Cytoplasmic Replacement Therapy for Poor Quality Human Oocytes

Background and Significance

Nuclear transfer (NT) has been previously applied to women who suffered from infertility, unrelated to Mitochondrial diseases. In 2003, the first pregnancy from human NT occurred after pronuclear transfer, for a 30-year old woman in China who had two failed IVF cycles due to persistent embryo arrest at the 2-cell stage (Zhang et al., 2003). In 2017, the pronuclear transfer technique was applied to a 34-year old woman in Ukraine who was also suffering from infertility and this resulted in a healthy birth. Nuclear transfer work led by researchers at the Nadiya Clinic in Ukraine and the Institute of Life in Greece has resulted in nine healthy babies from previously infertile women under age 41. In this clinical trial at the Darwin Life-Nadiya clinic, more cases will be investigated for the rescue of preimplantation embryonic arrest.

According to the statistics released by the Centers for Disease Control and Prevention (CDC), the birth rates for women 30 years of age or older continue to rise, and by 2016 they have resulted in a higher birth rate than for women aged 25–29 for the first time since 1940. However, older women are known to exhibit reduced birth rates due to their lower quality of eggs (Krey et al., 2001) as well as dramatically higher rates of aneuploidy (Webster et al., 2017; Nagaoka et al., 2012). The emerging trend of delaying having children combined with the fertility problems associated with advanced maternal age calls for the development of new IVF techniques and evaluation of their safety to meet growing needs. In this clinical trial, the regulatory mechanisms responsible for assembly of the meiotic spindle in the cytoplasm of the human oocyte are replaced with younger components from a young, healthy donor oocyte (Zhang et al., 2015).

Hypotheses and Objectives

A: Women 40 years old or younger who have failed four or more IVF cycles, and have a history of failure to form blastocysts. We hypothesize that nuclear transfer will help create normal blastocysts in a proportion of women experiencing preimplantation embryonic arrest. **B:** Women 41 years old or older, who have failed three or more IVF cycles, and still have a regular menstrual cycle. We hypothesize that nuclear transfer will help rescue meiotic errors in a proportion of women experiencing age-related infertility (marked by aneuploidy/chromosomally abnormal embryos).

Our objective is to evaluate the safety and efficacy of cytoplasmic replacement therapy for women seeking to have a genetically related child whose prior IVF treatment has failed to result in a healthy pregnancy.

Methods and Design

Human oocytes at metaphase II (MII) phase or germinal vesicle (GV) phase will be collected. All patients enroll by their own consent to participate in the clinical trial. In rare cases metaphase I (MI) oocytes may be processed in similar methods as described below.

A. <u>Rescue of preimplantation embryonic arrest:</u>

I. Transfer of patient nucleus to donor ooplasm.

Spindle nucleus from oocytes at MII phase or pronuclei from zygote stage after fertilization (NT group) will be removed by micropipette and placed into enucleated donor eggs. At the same time donor nucleus will be placed into patient ooplasm for reverse control (R-NT group). In order not to lyse the oocytes during removal of the patient nucleus or donor egg enucleation, cytoskeletal relaxants will be used such as Cytochalasin (Zhang et al., 2017a).

In order to maximize the number of embryos generated, Polar Body Genome Transfer (PBGT) may also be conducted for the first polar body of the MII oocytes, in parallel to Spindle Nuclear Transfer (SNT). PBGT is only possible when MII oocyte exhibits a viable polar body, i.e. before the degeneration of the first polar body. PBGT from first polar bodies has been previously applied successfully in generating human blastocysts with no adverse impact on preimplantation development (Zhang et al., 2017b; Ma et al., 2017). Furthermore, previous research has evidenced that the PBGT technique results in undetectable carryover levels or patient cytoplasm. The GV oocyte, MII spindle, MII polar body, or pronuclei will be combined with their retrospective donor cytoplasts using inactivated Cell Fusion Reagent GenomONE™-CF EX SeV-E (HVJ-E).

II. Post fertilization rescue NT procedures.

After SNT or PBGT, the reconstituted oocytes will be fertilized with partner/donor sperm via intracytoplasmic sperm injection (ICSI). In case of abnormal fertilization (e.g. 3PN) on Day 1 after ICSI, microsurgical correction will be applied by removal of the extra pronucleus to result in 1 female and 1 male pronucleus (Kattera and Chen 2003). Identification of female pronuclei will be conducted by tracking the PN(s) from the Polar body extrusion on the Embryoscope/MIRI time-lapse recordings. In case of the reconstructed oocyte exhibiting only one female PN on Day 1, the oocyte will be vitrified for pronucleus transfer (PNT) procedures into donor oocyte fertilized with the patient's partner/donor sperm.

III. Preimplantation Embryonic Development post nuclear transfer.

The preimplantation embryonic development will be monitored after SNT/PBGT or PNT by a senior embryologist. The embryonic development will be observed for fertilization, cleavage, and blastulation using standard morphological criteria (ALPHA Scientists In Reproductive Medicine; ESHRE Special Interest Group Embryology, 2011). At each checking point, the image or video will be taken. At blastocyst stage, the trophectoderm will be biopsied for aneuploidy analysis via Next Generation Sequencing (NGS) or array-based Comparative genomic hybridization (aCGH).

We anticipate that the SNT/PBGT or PNT would have no adverse effect on the human preimplantation embryonic development.

B. <u>Rescue of age-related meiotic errors:</u>

I. Transfer of patient immature nucleus to fresh donor germinal vesicle (GV) oocyte.

Nucleus from oocytes at GV stage (GVT) will be removed by micropipette and placed into young enucleated donor GV oocyte (Liu at al. 2017). Donor GV oocytes will be from healthy donors aged 32 years old or younger. Every effort will be made to use fresh donor GV oocytes for NT procedures by intending to thaw patient GV oocytes on the day of donor GV egg retrieval. A reverse control (R-GVT) group will be created whenever possible.

II. In Vitro Maturation (IVM).

GV oocytes will undergo IVM for 24h or 48h until the mature MII phase is reached, at which point matured oocytes with a visible birefringent spindle will undergo SNT into *in*

vivo matured donor MII oocytes (Liu at al. 2017; Liu et al., 2003). IVM is a standard procedure (Fesahat et al., 2017) which will be optimized for our conditions, by varying the culture drop size, the hours with cumulus cell attachment and cumulus cell co-culture, and the contents of the IVM media (Liu et al., 2018; Hirao et al., 2004). The conditions which result in maximum maturation rate in the control group will be used for in vitro maturation of GVs undergoing nuclear transfer.

Every effort will be made to apply pre-selection of donors for donor MII oocytes in the SNT procedures. Namely, the following criteria of will be applied for preselection of MII donors based on their cycle history: ≥18 retrieved MII oocytes with (i) minimum 80% normal fertilization rate, (ii) minimum 50% rate of blastulation, and (iii) minimum 60% rate of euploidy. In order to maximize the number of embryos generated, Polar Body Genome Transfer (PBGT) may also be conducted for the first polar body of the in vitro matured MII oocytes, in parallel to Spindle Transfer. PBGT is only possible when MII oocyte exhibits a viable polar body, i.e. before the degeneration of the first polar body. PBGT from first polar bodies has been previously applied successfully in generating human blastocysts with no adverse impact on preimplantation development (Zhang et al., 2017b; Ma et al., 2017). Furthermore, previous research has evidenced that the PBGT technique results in undetectable carryover levels or patient cytoplasm.

III. Post fertilization rescue NT procedures.

After GVT and SNT/PBGT, the reconstituted oocytes will be fertilized with partner/donor sperm via intracytoplasmic sperm injection (ICSI). In case of abnormal fertilization (e.g. 3PN) on Day 1 after ICSI, microsurgical correction will be applied by removal of the extra

pronucleus to result in 1 female and 1 male pronucleus (Kattera and Chen 2003). Identification of female pronuclei will be conducted by tracking the PN(s) from the Polar body extrusion on the Embryoscope/MIRI time-lapse recordings. In case of the reconstructed oocyte exhibiting only one female PN on Day 1, the oocyte will be vitrified for pronucleus transfer (PNT) procedures into donor oocyte fertilized with the patient's partner/donor sperm.

IV. Preimplantation Embryonic Development post nuclear transfer.

The preimplantation embryonic development will be monitored after GVT, SNT/PBGT, or PNT by a senior embryologist. The embryonic development will be observed for fertilization, cleavage, and blastulation using standard morphological criteria (ALPHA Scientists In Reproductive Medicine; ESHRE Special Interest Group Embryology, 2011). At each checking point, the image or video will be taken. At blastocyst stage, the trophectoderm will be biopsied for aneuploidy analysis via Next Generation sequencing (NGS) or array-based Comparative genomic hybridization (aCGH).

We anticipate that the GVT-SNT or GVT-PBGT procedures would have no adverse effect on the human preimplantation embryonic development. We anticipate that due to undergoing two sequential nuclear transfer procedures, the efficiency of viable embryos/blastocyst rates will be lower compared to the same rates for group **A**.

Materials

Oocytes will be collected from patients enrolled in the study who went through Informed Consents and doctor appointments with either Dr John Zhang or Dr Valery Zukin.

Data Management and Analysis

The results of the clinical trial will be analyzed and submitted in the form of manuscript(s), for independent peer-review in scientific journals.

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