Journal of Assisted Reproduction and Genetics (2022) 39:609–618
https://doi.org/10.1007/s10815-022-02401-7

ASSISTED REPRODUCTION TECHNOLOGIES

Human germline nuclear transfer to overcome mitochondrial disease and failed fertilization after ICSI

Maoxing Tang[1] · Annekatrien Boel[1] · Noemi Castelluccio[1] · Arantxa Cardona Barberán[1] · Antonia Christodoulaki[1] · Bieke Bekaert[1] · Mina Popovic[1] · Frauke Vanden Meerschaut[1] · Petra De Sutter[1] · Björn Menten[2] · Sofie Symoens[2] · Arnaud V. Vanlander[3] · Dominic Stoop[1] · Paul J. Coucke[2] · Björn Heindryckx[1]

Received: 1 September 2021 / Accepted: 15 January 2022 / Published online: 22 January 2022

Abstract

Purpose Providing additional insights on the efficacy of human nuclear transfer (NT). Here, and earlier, NT has been applied to minimize transmission risk of mitochondrial DNA (mtDNA) diseases. NT has also been proposed for treating infertility, but it is still unclear which infertility indications would benefit. In this work, we therefore additionally assess the applicability of NT to overcome failed fertilization.

Methods Patient 1 carries a homoplasmic mtDNA mutation (m.11778G > A). Seventeen metaphase II (MII) oocytes underwent pre-implantation genetic testing (PGT), while five MII oocytes were used for spindle transfer (ST), and one in vitro matured (IVM) metaphase I oocyte underwent early pronuclear transfer (ePNT). Patients 2–3 experienced multiple failed intracytoplasmic sperm injection (ICSI) and ICSI-assisted oocyte activation (AOA) cycles. For these patients, the obtained MII oocytes underwent an additional ICSI-AOA cycle, while the IVM oocytes were subjected to ST.

Results For patient 1, PGT-M confirmed mutation loads close to 100%. All ST-reconstructed oocytes fertilized and cleaved, of which one progressed to the blastocyst stage. The reconstructed ePNT-zygote reached the morula stage. These samples showed an average mtDNA carry-over rate of 2.9% ± 0.8%, confirming the feasibility of NT to reduce mtDNA transmission. For patient 2–3 displaying fertilization failure, ST resulted in, respectively, 4/5 and 6/6 fertilized oocytes, providing evidence, for the first time, that NT can enable successful fertilization in this patient population.

Conclusion Our study showcases the repertoire of disorders for which NT can be beneficial, to overcome either mitochondrial disease transmission or failed fertilization after ICSI-AOA.

Keywords Pronuclear transfer · Spindle transfer · Mitochondrial DNA disease · Female infertility · Fertilization failure

Introduction

Nuclear transfer (NT) is a novel in vitro fertilization (IVF)–based technique, also known as mitochondrial donation or mitochondrial replacement therapy [1, 2]. This technique involves the transfer of the nuclear genome from an oocyte or zygote (karyoplast) to an enucleated donor counterpart (cytoplast). The NT technique can be performed at various oocyte stages using techniques such as germinal vesicle transfer (GVT, isolation of the germinal vesicle, which is the oocyte nucleus at the at the prophase I stage), maternal spindle transfer (ST, isolation of the spindle, the microtubule-based structure enabling chromosome segregation at the metaphase II stage), and first polar body transfer (PB1T, isolation of the first polar body, whose extrusion marks the end of meiosis I), or at the zygote stage using a

Maoxing Tang and Annekatrien Boel are shared first authors.

✉ Annekatrien Boel
Annekatrien.Boel@UGent.be

[1] Ghent-Fertility and Stem Cell Team (G-FaST), Department for Reproductive Medicine, Ghent University Hospital, Corneel Heymanslaan 10, 9000 Ghent, Belgium

[2] Center for Medical Genetics Ghent (CMGG), Department of Biomolecular Medicine, Ghent University Hospital, Corneel Heymanslaan 10, 9000 Ghent, Belgium

[3] Division of Paediatric Neurology and Metabolic Diseases, Department of Paediatrics, Ghent University Hospital, Corneel Heymanslaan 10, 9000 Ghent, Belgium

technique known as pronuclear transfer (PNT, isolation of the male and female nucleus at the moment of fertilization), or second polar body transfer (PB2T, isolation of the second polar body, whose extrusion marks the end of meiosis II) (all techniques are reviewed in [1]).

Nuclear transfer has mainly been used as a method to overcome transmission of mitochondrial DNA (mtDNA) mutations. These maternally inherited mutations can cause a range of life-threatening mitochondrial disorders, affecting nearly 1 in 5000 individuals of the population [3]. MtDNA mutations can be present in either a homoplasmic form (all mtDNA copies are mutated) or a heteroplasmic form (mixture of wild-type and mutated mtDNA) [1]. The severity of mitochondrial disease is associated with the heteroplasmy level (proportion of mutant to wild-type mtDNA), referred to as the mutation load. Higher mutation loads are frequently associated with more severe clinical symptoms. The threshold for disease expression is generally thought to be 60–80%, but can vary across different tissues and the specific mtDNA mutation [4, 5]. Leber's hereditary optic neuropathy (LHON) is one of the most common mitochondrial diseases. Clinical manifestations of LHON mainly include progressively central vision loss, which often occurs in young adults (~ 10% of the females and ~ 50% of the males) [6]. Strikingly, predicting the risk of mtDNA disease transmission towards subsequent generations remains challenging due to the mitochondrial genetic bottleneck [7–9]. Possible treatment involves prenatal or preimplantation genetic testing for monogenic disorders (PGT-M) to select fetuses or embryos with low or no mutation loads, which is an option for some affected women [10–12]. Nevertheless, these approaches are not suitable for women harboring high mutation loads or a homoplasmic mtDNA mutation [13, 14], which highlights the need for alternative technologies to prevent mtDNA disease transmission from mother to offspring.

The potential of these NT techniques for preventing mtDNA disease has been evaluated in mouse [1, 15] and non-human primate models [16] by assessing the developmental competence of reconstituted embryos and the level of mtDNA carry-over (karyoplast-derived mtDNA). The (neuro)developmental effects on subsequent generations have also been assessed in both a mouse model [17] and a non-human primate model [18]. In human, GVT [19, 20] and PBT [21, 22] have been limitedly studied, while PNT [23, 24] and ST [25] are the most well explored to date. Aside from one report on the successful clinical use of ST in oocytes from a female carrier of Leigh syndrome (mtDNA mutation m.8993 T > G) 2, additional evidence on the performance of NT to reduce mtDNA mutation transmission in human is currently absent.

Besides preventing the transmission of mtDNA mutations, a direct consequence of NT is also the complete replacement of the oocyte cytoplasm. Therefore, NT additionally provides an opportunity for oocyte rejuvenation or to overcome certain genetically related ooplasmic defects [26, 27]. Replacing a low-quality cytoplasm with a competent one using NT may thus improve the oocyte competence, with the expectation of improving certain IVF outcomes, such as failed fertilization after intracytoplasmic sperm injection (ICSI). This condition is mainly caused by an oocyte activation deficiency, which could be sperm or oocyte related. In brief, oocyte activation is triggered after the fertilization process, where the sperm-related factor phospholipase C zeta (PLCζ) initiates a cascade reaction involving oocyte factors, ultimately evoking Ca^{2+} oscillations in the ooplasm, which leads to alleviation metaphase II arrest and the completion of the second meiotic division. Recently, mutations in both sperm (mainly *PLCZ1*) [28] and oocyte factor (reviewed in [29]) genes have been linked to failed fertilization.

The application of assisted oocyte activation (AOA) during ICSI, which is the artificial induction of a series of intracellular calcium rises, is in general very efficient to overcome failed fertilization after ICSI [30, 31]. Still, some patients do not benefit from this ICSI-AOA treatment [32]. It can be envisioned that the potential of AOA is dependent on the origin of the defect leading to failed fertilization. For instance, in two separate studies, it was shown that AOA was unable to overcome failed fertilization in patients with defective WEE2, which is an oocyte kinase involved in the release from metaphase II (MII) arrest that acts downstream of the Ca^{2+} oscillations [33, 34]. For these patients, potentially harboring defects in genes important for fertilization, but acting independently from or downstream of Ca^{2+} oscillations, we hypothesize that NT to a healthy donor oocyte could overcome failed fertilization.

One study has clinically applied PNT to overcome embryo developmental arrest in one patient [26], however, the application of NT to overcome other forms of female infertility such as fertilization failure has not been investigated to date.

In this study, we aimed to provide additional data on the efficiency of ST and early PNT (ePNT) to overcome the transmission of the pathogenic homoplasmic m.11778G > A mtDNA mutation. Additionally, we aimed to expand the repertoire of disorders for which NT could be beneficial and therefore investigated, for the first time, ST as a novel approach to overcome fertilization failure after ICSI-AOA.

Materials and methods

Patient description and clinical characteristics

The present study included a 30-year-old nulligravida woman (patient 1) with mitochondrial disease who

underwent the first ICSI cycle in our clinic. This patient was asymptomatic and carried a pathogenic and homoplasmic mtDNA mutation (m.11778G > A) in subunit 4 of NADH dehydrogenase (MT-ND4), which is known to cause LHON [35]. Clinical manifestations of LHON disease involve subacute bilateral loss of vision, sometimes accompanied by dystonia and cardiac pre-excitation syndromes [36] (Supplementary Table S1). In addition, we included two couples (patient 2 and 3) who experienced multiple failed ICSI cycles characterized by complete failed fertilization, which could be attributed to a female factor. All the patients consented to the application of NT, solely for research purposes. A schematic overview of the patients included in this study and the associated experiments performed is depicted in Supplementary Fig. 1.

Source and culture of human oocytes

Following controlled ovarian hyperstimulation, spare human oocytes were donated from patients undergoing IVF/ICSI cycles to be used as donor oocytes for NT. Different stages of human oocytes, including in vivo matured MII oocytes containing aggregates of smooth endoplasmic reticulum (SERa), collected 8 h after oocyte pick-up, germinal vesicle (GV)/prophase I oocytes, and metaphase I (MI) oocytes, were collected. GV oocytes were incubated for 24 h in in vitro maturation (IVM) medium [15], and MI oocytes were incubated for either 3 or 24 h in Cook Cleavage medium under mineral oil at 37 °C in 6% CO_2 and 5% O_2 (hereafter called standard culture conditions). The IVM medium was prepared in-house by supplementing tissue culture medium-199 (TCM-199) containing Earle's salt and bicarbonate with 10 ng/ml epidermal growth factor (EGF), 1 µg/ml estradiol, 10 mIU/ml follicle-stimulating hormone (FSH), 0.5 IU/ml hCG, 100 IU/ml penicillin G potassium salt, 100 µg/ml streptomycin sulfate salt, 0.3 mM sodium pyruvate, and 0.8% human serum albumin. SERa oocytes were vitrified following the protocol provided by the manufacturer (Irvine Scientific, USA), as previously described [37]. Before NT, oocytes were warmed following the manufacturer's instruction (Irvine Scientific, USA) and randomly assigned to NT experiments. Following NT, reconstituted embryos and control embryos were cultured in Cook Cleavage (days 1–3) or Cook Blastocyst (days 3–6) medium under mineral oil at standard culture conditions.

Assisted oocyte activation

For patients 2 and 3, at least 6 h after oocyte retrieval, ICSI-AOA was carried out as previously described [38]. In brief, a single sperm was injected into the patient's oocyte by human standard ICSI along with a limited volume of 0.1 mol/L $CaCl_2$ (Sigma-Aldrich), which was corresponding to the diameter of the oocyte. After incubation in Cook Cleavage medium for 30 min, the oocytes were exposed twice to 10 µmol/L ionomycin (I9657; Sigma-Aldrich) with an interval of 30 min. Finally, the oocytes were rinsed and cultured in Cook Cleavage (days 1–3) or Cook Blastocyst (days 3–6) medium under mineral oil at standard culture conditions. Fertilization outcomes were assessed 16–18 h following ICSI, based on the presence of 2 pronuclei (PN).

Maternal spindle transfer

Transfer of the spindle-chromosomal complexes was carried out using oocytes from patients 1, 2, and 3 according to the protocol established in our lab [15]. Briefly, human MII oocytes from the patient and donated recipient oocytes were placed into separate 10 µl manipulation droplets of gamete buffer containing 5 µg/ml cytochalasin B, which inhibits the polymerization of actin, in a glass-bottom dish. The droplets were covered with mineral oil, and the oocytes were then maintained at 37 °C for 15 min before spindle isolation. The dish was placed on the stage of an inverted microscope (Olympus IX71) equipped with a stage warmer, micromanipulators, Oosight™ Imaging System, and a laser objective. The oocyte was firstly secured by a holding pipette to place the spindle in an appropriate position (close to 1–3 o'clock). Then, the zona pellucida nearby the spindle was drilled with a laser, and a biopsy pipette with an inner diameter of 15 µm was inserted through the opening. The spindle was aspirated into the pipette with a small amount of cytoplasm. The karyoplast was briefly exposed to the fusogen, hemagglutinating virus of Japan envelope (HVJ-E) (GenomONE-CF EX, Cosmo Bio), and placed into the perivitelline space (PVS) of the enucleated recipient oocyte opposite to the first polar body (PB1). Finally, the reconstituted oocytes were washed with gamete buffer, transferred to Cook Cleavage medium and incubated at 37 °C in 5% CO_2 for 30 min until fusion occurred. The reconstructed oocytes were fertilized via ICSI using vitrified-warmed sperm within 2 h after ST. Fertilization outcomes were examined 18 h after ICSI, based on the presence of two pronuclei (2PN). Embryos were cultured in Cook Cleavage (days 1–3) and Cook Blastocyst (days 3–6) medium under mineral oil at standard culture conditions. Human blastocysts were scored according to the Gardner and Schoolcraft grading system [39]. Both blastocyst and good-quality blastocyst rates were calculated based on the 2PN embryos, with a good-quality blastocyst (AA, AB, BA); a fair-quality blastocyst (AC, BC, BB, CA); and poor-quality blastocyst (CB, CC).

Early pronuclear transfer

The ePNT procedure was conducted with an oocyte from patient 1, as previously described [24]. In short, ePNT was

performed under an inverted light microscope (Olympus, IX71) fitted with micromanipulators and a laser objective. An opening was created in the zona pellucida using a laser for insertion of the biopsy pipette during enucleation and ePNT procedures. The zygotes were exposed to Gamete buffer containing cytochalasin B (5 µg/ml), which inhibits the polymerization of actin, and nocodazole (10 µg/ml), which inhibits the polymerization of the microtubule cytoskeleton, for 15 min at 37 °C, before micromanipulation and throughout ePNT procedures. This increases elasticity of the cytoplasm to prevent lysis of oocytes during enucleation. Approximately 8 h after fertilization, the early 2PN was aspirated from a nuclear donor zygote into a biopsy pipette with an inner diameter of 22 µm and moved to a droplet containing the fusogen (HVJ-E) (GenomONE-CF EX, Cosmo Bio). After a brief exposure to HVJ-E, the karyoplast with a small volume of HVJ-E was gently expelled into the PVS of an enucleated recipient cytoplast. Karyoplast-cytoplast fusion usually occurred within 20–30 min following ePNT. The reconstituted zygotes were washed three times in Gamete buffer before being transferred to Cook Cleavage (days 1–3) and Cook Blastocyst (days 3–6) medium under mineral oil at standard culture conditions. Human blastocysts were scored according to the Gardner and Schoolcraft grading systems, as mentioned above [39].

Collection and culture of mouse oocytes and mouse oocyte activation test

B6D2F1 female mice (6–8 weeks of age) were injected with 7.5 IU pregnant mare's serum gonadotrophin (PMSG) followed by human chorionic gonadotropin (hCG) 48 h later, to induce follicular hyperstimulation. MII oocytes were collected from B6D2F1 females 14 h following hCG injection and were recovered into HEPES-buffered potassium simplex-optimized medium (KSOM-HEPES) supplemented with 4 mg/ml bovine serum albumin (BSA, Calbiochem, Belgium). Denudation of cumulus cells was performed through a brief exposure to 200 IU/ml hyaluronidase.

The mouse oocyte activation test (MOAT) was performed as previously described [31, 40]. Briefly, this procedure involves the injection of patient partner's sperm (patients 2 and 3) into mouse MII oocytes to test sperm activation potential. Fresh or vitrified-warmed sperm were washed and selected for the injection based on the swim-up method as earlier described [15]. Mouse oocytes were placed in KSOM-HEPES medium with the addition of 20% fetal bovine serum (Gibco BRL; Invitrogen, Belgium). Injection of immobilized sperm was conducted at 15–17 °C using piezo-driven ICSI. Four different groups were made for a complete MOAT: (i) ICSI with patient sperm (40 oocytes); (ii) ICSI with donated sperm with proven fertilization capability (40 oocytes) (positive control); (iii) sham ICSI with medium (20 oocytes) (negative control); and (iv) unmanipulated oocytes (20 oocytes) (medium control) to examine spontaneous parthenogenetic activation. Subsequently, oocytes were cultured in KSOM for 24 h and oocyte activation was assessed based on two-cell formation rate (ratio of two-cell embryos versus surviving injected oocytes) [41]. According to the MOAT result, the patient's sperm activation potential can be classified into three groups: MOAT 1 ($\leq 20\%$ activation, sperm-related activation deficiency), MOAT 2 (21–84% activation, diminished sperm activation capacity), and MOAT 3 ($\geq 85\%$ activation, normal sperm activation capacity but suspected oocyte-related activation deficiency).

Next-generation sequencing for mtDNA analysis

DNA was extracted from the individual oocytes and NT-generated embryos and blastocyst from patient 1. Specifically, individual oocytes, embryos, blastocysts, and isolated TE cells were transferred into a separately labeled 200 µl microcentrifuge tube (Westburg, Leusden, Netherlands) containing 10 µl of PicoPure DNA extraction buffer with proteinase K (PicoPure DNA extraction kit, Arcturus, Mountain View, CA, USA). All samples were incubated at 65 °C for 3 h, centrifuged briefly and heated at 95 °C for 10 min to inactivate proteinase K.

Following PCR amplification of the genomic region encompassing the pathogenic m.11778G > A mtDNA mutation, PCR amplicons were assigned to separate indices, and library preparation and sequencing were performed on an Illumina Miseq machine [42]. Next-generation sequencing (NGS) data processing was performed using CLC bio-genomics workbench software 9.0.1. Firstly, FASTQ files with input sequences were trimmed according to specified quality and length cut-offs. Subsequently, trimmed reads were aligned to GRCh37, including the revised Cambridge Reference Sequence (rCRS) for human mitochondrial DNA (NC_012920.1), using the CLC bio-aligner. After alignment, duplicate mapped reads resulting from PCR amplification were removed. In order to improve read mapping around indels, a local realignment step subsequently was performed. Following local realignment, sequence variants were determined using the CLC bio-algorithms for basic variant detection algorithm as well as low-frequency variant detection. The detected variants were further processed by filtering out reference variants, adding annotations with overlap information and amino acid changes. Finally, variant coverage was calculated by using the samtools depth command [43]. In order to account for overlapping read pairs, BamUtil clipOverlap was used to clip overlapping read pairs before calculating the coverage [44].

Results

ST and ePNT can minimize transmission of a homoplasmic pathogenic mtDNA mutation

A female carrier of LHON syndrome, harboring a homoplasmic pathogenic mtDNA mutation m.11778G > A, underwent ovarian stimulation, after which a total of twenty-six cumulus-oocyte complexes were retrieved: twenty-two MII oocytes, one MI oocyte, two GV oocytes, and one degenerated oocyte. The majority of the MII oocytes ($n = 17$) were fertilized via routine ICSI, and obtained blastocysts were subjected to PGT-M. Using mtDNA-sequencing analysis on trophectoderm (TE) biopsy samples, we confirmed a close to 100% (homoplasmic) mutation load in all embryos analyzed (Supplementary Table S2). Five randomly selected in vivo matured MII oocytes were donated for research, in addition to the GV and MI oocytes, to perform NT. While both GV oocytes failed to mature after 24 h in vitro culture, the MI oocyte successfully matured. The donated in vivo matured MII oocytes and in vitro matured oocyte were subjected to ST and ePNT, respectively. Enucleated healthy vitrified-warmed in vivo matured SERa oocytes and an enucleated healthy fresh IVM oocyte obtained from other women served as donor cytoplasts during ST and ePNT procedures, respectively. The ST was successful in all five in vivo matured oocytes from the patient, and after ICSI, 100% (5/5) of ST-oocytes were normally fertilized, 100% (5/5) cleaved, and 20% (1/5) progressed to the blastocyst stage (scored at day 5 as 4AA: expanding blastocyst, top score inner cell mass and trophectoderm), while the successfully reconstructed ePNT-zygote derived from the IVM MI oocyte from the patient, reached the compacted morula stage (Table 1). Data from targeted NGS revealed that the mtDNA carry-over in two TE biopsy samples from the ST-blastocyst and in the ePNT-morula was 3.2%, 2.9%, and 3.1%, respectively (Table 2), while all the patient's enucleated oocytes/zygotes (Supplementary Table S3), unmanipulated oocytes/zygotes (Supplementary Table S3), arrested embryos and blastocysts (Supplementary Table S4) from the PGT-M cycle showed mutation loads close to 100%. The negative control donated oocytes showed, as expected, a negligible m.11778G > A mutation load (Supplementary Table S5), which is below the sensitivity of NGS for the detection of mtDNA heteroplasmy, as we have previously determined in a mouse model [15]. Analyzing the same samples with a digital droplet PCR (ddPCR) approach, we confirmed m.11778G > A mutation loads of 0.00% in all three negative control samples [45]. The level of mtDNA carry-over in the remaining arrested ST-embryos ($n = 4$) was 2.9% ± 1.1% (mean ± SD) (Table 2). Overall, our findings confirm that NT can significantly reduce the transmission of pathogenic mtDNA mutations in human, with ST and ePNT showing comparable carry-over rates.

Table 1 Preimplantation development of ST and ePNT embryos

Group	No. of reconstructed embryos	Survival after recovery	Fertilization by ICSI	Two-cell	Eight-cell	Compact morula	Blastocyst	Good-quality blastocyst
ST	5#	5/5 (100%)	5/5 (100%)	5/5 (100%)	4/5 (80%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
ePNT	1#	1/1 (100%)	–	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)

ST spindle transfer, *ePNT* early pronuclear transfer

Table 2 Levels of mtDNA carry-over in individual embryos following NT

Oocyte/embryo ID ($n = 7$)	MtDNA carry-over	Coverage (reads)
PGT-M from the ST-generated blastocyst TE cells-1	**3.2%**	8126
PGT-M from the ST-generated blastocyst TE cells-2	**2.9%**	8126
Arrested embryo (sixteen-cells) after ST	**1.8%**	8131
Arrested embryo (eight-cell) after ST	**2.2%**	8130
Arrested embryo (five-cell) after ST	**3.2%**	8045
Arrested embryo (eight-cell) after ST	**4.2%**	8102
Morula arrest after ePNT	**3.1%**	8020

mtDNA mitochondrial DNA, *PGT-M* preimplantation genetic testing for monogenic disorders, *ST* spindle transfer, *ePNT* early pronuclear transfer, *TE* trophectoderm

ST can overcome fertilization failure after ICSI-AOA in two infertile couples

The first recruited patient involved a 26-year-old woman who experienced two failed cycles due to fertilization failure following ICSI. Specifically, in the first cycle, a total of thirteen in vivo matured MII oocytes were retrieved and subjected to routine ICSI, of which none showed 2 distinct pronuclei. Prior to the second cycle, we performed the MOAT to evaluate the sperm activation potential of the patient's partner, by injecting the partner's sperm into mouse oocytes. The MOAT results revealed that more than 85% of the mouse oocytes were activated, similar to a positive control sperm sample with normal activation capacity. Accordingly, this patient was classified in the MOAT 3 group, with a suspected oocyte-related problem, and was advised to undergo ICSI-AOA treatment, as AOA enables overcoming fertilization failure in most of the cases, even when an oocyte-related factor is involved [30]. For this procedure, a total of seventeen cumulus-oocyte complexes were retrieved after ovarian stimulation: twelve MII oocytes and five MI oocytes. When subjecting all MII oocytes to ICSI-AOA, only one out of the twelve MII oocytes was fertilized, but displayed vague 2PN formation and no further division. We further injected the partner's sperm into fresh IVM and 0PN oocytes obtained from other patients and found that most oocytes (83%, 5/6) were activated and cleaved after injection without the application of AOA, indicating that a sperm deficiency of the patient's partner could be excluded. Finally, we performed ST by transferring spindle-chromosome complexes from the patient's IVM oocytes retrieved at MI stage ($n=5$) into vitrified-warmed SERa oocytes donated by other women. Following ICSI in the absence of AOA, two out of the four ST-reconstituted oocytes were normally fertilized with 2PN and 2PB formation, the third oocyte showed 1PN formation and the fourth cleaved immediately. Subsequently, three out of four embryos cleaved to the four-cell stage, but did not progress further to blastocysts (Table 3).

The second case involved a 37-year-old nulligravida woman who had six failed ICSI cycles characterized by fertilization failure in almost all her oocytes. In the last three ICSI cycles, AOA was performed but only one oocyte showed normal fertilization (1/32). After MOAT, this patient was categorized in the MOAT 3 group, pointing to a suspected oocyte-related problem, as more than 88% of the mouse oocytes showed activation following injection of her partner's sperm. Moreover, the vitrified-warmed sperm of the patient's partner was injected into fresh IVM oocytes donated by other women. We found that most oocytes (2/3) were successfully fertilized and activated, indicative of normal activation potential of the partner's sperm. In the patient's final ICSI-AOA cycle, a total of twenty cumulus-oocyte complexes were retrieved: nine MII oocytes and eleven MI oocytes. ICSI-AOA was applied in all MII oocytes, while ST was carried out by transferring spindle-chromosome complexes from the patient's IVM oocytes retrieved at MI stage ($n=6$) into vitrified-warmed SERa oocytes donated by other women. After ICSI in the absence of AOA, 33% (2/6) of the ST-reconstructed oocytes were normally fertilized with 2PN, 33% (2/6) with 1PN, and 33% (2/6) with 3PN (Table 3). One out of two 2PN zygotes developed into a cavitating blastocyst showing poor quality, and the remaining 2PN, 1PN, and 3PN zygotes arrested before the ten-cell stage (Table 3). Taken together, these results indicate that ST may have the potential to overcome fertilization failure after ICSI associated with oocyte-related activation deficiencies.

Table 3 Preimplantation development following ST in both infertility patients (patients 2 and 3)

Patient	No. of reconstructed oocytes	Survival after recovery	Survival after ICSI	Fertilization outcomes	Development outcomes
Patient 2	5#	5/5 (100%)	4/5 (80%)	2/4 (50%) 2PN 1/4 (25%) 1PN 1/4 (25%) divided	2PN: both arrested at four-cell stage (day 3) 1PN: not divided Divided one: arrested at four-cell stage (day 3)
Patient 3	6#	6/6 (100%)	6/6 (100%)	2/6 (33%): 2PN 2/6 (33%): 1PN 2/6 (33%): 3PN	2PN: 1# three-cell arrested (day 3) 1# cavitating blastocyst but poor-quality (day 6) 1PN: 2# two-cell arrested (day 3) 3PN: 1# four-cell arrested (day 3); 1# ten-cell arrested (day 4)

Discussion

The present study provides additional proof that both ST and ePNT have the capacity to efficiently prevent the transmission of mtDNA disorders in human

In the context of prevention of mtDNA transmission, ST has been the method of choice [2]. However, interestingly, Hyslop et al. (2016) further optimized the PNT procedure using human in vivo matured MII oocytes, mainly including a modification in the timing, with PNT being performed shortly after meiosis II completion (around 8-h post-intracytoplasmic sperm injection (ICSI), early PNT) rather than before the first mitosis (around 16-h post-ICSI, late PNT). This adapted methodology, called ePNT, was shown to be highly beneficial in terms of blastocyst formation, with a concomitant minimal carry-over in generated blastocysts [24].

Our study compared ST and ePNT on a limited number of samples of a female patient carrying the homoplasmic m.11778G > A mutation, which is one of the three common LHON-causing mtDNA point mutations [6]. Unlike the majority of mtDNA disease-causing mutations, which are heteroplasmic, LHON mutations are often present in a homoplasmic form [46, 47]. Another distinct feature of LHON is that it displays incomplete penetrance, which is reflected by the fact that amongst all the matrilineal members of an LHON pedigree containing mutant mtDNA, only a portion of individuals develop optic neuropathy [48]. PGT-M was routinely carried out in this patient, and as expected, we found that no embryos were suitable for embryo transfer due to high mutation loads (~ 100%). In an effort to reduce transmission of the m.11778G > A mutation, we performed ST and ePNT.

The findings revealed that both ST and ePNT were capable of reducing the average level of mtDNA carry-over to 2.9% ± 0.8% (mean ± SD), which was consistent with previous studies in human showing < 2.2% and < 2% of carry-over in preimplantation embryos generated from ST and PNT, respectively [23–25, 49]. In addition to ST and PNT, other types of NT techniques, including PB1T and PB2T, are currently being developed for overcoming mitochondrial diseases [17, 21]. Consistently, recent mouse and human studies have revealed that both PB1T and PB2T were compatible with onward embryo development in vitro and led to an even lower carry-over rate of < 1% on average in the generated blastocysts [15, 50]. Even if mtDNA carry-over rates can indeed be further decreased by approaches such as PBT, it remains unclear whether, during the development of resultant offspring, the karyoplast-derived mutant mtDNA could still preferentially accumulate in various tissues, leading to a drift towards the mutant haplotype [1, 24]. Therefore, additional studies in, for instance, non-human primate models [18] and long-term follow-up of children born following NT are definitely a necessity.

In the UK, the NT technology is only recommended to women of childbearing age who have pathogenic mtDNA mutations and whose children are at risk of serious mitochondrial disease. Recently, using Bayesian methods, Pickett et al. (2019) proposed an algorithm based on a given maternal heteroplasmy (e.g. the mutation load in somatic cells) to predict the proportion of future children with safe heteroplasmy levels < 18%. This approach can forecast whether or not PGT-M is suitable as a first-line treatment, and which women would benefit from the germline NT treatment [51]. It has been estimated that, yearly, 152 births in the UK and 778 births in the USA can benefit from the NT technique to overcome mtDNA disorders [52].

In the second part of our study, we have shown, as a proof-of-concept, that ST might be able to overcome fertilization failure after ICSI in the case of an oocyte-related fertilization failure. Total fertilization failure following ICSI occurs in 3–5% of all ICSI cycles [53]. It may happen repeatedly even in cases with normal sperm parameters and optimal ovarian response, and a number of cases have been attributed to certain genetic factors. For example, mutations in the male gene responsible for oocyte activation (*PLCZ1*) [54] and mutations in different female genes (reviewed in [29]) have recently been reported in patients suffering from recurrent failed fertilization after ICSI.

In the current study, we included two female patients experiencing very low fertilization rates after ICSI. Using MOAT assessment, we classified both patients into MOAT group 3 (normal sperm activation capacity), hence suspected oocyte-related fertilization deficiencies. We have considered to apply mouse or human oocyte calcium analysis (MOCA or HOCA) to exclude a sperm-related factor of fertilization failure, but since injection in control oocytes, donated by other infertile women, showed normal fertilization capacity of the patient's sperm, an oocyte-related cause of failed fertilization is very likely. Even more, given the lack of any pronucleus formation after AOA, this rather points to a fertilization problem which acts downstream of the calcium-releasing machinery. Also, the AOA treatment, aiming to artificially trigger Ca^{2+} rises by Ca^{2+} ionophores, failed to induce oocyte activation and fertilization. It has been reported that AOA during ICSI is efficient to overcome fertilization failure in most of the treated couples [30, 38, 40], but especially when an oocyte factor is responsible for fertilization failure, AOA sometimes fails, suggesting defects downstream or independent from the calcium signaling pathway [30, 31]. For instance, ICSI-AOA failed to restore fertilization in female patients with gene mutations in *WEE2* (Wee1-like protein kinase 2) [55, 56], which is

an oocyte kinase involved in the release from MII arrest that acts downstream after the Ca^{2+} oscillations. Interestingly though, injection of *WEE2* wild-type complementary RNA (cRNA) molecules could restore fertilization potential. The treatment potential of cRNA is however limited to patients with confirmed molecular defects, and it remains to be determined whether the approach would be applicable to overcome molecular defects in other genes as well. NT on the other hand would be a more general approach applicable for all patients with abnormal cytoplasmic content, either due to defective proteins, or other anomalies. For instance, earlier work has shown that low mitochondrial DNA content, which could be due to cytoplasmic maturation or mitochondrial biogenesis defects, impacts fertilization [57]. Therefore, we investigated ST as an experimental treatment approach for both recruited patients, only for research purposes. Specifically, the spindle-chromosome complexes were removed from the patient's IVM oocyte and transferred into a vitrified-warmed enucleated SERa oocyte. Following ICSI, the results showed that most ST-reconstructed oocytes were activated, and some achieved normal fertilization, indicating that refreshing the whole oocyte cytoplasm by this technology has the potential to overcome certain fertilization failure after ICSI originating from cytoplasm deficiencies. The cytoplasm deficiencies may include downstream calcium signaling defects, accounting for the oocyte activation deficiency. Consequently, instead of oocyte donation, NT may offer a reproductive strategy for certain infertile couples to achieve genetically related children.

In this study, the majority of the ST-reconstructed oocytes and the ePNT-reconstructed zygote failed to reach the blastocyst stage. This might be primarily attributed to the lower developmental potential of in vitro matured and vitrified-warmed SERa oocytes used in our experiments, which were aged after collection (at least 8 h after oocyte pick-up). In addition, previously, reduced blastulation rates have been shown in SERa oocytes [24]. Therefore, to address whether ST to overcome fertilization failure has a positive influence on both fertilization potential, as well as embryonic development, future studies using increased numbers of human fresh in vivo matured MII oocytes from voluntary donors will be essential to obtain pre-clinical data for NT techniques.

To date, the number of published human studies on the use of NT treatment to treat infertility patients is limited to one case report. This involved a 30-year-old patient, who experienced two failed IVF cycles due to recurrent embryo arrest at the two-cell stage. This patient underwent PNT treatment and obtained a triplet pregnancy, however, without successful live-birth [26]. More recently, using a reproductive ageing (B6D2F1 mice) and an embryo arrest (NZB/OlaHsd mice) model, Tang et al. (2020) revealed that both ST and PNT could significantly increase blastocyst formation rates in such cases by replacing reproductive aged or embryo-arrest cytoplasts with competent ones [58]. These findings are in line with those of Costa-Borges et al. (2020), who reported that ST could rescue embryo developmental arrest caused by cytoplasm defects and lead to healthy offspring by transferring spindles from embryo-arrest mouse oocytes into non-arrest mouse cytoplasts [59]. Accordingly, as a means of "cytoplasmic rescue," the NT techniques may have the potential to rejuvenate dysfunctional oocytes resulting from cytoplasmic deficiencies. Nevertheless, as the molecular mechanism of these effects remains unclear, the use of PNT or ST for treating female infertility should be approached with caution. Therefore, whole-exome and whole-genome sequencing approaches to identify genetic abnormalities in known (for instance *WEE2*, *PATL2*, *TLE6*) and novel genes would further support this line of research.

Conclusion

Overall, this is the first evidence in human to show that NT is able to overcome fertilization failure after ICSI due to suspected inferior oocyte quality. Although more data are required to elucidate the efficiency and safety of NT, our study indicates the potential of NT techniques in avoiding mtDNA disease transmission as well as overcoming failed fertilization after ICSI. The scarce availability of human oocytes, particularly fresh in vivo matured MII oocytes donated for research purposes, is a limiting factor to obtain more pre-clinical evidence of this new NT technology. In addition, assessment of embryonic developmental potential, aneuploidy, and molecular analysis of reconstructed embryos following NT is still required to verify subtle and yet undetected safety concerns.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10815-022-02401-7.

Acknowledgements We thank the IVF lab team of the Department for Reproductive Medicine, Ghent University Hospital, for the collection of human oocytes.

Author contribution M.T., B.H., and A.B. designed and performed the experiments, collected and analyzed data, and wrote the manuscript. A.C.B., N.C., A.C., M.P., B.B., and F.V.M performed data acquisition and analysis. P.D.S., B.M., S.S., R.V.C., D.S., and P.C. conceived, designed, and supervised the experiments. All authors contributed to the interpretation of the results and revised the manuscript.

Funding This study was supported by grants from the China Scholarship Council (CSC) awarded to M.T. (Grant no. 201506160059) and Special Research Fund from Ghent University (Bijzonder Onderzoeksfonds, BOF) awarded to M.T. (Grant no. 01SC2916 and no. 01SC9518), the unrestricted educational grant of Ferring Pharmaceuticals (Aalst, Belgium), and multiple funds from FWO-Vlaanderen (Flemish fund for scientific research, Grant no. G051017N, G051516N and G1507816N). B.B. and A.C. are supported by FWO-Vlaanderen (Flemish fund for scientific research, Grant no. 11C2821N and 1S80222N, respectively).

Data Availability Data is available upon request.

Code availability Not applicable.

Declarations

Ethics approval The use of human oocytes/embryos was approved by the Ghent University Hospital Ethical Committee (EC 2016/0872) and the Belgian Federal Commission for medical and scientific research on embryos in vitro (FCE-ADV_071_UZ Gent).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Patients signed informed consent regarding publishing their data.

Conflict of interest The authors declare no competing interests.

References

1. Craven L, Tang MX, Gorman GS, De Sutter P, Heindryckx B. Novel reproductive technologies to prevent mitochondrial disease. Hum Reprod Update. 2017;23(5):501–19.
2. Zhang J, Liu H, Luo S, Lu Z, Chavez-Badiola A, Liu Z, et al. Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. Reprod Biomed Online. 2017;34(4):361–8.
3. Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, et al. Mitochondrial diseases Nat Rev Dis Primers. 2016;2:16080.
4. White SL, Collins VR, Wolfe R, Cleary MA, Shanske S, DiMauro S, et al. Genetic counseling and prenatal diagnosis for the mitochondrial DNA mutations at nucleotide 8993. Am J Hum Genet. 1999;65(2):474–82.
5. de Laat P, Koene S, van den Heuvel LP, Rodenburg RJ, Janssen MC, Smeitink JA. Clinical features and heteroplasmy in blood, urine and saliva in 34 Dutch families carrying the m.3243A > G mutation. J Inherit Metab Dis. 2012;35(6):1059–69.
6. Khan NA, Govindaraj P, Soumittra N, Sharma S, Srilekha S, Ambika S, et al. Leber's Hereditary optic neuropathy-specific mutation m.11778G>A exists on diverse mitochondrial haplogroups in India. Invest Ophthalmol Vis Sci. 2017;58(10):3923–30.
7. Hauswirth WW, Laipis PJ. Mitochondrial DNA polymorphism in a maternal lineage of Holstein cows. Proc Natl Acad Sci U S A. 1982;79(15):4686–90.
8. Jansen RP, de Boer K. The bottleneck: mitochondrial imperatives in oogenesis and ovarian follicular fate. Mol Cell Endocrinol. 1998;145(1–2):81–8.
9. Neupane J, Ghimire S, Vandewoestyne M, Lu Y, Gerris J, Van Coster R, et al. Cellular heterogeneity in the level of mtDNA heteroplasmy in mouse embryonic stem cells. Cell Rep. 2015;13(7):1304–9.
10. Sallevelt SC, Dreesen JC, Drusedau M, Hellebrekers DM, Paulussen AD, Coonen E, et al. PGD for the m.14487 T>C mitochondrial DNA mutation resulted in the birth of a healthy boy. Hum Reprod. 2017;32(3):698–703.
11. Heindryckx B, Neupane J, Vandewoestyne M, Christodoulou C, Jackers Y, Gerris J, et al. Mutation-free baby born from a mitochondrial encephalopathy, lactic acidosis and stroke-like syndrome carrier after blastocyst trophectoderm preimplantation genetic diagnosis. Mitochondrion. 2014;18:12–7.
12. Neupane J, Vandewoestyne M, Heindryckx B, Ghimire S, Lu Y, Qian C, et al. A systematic analysis of the suitability of preimplantation genetic diagnosis for mitochondrial diseases in a heteroplasmic mitochondrial mouse model. Hum Reprod. 2014;29(4):852–9.
13. Herbert M, Turnbull D. Progress in mitochondrial replacement therapies. Nat Rev Mol Cell Biol. 2018;19(2):71–2.
14. Mitalipov S, Amato P, Parry S, Falk MJ. Limitations of preimplantation genetic diagnosis for mitochondrial DNA diseases. Cell Rep. 2014;7(4):935–7.
15. Tang M, Guggilla RR, Gansemans Y, Van der Jeught M, Boel A, Popovic M, et al. Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants. Mol Hum Reprod. 2019;25(12):797–810.
16. Tachibana M, Sparman M, Sritanaudomchai H, Ma H, Clepper L, Woodward J, et al. Mitochondrial gene replacement in primate offspring and embryonic stem cells. Nature. 2009;461(7262):367–72.
17. Wang T, Sha H, Ji D, Zhang HL, Chen D, Cao Y, et al. Polar body genome transfer for preventing the transmission of inherited mitochondrial diseases. Cell. 2014;157(7):1591–604.
18. Ma H, Van Dyken C, Darby H, Mikhalchenko A, Marti-Gutierrez N, Koski A, et al. Germline transmission of donor, maternal and paternal mtDNA in primates. Human reproduction (Oxford, England). 2021;36(2):493–505.
19. Zhang J, Liu H. Cytoplasm replacement following germinal vesicle transfer restores meiotic maturation and spindle assembly in meiotically arrested oocytes. Reprod Biomed Online. 2015;31(1):71–8.
20. Zhang J, Wang CW, Krey L, Liu H, Meng L, Blaszczyk A, et al. In vitro maturation of human preovulatory oocytes reconstructed by germinal vesicle transfer. Fertil Steril. 1999;71(4):726–31.
21. Ma H, O'Neil RC, Marti Gutierrez N, Hariharan M, Zhang ZZ, He Y, et al. Functional Human oocytes generated by transfer of polar body genomes. Cell Stem Cell. 2017;20(1):112–9.
22. Zhang SP, Lu CF, Gong F, Xie PY, Hu L, Zhang SJ, et al. Polar body transfer restores the developmental potential of oocytes to blastocyst stage in a case of repeated embryo fragmentation. J Assist Reprod Genet. 2017;34(5):563–71.
23. Craven L, Tuppen HA, Greggains GD, Harbottle SJ, Murphy JL, Cree LM, et al. Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. Nature. 2010;465(7294):82-U9.
24. Hyslop LA, Blakeley P, Craven L, Richardson J, Fogarty NM, Fragouli E, et al. Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. Nature. 2016;534(7607):383–6.
25. Kang E, Wu J, Gutierrez NM, Koski A, Tippner-Hedges R, Agaronyan K, et al. Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations. Nature. 2016;540(7632):270–5.
26. Zhang J, Zhuang GL, Zeng Y, Grifo J, Acosta C, Shu YM, et al. Pregnancy derived from human zygote pronuclear transfer in a patient who had arrested embryos after IVF. Reprod Biomed Online. 2016;33(4):529–33.
27. Wolf DP, Mitalipov N, Mitalipov S. Mitochondrial replacement therapy in reproductive medicine. Trends Mol Med. 2015;21(2):68–76.
28. Cardona Barberan A, Boel A, Vanden Meerschaut F, Stoop D, Heindryckx B. Diagnosis and treatment of male infertility-related fertilization failure. Journal of Clinical Medicine. 2020;9(12):3899.
29. Sang Q, Zhou Z, Mu J, Wang L. Genetic factors as potential molecular markers of human oocyte and embryo quality. J Assist Reprod Genet. 2021;38(5):993–1002.
30. Bonte D, Ferrer-Buitrago M, Dhaenens L, Popovic M, Thys V, De Croo I, et al. Assisted oocyte activation significantly increases

fertilization and pregnancy outcome in patients with low and total failed fertilization after intracytoplasmic sperm injection: a 17-year retrospective study. Fertil Steril. 2019;112(2):266–74.
31. VandenMeerschaut F, Nikiforaki D, De Gheselle S, Dullaerts V, Van den Abbeel E, Gerris J, et al. Assisted oocyte activation is not beneficial for all patients with a suspected oocyte-related activation deficiency. Hum Reprod. 2012;27(7):1977–84.
32. Combelles CM, Morozumi K, Yanagimachi R, Zhu L, Fox JH, Racowsky C. Diagnosing cellular defects in an unexplained case of total fertilization failure. Human reproduction (Oxford, England). 2010;25(7):1666–71.
33. Yang X, Shu L, Cai L, Sun X, Cui Y, Liu J. Homozygous missense mutation Arg207Cys in the WEE2 gene causes female infertility and fertilization failure. J Assist Reprod Genet. 2019;36(5):965–71.
34. Dai J, Zheng W, Dai C, Guo J, Lu C, Gong F, et al. New biallelic mutations in WEE2: expanding the spectrum of mutations that cause fertilization failure or poor fertilization. Fertil Steril. 2019;111(3):510–8.
35. Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science. 1988;242(4884):1427–30.
36. Jacobs LJ, de Wert G, Geraedts JP, de Coo IF, Smeets HJ. The transmission of OXPHOS disease and methods to prevent this. Hum Reprod Update. 2006;12(2):119–36.
37. Van Landuyt L, Verpoest W, Verheyen G, De Vos A, Van de Velde H, Liebaers I, et al. Closed blastocyst vitrification of biopsied embryos: evaluation of 100 consecutive warming cycles. Hum Reprod. 2011;26(2):316–22.
38. Heindryckx B, De Gheselle S, Gerris J, Dhont M, De Sutter P. Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection. Reprod Biomed Online. 2008;17(5):662–8.
39. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. Toward reproductive certainty: Fertility and genetics beyond 1999 ed. London: Parthenon Publishing 1999;378–388.
40. Heindryckx B, Van der Elst J, De Sutter P, Dhont M. Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. Hum Reprod. 2005;20(8):2237–41.
41. Bonte D, Thys V, De Sutter P, Boel A, Leybaert L, Heindryckx B. Vitrification negatively affects the Ca(2+)-releasing and activation potential of mouse oocytes, but vitrified oocytes are potentially useful for diagnostic purposes. Reprod Biomed Online. 2020;40(1):13–25.
42. De Leeneer K, Hellemans J, Steyaert W, Lefever S, Vereecke I, Debals E, et al. Flexible, scalable, and efficient targeted resequencing on a benchtop sequencer for variant detection in clinical practice. Hum Mutat. 2015;36(3):379–87.
43. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16):2078–9.
44. Breese MR, Liu Y. NGSUtils: a software suite for analyzing and manipulating next-generation sequencing datasets. Bioinformatics. 2013;29(4):494–6.
45. Tytgat O, Tang MX, van Snippenberg W, Boel A, Guggilla RR, Gansemans Y, et al. Digital polymerase chain reaction for assessment of mutant mitochondrial carry-over after nuclear transfer for In Vitro Fertilization. Clin Chem. 2021;67(7):968–76.
46. Newman NJ, Lott MT, Wallace DC. The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation. Am J Ophthalmol. 1991;111(6):750–62.
47. Puomila A, Viitanen T, Savontaus ML, Nikoskelainen E, Huoponen K. Segregation of the ND4/11778 and the ND1/3460 mutations in four heteroplasmic LHON families. J Neurol Sci. 2002;205(1):41–5.
48. Bianco A, Bisceglia L, Trerotoli P, Russo L, D'Agruma L, Guerriero S, et al. Leber's hereditary optic neuropathy (LHON) in an Apulian cohort of subjects. Acta Myol. 2017;36(3):163–77.
49. Yamada M, Emmanuele V, Sanchez-Quintero MJ, Sun B, Lallos G, Paull D, et al. Genetic drift can compromise mitochondrial replacement by nuclear transfer in human oocytes. Cell Stem Cell. 2016;18(6):749–54.
50. Wu K, Zhong C, Chen T, Zhang X, Tao W, Zhang J, et al. Polar bodies are efficient donors for reconstruction of human embryos for potential mitochondrial replacement therapy. Cell Res. 2017;27(8):1069–72.
51. Pickett SJ, Blain A, Ng YS, Wilson IJ, Taylor RW, McFarland R, et al. Mitochondrial donation - which women could benefit? N Engl J Med. 2019;380(20):1971–2.
52. Gorman GS, Grady JP, Turnbull DM. Mitochondrial donation–how many women could benefit? N Engl J Med. 2015;372(9):885–7.
53. Bhattacharya S, Maheshwari A, Mollison J. Factors associated with failed treatment: an analysis of 121,744 women embarking on their first IVF cycles. PLoS One. 2013;8(12):e82249.
54. Yan Z, Fan Y, Wang F, Yan Z, Li M, Ouyang J, et al. Novel mutations in PLCZ1 cause male infertility due to fertilization failure or poor fertilization. Hum Reprod. 2020;35(2):472–81.
55. Dai J, Zheng W, Dai C, Guo J, Lu C, Gong F, et al. New biallelic mutations in WEE2: expanding the spectrum of mutations that cause fertilization failure or poor fertilization. Fertil Steril. 2019;111(3):510–8.
56. Yang X, Shu L, Cai L, Sun X, Cui Y, Liu J. Homozygous missense mutation Arg207Cys in the WEE2 gene causes female infertility and fertilization failure. J Assist Reprod Genet. 2019;36(5):965–71.
57. Reynier P, May-Panloup P, Chretien MF, Morgan CJ, Jean M, Savagner F, et al. Mitochondrial DNA content affects the fertilizability of human oocytes. Mol Hum Reprod. 2001;7(5):425–9.
58. Tang M, Popovic M, Stamatiadis P, Van der Jeught M, Van Coster R, Deforce D, et al. Germline nuclear transfer in mice may rescue poor embryo development associated with advanced maternal age and early embryo arrest. Hum Reprod. 2020;35(7):1562–77.
59. Costa-Borges N, Spath K, Miguel-Escalada I, Mestres E, Balmaseda R, Serafin A, et al. Maternal spindle transfer overcomes embryo developmental arrest caused by ooplasmic defects in mice. Elife. 2020;9.