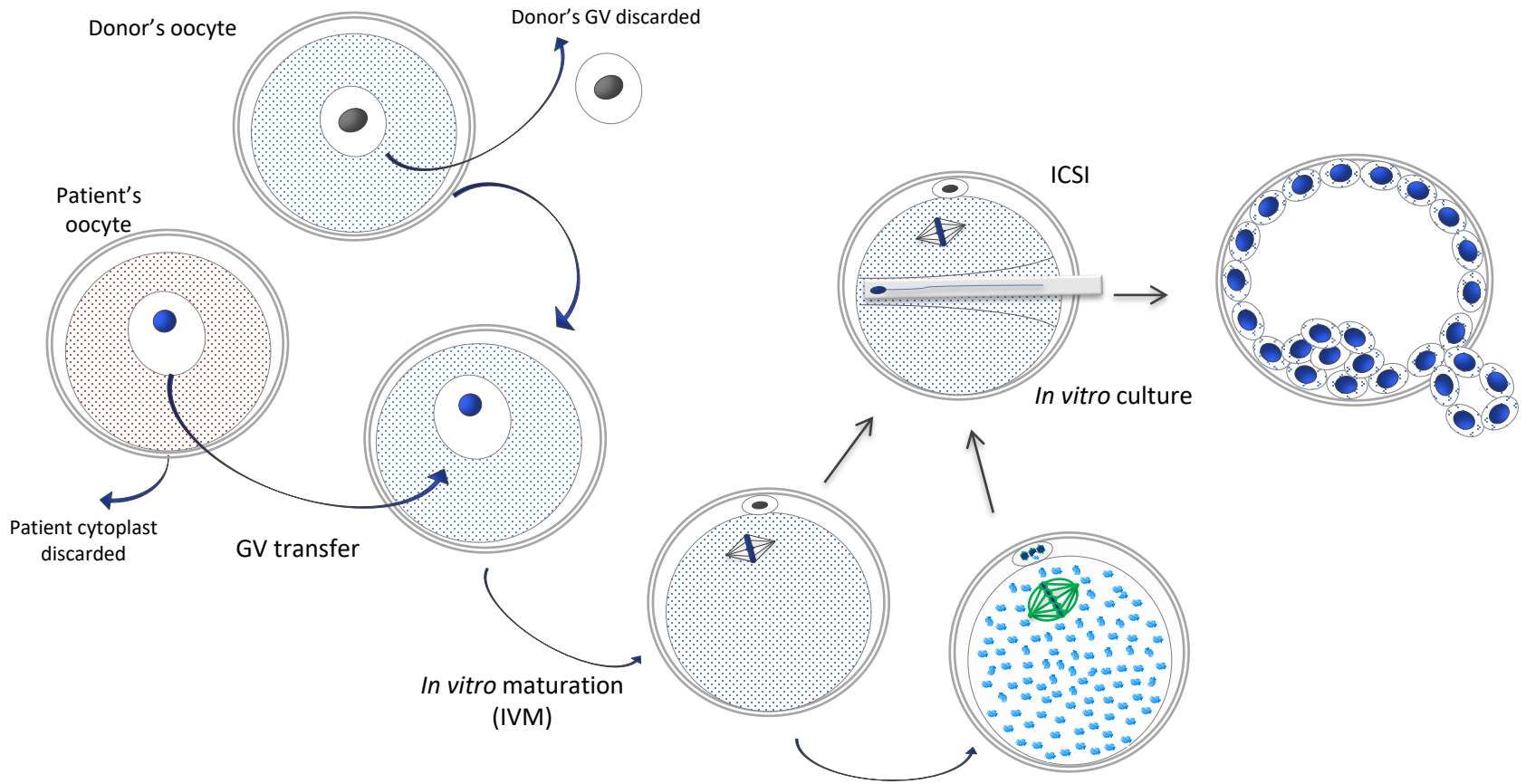
Current research

Germinal vesicle (GV) transfer alone or combined with spindle transfer?



Current research

Germinal vesicle (GV) transfer alone or combined with spindle transfer?



Questions & Answers

- Is MRTs considered genome editing?

- Genome editing refers to alterations in the sequence of the nuclear genome using techniques like CRISPR or TALENS, which can affect the germ line and therefore are likely to be transmitted or passed on to the following generations. With spindle transfer or MRT in general, we aim to do exactly the opposite, meaning that we aim to preserve and give chances to patients of transmitting there own nuclear genome, as it is.

- Are you going to follow up the babies?

- Yes, we dedicated a lot of time explaining the patients the importance of following –up the resultant babies, not only for them, but also for other couples that can benefit from the technique. They understood this and consented to do so, there actually an agreement with the pediatrics department of IASO hospital to follow the babies up to the age of 18 y/o. In parallel, we are creating an online platform to register all cases, which will be open to any other groups working on these techniques. This platform will represent an unique database with the health status of the MRT derived babies.

- Are you considering extending the indication of the MST technique to patients over 40 y/o?

- We are now conducting the trial with women under 40 y/o and we are seeing that patients that have a long history of unsuccessful IVF attempts with conventions technique due to poor embryo quality, can now have high quality blastocysts., however, but it is not clear yet, whether we would be able to rescue chromosomal errors with this technique. Most likely, if the meiotic spindle contains already errors, we may not be able to to correct them. However, if aged patients within their pool of oocytes still have a few oocytes with a spindle with the correct number of chromosomes, when put in a more competent cytoplasm, it may help to prevent errors occurring after oocyte activation (meiosis II) or during embryonic development (mitotic errors).

- Heteroplasmy and epigenetic concerns?

- Heteroplasmy with cytoplasmic replacement techniques (e.g. spindle or polar body transfer) can be avoided if the patient and donors' mtDNA haplotypes are matched; In addition, heteroplasmy does not mean disease, in particular, if we are not dealing with mtDNA mutations. We have learned from the paper of Chen et al., 2016 (Follow up of children resultant from cytoplasmic injection) that children with high heteroplasmy levels do not seem to present problems associated with heteroplasmy;
- Off course "Assisted reproduction techniques that today are a routine in IVF laboratories (IVF, ICSI, PGD/S) all involved a degree of uncertainty when they were first performed in humans. Actually, none of these techniques has been studied for so long as MRTs. All the scientific evidence, seem to indicate that there is no compelling reason to think that cytoplasm/mitochondrial replacement techniques, like spindle transfer, are unsafe. We reached the moment when it is important to move on and explore the feasibility of MST in carefully controlled clinical trials" – HFEA experts report, 2016
- Papers from Newcastle, Egli, Mitalipov's groups – reported normal global gene expression and DNA methylation patterns in blastocysts | Monkeys | Mice up to 5 generations | etc

Questions & Answers

- Isnt' it too premature to start experimenting with humans?

There is a huge amount of work involved to develop, optimize and demonstrate the effectiveness and safety of the technique, not only by our group, but also other groups in the US (Mitalipov's and Egli's) and in the UK . NewCastle's group – In our case, we took a very careful and conservative approach when translating the technology to humans:

1. Validations in the mouse model up to F5 generations;
2. Preclinical validation in donor oocytes, development of accurate molecular analysis for chromosomal analysis and mtDNA carryover, etc;
3. Pre-clinical trial. Only one embryo transferred and followed the first pregnancy very closely before doing more transfers; The second embryo was only transferred when the first pregnancy was already at 30 weeks;

Basic research is very important, but It reaches a moment that we cannot gather more information and we need to move on to understand whether the techniques are okay or not with carefully controlled clinical trials;

- Do you think these techniques could be implemented in the lab routine of IVFs center soon?

For the moment, only few labs over the world are ready to perform the technique successfully. It's a technology that requires very good micromanipulation skills, a long learning curve, special equipment, etc, as well as, accurate molecular techniques that can combine mtDNA and chromosomal analysis in a few cells. In our views, the logical way, would be to have an authorized by the regulatory bodies a few refence centrs in Europe, US, with demonstrated competence to perform these cases, that should be in charge of doing the cases in a controlled way, before the methods spread around;

- You announced a first pregnancy a few months ago, have you done more patients since then?

Yes, we have done more cases which resulted in the productions of blastocysts that are now being screened and are chromosomally normal – we did not want to rush, we wanted to ensure everything was okay with the first pregnancy, before doing any more transfers. All analysis, including: ultrasounds, amniocentesis, pre-natal diagnosis, all screening tests were normal, so we now transferring more embryos. Actually, we have already a second pregnancy out of 3 ET performed so far.

- "Three-parents baby"

Well, this is a sensationalist term, used in the past by some journalists, I think we should avoid using this term, as it is not fair for the parents or the individuals that may result from the procedures. When a person is transplanted with a bone marrow, a heart or other organ, we don't say it is a three-persons individual, why do we need to call them like that in this case?

Questions & Answers

- What are the main advantages of using this techniques?

This technique has a huge potential, the main advantage is that it can allow patients that have had several previous IVF cycles attempts new hopes and higher changes of having a child genetically related to them, instead of having to be enrolled in an oocyte donation program;
Also for donors, it offers advantages, as it would reduce the phycological concerns related to the creation of babies with a blood relationship, as it happens with current conventional oocyte donation programs;

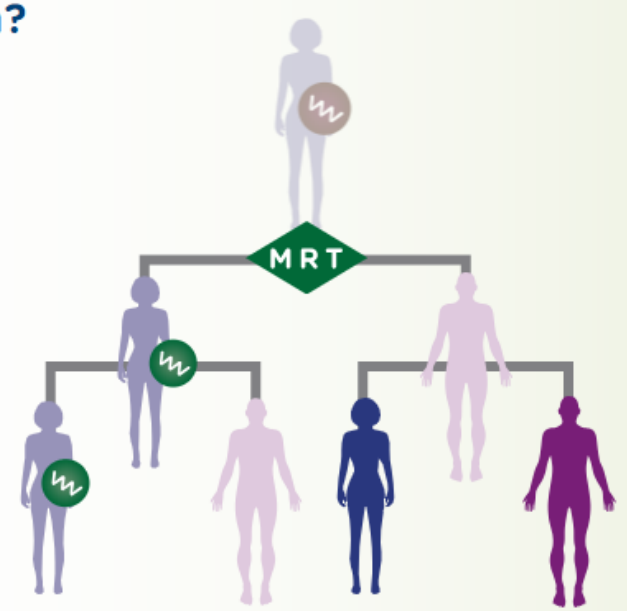
Is MRT germline modification?

‹ Previous

The following graphic illustrates heritable genetic change via MRT. Hover each figure in the graphic for more information.

Is this germline modification?

The committee concluded that MRT results in the genetic modification of germ cells (that is, eggs and sperm), but that it constitutes heritable genetic modification (germline modification) only if used to produce female offspring. Since mitochondrial DNA (mtDNA) is solely maternally inherited, **MRT to produce male offspring would not constitute heritable genetic modification.**



Why only males? ›

06

The National Academies of
SCIENCES · ENGINEERING · MEDICINE

Other points for discussion

- **Poor oocyte quality** is **not exclusive of advanced maternal age patients**;
- **Mitochondria injection does not show to** be **effective to correct cytoplasmic deficiencies** or **chromosomal abnormalities**;
- Mitochondria may not be the **only responsible** for poor oocyte quality – other cytoplasmic factors are likely to be **also involved**;
- **Heteroplasmy with cytoplasmic replacement techniques** (e.g. spindle or polar body transfer) can be **avoided** if the patient and donors' mtDNA haplotypes are matched[1];

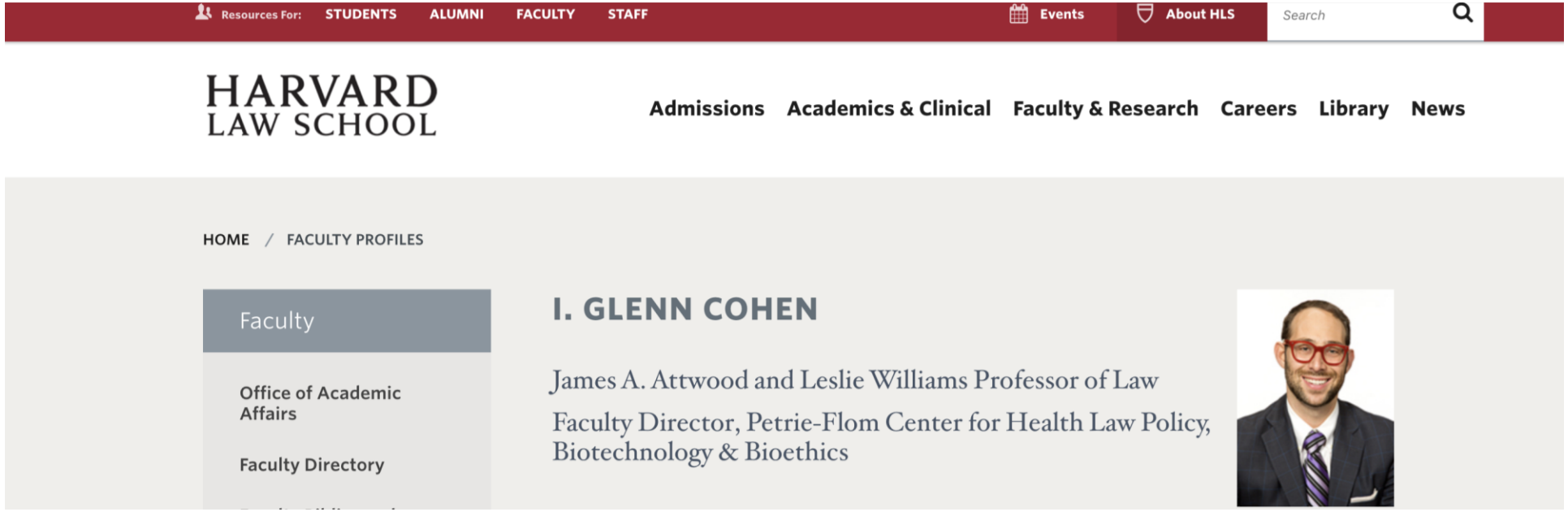
[1]Fischer et al., **Fertil Steril 2018** Vol 110, Issue 4, Suppl, Pages e423–e424.

Ethical / Moral issues

- “Humanity has always needed time to get over scientific breakthroughs. The fear about revising the laws of nature, altering the natural order - it’s resistant to cerebral arguments. It generally takes a generation or so to convince people that it’s okay.” by **Eli Adashi**[1], MD, Professor at Brown University, US.

[1]Preventing Mitochondrial Disease: A Path Forward. Adashi, Eli, Y., MD, MS; Cohen, I., Glenn, JD Obstetrics & Gynecology: March 2018 - Volume 131 - Issue 3 - p 553–556.

Feedback from other countries



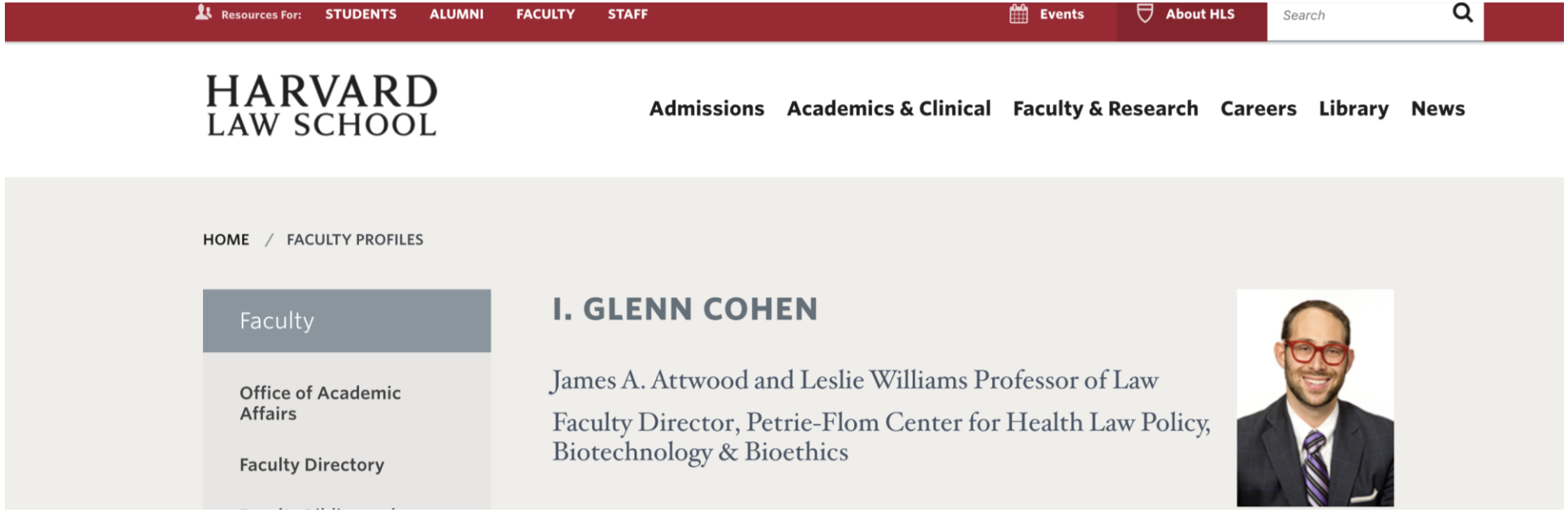
- *"This new trial in Greece may put pressure on the U.S. and other countries to change their policies for mitochondrial replacement therapy, noting these cases open the door to medical tourism"* – ***by***

  Professor I. GLENN COHEN [1,2], Harvard Law School

[1] *https://www.statnews.com/2019/01/24/first-trial-of-three-person-ivf-for-infertility/*

[2] *https://hls.harvard.edu/faculty/directory/10176/Cohen*

Feedback from other countries



"There is simply no way for any country to truly insulate itself from alterations through mitochondrial replacement therapy entering a country's gene pool" – ***by Professor I. GLENN COHEN [1,2], Harvard Law School***

[1]*https://www.statnews.com/2019/01/24/first-trial-of-three-person-ivf-for-infertility/*

[2]*https://hls.harvard.edu/faculty/directory/10176/Cohen*

Feedback from other countries

The Oxford Uehiro Centre for Practical Ethics

University of Oxford

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> Dr César Palacios-González

Dr César Palacios-González

"One of the ethical reasons presented so far against using MRTs for non-mtDNA related infertility hinges on the moral duty that doctors have not to offer futile courses of action. But whether MRTs (or some MRTs) can help to overcome certain types of infertility is an empirical question that is still open to empirical verification. And here is where a big difference between the Ukrainian case and the Spanish/Greek case seems to lie. Whereas there is no clear evidence (or at least no published evidence) that PNT, the technique that uses single cell embryos, can help to overcome infertility due to embryo arrest (for which it was used in the Ukraine instance), in the Spanish/Greek case, the scientists have already presented some evidence that MST "can enhance the potential of developmentally compromised oocytes to develop up to the blastocyst stage without compromising euploidy rates". And these findings seem to support the use of such technique to help the 32 year old woman who suffers from poor ovarian reserve, which "indicates a reduction in quantity and quality of oocytes in women of reproductive age group". All this being the case, we can say that at least this first ethical hurdle (not offering futile treatments) seems to have been overcome. And in doing so we are once more invited to revisit the ethics of MRTs. Let us close here by stating the obvious: until we have more information about this case we will not be able to make a complete ethical assessment of it."– ***by Dr. César Palacios-González [1,2], Oxford University***

[1]http://blog.practicalethics.ox.ac.uk/2019/01/a-third-mrt-baby-is-on-its-way/
[2] https://www.practicalethics.ox.ac.uk/people/cesar-palacios-gonzalez

Ethical / Moral issues

OHSU Healthcare

Paula Amato, M.D.

Healthcare
Who We Are
Healthcare Services
OHSU Near You

Paula Amato, M.D.

Faculty profile

Associate Professor of Obstetrics and Gynecology, School of Medicine

- *"We are hopeful that continued success abroad will lead Congress to reconsider its ban on mitochondrial replacement therapy trials in the U.S. so that Americans can have access to this promising technology"*
- *"Children will need to be followed into adulthood to make sure the technique doesn't have any side effects, but Amato said the limited data so far suggest that the procedure is feasible and safe in humans"* ***– by Paula Amato, OB-GYN at Oregon Health and Science University***

- "Three-parents baby" it is a sensationalist term and it should not be considered genome editing – it is exactly the opposite, it is a technique that is meant to patients that have had several previous IVF cycles attempts higher changes of having a child genetically related to them, instead of having to be enrolled in an oocyte donation program"; - ***by Nuno Costa-Borges, PhD***

- "Also for donors, it offers advantages, as it would reduce the phycological concerns related to the creation of babies with a blood relationship, as it happens with current conventional oocyte donation programs"; - ***by Nuno Costa-Borges, PhD***