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Members of the Jury

Prof. Dr. Fransiska Malfait (chairman)
Department of Biomolecular Medicine, Ghent University Hospital

Dr. Sofie Symoens
Department of Biomolecular Medicine, Ghent University Hospital

Prof. Dr. Claudia Spits
Research Group Reproduction and Genetics, Vrije Universiteit Brussel

Dr. Arnaud Vanlander
Department of Pediatrics and medical genetics, Ghent University Hospital

Dr. Frauke Vanden Meerschaut
Department for Reproductive Medicine, Ghent University Hospital

Dr. Lyndsey Butterworth
Wellcome Centre Mitochondrial Research, Newcastle University

PhD guidance committee

Prof. Dr. Dieter Deforce
Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University

Prof. Dr. Paul Coucke
Center for Medical Genetics Ghent (CMGG), Department of Biomolecular Medicine, Ghent University

Full text

Available: please contact Maoxing.Tang@UGent.be

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Scientific output

- Craven L†, **Tang MX†**, Gorman GS, De Sutter P, and Heindryckx B. Novel reproductive technologies to prevent mitochondrial disease. ***Hum Reprod Update*** 2017; 23: 501-19. (**†JOINT FIRST AUTHORS**)
- **Tang M**, Guggilla R R, Gansemans Y, Van der Jeught M, Boel A, Stamatiadis P, Ferrer-Buitrago M, Thys V, Van Coster R, Deforce D, De Sutter P, Van Nieuwerburgh F, Heindryckx B. Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants. ***Mol Hum Reprod*** 2019; 25: 797-810.
- **Tang M**, Popovic M, Stamatiadis P, Van der Jeught M, Van Coster R, Deforce D, De Sutter P, Coucke P, Menten B, Stoop D, Boel A, Heindryckx B. Germline nuclear transfer in mice may rescue poor embryo development associated with advanced maternal age and early embryo arrest. ***Hum Reprod*** 2020 (Accepted).
- **Tang M**, Boel A, Coucke P, Heindryckx B. Germline nuclear transfer technology to overcome mitochondrial diseases and female infertility. **Book chapter**: Manual of Intracytoplasmic Sperm Injection in Human Assisted Reproduction With Advanced Micromanipulation Techniques to Edit Genetic and Cytoplasmic Content of Oocyte. Editors: Gianpiero Palermo and Peter Nagy (*Under review 2020*)
- **Tang M**, Boel A, Guggilla R R, Bekaert B, Vanden Meerschaut F, De Sutter P, Menten B, Symoens S, Stoop D, Coucke P, Heindryckx B. Germline nuclear transfer to overcome mitochondrial disease and female infertility. (*In preparation for Nature Communications 2020*)

G-FaST Ghent Fertility and Stem cell Team

FACULTY OF MEDICINE AND HEALTH SCIENCES

Germline nuclear transfer to overcome mitochondrial diseases and female infertility

Mr. Mao-Xing Tang

Promoter: Prof. Dr. Björn Heindryckx
Co-promoter: Prof. Dr. Petra De Sutter

Ghent-Fertility And Stem cell Team (G-FaST)
Department for Reproductive Medicine
Department of Human Structure and Repair
Faculty of Medicine and Health Sciences
Ghent University Hospital

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Scientific research

Mitochondria are important cellular organelles responsible for generating ATP, containing their own genome (mtDNA), which is exclusively maternally inherited. Mutations or deletions in the mtDNA can cause a broad range of debilitating and life-threatening mitochondrial diseases, which reportedly affect approximately 1 in 5.000 individuals.

To date, no curative treatments for patients with mitochondrial diseases exist. While prenatal diagnosis and pre-implantation genetic diagnosis can be used to circumvent mtDNA diseases by selecting fetuses or embryos with low mutation loads, they are not suitable for women with high mutation loads or homoplasmic mutations. In such context, prevention of mitochondrial diseases may currently be possible using a novel *in vitro* fertilization (IVF) based technique called germline nuclear transfer (NT), such as maternal spindle transfer (ST), pronuclear transfer (PNT) and the more recently proposed first or second polar body transfer (PB1T or PB2T). These techniques involve the transfer of the nuclear genome from an oocyte or zygote carrying mtDNA mutations to an enucleated donor counterpart with wild-type mtDNA (please see Figure below).

In addition, mitochondrial dysfunction is also associated with poor outcomes of fertilization and pre-implantation development in oocytes or embryos. The NT technology has therefore been considered for certain female infertility indications, such as infertile women experiencing fertilization failure and embryo developmental arrest. This is mainly based on the rationale that replacing a low-quality cytoplasm with a competent one using NT, will improve oocyte competence, resulting in better IVF outcomes. Nevertheless, until now, there is only limited data available on the application of NT in human, either for avoiding mitochondrial disease transmission or for overcoming female infertility.

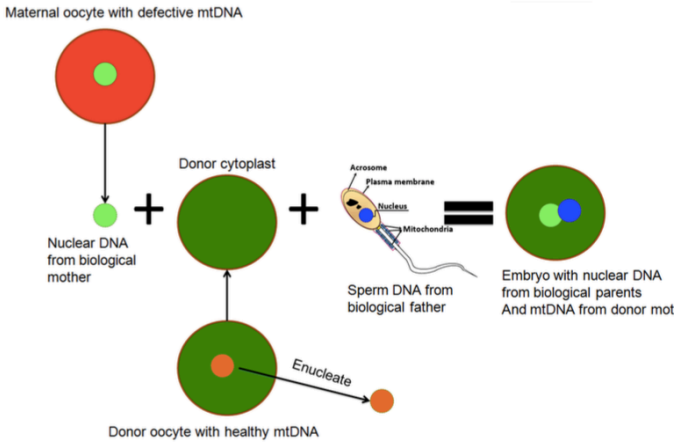

Figure: *Schematic diagram outlining the general procedure of NT technique. Chromosomal DNAs are inherited from both the biological parents, whereas mtDNAs are inherited from the donor mother.*

First, using the NZB/OlaHsd and B6D2F1 mouse model, we comparatively evaluated the efficiency of different NT techniques, including PB1T, PB2T, ST and PNT, to prevent the transmission of mtDNA variants in a mouse model. Furthermore, we attempted to optimize PB2T as a novel reproductive technology to overcome mtDNA diseases. Our data showed that both PB1T and a newly developed PB2T have the potential to reduce the mtDNA carry-over without compromising embryo development. Moreover, we successfully developed a novel method of PB2T, which is more suitable in human.

Next, using a reproductive-age and an embryo-arrest mouse model, we verified whether PNT and ST could be applied to improve fertilization rates and embryo developmental potential. The results revealed the therapeutic potential of both PNT and ST for improving oocyte competence and overcoming embryo developmental arrest in mice. These NT technologies may thus provide new potential avenues for genetic parenthood, for patients facing age-related infertility and early embryo developmental arrest.

Finally, in human, we assessed the efficiency of both ST and early PNT (ePNT) to reduce transmission of a pathogenic homoplasmic mtDNA mutation (m.11778G>A), which is known to cause Leber's hereditary optic neuropathy (LHON) disease, and investigated ST as a novel approach to overcome fertilization failure after ICSI. Furthermore, using a mouse model, we determined whether NT significantly alters gene expression patterns to verify the safety of this technique. Our findings indicated the potential of the NT technique in avoiding mtDNA disease transmission as well as overcoming failed fertilization after ICSI. Moreover, evaluation of NT-generated blastocysts revealed that this technology did not significantly affect the pattern of gene expression.

Overall, the NT techniques have the potential to circumvent mother-to-child transmission of mtDNA diseases. Moreover, these techniques may provide a novel reproductive strategy to overcome certain female infertility indications.

CONTACT
Maoxing.Tang@UGent.be
T +32 470406823