TMU-Joint Institutional Review Board

Study Protocol Synopsis

Synopsis Version: V3 Date: 20250212 TMU-JIRB Form057/20230801

1. Study Title

Investigation of the treatment of infertility patients with mitochondrial defects by artificial reproductive technology

2. Study Objective

To evaluate the proportion of mutant mitochondrial DNA carried by study participants following mitochondrial replacement therapy.

3. Investigational Drug

■ Not applicable

1. Drug name:
2. Dosage form:
3. Dose:
4. Administration:
5. Mechanism of action (if known):
6. Pharmacological classification:

4. Stage of Drug Development

Not applicable

5. Trial/Study Design

1. Control: ○ Placebo ○ Active drug (drug name, dosage form, administration): ○ Other: ● Non-controlled
2. Blinding: ● Open-label ○ Assessor-blinded ○ Single-blind ○ Double-blind ○ Double-dummy ○ Other:
3. Randomization: ○ Yes ● No
4. ○ Parallel design ○ Crossover design ○ Other: ● Not applicable
5. Treatment period: days / weeks / months / years ■ Not applicable
6. Dose adjustment: ○ Mandatory ○ Optional ○ None ● Not applicable
7. Study site: ● Single center ○ Domestic multicenter ○ International multicenter

6. Endpoints

1. Primary endpoint:

Following mitochondrial replacement therapy, the mutation ratio and heteroplasmy/carrying rate of mitochondrial DNA will be assessed in cumulus cells, polar bodies, and embryo biopsy specimens from recipients and donors. Pregnancy and birth rates for all participants will be calculated, and neonatal gestational age at birth, birth weight, and other relevant indices will be recorded to evaluate the effectiveness and safety of this approach.

2. Secondary endpoint:

Growth and development will be monitored through the routine 21-item government-funded newborn screening program and routine neonatal vaccinations. Because late manifestations of mitochondrial DNA defects may include type 1 diabetes mellitus and lipid accumulation, newborns will undergo blood glucose, blood cell/platelet, and blood Oil Red staining examinations every six months. Monitoring will continue until six years of age. The study will also continue annual health follow-up from age 6 through 15 years during childhood and adolescence.

7. Subject Eligibility Criteria

Inclusion criteria

participants meeting either of the following categories may be included:

Oocyte recipient:

(a) Women aged 20-45 y/o with mitochondria defects or mutation as oocyte recipient.

(b) Women aged 20-45 y/o with history of miscarriages or deliveries of progenies carried mitochondria defects or mutations as oocyte recipient.

Healthy oocyte donor: Healthy women aged 20-40 y/o as oocyte donor.

Exclusion Criteria

(a) History of cervical cancer
(b) Carriers for other diseases that might affect the examination of PGS
(c) Pregnant women
(d) HIV infections

8. Trial/Study Procedures

(1) Participant criteria:

Patients with a history of miscarriage or delivery of offspring with mitochondrial genetic defects, or carriers identified by preconception genetic testing, who will undergo in vitro fertilization combined with intracytoplasmic sperm injection (ICSI).

(2) Study design and procedures:

Participants will comprise two groups: recipients requiring mitochondrial replacement therapy and healthy oocyte donors. In the clinic, the principal investigator will ask about willingness to participate, explain the study procedures, and review the informed consent form. After informed consent is obtained, the study and specimen collection will proceed as follows:

(1) Collect cumulus cells surrounding the recipient's oocytes at oocyte retrieval for extraction of mitochondrial DNA.

(2) Collect fresh donor oocytes. During mitochondrial replacement (mitochondria transfer), remove the donor spindle containing chromosomes and collect medically discarded donor polar bodies, cumulus cells for mitochondrial DNA extraction.

(3) Thaw the recipient's oocytes, isolate the spindle, and transfer it into the enucleated donor oocyte.

(4) Perform sperm injection and culture the fertilized egg in an incubator to observe fertilization and development.

(5) Blastocysts generated through mitochondrial replacement will undergo trophectoderm (TE) biopsy and perform Preimplantation Genetic Testing for Aneuploidy (PGT-A) to assess chromosomal copy number (euploidy or aneuploidy). To determine the carrying rate of mutant mitochondrial DNA, donor-oocyte polar bodies and cumulus cells, recipient cumulus cells, and non-developing embryos after mitochondrial replacement will be cross-compared by next-generation sequencing (NGS) for mutant mitochondrial DNA. These analyses are intended to reduce concerns regarding unknown genetic backgrounds of donor oocytes and to improve the precision and clinical applicability of mitochondrial replacement therapy.

(3) Study duration and schedule:

The study is planned for five years. During the first four years, six participants will be recruited: three recipients requiring mitochondrial replacement therapy and three healthy oocyte donors. Successfully matched participants will undergo mitochondrial replacement, ICSI, embryo mitochondrial DNA testing, and embryo transfer. During the final year, embryo transfer for the last enrolled participants, subsequent mitochondrial DNA testing of amniotic fluid cells, testing of mitochondrial carrying rates in offspring, and preparation of the final results report will be completed.

(4) Follow-up or rehabilitation plan:

Participant data will be retained for 15 years after study completion. Following successful delivery after embryo transfer, neonatal umbilical cord blood will be collected to determine whether defective mitochondrial DNA remains. Within 48 hours after birth, routine newborn screening for congenital metabolic disorders and routine HCT examination will be performed to assess anemia and thrombocytopenia commonly observed in Pearson syndrome. Growth and development will subsequently be monitored together with the routine 21-item government-funded newborn screening program and neonatal vaccinations. Because large mitochondrial DNA deletions may be associated with lipid-droplet accumulation in bone marrow cells or peripheral leukocytes and later development of type 1 diabetes mellitus, it is recommended that, after birth, blood glucose and platelet measurements and Oil Red staining of peripheral blood smears be performed every six months. Monitoring is expected to continue until six years of age. Annual health follow-up will then continue from age 6 through 15 years.

(5) Evaluation and statistical methods:

Following mitochondrial replacement therapy, the mutation ratio and heteroplasmy/carrying rate of mitochondrial DNA will be assessed in recipient and donor cumulus cells, polar bodies, and embryo biopsy specimens. Pregnancy and birth

rates for all participants will be calculated, and neonatal gestational age at birth, birth weight, and routine developmental indices will be recorded to evaluate the effectiveness and safety of this approach.

9. Concomitant Treatment

(1) Permitted concomitant medical procedures:

Routine oocyte retrieval, hysteroscopy, hormone replacement therapy (HRT) cycles, intracytoplasmic sperm injection (ICSI), and preimplantation chromosomal screening. Concomitant medications used with assisted reproductive treatment in this trial may include routine medications such as those used for sedation and antibiotics.

(2) Prohibited concomitant medical procedures:

Cancer treatment and use of chemotherapeutic drugs.

10. Statistics

1. Primary trial/study hypothesis: ● Superiority trial ○ Non-inferiority trial ○ Equivalence trial ○ Other:

2. Sample size estimate:

Taipei Fertility Center: 0-6 participants;

Total: 0-6 participants.

3. Efficacy analysis population: ○ Intent-to-treat ● Per-Protocol ○ Other:

Safety analysis population: ○ Intent-to-treat ● Per-Protocol ○ Other:

Note: Intent-to-treat (ITT); Per-Protocol, PP population (PP).

4. Interim analysis: ● Yes ○ No

5. Statistical methods:

Mitochondrial heteroplasmy in embryos or specimens will be analyzed for qualitative and quantitative parameters using Student's t-test. A p value <0.05 will be considered statistically significant.

6. Handling of missing data:

The principal investigator and research personnel will follow the ethical principles for medical research of the Declaration of Helsinki and clearly explain the study objectives and scope of data collection to participants. Research personnel will also conduct data management and study procedures in accordance with medical ethics. Missing or unused data will not be included in the statistical analysis. If falsified data are identified, the principal investigator will be notified and an internal data verification will be conducted. If necessary, participants will also be contacted by telephone for verification.

11. English Abstract

Mitochondria are important organelles that generate energy (ATP) in human and mammalian cells. Currently, there are more than 50 kinds of diseases known to be related to mitochondrial defects in Taiwan. The incidence of mitochondrial diseases is very rare, about 1/10,000. The abundancy of mitochondria riches to 100,000 DNA copies in one oocyte, and the threshold of DNA mutation rate to cause pathogenicity is usually greater than 70%. Since mitochondria are inherited maternally, the pathogenic mitochondrial DNA in oocytes could be removed by modern artificial reproductive technology (ART). By treating with mitochondria replacement therapy (MRT), or so called spindle nuclear transfer, following artificial insemination, the ooplasm from healthy donor oocytes could be transferred into the chromosomes of patients to remove defected mitochondria, therefore creating the opportunity to produce healthy offspring. The United Kingdom officially legalized the technology in 2015, but it only allowed for the treatment of patients at high risk of mitochondrial defects. However, the official reports about the mitochondria heteroplasmy in their offspring of such patients are still very rare in the world. Therefore, in order to further detect the carrying rate of mutated mitochondrial genes in patients with mitochondrial defects after MRT, we aim to detect the mitochondria mutation in germ cells of patients in order to improve the specificity and operation procedures of this therapy. The following specimens are expected to be collected in this study: (1) cumulus cells that surrounded the oocyte during egg retrieval, (2) clinically discarded polar body of donor oocytes harvested during MRT (3) the patient's own ooplasm, and (4) blastocyst trophectoderm (TE) biopsy completed by MRT.

Those specimens will use next generation sequencing (NGS) to verify whether the mitochondrial DNA of the polar body from donor is normal, and cross-comparison with the non-developed embryos after the MRT for improvement of heteroplasmy of mitochondrial DNA. These study will generate the examination method to prevent the possibility of disease carried donating eggs with uncertain background accurately, and it helps to increase the precision and clinical applicability of MRT.

Taipei Medical University

Certificate of TMU-JIRB Approval

Issue Date: 2026/04/24

TMU-JIRB No.: N202304104
Protocol Title: Investigation of the treatment of infertility patients with mitochondrial defects by assisted reproductive technology
Principal Investigator: Tzeng Chii Ruey
CO- Investigator: Shu-Huei Kao, KUO, Yung-Ting, Ching Wen, Chang, Au Heng-Kien, Chih-Ping Chen
Study Member: Wei-Fang Chang, Yung-Ling Huang, Wan-Ju Liao

Study Site: Taipei Fertility Center
Sponsor：NA
Protocol:V6/20250118
Informed Consent Forms:V8/20250212
Informed Consent Forms(Neonatal Subject):V4/20250212
Case Report Forms:V3/20250217

The above study's continuing report has been approved by TMU-Joint Institutional Review Board in meeting #115-04-4(date:2026/04/23), duration of validity is from 2026/05/26 to 2026/11/25, and must be monitored by TMU-JIRB.

According to Ministry of Health and Welfare and the relevant regulations, follow-up procedures and requirements are as below:

1. Continuing Report: Frequency of the report of this trial/study is every 6 months, and should be submitted to TMU-JIRB for review 2 months prior to expiry date (2026-09-25) or the trial/study must be pending.
2. Final Report: The report should be submitted to TMU-JIRB for review once completed TMU-JIRB may withdraw the approval of the trial/study if the report didn't submitted within 3 months from the date of validity and will suspend PI from new application for 3 months per TMU-JIRB SOPs.
3. SAE: Serious Adverse Event(s) (SAE) Report: SAE report(s) should be submitted to related authorities according to "Regulations for Good Clinical Practice" as well as "Procedures for Reporting Serious Adverse Drug Reaction" by MOHW.

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Chairman

Chung Ming Chen

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本會組織與執行皆符合適用法規
The TMU-Joint Institutional Review Board performs its functions according to written operating procedures and complies with GCP and with the applicable regulatory requirements.